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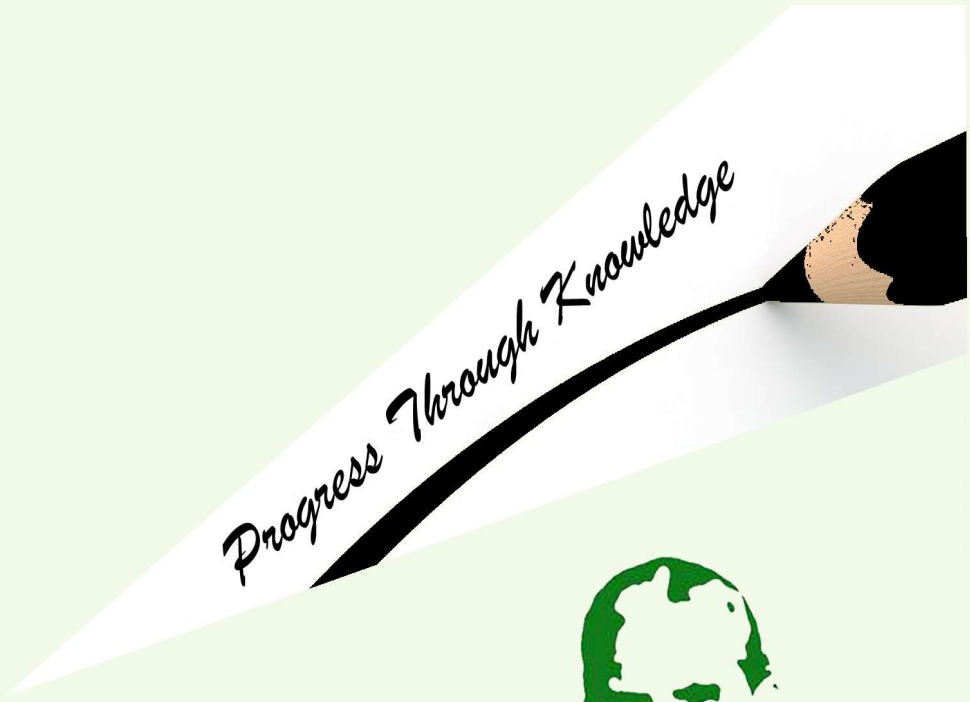

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EDITORIAL

Sleep: An Undervalued Privilege in the Contemporary Lifestyle

Muhammad Iqbal Afridi

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Sleep is an essential part of our daily routine. We spend about one-third of our lives sleeping. Quality sleep, which is of appropriate duration and at proper time, is equally fundamental to survival as food and water. Without sleep, our brain cannot form or maintain pathways for learning and creating new memories, and it becomes difficult to concentrate and respond quickly.

Sleep is important to a number of brain functions, including neuronal communication. Remarkably, our brain and body stay active while asleep. Recent findings suggest that sleep plays a housekeeping role that removes toxins in the brain that build up while we are awake. The sleeping brain has been shown to remove metabolic waste products at a faster rate than during an awake state.[1] It is further theorized that sleep helps facilitate the synthesis of molecules that help repair and protect the brain from these harmful elements generated during waking.[2]

Every individual needs sleep, and its diverse biological functions and mechanisms remain a paradox. Sleep affects almost every type of tissue and system in the body--from the brain, heart, and lungs to metabolism, immune function, mood, and cognition. Research shows that a chronic lack of sleep, or getting poor quality sleep, increases the risk of disorders including high blood pressure, cardiovascular disease, diabetes, depression, and obesity.[3]

Sleep architecture refers to the basic structural organization of normal sleep, and consists of two types of sleep, non-rapid eye-movement (NREM) sleep and rapid eye-movement (REM) sleep. NREM sleep is divided into stages 1, 2, 3, and 4, representing a continuum of relative depth. Each has unique characteristics including variations in brain wave patterns, eye movements, and muscle tone. Over the course of a period of sleep, NREM and REM sleep alternate cyclically. The function of alternations between these two types of sleep is not yet understood, but irregular cycling and/or absent sleep stages are associated with sleep disorders.[4]

An episode of sleep commences with a short period of NREM stage 1 progressing through stage 2, followed by stages 3 and 4 and finally to REM. However, individuals do not remain in REM sleep for the remainder of the night, but rather cycle between stages of NREM and REM throughout the night. NREM sleep constitutes about 75 to 80 percent of total time spent in sleep, and REM sleep constitutes the remaining 20 to 25 percent. The average length of the first NREM-REM sleep cycle is 70 to 100 minutes. The second, and later, cycles are longer lasting—approximately 90 to 120 minutes.[5] In normal adults, REM sleep increases as the night progresses and is longest in the last one-third of the sleep episode. As the sleep episode progresses, stage 2 begins to account for the majority of NREM sleep, and stages 3 and 4 may sometimes altogether disappear.

There is a great need to understand the physiology of sleep in the light of mental and behavioural disorders especially depression.

Advancing age is related to constant and considerable changes in Sleep Architecture. From infancy to adulthood, there are noticeable changes in how sleep is initiated and sustained, the percentage of time spent in each stage of sleep, and overall sleep efficiency (i.e., how successfully sleep is initiated and maintained). Sleep efficiency declines with age. Even though, its consequences are well documented, but the reasons are complex and poorly understood.

Most of us are surrounded by a culture that glorifies hectic lifestyles and task saturation. Thus, the lack of sleep has become a prevalent problem in modern society, affecting many individuals at some point in their lives.

Sleep deprivation occurs when an individual gets less sleep than needed to feel awake and alert. People differ in how little sleep is needed to be considered sleep-

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deprived. Some people, such as older adults seem to be more resistant to the effects of sleep deprivation, whereas others, especially children and young adults, are more vulnerable.

While occasional sleep interruptions are generally no more than a headache, but ongoing lack of sleep can result in excessive daytime sleepiness, emotional difficulties, poor job performance, obesity, and a reduced perception of quality of life.

While there is no doubt regarding the importance of restorative sleep, attention and emphasis is necessary to both manage and prevent sleep deficit. In 2015, the National Sleep Foundation (NSF) of the United States stated that there is a wide variation in durations of sleep required by different age groups[6] such as, newborns require 14-17 hours of sleep, teenagers (14 -17 years) require 8 to 10 hours of sleep and Adults (18-64 years) require 7 to 9 hours of sleep.

Quality of sleep is associated with the activity of pineal glands, also known as the third eye, that starts functioning according to the circadian rhythm, by releasing melatonin at 9:00 p.m. Due to our hectic schedules, sleeping at this time may not be possible for most of us which is why sleeping between 10 p.m. to 6 a.m. is highly recommended, as the quality of sleep is better during these hours.

Certain people including the prolific inventor Thomas Edison, consider sleep as wasted time, and deliberately deprive themselves of sleep to pursue entertainment activities, educational goals, or monetary interests. This intentional sleep deprivation is mostly seen in teenagers and young adults. However, others may unintentionally lose sleep because of shift work, family obligations, or demanding jobs especially medical students[7]. According to our local research, 33.5% medical students had insomnia[8].

Not all cases of sleep deprivation are voluntary. Insomnia, sleep apnoea, restless legs syndrome, night terrors, sleepwalking, and other medical problems, such as emotional disorders, hormonal imbalances, and chronic illnesses can also affect sleep. These conditions can be assessed and treated through consultation with a primary care physician and Sleep-medicine specialist. The treatment is mainly based on behavioural and cognitive measures, and pharmacotherapy. When non-medicinal treatment is ineffective, drugs are prescribed to induce sleep. There is a wide range of available options, which include benzodiazepines, non-benzodiazepine hypnotics, and melatonin receptor antagonists.

The good news is that most of the negative effects of sleep deprivation can be reversed with sufficient sleep. The treatment is to satisfy the biological sleep requirement, prevent deprivation and pay back accumulated sleep debt. Improved sleep hygiene can prevent sleep loss and also improve the quality of sleep. Good sleep hygiene involves:

- going to bed when tired,
- following a sleep schedule, even on weekends,
- avoiding eating meals and consuming caffeine or nicotine 2 to 3 hours before bedtime,
- if unable to fall asleep after 20 minutes of trying, going to another room and trying to read until feeling sleepy, then returning to bed,
- exercising daily,
- keeping the bedroom quiet, dark, and at a comfortably cool temperature,
- sleeping on a comfortable and supportive mattress and pillow,
- turning off electronic devices (televisions, tablets, smartphones, laptops, etc.) in the bedroom, especially blue-light emitting screens at least an hour before bed-time,
- maintaining a sleep diary or using smart technology, such as smartphone apps, bedside monitors, and wearable items (including bracelets, smart watches, and headbands) for monitoring sleep, and
- using apps and devices that make white noise, produce light that stimulates melatonin production, and use gentle vibrations to help us sleep and wake.

Repeatedly worrying and discussing loss of sleep is not fruitful. Along with nutrition and exercise, sleep is one of the pillars of health. We simply cannot achieve optimal health without taking care of our sleep. Sleep deprivation is a predominant issue, but it is very much treatable. Treating sleep as a priority, rather than luxury, is an important step towards preventing a number of chronic medical conditions. The cost of poor sleep is much greater than many people understand, as it may have profound consequences for our health in the long run. Thus, we need to become a society that values rest as much as we regard work and efficiency. As more of us prioritize sleep in our lives, this will slowly change, and optimize not only our health but also our productivity. So be part of the revolution and get adequate sleep today.

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Illustrations (Figures)

Figures should be either professionally drawn and photographed, or submitted as photographic quality digital prints. In addition to requiring a version of the figures suitable for printing, some journals now ask authors for electronic files of figures in a format (e.g., JPEG or GIF) that will produce high quality images in the web version of the journal; authors should review the images of such files on a computer screen before submitting them, to be sure they meet their own quality standard.

For x-ray films, scans, and other diagnostic images, as well as pictures of pathology specimens or photomicrographs, send sharp, glossy, black-and-white or color photographic prints, usually 127 x 173 mm (5 x 7 inches). Although some journals redraw figures, many do not. Letters, numbers, and symbols on Figures should therefore be clear and even throughout, and of sufficient size that when reduced for publication each item will still be legible. Figures should be made as self-explanatory as possible, since many will be used directly in slide presentations. Titles and detailed explanations belong in the legends, however, not on the illustrations themselves

(*Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication*)

ORIGINAL ARTICLE

Determination of Phenolic Compounds in *Callistemon Viminalis* L. and Its Role in Amelioration of Hyperglycemia in Alloxan-Induced Diabetic Rats

Khawaja Zafar Ahmed[1], Sadaf Naeem[1] and Assra Noor[2]

ABSTRACT

Objectives: The objective of the present study was to investigate the total contents and antidiabetic potential of Bottle Brush Callistemon viminalis L. leaves in alloxan-induced diabetic rats.

Methodology: The phenolic content of aqueous: methanolic extract of C. viminalis leaves were determined by High Performance Liquid Chromatography (HPLC) technique. The hypoglycemic activity of the aqueous: methanolic leaf extract of C. viminalis was investigated in alloxan-induced diabetic rats. For this purpose, aqueous leaf extract of C. viminalis at two different doses—200 mg/kg and 400 mg/kg—was orally administered after induction of diabetes mellitus with 150mg/kg alloxan monohydrate for seven days. Random blood glucose levels were measured before and after treatment of C. viminalis leaf extract for determining the acute hypoglycemic activity.

Results: Significant ($P<0.001$) dose dependent reduced blood glucose levels were achieved after 7 days of 200 and 400 mg/kg/day C. viminalis treatment in diabetic rats. HPLC results also suggested that C. viminalis is a potential source of flavonoidal compounds with antidiabetic activity.

Conclusion: It could be deduced from results obtained in this study that methanolic and flavonoid-rich leaf extracts of C. viminalis have antidiabetic potential in alloxan-induced diabetic rats.

Key words: Antidiabetic activity, C.viminalis *L*., flavonoids, alloxan, hypoglycemic effect

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عنوان: کیلسٹیمن ویمینالس (Callistemon Viminalis) میں فینولک (Phenolic) اجزاء کی مقدار معلوم کرنا اور ذیابیطس شدہ چوہوں میں Amelloratin کا عمل دخل۔

مقصد: اس تحقیق کا مقصد Callistemon Viminalis کے پتّے میں ذیابیطس کو ختم کرنے کی صلاحیت alloxan شدہ ذیابیطس چوہوں میں دریافت کرنا ہے۔

طریقہ: مائع میں فینولک کی مقدار: High Performance Liquid Chromatography طریقہ سے C.Viminalis کے پتّے سے methanolic لگایا گیا۔ مائع میں شوگر زیادہ مقدار کا عمل alloxan شدہ ذیابیطس چوہوں میں C.Viminalis پتّے کے مائع کا جائزہ لیا گیا۔ اس مقصد کے لئے C.Viminalis پتّے کے مائع کے دو طرح کی خوراک 200mg/kg اور 400mg/kg کو سات دنوں تک چوہوں کو منھ کے ذریعہ پلائی گئی۔ اس سے پہلے چوہوں میں ذیابیطس پیدہ کرنے کے لئے 150mg/kg کی alloxan monohydrate کی خوراک دی گئی۔ شوگر کی مقدار معلوم کرنے کے لئے اوپر لکھے خوراکوں کے دینے سے پہلے اور بعد میں خون کے نمونے لئے گئے۔

نتیجہ: خوراک میں شوگر کی کم مقدار سات دنوں بعد پر ہوئی۔ HPLC نے یہ بھی دکھایا کہ C.Viminalis کے پتّے میں ذیابیطس کم کرنے کی صلاحیت موجود ہے۔

حاصلِ مطالعہ: C.Viminalis کے methonolic اور flavonoid-rich پتّے کے مائع سے ذیابیطس کم کیا جاسکتا ہے۔

INTRODUCTION

Diabetes Mellitus (DM) is described as hyperglycemia, resulting from defects in insulin regulation that further lead to multiple anomalies including haemodynamic and metabolic abnormalities. This disease exists worldwide and is increasing at an escalating rate. The etiology of diabetes mellitus is unknown, however both hereditary and non-hereditary elements like obesity and lifestyle seem to play a part[1]. According to World Health Organization (WHO), by the year 2025, the population of diabetic patients will likely have increased above 300 million or more which will be a burden on public health organizations[2]. Hyperglycemia may result in an increased glucose oxidation and lipid peroxidation that may result in free-radical formation and oxidative degeneration[3]. Increased formation of free radicals

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such as reactive nitrogen and oxygen species (ROS, NOS), in turn, increases vascular complications of diabetes[4,5]. Several epidemiological studies have demonstrated that diabetes-induced oxidative stress is thought to play a vital role in the genesis of many irreversible complications such as coronary artery disease, neuropathy, nephropathy, retinopathy, stroke, cellular hypertrophy, and hyperplasia[6,7].

Oral hypoglycemic agents and insulin are the treatment options for hyperglycemia but these drugs have several adverse effects which may complicate the disease state[8]. Medicinal plants extend to yield worthy therapeutic agents, in both traditional systems and have always been a major concern for the treatment of different diseases as safer and most effective medicines[9]. Previous researchers have revealed that plant-derived polyphenols have greater anti-oxidant properties and have a role in the prevention of different degenerative diseases like diabetes mellitus, cardiovascular diseases, and malignancies. Plants that contain polyphenols are found to be effective against the inhibitory activities of á-glucosidase and aldose reductase, therefore they could be the alternative option for the development of a new class of probably safer antidiabetic agents[10, 11]. C. viminalis (Bottle brush) is a small flowering shrub that belongs to family Myrtaceae, used as a herbal tonic, tea, and health food in folk medicine[12,13]. Antioxidant, anti-inflammatory, hypolipidemic and antibacterial activities of the various parts of C. viminalis have been reported because of its phenolic, terpinoidal, and flavonoidal contents like 1,8-cineole and alpha-pinene[14]. In Pakistan, it has also been used to prepare a hot drink locally referred to as "tea" for the treatment of gastroenteritis, diarrhoea, and skin infections. However, there is no previous report on its anti diabetic activity with aqueous: methanolic C. viminalis leaf extract. Petroleum ether extract of C. viminalis plant have been used in only one previous research to explore its hypoglycemic effect[15]. In the light of evidence provided by the previous researches, we have designed the present study to explore the total phenolic contents in the aqueous: methanolic extract of the C. viminalis L. plant leaves and its hypoglycemic effect on alloxan-induced (150mg/kg) diabetic rats.

METHODOLOGY

Fresh leaves of the plant C. viminalis were procured from the botanical garden of the Department of Botany, University of Agriculture, Faisalabad, in the month of April 2013. The identity of the plant was confirmed by the taxonomist of the same University. Leaves were washed with distilled water and then dried in shade. Once dry, the leaves were ground into fine powder which was soaked for seven days in 3L of methanol: water (70:30) solvent in a sealed closed container. After seven days of soaking, this powder was filtered twice through a muslin cloth after which, Whatman#1 filtered paper was used for further filtration. Total solvent was evaporated from extract with the help of rotary evaporator to get a syrupy consistency. The residue was dried over anhydrous sodium sulphate to take away traces of alcohol and was kept in the refrigerator for further study[17,18].

Alloxan monohydrate was purchased from Sigma Aldrich, Limited, while the chemicals for plant extraction like methanol and n-hexane of analytical grade were purchased from Merck, Limited. Glibenclamide (Daonil) 5mg tablets were procured from Sanofi Aventis, Pakistan, Limited.

Aqueous: methanolic extract of C. viminalis leaves was tested for the presence of chemical components like terpenoids, alkaloids, saponins, tannins, flavonoids, and anthraquinon by standard procedure[17].

The phenolic contents of aqueous: methanolic extract of C. viminalis leaves were determined using HPLC technique. The extract was liquefied in methanol (24ml) and homogenized with homogenizer. Dilution of homogenized extract was made with addition of 10 ml of 6 molar HCl. After that, the mixture was thermostated for two hours on 95°C, then the solution was filtered with nylon membrane before examination. HPLC with Photodiode Array (PDA) detection (SHIMADZU Class VP system) analysis was carried out on an Inertsil ODS-3 (GL-Science, 5 µm, 4.6 × 250 mm) column (C-18) with oven temperature at 40 °C. The C. viminalis leaf extract was eluted at a flow rate of 1 ml/min with a mobile phase gradient consisting of a mixture of solvent A (H2O:methanol:1,4-dioxane, 50:15:35) and solvent B (acetonitrile:methanol:1,4-dioxane, 50:15:35) (0 min, 80:20; 20 min, 60:40; 25 min, 15:85; 25–30 min, 0:100; >30 min, 80:20). Flavonoids and phenolic compounds were separated with the help of ultraviolet-visible HPLC detector range 280 nm. The peaks of C. viminalis leaves' flavonoidal contents like chlorogenic acid, quercetin, Gallic acid, and syringic acid was identified by comparing the "retention time" and concurrence of UV-visible spectra with corresponding standard solutions, respectively[19,20].

Adult Wistar rats (200-300 g) of either sex for experimental work were procured from the animal house of the University of Agriculture, Faisalabad. The experimental Wistar rats were retained at normal room temperature and were handled as per the specifications provided by Helsinki Resolution. The animals were kept in typical metallic cages in animal house and provided adequate food and water ad libitum[18].

Intraperitoneal injection of aqueous alloxan monohydrate at a dose of 150 mg/kg of body weight was given for producing diabetes in adult Wistar rats[18]. Before administration of alloxan (150 mg/kg), the blood glucose levels of each rat were checked and animals were then kept fasting up to 12 hours with free access to drinking water. The animals were kept under observation and after 72 hours, they were tested for blood glucose levels to confirm the development of diabetes using Accu-Chek strips from Roche, Switzerland[18]. Blood glucose levels above 200 mg/dl were supposed to be diabetic, all animals having above 200 mg/dl glucose levels were separated and used for the present study[21].

Experimental rats (n=30) were equally divided into five groups, each group contained six rats. **Group I (Control):** This group received only distilled water (volume=10ml) by gavage technique[21]. **Group II (Diabetic Control):** This group was injected 120 mg/kg body weight alloxan in a single dose intraperitoneally and served as Diabetic Control (negative control). The rats in this group received only vehicle up to seven days. Rest of the animals were injected alloxan (120 mg/kg) according to body weight intraperitoneally, and allocated into three groups (six animals in each group) after one week of stabilization of diabetes. **Group III (Standard):** The first subgroup of alloxan-induced diabetic rats, treated with daily 0.5 mg/kg Glibenclamide (Daonil MR®), an oral hypoglycemic drug by gavage technique[22]. **Group IV (Treatment):** The second subgroup of diabetic rats received a daily dose of 200 mg/kg[23] of the aqueous: methanolic extract of the C. viminalis L. leaves by gavage technique. **Groups V (Treatment):** The third subgroup of diabetic rats received the aqueous: methanolic extract of C. viminalis L. leaves at a dose of 400 mg/kg daily by gavage technique[24].

After all drug administration, the rats in each of the groups were analyzed for alteration in random and fasting blood glucose levels and all groups were orally administered C. viminalis extract and standard drug (Daonil) up to one week. After a week of treatment, the animals were decapitated and by Accu Check® Glucometer, blood glucose levels were analyzed.

All the values were presented as Mean±S.D. Statistical analyses were performed by one-way ANOVA followed by post-hoc Tukey Multiple Comparison Test. Differences were considered statistically significant in relation to control and standard at the probability level of $p<0.05$.

RESULTS

Results of phytochemical test showed the presence of different kinds of phyto-constituents in aqueous: methanolic (30:70) extract of C. viminalis leaves. Preliminary Phytochemical Analysis confirmed the presence of different chemical constituent in the leaves as shown in Table 1.

Table 1: Preliminary Phytochemistry of Aqueous: Methanolic Extract of Callestimon Viminalis Leaves (Bottle Brush)

Phytochemicals	Name of Phytochemical Test	Aqueous: Methanolic Extract of C. Viminalis L.
Alkaloids	Mayers & Dragendorff's	+
Glycosides	Keller Kilani test	–
Saponns	Foam test	++
Taninis	Ferric chloride test	++
Flavonoids	Shinoda's andZn-HCl test	++

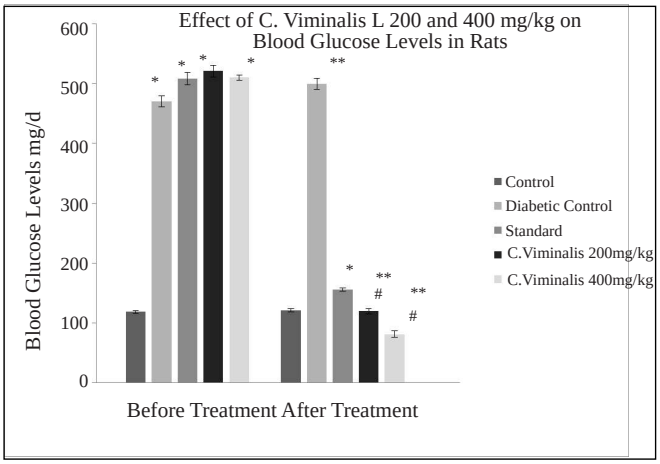
The amount of flavonoid extracted from fresh leaves of C. viminalis was shown in Table 2 which shows that the aqueous: methanolic extract contains flavonoids like chlorogenic acid (162.3), quercetin (22.4), gallic acid (21.3), syringic acid (14.15), and furulic acid (13.8).

Table 2. Flavonoids of C. Viminalis Leaves' Aqueous: Methanolic (30:70) Extract

Compound Name	Retention Time	Area%	Quantity (ppm)
Chlorogenic acid	2.653	6.1	162.3
Quercetin	2.987	5.9	22.4
Gallic acid	4.707	9.1	21.3
Syringic Acid	16.380	8.7	14.15
Ferulic acid	22.827	3.0	13.8

Graph 1 show the effects of C. viminalis leaves' aqueous: methanolic extract on blood glucose levels in diabetic rats after seven days of diabetes induction. Administration of alloxan (150 mg/kg, i.p.) led to 1.5-fold elevation of fasting blood glucose levels (510±6.75, 502±4.28), which was maintained over a period of one week. One week of daily treatment with C. viminalis leaves' aqueous: methanolic extract at 200 and 400 mg/kg/day led to a dose-dependent fall in blood sugar levels (121±2.26, 83.60±1.33) respectively.

As revealed by post-hoc Tukey's analysis, both doses (200 mg/kg and 400 mg/kg) of C. viminalis leaves' extract exhibited highly significant ($p< 0.001$) hypoglycemic effects in lowering the blood glucose level after seven days of treatment interval as compared to diabetic control. While 400 mg/kg/day dose of C. viminalis extract exhibited highly significant values ($P<0.002$) compared to the 200 mg/kg/day dose.



Graph 1. Hypoglycemic Effect of the Aqueous:Methanolic (30:70) Extract of the Callistemon Viminalis L. Leaves on Diabetic Rats

Values are expressed as Mean±S.D; n=10; * p<0.05, ** p<0.01, *** p<0.001 (as compared to Normal Control) # p<0.05, ## p<0.01, ### p<0.001 (as compared to Standard).

DISCUSSION

Aqueous: methanolic extract of C. viminalis (Family: Myrtacea) leaves showed presence of different phenolic compounds. Table 2 showed HPLC analysis of C. viminalis leaves that suggested presence of high percentage of flavonoidal contents like quercitin, gallic acid, syringic acid, ferulic acid, and chlorogenic acid. Our results are in accordance with previous researches that found same flavonoidal contents and phytochemicals in their studies[25,14]. Flavonoids possess antioxidant and antidiabetic activity. They have scavenging ability for superoxide radicals and exhibit antioxidant, analgesic, and antidiabetic activities by reducing oxidative stress[26].

Several researches have suggested that polyphenols, especially flavonoids, could be a better option for the management of diabetes mellitus and its complications due to their antioxidant value[27,28]. The current study confirms the availability of flavonoidal contents in C. viminalis leaf extract and suggests presence of flavonoidal contents exhibiting anti diabetic effect. Stress, hypertension, and imbalanced diet may produce reactive species in human body[29] that may produce imbalance between the free radicals (ROS/NOS) and antioxidants, thus producing 'oxidative stress' which may be responsible for producing certain pathological conditions including diabetes[30]. Oxidative stress induces diabetes mellitus by producing variations in enzymatic systems, lipid peroxidation, impaired glutathione metabolism, and decreased vitamin C levels[31]. Studies have shown that antioxidants are able to improve insulin action and can decrease the risk of diabetes mellitus[32]. Sarian et al[33] reported that flavonoids exhibit antioxidant property through which they exert hypoglycemic effect and enhance glucose metabolism and uptake, so these could be considered as a chief alternative in reducing blood glucose levels in diabetes mellitus and associated vascular complications. Results of the present study were in accordance with previous studies that explained the presence of flavonoids, tannins, saponins and phenolic compounds in the leaves of C. viminalis, which are considered as bioactive constituents in the management of diabetes[16,34]. C. viminalis leaves' extract at doses of 200 and 400 mg/kg produced significant ($p<0.05$) reductions in blood glucose levels after seven days of treatment. However, the hypoglycemic effect was dose dependent and similar to standard hypoglycemic drug Glibenclamide. C. viminalis extract has been reported as a potent antioxidant for treating antibacterial infections and other inflammatory disorders so, due to its antioxidant and scavenging property, we suggest it would be a potent neutraceutical for treating diabetes and its associated complications.

CONCLUSION

The phytochemical analysis showed that the extract of Callestemon viminalis L. plant leaves contains a mixture of phytochemicals as alkaloids, saponins, tannins, and flavonoids. The quantitative HPLC assay indicated that the plant extract has potential antioxidant agents which can be an excellent option for biological and chemical activities and can be further subjected for the isolation of the therapeutically active compounds. C. viminalis plant must be considered as an excellent candidate for future studies on diabetes mellitus. We suggest that the antidiabetic potential of methanolic extract of C. viminalis is due to the presence of flavonoid. Further in vivo studies are required to substantiate our findings.

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ORIGINAL ARTICLE

Evaluation of Antidepressant Like Effect After Single Dose Administration of Escitalopram and Amitriptyline in Rats: A Comparative Study

Humaira Anser[1], Sadaf Naeem[1], Humera Khatoon[2], Saira Saeed[3], Fouzia Israr[1], Shehlla Imam[1], Saad Sheikh[1] and Bushra Abdul Ghafoor[1]

ABSTRACT

Objective: The objective of the study was to evaluate the antidepressant effect of different doses of amitryptyline and escitalopram in animal depression model after acute administration.
Methodology: We assessed the acute effects of different therapeutic doses of two antidepressant drugs amitryptyline and escitalopram on swimming using forced swim test in healthy rats. Five groups of rats were administered distilled water, amitryptyline (50 mg), amitryptyline (100 mg), escitalopram (10 mg), and escitalopram (20 mg) before 60 minutes of performance. The swimming time was observed in all groups.
Results: The study showed highly significant ($p<0.001$) increase in swimming time in amitryptyline 100 mg treated group as compared to control. The amitryptyline 50 mg treated group also showed an insignificant increase in swimming time as compared to control. However, it was also superior to other groups. Insignificant increases in swimming time in animals treated with escitalopram 10 mg and 20 mg as compared to control were observed.
Conclusion: The study found that after 60 minutes of amitryptyline 100 mg, the swimming time was found significantly increased and thus can be effective in acute alleviation of symptoms in moderate to major depressed patients.

Key words: Selective serotonin reuptake inhibitors (SSRI), Tricyclic antidepressant (TCA), Forced swim test (FST)

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ایسیٹالوپریم اورایمیٹر پٹائیکن کی ایک خوراک کے چوہوں میں مانع افسردگی اثرات کا تجزیہ۔ایک تقابلی مطالعہ

خلاصہ: مقصد:اس مطالعہ کا مقصدایسیٹالوپریم اورایمیٹر پٹائیکن کی ایک خوراک کا جانوروں میں پائے جانے والے ڈپریشن پر ہونے والے مانع افسردگی اثرات کا جائزہ لینا تھا۔

طریقہ: اس مطالعے میں دو مانع افسردگی ادویات،ایسیٹالوپریم اورایمیٹر پٹائیکن ،کی مختلف خوراکوں کے اثرات کا صحتمند چوہوں پر جبری تیراکی کے امتحان کے ذریعے جائزہ لیا گیا۔چوہوں کے پانچ گروہوں کوصاف شدہ پانی, ایمیٹر پٹائیکن پچاس اورسولی گرام، ایسیٹالوپریم دس اوربیس ملی گرام دیے گئے جس کے بعدانہیں 60 منٹ کا امتحان کروایا گیا۔تمام گروہوں میں تیراکی کے وقت کا مشاہدہ کیا گیا۔

نتائج: کنٹرول گروہ کے مقابلے میں سولی گرام ایمیٹر پٹائیکن لینے والے گروہ کے تیراکی کے وقت میں بہت اہم اضافہ دیکھنے میں آیا۔ایمیٹر پٹائیکن پچاس ملی گرام لینے والے گروہ میں کنٹرول سے زیادہ مگرکم اہم اضافہ پایا گیا لیکن یہ اضافہ دیگر گروہوں سے بڑھ کر تھا۔کنٹرول کی نسبت کم اہم اضافہ ایسیٹالوپریم دس اوربیس ملی گرام لینے والے گروہوں میں بھی دیکھا گیا۔

حاصل مطالعہ: اس مطالعہ کے مطابق سولی گرام ایمیٹر پٹائیکن لینے کے ایک گھنٹے بعدتک تیراکی کے وقت میں بہت اہم اضافہ پایا گیا،لہذا ایمیٹر پٹائیکن کودرمیانے سے لے کرشدید درجے کے ڈپریشن کے مریضوں کی علامات کے علاج میں استعمال کیا جاسکتا ہے۔

INTRODUCTION

Affective disorders or mood disorders are a group of psychotic illnesses that include depressive disorder, anxiety disorder, bipolar disorder, schizophrenia,etc. Depression is one of the most debilitating illnesses that creates burden on the economy of a country[1]. Women are more prone to be affected by depression. The symptoms manifest at psychological, behavioural, and physiological levels[2]. Different hypotheses have been proposed by researchers to understand the

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pathophysiology of depression which include monoamine hypothesis, neurotrophic hypothesis, and neuroendocrinologic hypothesis[3].

Various antidepressants are available in the market with varying efficacy and indications. These include selective serotonin reuptake inhibitors (SSRI), serotonin and norepinephrine reuptake inhibitor (SNRI), tricyclic antidepressants (TCA), and monoamine oxidase inhibitor (MAOI) etc. Among these, SSRIs are the drugs of choice for treating moderate to major depressive disorders[4].

The majority of agents in SSRIs are serotonin transporter inhibitors only and have no affinity for norepinephrine transporters as well as dopamine transporters. Only some agents have negligible affinity for other receptors, for example fluoxetine. For this reason, they possess very limited number of adverse effects as compared to other antidepressant class agents, for example SNRIs and TCAs[4]. Escitalopram is considered superior to other SSRIs which is the S-enantiomer of citalopram[5]. The superiorityof escitalopram is due to its binding to the orthosteric binding site of serotonin transporter (SERT) as well as its affinity to the allosteric site of SERT[6] resulting in pronounced blockade of serotonin transporters. This gives the reason for efficacious effect of escitalopram as compared to citalopram.

Amitryptyline is an older TCA which was considered the mainstay and was used widely for the treatment of major depressive disorder many decades ago[6]. It is a mixed serotonin and noradrenaline reuptake inhibitor through inhibition of membrane transporter responsible for the reuptake of these amines. This effect underlies the antidepressant effect of amitryptyline,but due to its antagonistic action on various receptors like histamine, muscarinic cholinergic receptors and adrenergic receptors, it produces adverse effects which patients do not tolerate[7].

The antidepressant effects of all drugs mentioned above are produced after chronic administration (more than four weeks of administration), therefore the purpose of this study is to evaluate the acute antidepressant-like effect of different therapeutic doses of escitalopram (SSRI) and amitriptyline (TCA) after 60 minutes of drug administration, performing despair swim test using rats as test animals for assay. The antidepressant effect of these drugs is usually observed after chronic dosing (approximately four weeks of dosing).

METHODOLOGY

Fifty albino rats of 150-180 gms of average body weight were randomly assigned into five groups of ten animals each with equal sex distribution. Group-I served as a control group. Other four groups of animals are as follows:

Group-II served as treated for amitryptyline 50 mg. Group-III served as treated for amitryptyline 100 mg. Group-IV served as treated for escitalopram 10 mg, Group-V served as treated for escitalopram 20 mg. For behavioral analysis of animals, specifications of Helsinki resolution 1964 were followed.

Albino rats were kept in acrylic home cages, at 22+3^{o}C room temperature with 12-hour light and 12-hour dark cycle at the Department of Pharmacology, Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University. Rodents had access to water and food ad libitum. They were housed two per cage under standard conditions. The animals were deprived of food overnight before the test.

Escitalopram manufactured by Hilton Pharma (Pvt Limited), and amitryptyline manufactured by OBS Pharma (Pvt Limited) were purchased from a local pharmacy in Karachi. All the drugs were administered orally. A fixed-dose study was done, escitalopram 10 mg/day and 20 mg/day and amitryptyline 50mg/day and 100mg/day were compared with control group. The doses were calculated based on the rodents' body weight. Feeding tube was used for dosing the rats. All drugs were dissolved in distilled water. Two different potencies of each drug were chosen in order to compare their antidepressant-like effect after 60 minutes of dosing. After 60 minutes, every rat was introduced in the swimming tank and was allowed to swim till it started floating on the surface of water, with minor movements that helped the rat in floating. Dose calculation of amitryptyline: 50/60 x0.15= 0.125 mg, 100/60 x0.15= 0.25 mg; Dose calculation of escitalopram:10/60 x0.15= 0.025 mg , 20/60 x0.15 = 0.05 mg

Forced Swim Test (FST) was performed in an acrylic glass cylinder of 20 cm height with a diameter of 6 cm. Water was filled at a specific level of the apparatus (12 cm high). Water temperature was set at 25±2^{o}C. After 60 minutes of dosing of all the drugs[8], animals of both control and treated groups were placed into the cylinder separately and the struggling time was noted for five minutes. After each swimming session, the water was replaced to avoid bacterial and fecal contaminations[9].

Statistical analysis: Behavioural scores of the escitalopram, amitriptyline, and control groups have been expressed as a mean+standard deviation. Significant differences among the groups were assessed by one way ANOVA. The statistical analysis was

performed using Social Sciences version 20 (SPSS, Inc., Chicago, IL, USA). Post hoc analysis was done by using tuckey test. $p < 0.05$ was considered statistically significant.

RESULTS

Results showed significant differences in swimming time after acute administration of drug between and within groups (P = 0.002). Post-hoc test revealed insignificant increase in swimming time in animals treated with escitalopram 10 mg and 20 mg as compared to control after 60 minutes of dosing. Amitriptyline 50 mg treatment showed insignificant increase in swimming time as compared to control. However, Amitriptyline 100 mg treated group when compared with control showed highly significant increase in swimming time (p = 0.001). Moderately significant (p = 0.01) increase in swimming time was observed when amitriptyline 50 mg was compared with amitriptyline 100 mg treated group. Amitriptyline 100 mg showed a moderately significant increase in swimming time (p = 0.01) when compared with escitalopram 10 mg and 20 mg.

Table1: Acute Effects of Drugs on Swimming Time in FST

Groups	Swimming time (Mean + SD)	P value
Control	5.30+ 2.92	0.002
Amitryptyline (50 mg)	5.68+2.06	
Amitryptyline (100 mg)	9.05+2.43***!!!##^^	
Escitalopram (10 mg)	6.10+2.42	
Escitalopram (20 mg)	6.35+ 0.445	

***p=0.001 highly significant difference as compared to control. !!!p=0.001, ## p=0.01, ^^ p=0.01 showed a significant difference as compared to amitryptyline 50 mg, escitalopram 10 mg, and escitalopram 20 mg respectively.

DISCUSSION

Forced Swim Test model is a valid tool for the assessment of antidepressant-like effect of various pharmacological agents mainly selective serotonin reuptake inhibitors, tricyclic antidepressants, monoamine oxidase inhibitors, etc. The rodents, when subjected to immersion and forced to swim, developed a state of behavioural despair or hopelessness and lowered mood, a common trait of depression in humans as well. The rodents, after struggling to escape, lost hope, became immobile, and only made moves that were required to help them in floating[10,11].

The antidepressants used as the standard in treatment of depression are SSRIs, TCAs, MAOI etc. All of them produce their antidepressant effects after four weeks of administration[12,13]. But in this study, we had observed the acute effects of two different therapeutic doses of escitalopram and amitryptyline after 60 minutes of administration. As per results of this study, after one-time administration of 100 mg adult dose of amitriptyline, the swimming time was found to be increased significantly. This effect could be attributed to the fact reported by Maj et al[14] that high doses of amitryptyline increase the level of dopamine in the nucleus accumbens which is attributed to its antidepressant-like effect in the FST. However amitryptyline is a most commonly used tricyclic antidepressant and a potent inhibitor of serotonin and noradrenaline reuptake. Amitryptyline immediately raises the levels of noradrenaline and serotonin leading to alleviation of anhedonia and hopelessness[15]. Antidepressant activity of amitriptyline is also supposed to be due to its more potent inhibition of serotonin reuptake and over all increase in serotonin neurotransmission.This possible mechanism was also supported by Sellinger-Barnette, Mendels, and Frazer[16] recently and reported serotonin-toxicity like behaviour in adult zebrafish after acute administration of different doses of amitriptyline.The study also reported dose-dependant increase in serotonin turnover (but not noradrenaline levels) in zebrafish's whole brain[16].

However, escitalopram, which is considered as first line agent in treatment of depression, showed insignificant results after single dose administration. Therefore, on the basis of severity of depression, other potent agents like amitryptyline, which is efficacious, can be used as first line agent[17].

CONCLUSION

This study concludes that amitryptyline can be given in case of acute depression in order to relieve the patient from depressive symptoms and then it may be substituted with safer antidepressant drugs like SSRIs to avoid its adverse effects on chronic use.

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(*Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication*)

ORIGINAL ARTICLE

Protective Efficacy of *Calotropis Procera* Leaf Hexane Extract Against Ibuprofen Induced Kidney Toxicity in Albino Rats

Abid Ali[1] and Tabassum Mahboob[2]

ABSTRACT

Objective: Plants are a natural source of medicinally important active ingredients especially phenols. In-vivo experiments were performed to determine the therapeutic ability of Hexane soluble fraction of *Calotropis procera* leaf against (NSAID) ibuprofen-induced nephrotoxicity in rat model.
Methodology: Nephrotoxicity was determined by evaluating renal function markers (urea and creatinine), plasma measure of enzymes SOD and CAT alongwith the determination of tissue lipid peroxidation markers, i.e. aldehyde products MDA and 4-HNE. Phytochemical analysis was also carried out to determine phenolic constituents in test plant.
Result: The imbalance in oxidative status was determined by evaluating decreased level of CAT, SOD, and GSH alongwith increased levels of MDA and 4-HNE, which was neutralized by the co-administration of *C. procera* hexane extract with ibuprofen. The cell sustainability was maintained and indicated nephro-protective activity of *C. procera*. This protective efficacy can be corelated with the enough quantity of phenolic compounds found in *C. procera* leaf.
Conclusion: This data is of immense value for the development of an effective therapeutic agent from natural resources.

Key words: NSAIDs, kidney, toxicity, nephrotoxicity, hexane extract, antioxidant, free radicals, ibuprofen, catalase, superoxide dismutase, glutathione, malonyldialdehyde, 4hydroxynonenal, lipid peroxidation, in vivo

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کیلوٹروپس پروسرا کے پتے کے نچوڑ سے آئی بی یو پروفن کے زہریلے مواد کے گردے میں داخلے میں کمی کا سفید چوہوں پر تجربہ

خلاصہ:

مقاصد: پودے طبی طور پر اہمیت رکھنے والے سرگرم اجزا خاص کر فینولز کا ایک قدرتی ذریعہ ہیں۔ کیلاٹروپس پروسرا کے پتے میں زہریلے مواد کی حوصلہ افزائی کی روک تھام کے لیے سفید چوہوں پر تجربہ کیا گیا۔

طریقہ کار: گردوں میں زہریلے اثرات کے تعین کا جائزہ لینے کے لیے یوریا اور کریٹینین کے پلازما کے اینزائمز ایس او ڈی اور سی اے ٹی کی ٹشو لیپڈ پر آکسیڈیشن کے ساتھ پیمائش کی گئی۔ تجرباتی پودے میں فینولک کونسٹی ٹیونٹس کا تعین کرنے کے لیے فائٹوکیمیکل تجزیہ بھی کیا گیا۔

نتائج: آکسی ڈیٹو صورت میں توازن کا تعین کرنے کے لیے سی اے ٹی اور ایس او ڈی اور جی ایس ایچ کی سطح کم کی گئی جبکہ ایم ڈی اے اور فوراچ این ای کی سطح بڑھائی گئی۔ خلیے کی مضبوطی کو برقرار رکھا گیا۔

خلاصہ: گردوں کی حفاظت کے لیے تیار کیے جانے والے قدرتی ذریعہ کے طور پر اس ڈیٹا کی بے حد اہمیت ہے۔

INTRODUCTION

Toxicity is the degree to which a substance is able to damage an organism. Toxicity can affect the whole organism or substructure of the organism and it may occur due to certain biological, physical, or chemical effects. Drug induced toxicity can damage any tissue depending on dosage such as acute dosage of a drug can produce toxicity for nervous system and its chronic exposure may cause serious injuries to other organs. Toxicity can also be produced by the medicines which

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are normally used for curative purposes. Sometimes, use of over-the-counter medicines and long term use of overdoses of drugs may also cause toxicity to certain specific organs. The process of oxidation continuously takes place in all aerobic living bodies, due to this ROS including O_2, H_2O_2 , OH and NO/NOO^- are constantly formed within the cells. The overproduction of these substances may cause oxidative load in the cells. This oxidative stress produces deleterious effects on cells of DNA, proteins, and lipids. Lipids are specifically more damaged due to the formation of lipid peroxidation products.

There are several substances which can initiate toxicity to the kidneys, called nephrotoxic agents. These substances include antibiotics, anticancer drugs, heavy metals, herbicides, pesticides, excess amount of uric acid, and long-term use and high doses of analgesics may also cause nephrotoxicity. These analgesics usually include aspirin and ibuprofen.

Likewise, NSAIDs are the commonly used over the counter drugs. These are pain relievers, help in reducing inflammation, and lower fever. They also prevent blood from clotting. Ibuprofen is selected for present experimental study, which is said to be an inhibitor of prostaglandin synthesis. The exact mechanism of action is still unknown. Ibuprofen is an inhibitor of an enzyme (cyclooxygenase). This enzyme converts arachidonic acid to prostaglandins. Prostaglandins are the initiators of inflammation, fever, and pain. There are two types of cyclo-oxygenase, one is COX-1 which protects the lining of the stomach from digestive chemicals and also maintains kidney function, whereas, COX-2 is released when joints are injured or inflamed[1].

Plants are a major source of traditional medicine. Even modern medicine depends on pharmacologically active agents from plants to obtain useful drugs. *Calotropis procera* (Aiton) R. Br. is a famous medicinal plant with bioactive molecules which may serve against various ailments. *C. procera* is usually known as "Aak" and treated as the member of Asclepiadaceae, Plant is widely found in Middle Eastern, African, and Asian countries[2]. *C. procera* is characterized by the presence of opposite and decussate leaves[3].

Biologically active compounds derived from plants are usually categorized in secondary and primary metabolites. While primary metabolites are parts of metabolic pathways, secondary metabolites are waste products or byproducts of metabolic pathways. Regarding the medicinal uses of *Calotropis procera*, secondary metabolites namely phenolic compounds tannins, terpenoids, and saponins have received immense attention[4,5]. Researchers have evaluated roots, leaves, and stem bark of *C. procera* with aqueous, hexane, petroleum ether extracts for the detection of phytochemicals where leaves and roots showed the presence of glycosides, saponins, triterpenoids, steroids, alkaloids, tannins, and flavonoids while stem bark showed flavonoids, triterpenoids, and saponins[6]. Similarly, others have observed that the flower ethanol extract of *C. procera* has strong antioxidant potential due to its Quercetin related flavonoids[7]. Similarly, scientists have screened ethanolic samples of flower, leaves, and stems of *C. procera* and detected alkaloids, glycosides, saponins, triterpenoids, phenols, and tannins in almost all parts of this plant[8-10]. Phytochemicals investigated in *C. procera* flower extracts of chloroform, acetone, methanol, record the presence of phenols, alkaloids, asteroids, tannins, glycosides, saponins, and flavonoids[11]. Two flavonoids isorhamnetin-3-o-rubinobioside and isorhamnetin-3-o-rutinoside were isolated from n-butanol and ethyl acetate extracts of *C. procera*[12]. Scientists have investigated five different latex fractions (n-butanol, hexane, dichloromethane, water and ethyl acetate) of *C. procera* and concluded that dichloromethane and ethyl acetate sample showed anti-inflammatory properties[13]. Researchers have evaluated phytochemical profile of *C. procera* using various solvents[14,15]. Verma also found out the cardiac glycosides, saponins, triterpenoids, alkaloids and tannins and absence of flavonoids in ethanolic and chloroform samples of *C. procera*[16]. According to Shrivastava, *C. procera* is the storehouse of secondary metabolites[17]. Joshi and Kaur analysed *C.procera* for the presence of bioactive compounds[18]. While, a study detailed stem powder of *C. procera* with different extracts of hexane, chloroform, methanol, and sterile water for detection of saponins, flavonoids, sterols, tannins, and alkaloids[19]. The study by Tiwarie valuated the phytochemicals of petroleum ether and methanol leaf extracts of *C. procera,* determined the presence of protein, glycosides, triterpenoids, flavonoids, and steroids and suggested that these chemicals may be a significant indicator for the medicinal importance of the plant[20]. Moreover, others have also observed the leaf, flower, fruit, and latex samples of *C. procera* with the detection of phenolic constituents and proved it as a strong antioxidant plant, which could be utilized in food and drug industry[21]. While, Al Snafi has suggested that *C. procera* exhibits many pharmacological aspects due to the presence of biologically active constituents[22].

METHODOLOGY

Plant Material and Sample

Fresh leaves of *Calotropis procera* were collected and authenticated by Karachi University Herbarium. Leaves material was washed with tap water and air dried for 20 days. To obtain a crude extract, material was soaked

in 80% ethanol for about 10 days then filtered through filter paper. The extract was concentrated by using RE. The sample residue was stored in freezer until further use. By using separating funnel, hexane fraction of leaf was separated from ethanolic extract and concentrated on rotary evaporator.

Chemicals

NSAID (Ibuprofen) oral suspension 200mg/5ml approximately for 20-30mg/kg body weight of rats was purchased from market, manufactured by Abbott Laboratories Ltd. Karachi, Pakistan. Methyl cellulose (CMC) was purchased from Sigma Aldrich.

Animals

Wistar white male rats (180-250 g body weight) were bought from the animal house of Dow University of Health Sciences, Karachi, Pakistan. Before starting experiments, rats were adjusted to the laboratory atmosphere and accommodated separately in artificially maintained temperature (22-26^{o}C). Water and diet were provided to rats.

Proper Recommendations

The scientific procedures were conducted in compliance with the ethical recommendations of ERB (Ethical Review Board) for laboratory use and care in animal research (Health Research Extension Act, 1985)[23].

Study design

Animals were divided into three different groups (n= 6) Each group consisted of six rats and used as follows: **Group I** consisted of healthy animals, remained untreated and termed as Control. They were weighed and recorded at 10:00 -11:00 hours for 10 alternate days. **Group II** treated with prepared Ibuprofen suspension orally at a dose of 2ml /200gm b.w. for 15 days, termed as Ibuprofen Treated +ve Control. They were weighed before administration of oral dose daily. **Group III** treated with only hexane suspension orally at a dose of 2ml/200gm b.w. **Group IV** treated with Ibuprofen + Hexane extract treated group, received hexane extract orally according to the recommended doses i.e., they were weighed before hexane extract treatment. Hexane extract was given 30 minutes prior to ibuprofen administration.

Blood and kidney samples

Animals were decapitated after 24 hours of the last dose of treatment, and blood was collected from the head in lithium-heparin coated tubes. A portion of blood was used to collect plasma. The plasma was separated and collected in disposable Eppendorf tubes and stored at -70^{o}C till analysis. The kidneys were rinsed with saline, dried, and weighed then kept in the freezer at 70^{O}C for biochemical analysis. Plasma urea was estimated by using Diacetyl monoxime method by Butler[24]. Plasma creatinine was estimated by Modified Jeff's method[25]. Kidney homogenate was used for the estimation of MDA Okhawa[26], 4HNE Kinter[27], Catalase Sinha[28], SOD Kono[29] and GSH was determined using the method of Carlberg and Mannervik[30].

Phytochemical analysis

Phytochemical constituents were determined in leaf extract of *Calotropis procera*. The method of Swain and Hillis[31] was used for the determination of phenolic compounds. Phenolic acids were also detected using Harborne[32] method.

Statistical analysis

Data were analyzed statistically by using mean values ± SE at $P<0.05$ through One-way ANOVA and Duncan's multiple comparison test (DMCT) with the help of a computer software Statistical Package for Social Sciences (SPSS)[33] for windows version 14.0.0.

RESULTS

Amongst the primary metabolites, value of amino acids in leaf was (i.e. 110.33 µg/ml). Enough amount of carbohydrates, reducing and non-reducing sugars, and protein were also recorded in leaf extract (Table 1).

Leaf exhibited 17.5 µg/ml phenols. Similarly, the qualitative determination of phenolic constituents are observed in (Table 2).

According to one way ANOVA, values of body and kidney weight differ significantly ($P<0.05$) (Fig. 1). Duncan's Multiple Comparison Test (DMCT) showed a marked decrease in body weight in treated rats as compared to control ($P<0.05$). Similarly, marked decrease was observed in Ibu+hex and Ibuprofen respectively ($P<0.05$) (Fig. 1). According to DMCT, kidney weight of animals was significantly higher compared to control ($P<0.05$). A marked increase in kidney weight in hexane, Ibu+hex and Ibuprofen was observed respectively ($P<0.05$).

However, values of kidney weight were insignificant between hexane and Ibu+hex ($P<0.05$) (Fig. 2). One way Anova showed the values of plasma urea and plasma creatinine differing significantly ($P<0.05$) (Fig. 3). According to Duncan's Multiple Comparison Test, Ibuprofen rats showed a marked increase in plasma urea level as compared to control, hexane, and Ibu+hex

respectively (P<0.05). Similarly, plasma creatinine level was significantly increased in Ibuprofen treated rats as compared to hexane and control (P<0.05). While, a significant decrease in Ibu+hex than all the other treated and control rats was observed (P<0.05) (Fig. 4). According to one way ANOVA, values of SOD and catalase differ significantly (P<0.05) (Figs. 5, 6). While, DMCT showed significant increase in SOD level in Ibu+hex treated rats as compared to control, hexane, and Ibuprofen respectively (P<0.05) (Fig. 5). However, increased level of CAT was observed in Ibu+hex treated rats as compared to control, hexane, and Ibuprofen treated rats respectively (P<0.05) (Fig. 6). One way ANOVA showed significant differences in plasma MDA, tissue MDA, tissue 4-HNE, and GSH levels (P<0.05). (Figs. 7-10). DMCT showed a significantly increased level of plasma MDA in Ibuprofen treated rats (P<0.05), whereas, level of plasma MDA was significantly decreased in control followed by hexane and Ibu+hex treated rats (P<0.05). Similarly, tissue MDA level in Ibuprofen treated rats increased significantly as compared to hexane, control, and Ibu+hex respectively (P<0.05) (Fig. 8). The value of tissue 4-HNE showed a significant increase in Ibuprofen treated rats (P<0.05). While, the level of tissue 4-HNE gradually decreased in control, hexane, and Ibu+hex treated rats respectively (P<0.05) (Fig. 9). On the other hand, level of tissue GSH was significantly higher in Ibu+hex treated rats as compared to control, hexane, and Ibuprofen treated rats (P<0.05) (Fig. 10).

Table 1: Phytochemical Estimation of Calotropis Procera Leaf Extract

Constituents	Leaf µg/ml
Reducing Sugar	145.0±5.0
Non-reducing sugar	335.0±73.65
Proteins	373.33±25.16
Carbohydrates	480.0±72.4
Amino Acids	110.33±9.073
Phenols	17.66±0.57

Table 2: Phenolic Constituents of *Calotropis Procera* Leaf Extract

Phenolic Compounds	Class of Phenolic Compounds	Leaf Extract	Leaf Acid Hydrolysis Extract
Apigenin	Flavones	-	-
Luteolin	Flavones	-	-
Tricin	Flavones	+	-
Orientin	Glycosyl Flavones	-	+
Iso-orientin	Glycosyl Flavones	-	+
Vitexin	Gylcosyl Flavones	+	-
Isovitexin	Glycosyl Flavones	+	-
Azaleatin	Flavonols	-	-
Gossypetin	Flavonols	+	-
Kaempferol	Flavonols	+	-
Myricetin	Flavonols	+	-
Quercetin	Flavonols	-	-
3-Glucoronide (Quercetin Glycoside) Isoquercitrin	Flavonols	-	-
Dihydrokaempferol	Flavanol	+	-
Dihydromyricetin	Flavanol	+	-
Naringenin	Flavanones	+	-
Dihydroquercetin	Flavanones	-	-
Mangiferin	Xanthone	+	-
Isovanillin	Phenolic Aldehyde	-	-
Unknown Rf=10	-	+	-
Unknown Rf=15	-	+	+
Unknown Rf=20	-	-	-

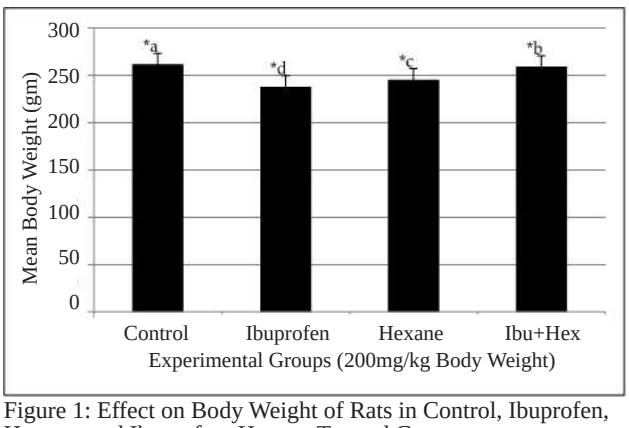

Figure 1: Effect on Body Weight of Rats in Control, Ibuprofen, Hexane, and Ibuprofen+Hexane Treated Groups

n=6, mean values ± SE, *= P<0.05, a-d= according to DMCT, ranks of mean values, sharing of same letter do not differ significantly.

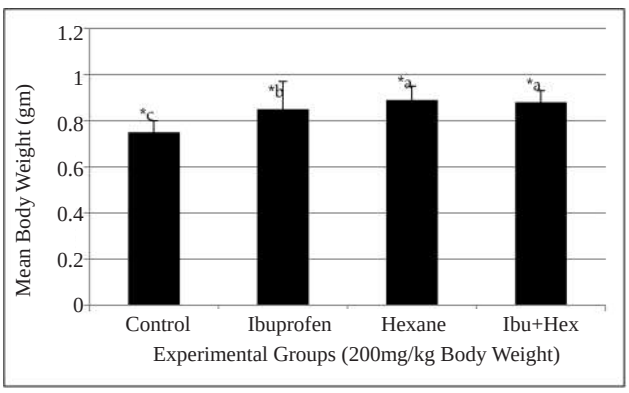

Figure 2: Effect on Kidney Weight of Rats in Control, Ibuprofen, Hexane, and Ibuprofen+Hexane Treated Groups

n=6, mean values ± SE, *= P<0.05, a-c= according to DMCT, ranks of mean values, sharing of same letter do not differ significantly.

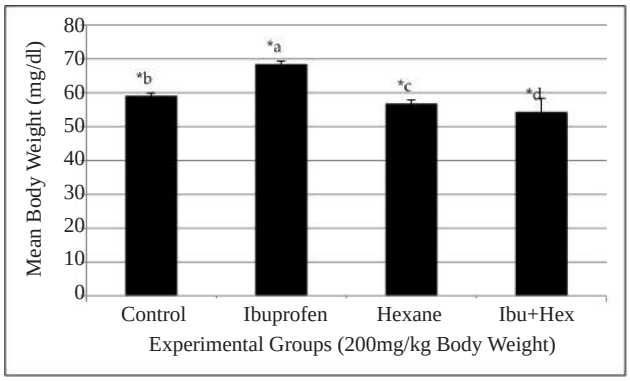

Figure 3: Effect on Plasma Urea Level of Rats in Control, Ibuprofen, Hexane, and Ibuprofen + Hexane Treated Groups

n=6, mean values ± SE, *= P<0.05, a-d= according to DMCT, ranks of mean values, sharing of same letter do not differ significantly.

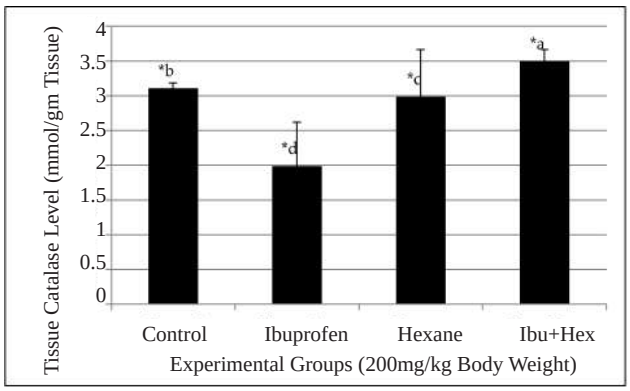

Figure 6: Effect on Tissue Catalase Level of Rats in Control, Ibuprofen, Hexane, and Ibuprofen + Hexane Treated Groups

n=6, mean values ± SE, *= P<0.05, a-d= according to DMCT, ranks of mean values, sharing of same letter do not differ significantly.

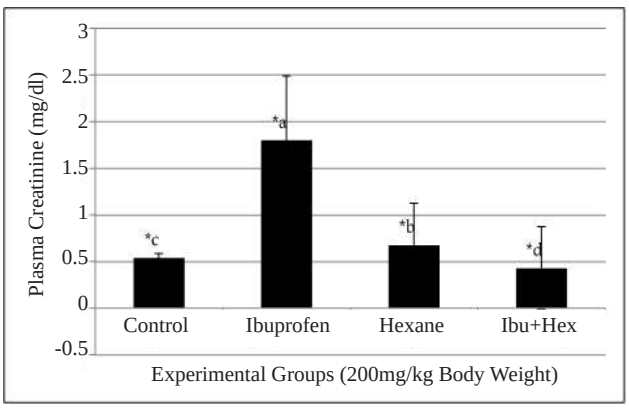

Figure 4: Effect on Plasma Creatinine Level of Rats in Control, Ibuprofen, Hexane, and Ibuprofen+Hexane Treated Groups

n=6, mean values ± SE, *= P<0.05, a-d= according to DMCT, ranks of mean values, sharing of same letter do not differ significantly.

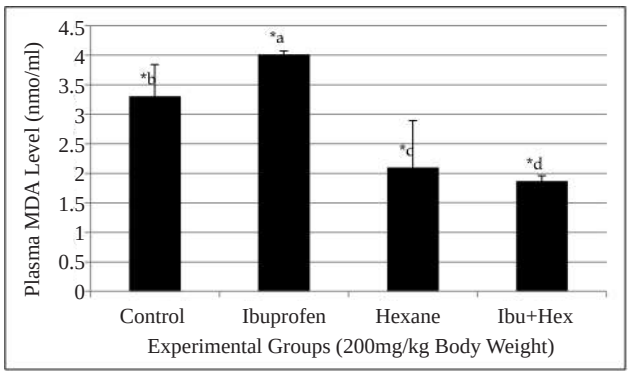

Figure 7: Effect on Plasma MDA Level of Rats in Control, Ibuprofen, Hexane, and Ibuprofen + Hexane treated groups

n=6, mean values ± SE, *= P<0.05, a-d= according to DMCT, ranks of mean values, sharing of same letter do not differ significantly.

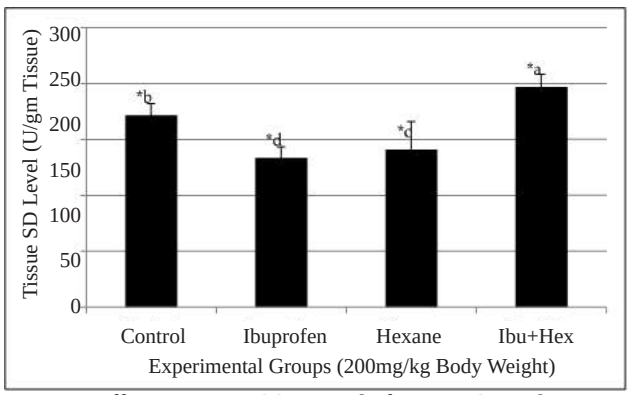

Figure 5. Effect on Tissue SOD Level of Rats in Control, Ibuprofen, Hexane, and Ibuprofen+Hexane Treated Groups

n=6, mean values ± SE, *= P<0.05, a-d= according to DMCT, ranks of mean values, sharing of same letter do not differ significantly.

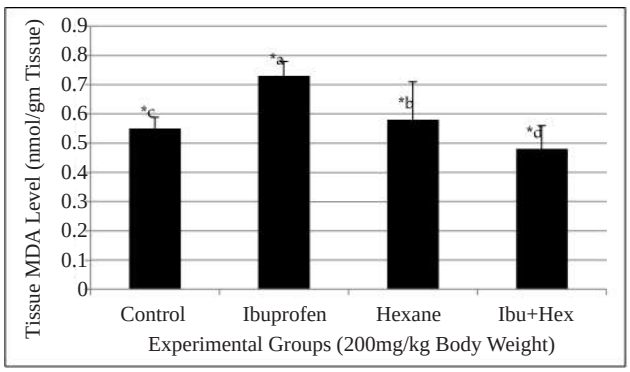

Figure 8: Effect on Tissue MDA Level of Rats in Control, Ibuprofen, Hexane, and Ibuprofen + Hexane Treated Groups

n=6, mean values ± SE, *= P<0.05, a-d= according to DMCT, ranks of mean values, sharing of same letter do not differ significantly.

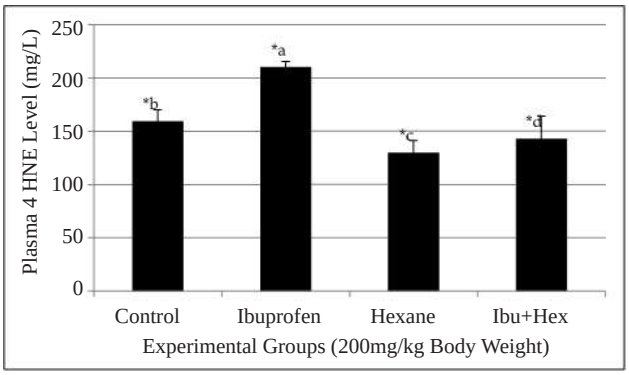

Figure 9: Effect on Tissue 4HNE Level of Rats in Control, Ibuprofen, Hexane, and Ibuprofen + Hexane Treated Groups

n=6, mean values ± SE, *= P<0.05, a-d= according to DMCT, ranks of mean values, sharing of same letter do not differ significantly.

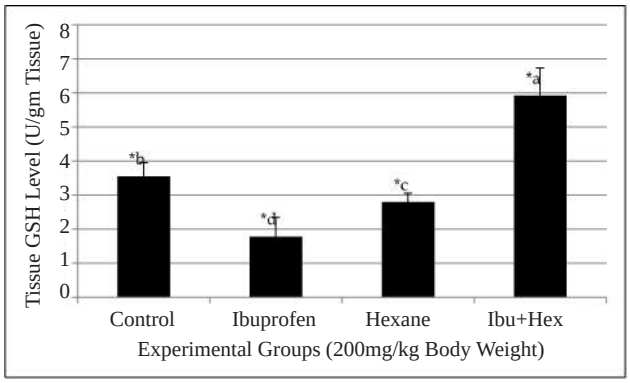

Figure 10: Effect on Tissue GSH Level of Rats in Control, Ibuprofen, Hexane and Ibuprofen + Hexane Treated Groups

n=6, mean values ± SE, *= P<0.05, a-d= according to DMCT, ranks of mean values, sharing of same letter do not differ significantly.

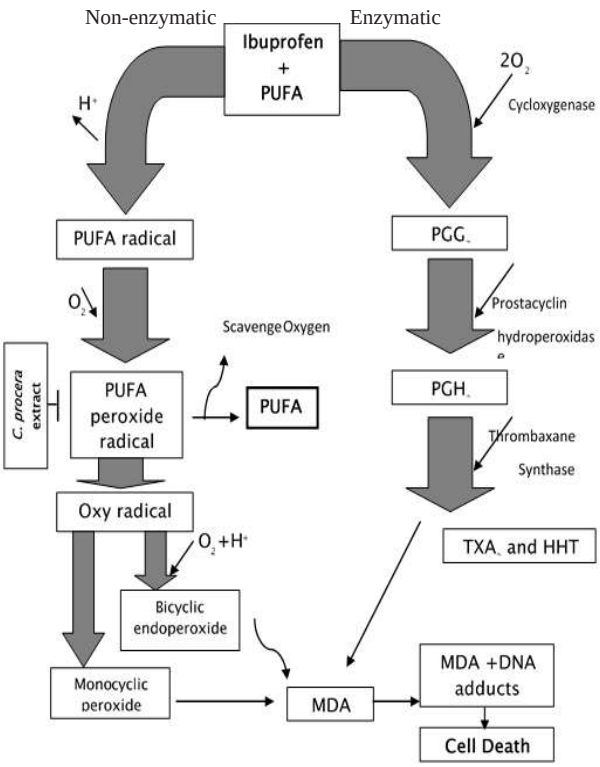

Figure 11: Proposed MDA Formation by Ibuprofen and Inhibition by C. Procera

Propose MDA formation and inhibition; Ibuprofen may form MDA by enzymatic or non-enzymatic pathways. Formation may be inhibited by the action of phenolic compounds present in *C. procera* extract and convert lipid peroxide into non-toxic form of PUFA.

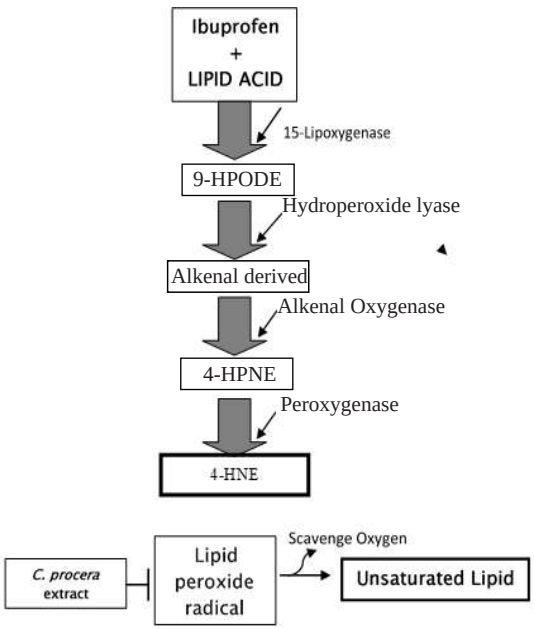

Figure 12: Proposed 4HNE Formation by Ibuprofen and Inhibition by C. Procera

Propose 4HNE formation and inhibition; Ibuprofen may form 4HNE by enzymatic pathway. Formation may be inhibited by the action of phenolic compounds present in *C. procera* extract and convert lipid peroxide into on-toxic form of Unsaturated Lipid.

DISCUSSION

Amongst the detected phenolic compounds, especially flavanols and flavones of the class flavonoids have received considerable attention[4,21,34-38,41] to prove their medicinal importance. Quercetin, luteolin, and kaempferol are the most commonly consumable and natural flavonoids and are reported to have antibacterial and antioxidant properties[39]. Similarly, Mangiferin is a natural phenolic compound exhibiting antimicrobial and antioxidant activities[40]. The dihydromyricetin is a flavanol and used as anti-inflammatory agent[42]. Isoorientin is a flavone showing hypoglycaemic activity[43,44]. So there seems to be a good correlation between phenolic compounds and antioxidant potential.

NSAIDs are among the sources for pre-renal failure[42]. Ibuprofen (NSAID) is used to induce nephrotoxicity in rats with pretreatment of *C. procera* leaf hexane extract. A marked decrease was observed in body weight of Ibuprofen treated rats as compared to control ($P<0.05$) (Fig. 1). This weight loss was restored by the administration of pretreatment of *C. procera* leaf hexane with Ibuprofen ($P<0.05$) (Fig. 1). Previously, it was reported that Ibuprofen may cause gastrointestinal disturbance damaging gastrointestinal mucosa that results in reduced ingestion of food[45]. This is the consequence of inhibition of cyclooxygenase-1 enzyme system which protects gastrointestinal lining from digestive chemicals.

Previously, it was believed that accumulation of urea and creatinine is the indicator of improper renal function[46]. In the present study, serum urea and creatinine levels were significantly increased ($P<0.05$) after the administration of Ibuprofen showing renal disorder. This result is also in accordance with the previous findings[45] when ibuprofen was used as NSAID. The significant decrease in urea and creatinine values was observed after treatment of Ibuprofen with *C. procera* hexane extract, which also provides the evidence of positive role to inhibit the toxicity in rats produced by Ibuprofen ($P<0.05$).

The Reactive Oxygen Species (ROS) initiates the contraction of mesangial cells which change the filtration surface area and alter the ultrafiltration coefficient factor that decreases the rate of glomerular filtration[47-50]. However, accumulation of urea and creatinine in plasma induces decrease in kidney function which is an indication of decreased glomerular filtration rate due to nephrotoxicity[46]. Increased Ca^{+2} movement in the mesangial cells may also be a cause of reduced glomerular filtration rate[46,51]. In the present study, these increased levels are neutralized by the administration of *C. procera* leaf hexane extract with Ibuprofen to prevent normal kidney function. While, Kaneko[52] found an opposite relation between quantity of absorbed urea and rate of tubular urine flow.

There was an imbalance between oxidative stress in Ibuprofen treated rats, which is responsible for the formation of ROS. This was determined by evaluating decreased levels of catalase, super oxide dismutase (SOD), Glutathione (GSH), and increased levels of malonyldialdehyde (MDA) and 4 hydroxy nonenal (4HNE) (Fig. 5-10). In order to counteract oxidative imbalance due to administration of Ibuprofen, *C. procera* leaf hexane extract, co-administered with Ibuprofen to test rats, significantly restored decreased levels of catalase and super oxide dismutase (SOD) (Fig. 7.5-6). Whereas, catalase, glutathione peroxidase, and glutathione reductase belong to the endogenous type of antioxidant defense system. While, SOD acts as a first line of defense against ROS which traps superoxide radical and converts it into H_2O_2[53]. However, increased amount of H_2O_2 and hydroxyl radicals and decreased amount of GSH are responsible to initiate Ibuprofen nephrotoxicity[54].

The higher amount of glutathione peroxidase may reduce H_2O_2 to H_2O with oxidation of (GSH) and due to reducing nature, GSH is one of the important substances for maintaining cell sustainability[53,55]. Presently, a decrease in renal GSH level is observed in Ibuprofen treated rats (Fig. 10). These results are supported by previous studies[55]. However, some previous studies have also observed that kidney damage may occur due to increase in GSH levels[56]. *C. procera* hexane extract prevented Ibuprofen-induced decline in (GSH) activity in the renal mitochondria of rats ($P<0.05$) which is also supported by the report[46] in which *C. procera* flower extract increased the depleted concentration of GSH. Due to Ibuprofen administration, different complexes formed are taken up by renal cells and stabilized by intracellular GSH. GSH Peroxidase present in the cytoplasm of the cells, removes H_2O_2 by coupling its reduction to H_2O with oxidation of GSH. In case of intracellular decrease, the complexes undergo rapid transformation to receive metabolites. This decrease seems to be the main factor that impairs antioxidant enzyme[57]. It is also noteworthy that the presence of flavonoids in *C. procera* reveal the correlation of antioxidant properties[46,58] which may ultimately be useful for the treatment of kidney toxicity.

It is also shown that Ibuprofen administration is linked with heavy oxidative stress and increased formation of free radicals[46,59]. This will lead to oxidative damage to cell components like proteins and nucleic acids[60].

Similarly, in vivo lipid peroxidation can be evaluated

by estimating MDA and 4-HNE. However, lipid peroxidation is an important oxidative damage mechanism to cell structure which may become the reason of cell disintegration. The presence of lipid peroxidation involves generation of lipid radicals, collection of oxygen, and shifting of double bonds in unsaturated lipids, which leads to abolition of membrane lipids. After this process, a number of products are obtained which include alkanes, ethers, alcohols ketones, and aldehydes[61].

4-HNE and MDA cause protein damage by reaction with lysine amino group, cystein sulfhydryl group, and histidine imidazole group[62]. In the present study, MDA and 4-HNE levels were found in higher levels in ibuprofen treated rats (Fig.7-9), which may cause damage to kidney tissues. While, co-administration of *C. procera* leaf hexane extract with ibuprofen showed decrease levels of MDA and 4-HNE (Fig.7-9) which was the indication of nephro-protective property of *C. procera*.

The high concentration of malonyldialdehyde (MDA) in kidney tissues may cause malfunction. Present findings showed decreased value of MDA in *C. procera* treated tissue and plasma ($P<0.05$). These results are also supported with previous findings[55,63] where, decrease in MDA level treated with *C. procera* latex was observed to correlate with antioxidant activity. 4-HNE is an unsaturated aldehyde considerably more toxic for cell in vivo than MDA. It is very important to measure 4-HNE levels[64-66]. In present study, treatment by *C. procera* with ibuprofen decreased the level of 4-HNE ($P<0.05$). The reason behind this decreased level of toxicity was the presence of phenolic compounds in leaves of *C. procera*. Table 2 is also evidence about the detection of phenolic compounds in present studies. A proposed mechanism of ibuprofen-induced MDA and 4HNE formation and counter effect of phenolic compounds detected in *C. procera* is also diagrammatically presented in Figure 11 and 12.

CONCLUSION

It is concluded that ibuprofen-induced nephrotoxicity in rats is the result of overproduction of hydrogen peroxide and hydroxyl radical that may finally cause renal oxidative stress. However, with the supplementation of *C. procera*, oxidative stress was significantly decreased by regularizing the levels of superoxide dismutase, catalase, 4HNE, and MDA and also found effective in inhibiting lipid peroxidation in vivo. Results reveal that *C. procera* leaf extract has potential and this could be well correlated with that of the presence of phenolic compounds in leaf extracts. While, it is also established that *C. procera* could completely protect nephrotoxicity induced by the long-term use of NSAIDs (ibuprofen) in a rat model. Therefore, *C. procera* could be proposed as an antioxidant source of natural origin, which may ultimately be more efficient than any synthetic drug.

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ORIGINAL ARTICLE

Influence of Education on Reproductive Health Indicators Among Women in Sindh, Pakistan

Tanzil Jamali[1], Sana Tanzil[2] and Syed Sanowar Ali[1]

ABSTRACT

Objective: In Pakistan, the role of women's education and its impact on the use of contraception methods and reproductive health indicators needs to be identified. Therefore, the objective of this study was to find out the association of education levels of women with family planning and reproductive health indicators in Sindh.

Methodology: Secondary data analysis of Pakistan Demographic and Health Survey (PDHS) 2012-13 was performed on the data set consisting of 2932 females aged between 15 and 49 years, belonging to the province of Sindh, Pakistan. Descriptive frequencies and logistic regression were performed with different types of model formation. We included independent variable as women's education while dependent variables were indicators of reproductive health included in the study which we: current contraceptive use, any use of contraceptives ever, antenatal visits, unmet need of family planning, and desire for last child.

Results: The mean age of the women was 32.5 years. Over all, 46.1% of the women in Sindh were shown as educated. The current contraception use by women was 30.4%. Out of whom, 4.8% were using traditional methods and 25.6% were using modern methods for family planning. Odds ratio of contraception use among educated women was (aOR: 1.50, CI: 1.09, 2.05), any use of family planning ever (aOR: 1.85, CI: 1.38, 2.48), and antenatal visits (aOR: 1.65, CI: 1.04, 2.62).

Conclusion: The increasing trend of odds was also seen in reproductive health indicators as the level of education increased. Therefore, the study concludes that the level of education can influence women in choosing different family planning methods.

Key words: Influence, family planning, education, women, Sindh

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عنوان: سندھ کی خواتین میں تولیدی صحت کے استعمال کے اشاریوں میں تعلیم کے اثرات

خلاصہ

مقاصد: پاکستان میں مانع حمل طریقوں کے استعمال اور تولیدی صحت سے متعلق اشاریوں میں خواتین کے تعلیم یافتہ ہونے کے اثرات واضح کرنے کی ضرورت ہے۔اس مطالعے کے دوران سندھ کی خواتین کی تعلیمی قابلیت، خاندانی منصوبہ بندی اور تولیدی صحت کے درمیان ایک ربط پایا گیا۔

طریقہ کار : پاکستان ڈیموگرافک سوسائٹی کے سال ۱۳-۲۰۱۲ کے ہیلتھ سروے کے ڈیٹا کے ثانوی تجزیے کے دوران، جس میں سندھ سے تعلق رکھنے والی ۵۱ سے ۹۴ سال کی دو ہزار ۹ سو ۳۲ خواتین کو شامل کیا گیا تھا، ڈیٹا میں ماڈلز کی تیاری میں رجعت رکھی گئی۔اس مطالعے کے دوران ہم نے آزاد وبری ایبل خواتین کی تعلیم اور غیر آزاد وبری ایبل تولیدی صحت کے اشاریے جیسے مانع حمل ادویات کا استعمال ،خاندانی منصوبہ بندی اور مزید اولاد کی خواہش نہ ہونا رکھے۔

نتائج: مطالعے میں خواتین کی عمر کا اوسط ۳۲ اعشاریہ ۵ سال نکلا ۔مجموعی طور پر سندھ میں چھیالیس اعشاریہ ایک فیصد خواتین تعلیم یافتہ تھیں جن میں مانع حمل ادویات کا استعمال تیس اعشاریہ چار فیصد تھا ۔چار اعشاریہ آٹھ فیصد خواتین خاندانی منصوبہ بندی کے روایتی طریقے جبکہ پچیس اعشاریہ چھ فیصد خواتین جدید طریقے استعمال کرتی تھیں۔

حاصل مطالعہ: اس مطالعے کے دوران تعلیم یافتہ خواتین میں تولیدی صحت کے استعمال کے اشاریوں میں اضافہ دیکھنے میں آیا۔مطالعے سے یہ بات بھی سامنے آئی کہ تعلیم میں اضافہ خواتین میں خاندانی منصوبہ بندی کے مختلف طریقوں کے استعمال میں مددگار ثابت ہوگا۔

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INTRODUCTION

Family planning is one of the essential interventions considered around the world for the development of population policy[1]. Around 222 million women worldwide in low and middle income countries want to delay or stop childbearing but are not using any

methods of contraception[2]. Over all, contraception rate around the world is 63.3%, while unmet need for family planning is 12.1%[3]. However, among half of the low and middle-income countries which include Asian and African countries, contraceptive practices remain on the lower side and population growth rate and unmet needs for family planning are high[4]. In Africa, the unmet need for modern contraception among women of child bearing age (CBA) is around 53%. Similarly, in Asia and Latin America, the unmet need for family planning is 21% and 22% respectively, while rate of contraception use is on the lower side[2,5]. It is estimated that by providing these women of reproductive age access to modern contraceptives, maternal mortality can be reduced by 25%[5]. By providing contraception and reducing the unmet need for family planning, newborn deaths can be reduced by 18%, and unintended pregnancies reduced by 73 percent[5]. Moreover, 52% of pregnant women in high income countries attended at least four antenatal care visits in 2012, however, only 38% of pregnant women in low-income countries made antenatal care visits at least four times[6].

Women's education, mainly completion of primary school education and secondary school education, has emerged to be strongly related to lowered fertility[7]. Even though the usage of contraceptives has increased over a period of time, there is still a gap in knowledge regarding seeking contraception and antenatal care[8,9]. Many studies have reported reasons cited for not using any method of family planning, which include: lack of education/ knowledge, religious beliefs, and the fear of side effects[9,10].

Many population-based surveys, for example, Demographic and Health Survey (DHS) from Kenya, Malawi and Nigeria, have reported the relationship of education level with seeking antenatal care, by mediating the effect of women's education level through the number of antenatal visits[11]. Furthermore, demographic data from India in 2001 reports that highly educated women tend to show lower desire of fertility and higher use of contraception[7,12]. Many studies report that married women who have attained a certain level of education, show a higher probability of using family planning practices[13]. Similarly, studies from India and Bangladesh show that working women consult their doctors more often about family planning than do the unemployed ones[13,14].

In Pakistan, the literacy rate among women is cited to be less than 50%[15]. Previously, Pakistan Demographic and Health Survey 2006-7 had reported around 22% of married women of reproductive ages in Pakistan to be using modern contraceptive methods, 8% using traditional methods, while 24% wanting to use family planning methods but unable to do so[15,16]. However, there is limited information associating women's education with family planning methods. A few studies have been conducted with different aims and objectives, which have identified a relationship between family planning and education. A study conducted in urban Karachi has reported that women with secondary education and employed in professional occupations, have an increased contraceptive prevalence rate of 31%[17]. The Pakistan Reproductive Health and Family Planning Survey 2000 data reported that contraceptive use among women was strongly correlated with their education; however this relation was not mediated by women's autonomy[18]. According to Pakistan Demographic Health Survey 2012-13, health indicators were the worst in Sindh as compared to other provinces, more than 70% of the population lived in rural areas, and around 53.9% of women were illiterate[19].

Consequently, it is necessary to identify the role of women's education and its impact on family planning and reproductive health indicators, in the context of Sindh to develop interventions to improve education as well as effective strategies to support women with poor educational backgrounds. Therefore, the purpose of this study is to determine the association of education with family planning and reproductive health indicators and antenatal care seeking behaviour among women (aged 15–49 years) in Sindh.

METHODOLOGY

This was a secondary data analysis of Pakistan Demographic and Health Survey (PDHS) 2012-13 performed on data from the province of Sindh. The National Institute of Population Studies (NIPS) conducts PDHS in Pakistan every five years. The current PDHS 2012-13 was the third round after the surveys of 1990-91 and 2006-07. The aims and objectives of the current survey were to monitor and evaluate health status (particularly family planning activities) for providing guidelines to the policy makers and programme managers, to plan effective interventions[19]. The PDHS gathered information on maternal and child health, fertility levels, family planning, nutritional statuses of mothers and children, infant and child mortality, and immunization status[19].

Sampling Strategy:

The PDHS followed a two-stage, stratified, random sample design. They selected a total of 1,000 clusters proportional to the size of 390 in urban areas and 610 in rural areas for the first stage. The clusters were selected in urban areas from a frame consisting of 26,800 enumeration blocks being maintained by the

Federal Bureau of Statistics (FBS). Each cluster included 200-250 households. Whereas for rural areas, the frame consisted of 50,588 villages list being enumerated in the census of 1998.

For second stage sampling, households were selected through a systematic random sampling technique. Each cluster had 105 selected households. A total of 10 households per cluster were selected by utilizing systematic random sampling so as to conduct Long Household and interviews of women through questionnaires. In PDHS data set, the maternal data consisted of 2932 ever married females aged 15-49 years, representing Sindh, Pakistan.

List of variables included in the study:

Exposure or independent variable was womens education while dependent variables were indicators of reproductive health included in the study which are: current contraceptive use, any use of family planning ever, antenatal visits, unmet need of family planning, and desire for last child (wanting last child). The variable of educational status had four categories i.e., primary, secondary, higher, and no formal education. It was changed into binary categories as educated and uneducated. Educated variable included women having primary, secondary, and higher education levels. Categories of current contraceptive usage were transformed into binary categorical variables of using and not-using contraception.

Statistical analysis:

Dataset obtained from PDHS 2012-13 was analyzed using SPSS version 19. Descriptive analysis of the data set was run, including the variables: respondent's age, urban-rural residence, respondent's education, wealth index, current contraceptive use, etc. Weighted analysis was done as the sampling was done with probability proportional to size in the survey**.** Multiple logistic regression was carried out to calculate the odds ratios and all independent variables mentioned above were entered in the model to adjust for confounding variables including: age of the respondent, community wealth index, urban-rural residence, spouses education status, respondent's working status, place of delivery, and person's decision on healthcare visit. Variables with p values < 0.05 were considered significant.

RESULTS

The mean age of the women included in the survey was 32.5 years (SD +8.7) and mean age at first birth was 20.7 years (SD +3.7). Over all, 46.1% of the women who were educated, were included in the study, in whom levels of education completed were: primary 12.7%, secondary 17.6%, and tertiary 15.9%. (Figure 1)

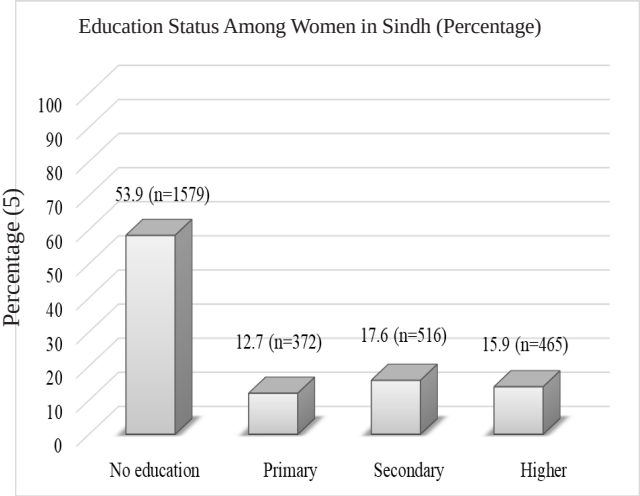

Figure 1: Frequency Distribution of Education Status Among Women in Sindh. PDHS 2013 (n=2932)

Around 36.1% of the participants were Sindhi speaking while 26.1% belonged to Urdu speaking group. Remaining were from other ethnic groups including Punjabi, Saraiki, Pushto, Balochi etc. In our study, 27.1% of the population consisted of working women. As many as 56.3% of the population lived in rural settings. The family planning indicators showed current rate of contraception use among women in Sindh as 30.4%, any contraception use ever was 45.8%, unmet need for spacing 10.2%, and unmet need for limiting 10.4%. Moreover, 48% of the population in Sindh had had four antenatal visits during their last pregnancy. Around 65.5% of the population in Sindh utilized the services of childbirth at a health facility (Table 1).

Among different ethnic groups, majority of the Urdu speaking (79.2%) were educated. Total 36.4 % of the participants belonged to other ethnic groups including Pushto, Balochi, Saraiki, Punjabi etc. and 32.4% of Sindhis were found to be educated. Around half of the educated women were homemakers, while only 26% of the working women were educated. Most of the educated women were residing in urban areas (69.4%), while the proportion of educated women in rural areas was low at 16.2%. Similar trends were observed in community wealth index which included highest education level among the richest wealth quintile (86.8%), while lowest in the poorest wealth quintile 6.9% ($p<0.05$) (Table 2).

Among current contraceptive users, 63% were educated and among non-users 38.8% were educated. Majority of the traditional method users and modern method users were educated i.e. 80.7% and 59.2% respectively. Among educated women, the unmet need for spacing through family planning was 44.8% and the unmet need for limiting was 35.1%. The frequency of antenatal

visits (four visits during the pregnancy) was found to be higher among educated women of Sindh (47%) while only 37.6% educated women did not visit for antenatal care. Half of the respondents independently took personal decisions for seeking healthcare. While majority of the educated respondents' partners decided for them to seek health care (p<0.05) (Table 2).

Table 1: Frequency Distribution of Socio-Demographic Characteristics Among Women in Sindh (n=2932)

Characteristics	Frequency (n)	Percentage (%)(95% CI)
Age (mean + SE)	32.7 +0.16	(32.4-33.0)
Ethnicity		
Urdu	765	26.1 (24.5, 27.7)
Sindhi	1058	36.1 (34.3, 37.8)
Others	1109	37.8 (36.0, 39.5)
Women's Occupation		
Homemaker	2140	72.9 (71.3, 74.5)
Working women[a]	792	27.1 (25.4, 28.6)
Husband's Education	1967	67.1 (65.3, 68.7)
Wealth Index		
Poorest	728	24.8(23.3, 26.4)
Poorer	406	13.8 (12.6, 15.1)
Middle	319	10.8 (9.8, 12.6)
Richer	525	17.9 (16.5, 19.3)
Richest	954	32.5 (30.8, 34.2)
Place of Residence		
Rural	1650	56.3 (54.4, 58.0)
Urban	1282	43.7 (41.8, 45.5)
Current Contraception Use	890	30.4 (28.7, 32.0)
Any Use of Contraception Ever	1345	45.8 (44.0, 47.6)
Unmet Need for Spacing	286	10.2 (9.1, 11.4)
Unmet Need for Limiting	293	10.4 (9.3, 11.6)
Using for Spacing	261	9.3 (8.3, 10.4)
Using for Limiting	629	22.4 (20.9, 24.0)
Delivery at Home[*n=1584]	545	34.4 (32.1, 36.7)
Delivery at Health Facility[*n=1584]	1039	65.5 (63.2, 67.8)
Antenatal Visits[b* n=1584]	761	48.0 (45.5, 50.5)

a: Working women include professional, clerical, agricultural, sales, domestic household, self-employed, skilled manual, unskilled
b: Antenatal visit: four visits during last pregnancy
*: missing values

Two models of multiple logistic regression analysis with unadjusted and adjusted Odds ratios were calculated. Model 1 performed on any level of education among women of Sindh with various characteristics. The odds of contraception use among literate women were (aOR: 1.50, CI: 1.09, 2.05); any use of family planning ever among literate women were (aOR: 1.85, CI: 1.38, 2.48); and antenatal visits among literate women were (aOR: 1.65, CI: 1.04, 2.62) (p<0.05). In model 2, adjusted odds ratios were calculated for each level of education i.e. primary, secondary and tertiary.The indicators of family planning showed the increasing trend of odds as the level of education increased i.e. for any use of family planning ever, significant results were seen in the association with primary education level (aOR: 1.64, CI: 1.15, 2.34) and secondary education level (aOR: 2.48, CI: 1.62, 3.79) (p<0.05).While at tertiary education level, result was insignificant (aOR: 1.73, CI: 1.00, 3.11) (p=0.05). (Table 3)

Table 2: Relation of Socio-Demographic Characteristics Between Educated and Uneducated Women in Sindh (n=2932)

Characteristics	No Education n (%)	Education n (%)	p-value
Ethnicity			
Urdu	159 (20.8)	606 (79.2)	
Sindhi	715 (67.6)	343 (32.4)	<0.01
Others	705 (63.6)	404 (36.4)	
Women'sStatus			
Homemaker	993 (46.4)	1147 (53.6)	
Working Women	586 (74)	206 (26)	<0.01
Place of residence			
Urban	505 (30.6)	1145 (69.4)	
Rural	1074(83.8)	208 (16.2)	
Wealth index			
Poorest	678 (93.1)	50 (6.9)	
Poorer	331 (81.5)	75 (18.5)	
Middle	224 (70.2)	95 (29.8)	<0.05
Richer	220 (41.9)	305 (58.1)	
Richest	126 (13.2)	828 (86.8)	
Current Contraception Use			
No	1250 (61.2)	792 (38.8)	
Yes	329 (37)	561 (63)	<0.001
Types of Contraception Use			
No Method	1250 (61.2)	792 (38.8)	
Traditional Methods	29 (19.3)	125 (80.7)	<0.001
Modern Methods	300 (40.8)	436 (59.2)	
Status of Users Ever			
Never Used	1041 (65.6)	546 (34.4)	
Use Traditional Methods	30 (25)	94 (75)	<0.05
Use Modern Methods	508 (41.6)	713 (58.4)	
Visited Family Planning Center Last Year			
No	131 (41.7)	183 (58.3)	
Yes	1448 (55.3)	1170 (44.7)	<0.01
Unmet Need			
No Unmet Need	992 (61.5)	621 (38.5)	
Unmet Need for Spacing	153 (55.2)	124 (44.8)	
Unmet Need for Limiting	189 (64.9)	102 (35.1)	<0.05
Using for Spacing	66 (25.3)	194 (74.7)	
Using for Limiting	263 (41.8)	366 (58.2)	
Antenatal Visits*			
No	552 (62.4)	271 (37.6)	
Yes	403 (53)	358 (47)	<0.05

p<0.05
*(n=1584)

Table 3: Multiple Logistic Regression Model Showing the Association of Education on Reproductive Health Indicators Among Women in Sindh

Model 1			
Characteristics	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	p-value
Current use of FP	2.73 (2.33,3.20)	1.50 (1.09, 2.05)*	<0.01
Antenatal visit	1.47 (1.27,1.69)	1.65 (1.04, 2.62)*	<0.05
Ever use of FP	3.00 (2.59, 3.48)	1.85 (1.38, 2.48)*	<0.05
Unmet need	0.75 (0.62, 0.90)	1.11 (0.82, 1.51)	0.11
Desire for last child	1.49 (0.97, 2.29)	1.42 (0.89, 2.24)	0.61
Birth interval[2]	1.34 (1.11, 1.62)	1.42 (0.76, 2.67)	0.62
Model 2			
	Adjusted OR (95% CI)		
Characteristics	Primary	Secondary	Higher secondary
Current use of FP	1.20 (0.81, 1.77)	2.16 (1.42, 3.27)*	1.73 (1.0, 3.11)
Antenatal visit	1.46 (0.78, 2.18)	3.36 (1.31, 8.59)*	1.68 (0.41, 6.85)
Unmet need for FP	1.22 (0.86, 1.73)	0.78 (0.49, 1.24)	0.63 (0.34, 1.17)
Ever use of FP	1.64 (1.15,2.34)*	2.48 (1.62, 3.79)*	1.73 (1.00, 3.11)
Birth interval[b]	1.43 (0.81, 2.53)	1.25 (0.66, 2. 37)	1.74 (0.65, 4.62)
Desire for last child	1.20 (0.55, 2.59)	1.49 (0.62, 3.52)	3.66 (1.15, 11.59)*

*p<0.05
Adjusted for age, ethnicity (others vs Urdu and Sindhi), residence (rural vs urban), decision of health care= respondent, husband alone, both and others, place of delivery (healthcare facility vs home delivery) and wealth index
Model 1= No education as a reference vs any education
Model 2 = No education as a reference vs education levels (primary, secondary, and higher secondary)
b. Birth interval > 2 years as reference vs < 2 years

DISCUSSION

This study draws attention to an important social determinant associated with different family planning and reproductive health indicators. The results showed an association of women's education with their current use of contraception, antenatal visits, and any use of methods of family planning ever. As all these parameters play a pivotal role in reducing maternal mortality and improving maternal health, therefore education is turning out to be an important factor in the situation. The precise process which explains the mentioned relationship needs to be explained. It is possible that education leads to empowerment of women through employment and that then leads to health seeking behaviour. Another possibility is improved decision making ability among educated women which leads to improved insight about health problems and therefore an increase in health seeking behaviour.

The findings of our study are consistent with the findings of other studies performed to explore the mentioned relationship[20]. A study conducted in Pakistan reported that improved utilization of family planning services is associated with economic stability and empowerment of women[21]. Many other studies have reported similar findings for the relationship of contraception rate and education[21,24].

The unavailability or dearth of formal education among women may limit their capability of making independent choices about family planning and other health seeking behaviour. In low income and low and middle-income countries, the discussion about contraception and family planning practices has found the male partner's (husband's) role as crucial for wider acceptance of contraceptive practices and reducing the partners' fertility goals[25]. Our findings also reported higher percentage of modern contraceptives users among those women who had discussed family planning methods and contraception use with their husbands, than among those who had not had such discussions. This is consistent with other studies conducted in developing countries including Africa and Asia[14,25,26].

Strengths and Limitations:

The major strength of this study is that it was a large representative sample of the province of Sindh, which was used to conduct the PDHS survey. Availability of a large sample size and comprehensive information on participating women and their households were the strengths of this study. We used advance level of analysis by using different models of logistic regression and calculated adjusted odds ratio for different categories of educational status.

However, there are a few limitations of our study. Firstly, it was a cross-sectional survey and it could be difficult to establish the cause-effect relationship. Secondly, the scope of this study did not include examining the literacy rate among males who were using contraceptive methods. However, it is a probability that increased rate of literacy among males may also result in acceptance of women's role in deciding to use family planning methods and also in other aspects of life in society. Third limitation of this study was that men were not included as study subjects to determine the differences in practice between men and women and for the reasons behind these differences. Fourthly, in the regression model, women's age at marriage was not adjusted, which might affect the findings.

Recommendations:

It is recommended to design strategies that should target the most vulnerable and disadvantaged population. A comprehensive plan is required for the general population to create attitude and behaviour change towards fertility patterns and contraceptive usage. The programmes directly or indirectly related to maternal and child health in the country should be evaluated for these factors. Scaling up implementation of poverty reduction strategies, inter-sectorial collaboration, and social sector investment will help in achieving better reproductive health outcomes. Moreover, in Pakistan, promotion of educational interventions about family planning which include both husband and wife as well as communication involving both, will be effective in increasing contraceptive use among couples. Considering cultural and religious opportunities to reinforce family planning education, may strengthen the already existing family planning service delivery system in Pakistan.

CONCLUSION

The study concludes that the level of education among women was found to be significantly related to their use and practices of different family planning methods. Increase in literacy rate and education level can play a pivotal role in the implementation of family planning policies. In addition, use of modern contraceptive practices was found to be low among married women in the province of Sindh in contrast to the demand of family planning which was found to be very high. This points to a dire need to promote the use of modern methods for family planning so that unwanted pregnancies and reduction in the number of abortions could be achieved. This will improve women's reproductive health.

Competing interests: None declared

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Units of Measurement

Measurements of length, height, weight, and volume should be reported in metric units (meter, kilogram, or liter) or their decimal multiples.

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(*Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication*)

ORIGINAL ARTICLE

Effect of Dietary and Oral Hygiene Pattern on Incidence of Dental Caries among a Population from Riyadh, Saudi Arabia

Komal Zia[1], Julie Susan Rajan[2], Soban Qadir Khan[3] and Talal Siddiqui[4]

ABSTRACT

Objective: The aim of this study was to evaluate and compare the effects of dietary and oral hygiene maintenance patterns on the incidence of dental caries among a population from Riyadh, Kingdom of Saudi Arabia (KSA).

Methodology: A self-administered questionnaire was prepared (according to World Health Organization (WHO) criteria, and intra-oral examination was conducted on 201 random male and female patients (n=201) who visited the clinics of Riyadh College of Dentistry and Pharmacy (RCDP) between August 2014 and January 2015. The participants were divided into three groups: Group 1—involving children and teenagers (6-19 years), Group 2—consisting of middle aged people (20-40 years) and Group 3—including older patients (41-60 years). Data were collected and analyzed statistically using T-test.

Results: The participants of Group 1 were found to be more prone to caries due to their unhealthy dietary pattern and negligence in maintaining oral hygiene. Group 3 participants showed similar results probably due to their poor general health leading to inferior oral health. In spite of an unhealthy diet, Group 2 participants still showed less prevalence of caries because of their good oral hygiene maintenance. Prevalence of dental caries in the sample was 85.6% and increase in age was significantly associated with increased caries prevalence which was statistically significant (p=0.000, ANOVA). Post Hoc test was used to compare age groups with DMFT and significance was found when Group 1 was compared to Group 2 (0.016), Group 1 to Group 3(0.005), and Group 2 to Group 3 (0.000).

Conclusion: This study showed that unhealthy dietary patterns, along with poor oral hygiene maintenance, can lead to dental caries. Thus, a proper balanced diet and preventive measures for proper oral hygiene maintenance must be adopted to prevent or reduce incidence of dental caries.

Key words: Dental caries; oral hygiene; dietary habits

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ریاض، سعودی عرب کی ایک آبادی میں غذائیت اور منہ کی صفائی برقرار رکھنے کے طریقہء کار کے دانتوں میں کیڑا لگنے کے عمل پر اثرات

مقصد: اس تحقیق کا مقصد ریاض، سعودی عرب کی ایک آبادی میں غذا اور منہ کی صفائی برقرار رکھنے کے طریقہء کار کا دانتوں میں کیڑا لگنے کے عمل پر اثرات کا اندازہ لگانا اور ان کا موازنہ کرنا تھا۔

طریقہء کار: WHO کے معیار کے مطابق ایک خود مکمل کرنے والا سوالنامہ تیار کیا گیا اور ریاض کالج آف ڈینٹسٹری اور فارمیسی کے کلینک میں اگست 2014 سے جنوری 2015 کے درمیان آنے والے 201 مرد و خواتین مریضوں کا معائینہ کیا گیا۔ شرکاء کو تین گروہوں میں تقسیم کیا گیا۔ گروہ ایک: جس میں بچوں اور نوجوانوں کو شامل کیا گیا۔ گروہ دو: جس میں درمیانی عمر کے لوگ تھے۔ گروہ تین: جس میں گروہ دو والوں سے بڑی عمر کے لوگ تھے۔ اعداد و شمار کو جمع کیا گیا اور ٹی۔ ٹیسٹ کے ذریعے ان کا تجزیہ کیا گیا۔

نتائج: پہلے گروہ کے شرکاء میں دانتوں میں کیڑا لگنے کا رجحان ان کے غیر صحتمندانہ کھانے کی عادات اور منہ کی صفائی برقرار رکھنے میں غفلت کی وجہ سے تھا۔ گروہ تین کے شرکاء میں بھی اسی طرح کے نتائج ملے جو کہ شاید ان کی صحت کی عموماً کمتر صورتحال کی بنا پر تھے۔ غیر صحتمندانہ غذا کے باوجود گروہ دو کے شرکاء میں دانتوں میں کیڑا لگنے کا کم رجحان ان کے دانتوں کی اچھی صفائی برقرار رکھنے کا نتیجہ تھا۔ تمام شرکاء میں سے 85.86 فیصد کے دانتوں میں کیڑا تھا جو کہ عمر میں اضافے کے ساتھ بطور خاص پایا گیا۔

حاصل مطالعہ: اس تحقیق میں دیکھا گیا کہ غیر صحتمندانہ غذا استعمال کرنا اور دانتوں کی غیر معیاری صفائی بڑھتی عمر کے ساتھ دانتوں میں کیڑا لگنے کی وجہ بن سکتی ہے۔ چنانچہ دانتوں میں کیڑا لگنے کو روکنے یا کم کرنے کے لیے درست متوازن غذا اور منہ کی درست طریقے سے صفائی کا اہتمام ضروری ہے۔

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INTRODUCTION

Dental caries is a localized, progressively destructive disease of the teeth that starts on enamel with the apparent dissolution of the inorganic components by organic acids that are produced by the enzymatic action of microorganisms (plaque) on carbohydrates. In the year 1890, [1]Miller was the first to describe dental caries as one of the world's most common health problems being faced by the people. In a longitudinal study done by Berman DS et al, the common factors causing

damage to mineralized portions of the teeth causing cavitation and leading to progression of caries included presence of cariogenic bacteria in the oral cavity, unhealthy snacking habits, and increased sugar intake.[2]

Dental caries is still a common disease in the developing countries, even though its prevalence has decreased in the developed countries. According to a study done by Suddick RP et al[3], with increasing sugar plantation and consumption in the 1700's, tooth decay became extensive and by 1800's, dental caries had progressed among the citizens of Europe. Currently, controlling and preventing dental caries especially in the developing countries is being emphasised. It is a disease which is caused by loss of minerals from the tooth structure, thus weakening the tooth structure. Hollway PJ, Moore et al, evaluated in their study that Streptococcus Mutans and Lactobacillus species are the most common cariogenic bacteria that break down the dietary carbohydrates by releasing lactic acid and forming sucrose through a process called fermentation, thereby lowering the pH of the oral cavity and making the environment acidic causing loss of tooth minerals.[4]

Dietary habits of an individual determine the kind of food the bacteria will act on. Therefore, diet plays a major role in caries progression. In a study, H.Kalsbeek et al, reviewed that sugar and sugar substitutes played an important role in causing dental caries along with other factors like saliva, teeth, plaque, diet, and time.[5] This study was also supported by Riva Touger Decker et al that a diet rich in sugars creates an acidic environment in the oral cavity which is suitable for bacteria to act on. To counteract the progression of caries, G.Z.Wright et al evaluated in their study that preliminary methods like proper tooth brushing and using other aids like mouthwashes, interdental cleansers to maintain proper oral hygiene must be adopted[20]. According to J. Ainwright et al, the most favourable and suggested tooth brushing technique to maintain proper oral hygiene is the Modified Bass Technique.[6]

Many studies were done regarding the dietary and oral hygiene pattern in relation to dental caries. Lack of awareness and negligence in the utilization of the oral healthcare techniques along with unhealthy diet are some major issues associated with bad oral hygiene leading to tooth decay. Therefore, the current study was done to evaluate and compare the effects of diet and oral hygiene patterns on the incidence of dental caries in patients visiting Riyadh Colleges of Dentistry and Pharmacy (RCsDP).

METHODOLOGY

The Study was performed between August 2014 and January 2015. Ethical approval was taken before initiating this study and it was registered with the research center of RCDP under the registration number USRP/2013/144. The study was performed on 201 random male and female patients (n = 201) visiting the clinics of RCDP, KSA. The participants were divided into three groups as follows: Group 1—ages group ranging from 6-19 years, Group 2—ages ranging from 20-40 years, and Group 3—ages ranging from 41-60 years.

This division was made to benefit the questionnaire so that the dietary patterns and oral hygiene maintenance pattern of the people of Riyadh could be analyzed. A questionnaire was prepared according to World Health Organization (WHO) criteria with random questions concentrating on their diet pattern and oral hygiene habits to collect the required data. A team of six members was formulated, which comprised two dentists and four dental students. Team members were involved in asking questions, examining the participants, and recording the data on a WHO prescribed form. The data was completed with cooperative patients and an Informed Consent form was filled out assuring the confidentiality of the collected data. On observation, it proved that most of the patients were unaware of the importance of proper oral hygiene maintenance and preventive measures. The data was completed with intraoral examinations performed in the clinics of RCDP.

Four investigators were trained to approach patients with proper patient-management techniques to receive complete cooperation. The whole procedure and its importance were explained to the patient before the questions were asked and before the intraoral examinations were performed. The patients were examined using a plane mouth mirror, community periodontal index (CPI) probe, and proper lighting (wherever necessary) according to WHO caries diagnostic criteria (World Health Organization,1993).[7]

The collected data were reviewed and analyzed statistically using T-test to analyze any statistically significant difference between dietary and oral hygiene maintenance habits with prevalence of caries. To record the dental caries, DMFT (decayed, missing, filled teeth) caries index was used.

The diet variables were recorded in relevance to the prepared questionnaires during intraoral clinical examinations. The diet pattern chart included the balanced and unbalanced diet pattern, snacking sugars in between meals, and drinking coffee or tea or sweetened milk before bedtime. The oral hygiene chart included proper oral hygiene habits like tooth brushing, use of interdental cleansers, mouthwashes, and frequency of visits to dentists.

RESULTS

This study was done on 201 random male and female patients who visited the clinics of RCsDP. The participants were divided into three groups and the DMFT scale of all the three groups was recorded (Table 1).

Table 1: Showing Age and DMFT Value from All Groups

Group	Age	Decayed	Missing	Filled	DMFT
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
1	6-19	1.69(1.3)	.16(.45)	.52(.77)	2.37(1.6)
2	20-40	0.9(0.96)	0.15(0.43)	0.58(0.65)	1.63(1.4)
3	41-60	1.76(1.14)	0.7(1.17)	0.76(1.1)	3.22(1.67)
Over all		1.45(1.2)	0.34(0.8)	0.62(0.84)	2.41(1.68)

The prevalence of dental caries in the sample was 85.6% and the age was significantly associated with caries prevalence and p-value was 0.000 (ANOVA). Post Hoc test was used to compare age groups with DMFT and statistical significance was found when comparison was made between the Groups 1 and 2, 1 and 3, and 2 and 3 (Table 2).

Table 2: Showing Comparison Between Groups

Groups	P-value
1 and 2	0.016
1 and 3	0.005
2 and 3	0.000

Post Hoc test significant at $p < 0.05$

Inter-examiner agreement was analyzed using Kappa statistics, and a high degree of agreement (Kappa index: 92.4%) was observed between the two examiners. It was observed from the questionnaire that the patients belonging to Group 1 (children and teenagers) were on a high carbohydrate diet consisting of sugary and sticky food like chocolates, sweets, candies, fast food, biscuits, pastries, and carbonated drinks. Also, a few children in this group took sweetened milk before bed time. Group 2 patients proved to be the second most frequent carbohydrate consuming group, whereas Group 3 patients showed the least carbohydrate consumption. The results showed a p-value of 0.29, indicating that carbohydrate dietary pattern plays a significant role in causing caries (Table 3).

It was also observed that carbonated drinks were frequently used by Group 1 patients when compared to Group 2 and Group 3 (Table 3). According to the questionnaire, it was observed that the Group 3 patients were mostly on a fibrous diet in the form of fruits, vegetables, and legumes indicating a low carbohydrate diet. Therefore, loss of tooth structure would be mainly due to age related issues. During intraoral examination, Group 1 participants showed high caries risk mainly due to their high carbohydrate diet pattern and improper brushing habits mostly leading to pit and fissure caries.

Table 3: Showing Dietary Patterns and Oral Hygiene Maintenance Habits

Variable	Responses	Mean DMFT(SD)	P-value
Toothbrush	Yes=171	2.4(1.8)	0.16
	No=30	2.67(1.7)	
Toothpaste	Yes=175	2.27(1.7)	0.003*
	No=26	3.31(1.5)	
Dental Floss	Yes=89	2.13(1.5)	0.04*
	No=112	2.63(1.8)	
Mouthwash	Yes=59	2.1(1.7)	0.096
	No=142	2.5(1.68)	
Fissure Sealant	Yes=9	2.33(1.8)	0.89
	No=192	2.4(1.68)	
Carb. Con	Yes=155	2.5(1.9)	0.29
	No=46	2.2(1.5)	
Carbonate Drink	Yes=160	2.47(1.8)	0.69
	No=41	2.34(1.6)	

Students' T-test was used, * significant at $P < 0.05$

Group 2 participants showed better oral hygiene with frequent dental visits. Most of the participants were aesthetically conscious. The P-value of tooth brushing in this study was found to be 0.16 indicating the importance of maintaining oral hygiene to minimize dental caries.

The changing of toothbrushes after every three months was recorded in the data from most of the participants in the three groups. All the groups used fluoride in the form of fluoridated water or fluoride-based toothpastes for brushing their teeth. Whereas, few participants from Group 3 were found using baking soda for brushing their teeth. Use of toothpaste affected significantly on caries prevalence showing the P-value of 0.003 (table 3). The participants of Group 2 mostly used fluoride-based mouthwashes and other inter-dental cleansers like dental floss and inter-dental brushes. Use of dental floss significantly reduced the chances of proximal caries showing a P-value of 0.04 (table 3). Few participants of Group 2 also showed fissure sealant application.

From the collected data and intra-oral examinations, it was observed that the older Group 3 participants were partially or completely edentulous and had many systemic health issues like diabetes, renal problems, cholesterol, and cardiac problems. Some of these patients were on continuous medications. Their intra-oral examination showed poor oral hygiene with less motivation towards oral hygiene maintenance. This group was mostly affected by root caries, gingivitis, and periodontitis leading to gingival recession and halitosis.

Table 2 shows the difference in caries prevalence between Groups 1 and 2, 1 and 3, and 2 and 3 which was significant at p-values 0.016, 0.005, and 0.000 respectively.

DISCUSSION

The probable reason for the prevalence of caries among the observed patients could be their unhealthy diet pattern, as sugary diet is one of the main factors involved in the caries progression. Carbohydrates create an acidic environment in the oral cavity and they have a propensity for making conditions appropriate for caries progression by lowering the pH of oral cavity.[5]

In this study, it was found that most of the children were in the habit of consuming carbohydrates mostly in the form of sweets with a sticky consistency. This finding is in agreement with the results of some previous studies showing that the foods which have semi-solid and liquid sugar can be extremely cariogenic. In a previous study conducted in 1966, it was concluded that regular intake of sugars can lead to uncontrolled tooth decay[8].

In 1994, H. Kalsbeek and G. H. Verrips confirmed that the rate of sugar consumption is one of the major caries causing factors in primary school children[9]. Similarly in 1954, Vipeholm studied and emphasized that the caries incidence was very low in basic diet and the caries risk increased when sucrose was given at meal times and in between meals[10].

The high incidence of caries recorded in this study among the groups, could also be due to the consumption of fizzy or carbonated drinks which can cause dental caries and acid erosion. The carbonated drinks have inherent acids and sugars in them, leading to higher incidence of dental caries. The intraoral examinations performed in this study showed tooth loss due to erosion especially in Group 1. Several researchers have confirmed a positive connection between caries and dental erosion with the use of soft drinks[11].

In all age groups studied, the most common site for caries attack was the pit and fissures of molars, probably because dental plaque can easily accumulate in these areas if dental hygiene is not proper. Chestnutt IG et al has mentioned that the occlusal surfaces are more at risk of getting caries than the smooth (labial and lingual) surfaces.[12]

This study also showed that the older participants mostly had root caries. The possible reason for this could be gingival recession with increasing age which leads to poor oral hygiene and eventually, initiation of periodontal disease. Previously, it has been explained by Warren et al, that caries beginning in this area, progress rapidly and are more difficult to restore as they are very close to the pulp.[13]

Plaque control is the basic goal of limiting and controlling the progression of periodontal disease and caries activity before tooth destruction. Preliminary method used for cleaning the oral cavity is toothbrushing. This study shows evidence that patients with good oral hygiene habits (Group 2) following a carbohydrate-rich meal were found to be at reduced risk of caries. Toothbrushing mainly depends on how it is used by an individual. In this study, it was found that most of the Group 1 and Group 3 participants were using improper brushing techniques. Only majority of the adults from Group 2 were using the proper brushing technique, which is the Bass method. It could be because adults are more aware and conscious about their oral hygiene. The Bass technique is widely accepted as an effective method for removing plaque not only at the gingival margin, but also approximately 1 mm subgingivally.[14]

A study conducted by Addy M et al also confirmed the importance of toothbrushing to remove plaque. It was evident from his results that there was inflammation and exudate present in the gingival tissues due to increased bacterial colonization, when the subjects were not allowed to brush their teeth for four days.[15]

Tooth brush also acts as a carrier for anti-cariogenic agents, such as fluoride. In this study, we found out that most of the population studied was using fluoride toothpastes especially the Group 2 adults. This could be one of the reasons for low prevalence of caries in young adult participants.

A longitudinal analysis done on consumption of fluoride also supports the results of this study showing that the spread of proximal caries is moderately low, mainly in the adult patients who are regularly taking fluoride as treatment or through utilization of fluoridated water.[16] Numerous clinical analyses, which include a Cochrane systematic review, have revealed that usual toothbrushing with fluoride toothpaste decreases the growth of coronal tooth decay by up to roughly 25% in contrast to toothbrushing with non-fluoride toothpaste.[17]

An interesting observation from Group 3 participants in this study was that some of them were using baking soda to clean their teeth. These patients, when examined, showed some arrested caries and low plaque index. This observation is also supported by a clinically controlled study with 270 reported subjects showing that toothpastes with baking soda showed more reduction in oral plaque ($p<0.01$) when compared to toothpastes without baking soda. Thus, the results proposed an affirmative link between plaque reduction and baking soda concentration.[17]

Mostly Group 2 participants were using mouthwash, to protect against dental caries and sensitivity. A study conducted by Augusto R et al on different mouthwashes, confirms the finding that mouthwashes offer a considerable and better reduction in dentine hypersensitivity, especially those mouthwashes that contain arginine.[18]

It was observed that participants in Group 2 were frequently using dental floss after meals. The use of dental floss appears to add additional advantages in decreasing plaque, when they are associated with conventional manual brushes. This observation was supported by a clinical study conducted by G.Z. Wright et al on school children in 1976 when a significant reduction of proximal caries was observed.[19]

A current assessment done on the effects of pits and fissure sealants found that sealants considerably decreased bacterial rate in carious lesions[20], keeping up the results of a new meta-analysis that sealants reduce caries advancement.[21] It was observed in this study that majority of the population being studied was unaware of fissure sealants and their benefits.

CONCLUSION

Unhealthy dietary patterns, alongwith poor oral hygiene maintenance, can lead to dental caries. Thus, a proper balanced diet and preventive measures for proper oral hygiene maintenance must be adopted to prevent or reduce incidence of dental caries.

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ORIGINAL ARTICLE

Awareness Regarding Emergency Management of Avulsed Teeth Among Medical Professionals Working in Emergency Departments of Select Hospitals in Karachi

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ABSTRACT

Objective: The aim of the study was to assess the knowledge of physicians working in emergency departments/ rooms (ED/ ER), regarding emergency, first-aid handling, and management of dental avulsion cases.
Methodology: This cross-sectional survey was conducted at the ERs of private and public sector hospitals of Karachi, Pakistan from August 2017 to January 2018. Data was collected using a survey questionnaire form with multiple-choice format, from 62 participants working in ERs of private and public sector hospitals of Karachi, Pakistan. The survey form included questions on sociodemographic details and questions on knowledge, confidence, and skill for handling of dental avulsion, prior experience, and interest in improving current relevant knowledge.
Results: More than 80% of respondents were not satisfied with their current knowledge regarding management of dental avulsion and wanted to take continuing medical education courses to improve it. Only 12.9% of respondents could identify milk as the best storage medium to store avulsed teeth. The majority of respondents would rather refer these cases to a dentist than perform emergency management.
Conclusion: In order for physicians working in emergency rooms to better handle cases of dental avulsion, it is essential that basic knowledge regarding management of dental avulsion is communicated to them with the help of continuing medical education courses, workshops, and incorporation of dental trauma management in the medical curriculum.

Key words: Tooth avulsion, emergency room, management

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کراچی کے منتخب ہسپتالوں میں ہنگامی شعبوں میں کام کرنیوالے ڈاکٹروں کے درمیان چوٹ سے نکلے دانتوں کے ہنگامی انتظام کے بارے میں آگاہی:

خلاصہ:

مقاصد: اس تحقیق کا مقصد ایمرجنسی، ابتدائی طبی امداد اور حادثاتی طور پر ٹوٹنے والے دانتوں کے بارے میں ہنگامی شعبوں میں کام کرنے والے معالجین کی معلومات جانچنا تھا۔

طریقہ کار: یہ کراس سیکشنل سروے اگست 2017 سے جنوری 2018 کے دوران کراچی کے پبلک اور پرائیوٹ سیکٹر کے ہسپتالوں کے ایمرجنسی رومز میں کیا گیا۔ ڈیٹا جمع کرنے کے لیے ایک سروے پر مشتمل سوالنامہ استعمال کیا گیا تھا جس میں سے انتخاب کرنے کے لیے آپشنز دیے گئے تھے۔ اور یہ سوالنامہ کراچی کے باسٹھ ہسپتالوں کے ایمرجنسی رومز میں کام کرنے والے ملازمین سے بھروایا گیا تھا۔ سوالنامہ معلومات عامہ، خود اعتمادی اور ٹوٹے دانتوں کے کیسز ہینڈل کرنے سے متعلق تھا۔

نتائج: نتائج میں یہ بات سامنے آئی کہ ۸۰ فیصد سے زائد افراد حادثاتی طور پر ٹوٹ جانے والے دانتوں کے علاج کے حوالے سے اپنی معلومات سے مطمئن نہیں تھے اور اس سلسلے میں مزید تعلیم حاصل کرنے کے خواہشمند تھے صرف 12 فیصد افراد یہ شناخت کر پائے کہ ٹوٹے دانتوں کو محفوظ رکھنے کا سب سے بہترین ذریعہ دودھ ہے۔ جواب دہندگان کی اکثریت ایسے کیسز کو دندان ساز کو بھیج دیتی تھی۔

تحقیق کے نتائج یہ سامنے آئے کہ ایمرجنسی رومز میں ایسے کیسز کو ہینڈل کرنے والے معالجین کی بنیادی معلومات میں اضافے کے لیے ان کو متعلقہ طبی کورسز، ورک شاپس کروانے چاہئیں۔

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INTRODUCTION

Dental trauma is frequently encountered in clinical practice. It may present in isolation or as part of poly-trauma due to road traffic accidents, assaults or sports injuries.[1] Commonly, patients suffering from injuries that have associated dental trauma, report to the emergency departments / rooms (ED/ ER) seeking treatment. It is not uncommon for dental injuries to be neglected in the face of other life threatening

complications.[2] Even when encountering isolated dental injuries, many a times necessary steps are not undertaken to prevent further damage to the teeth or improve the prognosis of such trauma, considering them to be trivial.[3] Dental avulsion is a type of dental trauma that comprises 16% of all traumatic dental injuries.[4] Medical physicians working in the ER are usually the first responders to deal with such dental injuries.[2]

The prognosis of an avulsed tooth is greatly dependent on how it is handled and stored before re-implanting into the socket.[5-7] It is an established fact that certain storage media have the ability to maintain periodontal ligament (PDL) cell viability until tooth re-implantation.[6] If the tooth is not placed in an appropriate medium while it is out of the socket, PDL cells undergo necrosis, resulting in replacement resorption and ankylosis in the coming years.[7] Ultimately, the tooth is lost and patients have to undergo the emotional and financial burden of replacing teeth in the aesthetic zone of the mouth.[8,9] Majority of the dentists are familiar with these media as well as with the first-aid steps necessary to handle dental avulsion. But it is the medical professionals working in emergency departments that are frequently the first healthcare providers that tend to patients of dental avulsion.[10] To ensure proper and appropriate treatment for such patients, it is essential that emergency medical professionals have sufficient training in basic principles of dental trauma management.[6] As physicians working in the emergency department attend to dental trauma patients before the dentist, it is vital that they possess sufficient knowledge on primary management of tooth avulsion, including handling of the tooth, placement in appropriate storage medium and re-implantation of tooth, before referring to dentists, as a dentist is usually not available at all hours in the ER.[10] This is important because if dental avulsion patients are managed appropriately, the economic value of retaining the tooth versus a prosthetic replacement can prove to be considerable over the person's lifetime. The replacement of the lost tooth later on with artificial means and its life-long maintenance places a considerable financial burden on the patient.[9] Loss of tooth has a negative influence on facial growth, function, aesthetics, and psychology, especially in young patients in which dental avulsion is a frequent occurance.[8,11,12]

Studies have been undertaken globally to assess the knowledge of ER personnel regarding emergency management of avulsed teeth[4,13,14] and one local study also examined the knowledge of different cadres of society regarding management of dental avulsion.[15] To the best of our knowledge, no local study has been conducted to determine the level of knowledge, exclusively of ER physicians regarding first-aid management of dental avulsion. This study was undertaken to assess the level of understanding and knowledge of ER physicians working in various private and public sectors hospitals of Karachi, regarding the basic and first-aid management of avulsed teeth.

METHODOLOGY

The study was conducted at Sindh Institute of Oral Health Sciences, Jinnah Sindh Medical University, Karachi, Pakistan. The protocol of the study was approved by the University's Institutional Review Board (JSMU/IRB/2017/-57). A survey form with questions related to dental avulsion and its management was developed and tested in a pilot survey of six individuals. The form was modified accordingly and finalized. Informed consent was obtained from all the participants. The questionnaire had three sections. The first section was reserved for the demographic information of the respondent including age, gender, educational qualification, and duty hours. The names of the participants were not recorded in order to ensure anonymity. The other two sections had questions with multiple options regarding physicians' basic knowledge and confidence in their skills for management of dental avulsion. Questions were directed towards the participants' familiarity with dental avulsion, their current knowledge and concepts of its management, personal and professional experience of dealing with avulsion, and their interest in increasing their knowledge regarding its management. Various private and public sector hospitals of Karachi with well-established ERs were targeted for data collection from their ER physicians. All physicians (including interns, house officers, medical officers, residents, and consultants) and medical students dealing directly with patients coming to ER, were included in the study. ER staff and physicians who refused to consent were excluded. Any one of the authors was always present while the participants filled the survey form, in order to clarify any ambiguity. Data was collected over a period of three weeks.

The data obtained from the completed survey forms were analyzed using SPSS version 20.0 (SPSS Pvt. Ltd., Chicago, IL, USA).

RESULTS

A total of 62 forms were collected, with 100% response rate. There were 42 (67.7%) females and 20 (32.3%) males who participated in the survey. The results from the section judging the confidence of the ER physicians regarding their knowledge and experience in dental

avulsion are given in Table I. Even though majority of the respondents knew the correct definition of dental avulsion, 67.7% of them confirmed that they did not have any prior relevant knowledge regarding its management. Regarding their knowledge and confidence about managing cases of dental avulsion, majority of respondents were found to be unsatisfied with their current knowledge. Almost 80% of the survey participants responded positively to the idea of attending Continuing Medical/Dental Education (CME/CDE) courses to change or improve their current knowledge about dental avulsion management. Around 30% of the respondents had seen 1-5 cases, while 32.3% of respondents had seen more than five cases during their professional careers. The rest had not come across any cases of dental avulsion in the ER.

More than half (56%) of the respondents thought that saline would be the best medium to preserve an avulsed tooth (Fig. 1). Only 44% respondents thought that upto one hour is an acceptable time until the tooth could be replaced after avulsion when kept dry. Results of other questions regarding management of dirty avulsed tooth in ER, handling of the avulsed tooth and avulsion trauma outside of hospital settings have been tabulated in Table II.

Table I: Confidence in Handling an Avulsed Tooth Based on Knowledge and Experience

	n (%)
Are you satisfied with your current knowledge regarding management of dental avulsion?	
Yes	5 (8.1)
No	50 (80.6)
Maybe	7 (11.3)
Would you consider taking CME to improve your knowledge?	
Yes	50 (80.6)
No	8 (12.9)
Maybe	4 (6.5)
How many cases of dental avulsion you may have seen?	
1 -5	19 (30.6)
6-10	10 (16.1)
11-15	3 (4.8)
>20	7 (11.3)
None	23 (37.1)
If you have had any personal experience regarding dental trauma, do you feel it has helped improved your ability/knowledge to manage dental trauma?	
Yes	10 (16.1)
No	24 (38.7)
Maybe	5 (8.1)
No personal experience	23 (37.1)

Table II: Management of Avulsed Teeth by Emergency Room Physicians

	n (%)
If the tooth is not placed in any storage medium, what is the maximum time when it can be re-implanted without any special surface treatment?	
up to 15 mins	12 (19.4)
up to 30 mins	20 (32.3)
up to 45 mins	3 (4.8)
up to 1 hour*	27 (43.5)
If you were at a site where someone knocked out tooth, you would	
Not take any action	3 (4.8)
Not take action because of medico-legal consequences	6 (9.7)
Be confident and replant the tooth*	3 (4.8)
Refer the patient to dentist*	50 (80.6)
The patient brings a knocked-out tooth in the ER and it is dirty, will you	
Wipe the tooth with a tissue paper	1 (1.6)
Rinse the tooth gently under running tap water for a few seconds without scrubbing it*	17 (27.4)
Discard the tooth saying it is of no use	8 (12.9)
Contact a dentist*	36 (58.1)
How would you hold the tooth while handling it?	
From the crown	47 (75.8)
From the root	5 (8.1)
Anywhere (crown or root)	10 (16.1)

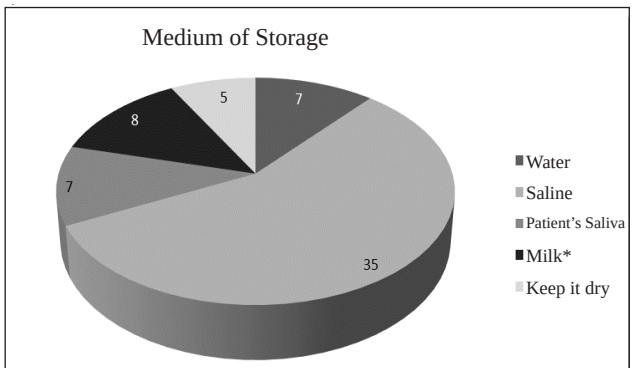

Figure I: Preferred Medium by Physicians for Storage of Avulsed Teeth

DISCUSSION

The importance of appropriate handling of an avulsed tooth cannot be overly emphasized. Every minute the tooth is left to dry results in death of the crucial PDL cells, without which tooth attachment to the socket wall is greatly compromised.[16,17] Every effort should be made to decrease this time period. Knowledge of appropriate handling and placement in appropriate

media is essential.[17] Even though this knowledge is considered basic for the dental fraternity; unfortunately medical physicians may not have been taught this during their formal education. They do, however, routinely encounter such patients,[18] and before the on-call dentist can be summoned to assess the patient, small measures such as placing the tooth in an appropriate storage medium, can go a long way in improving the prognosis of the tooth. Although establishment of dental specialty hospitals has increased in recent years, it is usually tertiary care hospitals with emergency rooms that the patients with dental and/or maxillo-facial injuries, go to for treatment.[18] These injuries may be accompanied by poly-trauma where the patient may be suffering from other injuries as well.[2] Management of dental trauma cannot be ignored or delayed in view of other injuries present.[3]

It is important to note that majority of the survey responders were familiar with the definition of tooth avulsion. This is an indication that very basic aspects of dental trauma are studied under the umbrella of general trauma during undergraduate medical education. But their prior knowledge was limited to being familiar with just defining dental avulsion.[1] In terms of first aid emergency management of avulsed teeth, participants were not familiar with the required protocols. Their responses depicted that they would rather refer avulsion cases to dentists than attempt any management, as they were uncertain of appropriate measures to be taken. In fact, the participants themselves were aware of this concern and professed that they were unsatisfied with their skills in management of avulsed teeth.

A large number of physicians who participated in the survey showed an interest in acquiring more knowledge regarding proper handling of the avulsed tooth, which is very encouraging. It is essential that there be an assimilation of dental trauma and its management in the medical education, or CME courses and workshops be held for medical colleagues by dental faculty, in order to update them on current practices in dental trauma management.[19,20] Medical undergraduates can be engaged in interdisciplinary seminars, case discussions, and clinical postings in dental or oral and maxillofacial departments to update them regarding basic guidelines of emergency management of dental avulsion. This will help the ER physicians to handle the avulsed teeth better, resulting in improved prognosis of these teeth. Since only adequate knowledge, skill, and very basic material is needed for managing dental trauma,[21] striving towards familiarizing the ER physicians in this aspect should present with minimal logistical hindrances.[22]

The respondents were not questioned regarding a relatively complex procedure that usually follows re-implantation of avulsed tooth, which is splinting. This is because splinting requires additional skill, training, and armamentarium. Therefore, the emphasis in this study was to assess only first-aid knowledge limited to handling, storing, and re-implanting the avulsed tooth, which are reasonably simpler procedures. This would encourage the ER physicians to take initiative in managing such cases, without getting hindered by lack of facilities or paraphernalia.[23]

Majority of the ER physicians had seen cases of avulsion during their practice, which highlights the importance of imparting knowledge regarding handling of avulsed teeth. Cases of dental avulsion had been seen in variable numbers by the participants. It is important to note that majority of the participants was less than 30 years old, thus possessing limited work experience and encountering fewer cases of avulsion. Even physicians with personal experience of avulsion also felt that this had not helped improve their skills. This could be attributed to the stressful environment in the ER, where critical patients with life-threatening injuries might hamper the physicians' concentration on management of dental avulsion, without appropriate background knowledge.

Dental trauma is not considered to be of primary concern in view of other life threatening injuries that the patient may present with. Yet, it is later during patient rehabilitation, that the loss of teeth sustained during previous injuries is acutely felt.[10-12] Hence, even if no active intervention can be planned immediately, merely placing avulsed teeth in an appropriate medium can improve their prognosis after re-implantation.

Even for physicians who have yet to encounter any such cases, their line of work makes it imperative that they will deal with avulsed teeth at one point or another during their job.[10] It is important to teach dental avulsion management to ER physicians in a stress-free simulated environment, in order to impart the relevant skills to them. Equipped with the right knowledge, these physicians can help to improve the prognosis of avulsed teeth.[15]

In terms of re-implanting the avulsed tooth back in the socket, very few physicians were confident about undertaking the procedure. Ideally, if the patient brings the avulsed tooth to the ER in a dry state, the first line of action should be to gently clean it under running water for 10 seconds, followed by immediate re-implantation of tooth into the empty socket.[5] The lack of confidence to undertake this procedure could be related to deficient knowledge and clinical experience in this regard.[13,15,24]

The majority of physicians correctly identified how to hold an avulsed tooth, but there was deficient knowledge regarding the window of opportunity to re-implant a tooth that has been brought to the ER in a dry state, which is upto 60 minutes from the time of actual avulsion.[5] This fact was not known to the majority of the study participants, who considered a time shorter than one hour from the time of trauma, a lost opportunity to re-implant the tooth. This gap in knowledge can be bridged with the help of continuing medical education courses, which would aid in keeping the ER physicians abreast with the current recommendations for first aid management of avulsed teeth.[25] Furthermore, brochures and informative posters could also be distributed to ER setups, to be kept for record and prominently displayed so they can easily be referred to when the need arises.

The main requirement for the maintenance of PDL cell viability is the timely placement of the tooth in an appropriate storage medium.[6,7] Drying of the PDL cells is detrimental to the prognosis of the tooth after re-implantation, resulting in ankylosis and ultimately loss of the tooth.[17] The knowledge of ER physicians regarding storage medium revealed that majority of the participants considered saline to be the best medium. This is consistent with findings from other studies,[10,18] which confirms the fact that knowledge about milk as the most recommended storage medium[17] is not widely known among the ER physicians. Although other storage media such as Hank's balanced salt solution and Propolis have also been recommended as storage and transport media for avulsed teeth, but milk remains the first option because of its low cost, ease of availability, and its ability to maintain the viability of PDL cells.[6] Packaged milk can easily be included in the inventory of any ER set up, to be immediately available in case a patient arrives with avulsed teeth. Although this study has been conducted in only one city of Pakistan, it can be assumed that similar responses regarding knowledge about management of avulsed teeth would be observed in other cities of the country, as similar curriculum for medical studies is followed as directed by PMDC.[15] This assumption does not account for individual personal experience or effort to gain additional knowledge in the field of dental trauma by an ER physician. Since the steps necessary to ensure survival of the PDL cells of avulsed teeth are simple and inexpensive, it is relatively easy to carry out these straightforward measures by the first respondents. The findings of this survey are consistent with similar studies evaluating the knowledge of ER physicians regarding management of avulsed teeth in different regions of the world.[10,14,17]

CONCLUSION

Dental avulsion is a common form of dental trauma, encountered routinely in the ERs. Physicians working in the ER are usually the first responders to such trauma, so it is essential that they acquire the basic first-aid knowledge on appropriate handling and management of avulsed teeth. This will help to greatly improve the prognosis of avulsed teeth, resulting in patient's psychological and financial benefit. The knowledge and understanding of physicians in management of dental avulsion can be improved by the help of incorporation of dental trauma management into the undergraduate medical curriculum, continuing medical education courses, and workshops.

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Technical information

Identify the methods, apparatus (give the manufacturer's name and address in parentheses), and procedures in sufficient detail to allow other workers to reproduce the results. Give references to established methods, including statistical methods (see below); provide references and brief descriptions for methods that have been published but are not well known; describe new or substantially modified methods, give reasons for using them, and evaluate their limitations. Identify precisely all drugs and chemicals used, including generic name(s), dose(s), and route(s) of administration.

Authors submitting review manuscripts should include a section describing the methods used for locating, selecting, extracting, and synthesizing data. These methods should also be summarized in the abstract.

(*Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication*)

ORIGINAL ARTICLE

A Retrospective Analysis of Gynaecological Polyps at a Tertiary Care Hospital in Karachi

Saba Sattar[1], Nazish Jaffar[1], Ayesha Iftikhar[2], Shahnaz Imdad Kehar[2], Syed Mehmood Hasan[1], Abdul Sattar[3] and Muhammad Ammar Yonus[4]

ABSTRACT

Objectives: To analyze the spectrum of various histological types of the gynaecological polyps in a tertiary care hospital in Karachi and to identify prevalent age group as well as the common anatomical location of these polyps.

Methodology: This retrospective study was carried out on all histopathologically proven cases of gynaecological polyps received in Pathology Department, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, between January 1, 2016 to December 31, 2017. The prevalence of various types of polyps was analyzed in simple frequencies, and prevalence of each histological type of the polyps was calculated according to the age and their common anatomical location.

Results: Mean age of the patients was 41.80 ± 11.87 years and most of the cases (40.96%) were in the fourth decade, followed by fifth (25.5%) and third (15.6%) decades respectively. Frequently encountered polyps were endometrial (33; 39.7%), followed by inflammatory (28; 33.7%), leiomyomatous (12; 14.45%), and adenomyomatous polyps (07; 8.4%) respectively. Only a single fibroepithelial polyp was found. Two polyps (2.4%) were identified as atypical polypoidal lesions.

Conclusion: Gynaecological polyps were common in fourth decade. Most of the polyps were located in the endometrium followed by the endocervix. Only two of the polypoidal lesions exhibited atypical morphology.

Key words: Gynaecological polyps, endometrial polyps, endocervical polyps, adenomyomatous polyps, leiomyomatous polyp, fibroepithelial

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کراچی میں تیسرے درجے کے اعلیٰ ہسپتال میں جینیاتی پولپس کا پچھلے حالات کا تجزیہ

مقصد: کراچی میں ایک ہسپتال میں جینیاتی پولپس کے مختلف حیاتیاتی اقسام کی اسپیکٹرم کا تجزیہ کرنا اور زیادہ تر مریضوں کی عمر کے گروپ کے ساتھ ساتھ ان پولپس کے عام جسمانی مقام کی شناخت کرنا۔

طریقہ کار: یہ پچھلے حالات کا مطالعہ پہلی جنوری 2016 سے اکتیس دسمبر، 2017 کے درمیان پیتھالوجی ڈیپارٹمنٹ کے بنیادی میڈیکل سائنس انسٹی ٹیوٹ، جناح پوسٹ گریجویٹ میڈیکل سینٹر، کراچی میں حاصل ہونے والی جینیاتی پولپس کے تمام ہسٹرولوجیکلی ثابت کیسز پر کیا گیا۔

نتائج: اس تحقیق میں مریضوں کی عمر 41.80 سے 11.87 سال کے درمیان تھی اور زیادہ تر مریض عمر کی چوتھی دہائی میں تھے، اس کے علاوہ بالترتیب پانچویں اور تیسری دہائیوں میں بھی تھے۔ اکثر متضاد پولپس اینڈومیٹریل تھے (39.7÷33)، اس کے بعد سوزش (33.7÷28)، leiomyomatous (14.45÷12)، اور adenomyomatous پولپس (8.4÷07) بالترتیب صرف ایک فبروایپی تھیلیل پولپ پایا گیا تھا۔

نتیجہ: تحقیق میں یہ بات سامنے آئی کہ چوتھی دہائی میں جینیاتی پولیپ عام تھے. سب سے زیادہ پولپس اینڈومیٹریلم میں واقع تھے اور اس کے بعد endocervix میں۔

INTRODUCTION

Around the world, abnormal uterine bleeding is a frequently encountered gynaecological complaint in females of all ages[1]. The International Federation of Gynaecology and Obstetrics (FIGO) has introduced a simple and uniform classification system PALM-COEIN (polyp; adenomyosis; leiomyoma; malignancy and hyperplasia; coagulopathy; ovulatory dysfunction; endometrial; iatrogenic; and not yet classified) for precise treatment and to correlate clinical and scientific

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parameters[2]. The polyps of endometrium are a frequent gynaecological problem with incidence varying from 7.8%-34.9% respectively. Endometrial polyps are localized hyperplastic stromal growths of endometrium covered by epithelium. Generally, these polyps are benign but, occasionally may exhibit premalignant, malignant and/or metastatic disease[3,4]. The polyps arising from uterine cervix result from hyperplasia of endocervical columnar epithelial cells and are usually associated with inflammation. Endocervical polyps are also referred to as inflammatory polyps and affect approximately 2-5% of the female population[5]. Uterine leiomyomas are the most frequently encountered benign neoplasms, attributing 30% of females with abnormal uterine bleeding[6]. A rare entity referred to as adenomyomatous polyps contributes for only about 1.3% of all endometrial polyps and predominantly contains smooth muscle component covered with epithelium[7]. The present study was conducted to analyze the spectrum of various histological types of gynaecological polyps in a tertiary care hospital in Karachi and to identify the prevalent age group as well as the common anatomical location of these polyps.

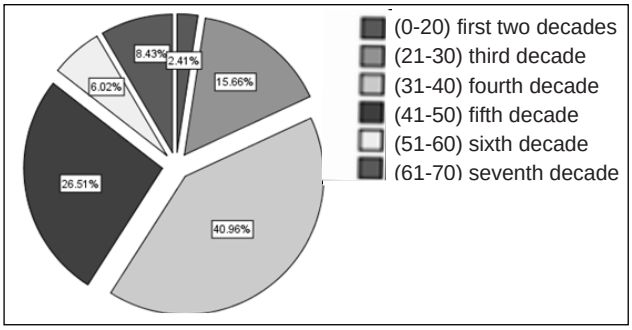

Figure 1: Age Distribution of the Included Subjects

Table 1: Frequency of Various Gynaecological Polypoidal Lesions (N=83)

Types of Polyps	No. of Polyps	%
Inflammatory Polyps	28	33.7
Endometrial Polyps	33	39.7
Leiomyomatous Polyps	12	14.45
Adenomyomatous Polyps	07	8.4
Fibroepithelial Polyps	01	1.21
Atypical Polypoidal Lesions	02	2.4
Total	83	100

METHODOLOGY

This retrospective study was carried out in the Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi from January 1, 2016 to December 31, 2017. The study included all histopathologically proven (n=83) cases of gynaecological polyps received at the pathology department. The specimens were retrieved and re-analyzed for the morphological typing according to the FIGO PALM-COEIN classification system. The demographical values were collected from the records. Data analysis was performed by SPSS windows version 22. Frequencies of various gynaecological polyps were calculated in percentages, and the prevalent age groups were identified. Moreover, the prevalence of each histological type of the polyps was calculated according to the age and the anatomical location. Results were expressed in terms of charts and tabulations.

RESULTS

A total of 83 gynaecological polyps were received during the above-mentioned duration. The mean age of the patients in present study was 41.80 ± 11.87 years.

Among the age groups, the maximum number (40.96%) belonged to the fourth decade, followed by fifth (25.5%), third (15.6%), and seventh (8.4%) decades respectively. Figure 1 shows the age distribution of the included subjects.

Out of 83 polypoidal lesions, the highest values 33 (39.7%) were of endometrial polyps followed by 28 (33.7%) polyps of inflammatory type, 12 (14.45%) leiomyomatous and 07 (8.4%) adenomyomatous polyps respectively. There was only one (01) fibroepithelial polyp found in this study. In present study, two cases (2.4%) were found to have atypical lining, and were identified as atypical polypoidal lesions. Table 1 shows the frequency of histological types of gynaecological polyps.

We also assessed the prevalence of histological types of the polyps with the patient's age (Table 2). The frequency of the endometrial polyp was significantly higher among participants in fourth decade (13.2%) of life, followed by those in fifth (10.8%) and seventh decades (7.2%) respectively. The inflammatory polyps were common in fourth decade (15.6%), followed by third (8.4%) and fifth decades (7.22%) respectively. Fourth decade was also common for leiomyomatous polyps (8.4%), however the frequency of adenomyomatous polyps was high among fifth decade (3.36%) followed by 2.4% cases in fourth decade respectively. The only case of fibroepithelial polyp belonged to a participant in the third decade of life. Out of the two atypical polyps, one polyp was seen in fourth decade while the other was in sixth decade.

The anatomical location and histological type of the polyps were also assessed in the study (Table 3). In the current study, all endometrial polyps (39.7%) were

Table 2: Distribution of Gynaecological Polyps According to the Age (N=83)

Age in Years	Types of Polyps N (%)					
	Inflammatory Polyps	Endometrial Polyps	Leiomyomatous Polyps	Adenomyomatous Polyps	Fibroepithelial Polyps	Atypical Polyp
<20	01 (1.21)	01 (1.21)	00	00	00	00
21-30	7(8.4)	3 (3.6)	1 (1.21)	1 (1.21)	1 (1.21)	00
31-40	13(15.6)	11 (13.2)	7 (8.4)	2 (2.4)	00	01 (1.21)
41-50	6 (7.22)	9 (10.8)	4 (4.8)	3 (3.6)	00	00
51-60	01 (1.21)	3 (3.6)	00	00	00	01 (1.21)
61-70	00	6 (7.2)	00	01 (1.21)	00	00
Total	28 (33.7)	33 (39.7)	12 (14.45)	7 (8.4)	01 (1.21)	2 (2.4)

Table 3: Frequency of Gynaecological Polyps According to the Anatomical Location (N=83)

Type of Polyp	Anatomical Site of Polyp N (%)			
	Endocervix	Endometrium	Vulva/vagina	Total
Inflammatory Polyps	26 (31.3)	2 (2.4)	00	28 (33.7)
Endometrial Polyps	00	33 (39.7)	00	33 (39.7)
Leiomyomatous Polyps	04 (4.81)	8 (9.63)	00	12 (14.45)
Adenomyomatous Polyps	01 (1.21)	06 (7.22)	00	7 (8.4)
Fibroepithelial Polyps	00	00	01 (1.21)	01 (1.21)
Atypical Polypoidal Lesions	01 (1.21)	01 (1.21)	00	02 (2.4)
Total	32 (28.55)	50 (60.24)	01 (1.21)	83 (100)

located within the uterine cavity. Most of the inflammatory polyps (26; 31.3%) arose from the endocervix, while only 02 (2.4%) were derived from endometrium. Amongst the twelve leiomyomatous polyps, majority (9.63%) had originated from uterine corpus while 4.81% arose from the uterine cervix. Almost all adenomyomatous polyps were placed within the uterine cavity and only one (1.21%) had endocervical origins. The only case of fibroepithelial polyp had originated in vulva. One of the atypical polyps was identified as endocervical atypical polyp whereas the other had an atypical endometrial lining.

DISCUSSION

Genital tract polyps are a common gynaecological problem. They are a known cause of menorrhagia in women. Other common causes include leiomyoma and adenomyosis[8]. Female genital tract polyps are frequently reported in endometrial and cervical biopsies. They are also incidentally found in hysterectomy specimens. The current study was aimed at observing the spectrum of female genital tract polyps and their association with site and different age groups.

The mean age of the subjects of the study was 41.80 ± 11.87 year. This is comparable to 37 years of mean age reported by Barati, Masihi, and Ilkhan in an Iranian study.[9] Age range recorded was 19-70 years in the present study. Dreisler *et a*l. in their study also reported a similar age range of 20-74 years. Maximum number of polypoidal lesions presented between 31-40 years age group in the current study. This finding is supported by a study of Dreisler *et al*. which showed maximum number of cases above the age of 30 years.[10] Furthermore, in the present study, only two cases, one each of endometrial and inflammatory polyp, were noted in less than 20 years age group. This finding suggests that prevalence of gynaecological polyps increases with age. To our knowledge, none of the studies observed polypoidal lesions of female genital tract in the second decade.

In the current study, endometrial polyps from uterine corpus were recorded in majority 50 (60.2%). This finding is comparable to a study conducted at Aga Khan University Hospital (AKUH) which reported 42.7% endometrial polyps. This slight discordance in findings may be due to inclusion of benign as well as malignant cases in their study. Endometrial polyps are mucosal outgrowths comprising endometrial glands and stroma. It is critical to identify the histological subtype of endometrial polyps because on ultrasound, they may occasionally give an impression of endometrial hyperplasia or frank malignancy.[11] Cervical polyps constituted 32 (28.5%) cases in the current series. This is supported by an Iranian study of Barati, Masihi, and Ilkhan which recorded 34.7% endocervical polyps.[9,12]

It has been reported that occasionally lesions like leiomyoma and adenomyoma of uterus can give rise to a polypoidal lesion within the endometrial cavity. On gross examination, they may mimic endometrial polyp. Biopsy is essential to identify the morphological subtype of polypoidal lesions. In the present study, out of 83 cases, 12 (14.4%) were leiomyomatous polyps. A Danish study agrees with this finding by observing 13% of these lesions. Adenomyomatous polyps represented 07 (8.4%) cases in our study. This is comparable with a study by Sheth, Hamper, and Kurman which recorded 2.85% cases. This difference may be due to inclusion of only postmenopausal women in their study[10,11,14].

Inflammatory polyps constituted 28 (33.7%) cases in the current series. A study by Filomena *et al*. observed the association of endometritis (inflammation) with endometrial polyps. This study recorded a comparable number of 27.4% of inflammatory polyps. It also analyzed the possible association of vascular changes with inflamed endometrium[14]. This result suggests that a significant number of endometrial polyps develop in the background of inflammation.

Atypical polypoidal lesions were recorded in only 02 (2.4%) cases in the present study. This observation is supported by a Danish study which recorded 1.5% polypoidal lesions with atypical changes. Similarly, Pujani *et al*. agrees with our finding by reporting 2.2% of atypical lesions in their series. This fact highlights that the risk of malignancy is low in uterine polypoidal lesions. Only 01 (1.21%) case of fibroepithelial polyp was diagnosed in the vulval/vaginal region in the current study. In literature, this entity has been reported as a benign and rare subtype of polyps of this region[10,11,15].

It is important to accurately diagnose the histopathological types of polypoidal lesions on endometrial and cervical curettings. Correlation with clinical history is helpful in majority of cases. Various treatment modalities including non-surgical methods as well as minor procedures like polypectomy, myomectomy, or surgical ablation are available[16]. Correct diagnosis on small biopsies may prevent the burden of unnecessary hysterectomies in females of reproductive age group.

Further detailed studies with more defined criteria should be conducted to establish a clear association between the age groups and the subtype of polyps and also to rule out any premalignant or malignant transformation within the polypoidal lesion in females.

Limitation of study: This study is a uni-centred analysis and does not reflect the prevalence of population.

CONCLUSION

The study concludes that gynaecological polyps are common in fourth decade. Endometrial polyps are the most common histological type followed by inflammatory polyps. Majority of the polyps arise from uterine corpus and subsequently from the uterine cervix. Most of the gynaecological polyps are benign, however, only two lesions exhibited atypical morphology in present study.

Conflict of interest: The authors declare no conflict of interest for this study.

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Tables

Tables capture information concisely, and display it efficiently; they also provide information at any desired level of detail and precision. Including data in tables rather than text frequently makes it possible to reduce the length of the text.

Type or print each table with double spacing on a separate sheet of paper. Number tables consecutively in the order of their first citation in the text and supply a brief title for each. Do not use internal horizontal or vertical lines. Give each column a short or abbreviated heading. Authors should place explanatory matter in footnotes, not in the heading. Explain in footnotes all nonstandard abbreviations. For footnotes use the following symbols, in sequence:

*,†,‡,§,||,¶,**,††,‡‡

Identify statistical measures of variations, such as standard deviation and standard error of the mean.

Be sure that each table is cited in the text.

(*Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication*)

ORIGINAL ARTICLE

A Comparison of Smoking Habits Between Medical and Non-Medical University Students and Effect of University Related Factors on Their Habits

Saima Akhter[1], Usman Ali Warraich[2], Sajeer Bhura[3], Hassan Mustafa[3] and Nadeem Rizvi[4]

ABSTRACT

Objective: This study has determined the prevalence of tobacco smoking among medical and non-medical students alongwith the influence of university related factors on smoking habits.
Methodology: We surveyed 1487 students from 18 universities across Pakistan from September to December, 2014. A self-administered questionnaire was filled out by the students. Chi square and binary logistic regression was used through SPSS 22.
Results: Mean age of study population was 20.17 years. Overall prevalence of smoking was 21.5% with 13%, 28.5%, and 30.6% among Medical, Engineering, and Social Sciences students respectively. Among smokers' group, 69.9% started smoking in the age bracket of 16-20 years. In all universities, cigarette smoking was popular over shisha. There was an exponential rise in frequency of smokers as we examined successive academic years in non-medical universities ($p<0.05$). Allocation of specific place for smoking within campus, presence of shisha bar nearby, anti-smoking literature on social media, and health awareness seminars conducted by teachers have a strong impact on smoking habits ($p<0.05$).
Conclusion: All kinds of smoking were found to be more prevalent in non-medical universities. There is a strong relationship between university-related factors and smoking prevalence. Smoking-related health education must be a part of the academic curriculum in all types of institutions.

Key words: Shisha, non-medical university, medical university

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طبی اور غیر طبی یونیورسٹی کے طالب علموں کے درمیان تمباکو نوشی کی عادتوں کے موازنے اور ان کی عادات پر یونیورسٹی کے متعلقہ عوامل کے اثرات کا جائزہ:

خلاصہ: مقاصد:: اس مطالعہ میں طبی اور غیر طبی طالب علموں میں تمباکو نوشی کی عادات اور طالبعلموں پر یونیورسٹی کے ماحول کے اثرات کا تمباکو نوشی کی عادت سے تعلق کا جائزہ لیا گیا

طریقہ کار: ہم نے ستمبر 2014 سے دسمبر 2014 کے دوران پورے پاکستان سے اٹھارہ جامعات کے 1487 طلبا پر سروے کیا۔ ایک خود سے تیار کردہ سوالنامہ طلبا سے پُر کروایا گیا۔

نتائج: تحقیق میں شامل طلبا کی عمر کا اوسط 20.17 سال تھا، تمباکو نوشی کا مجموعی پھیلاؤ 21.5 فیصد تھا جس میں میڈیکل کے طالبعلموں میں 13 فیصد، انجینئرنگ میں 28.5 فیصد اور سماجی سائنس کے طالب علموں میں 30.6 فیصد تھا۔ تمباکو نوش گروپس میں سولہ سے بیس سال کی عمر کے لوگوں میں تمباکو نوشی کا رجحان 69.9 فیصد تھا۔ تمام جامعات میں سگریٹ نوشی شیشے کے مقابلے میں زیادہ مقبول تھی تمباکو نوشی کرنے کے لئے مخصوص جگہ کا کیمپس کے اندر ہونا، قریب میں شیشا بار کا ہونا، سوشل میڈیا پر انسداد سگریٹ نوشی مہم، اور اساتذہ کی طرف سے کئے جانے والے صحت کے بارے میں بیداری کے سیمینار کے تمباکو نوش افراد پر زبردست اثرات نظر آئے۔

نتائج: تمام اقسام کی تمباکو نوشی غیر طبی جامعات میں زیادہ مقبول نظر آئی۔ یونیورسٹی مین موجود عوامل اور سگریٹ نوشی کی کھپت کے درمیان خاصا مضبوط تعلق پایا گیا۔ تمباکو نوشی سے متعلق صحت کی تعلیم کو ہر قسم کے اداروں میں تعلیمی نصاب کا حصہ بننا چاہئے۔

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INTRODUCTION

With one billion tobacco smokers[1], the world faces an epidemic that would take decades to control. World Health Organization (WHO) predicts that tobacco smoking rate will rise to 1.5 times of its current value by 2025[2]. Tobacco smoking does not refer to cigarette smoking per se; other types like pipe, *bidi*, water pipe (*shisha*), and cigar are also included.

Smoking behaviour formed in the second decade of life has long lasting impact on individual as well as for public health in general. It is a complex behaviour and several factors influence this habit. In the past, investigators have pointed out many social factors: smoking by peers and family, spending leisure time outside home[3], poor educational performance, availability of pocket money[4], and relieving boredom[5]. The author postulated that students from non-medical faculties would indulge more in smoking when compared to medical students. Several studies on smoking habits among students around the world have been reported, but none, to the authors' knowledge, has established the connection between smoking attitude and university-related factors. Therefore, this study was conducted to find the correlation between these factors.

The aim was to describe the overall prevalence of smoking in students from 18 medical and non-medical universities across Pakistan and also elicit preference between cigarette and shisha among these students. We also evaluated the influence of university-related factors on their smoking habits and pattern of smoking over successive years of graduation.

METHODOLOGY

This cross-sectional survey was conducted in 18 universities across the country. Medical and non-medical universities located in four large cities: Karachi, Multan, Hyderabad, and Lahore were selected. Non-medical universities were further divided into Engineering and Social Sciences. Students from all graduation years, from the first year to the final year, were requested to take part in survey. Non-medical universities offer four-years while medical universities offer five-years graduation programmes in Pakistan. Convenient sampling technique was used to collect the data. Ethical approval was taken from the Chest Health and Education Society, an individual body from Pakistan including leading pulmonologists and statisticians.

Self-made questionnaire was use as the tool for data collection. Questionnaire contained three sections. First section covered the demographic data including age in years, gender, university name, and year of graduation. Second section contained questions regarding personal smoking habits, and third section was related to university factors influencing the smoking habits. In second and third sections, five and seven questions were asked respectively and were measured in dichotomous scale, except age of initiation that was measured in ratio scale.

Investigators visited selected universities from September 2014 to December 2014 and permission was taken from university management. Students were approached in their classes during lectures. The aim of the study was explained to students and they were assured regarding the confidentiality of responses and consent was taken. The college faculty members were not involved in handling the questionnaire to ensure confidentiality. The questionnaires were filled in average time period of 10 to 20 minutes in the presence of the investigator to ensure complete comprehension by respondents and avoid misunderstandings.

SPSS 22 was used for data analysis. P value of less than 0.05 was considered as statistically significant. In the descriptive analysis, mean ± standard deviation and proportions were used for continuous variables and categorical variables respectively. Chi-square test of association was applied for association between two nominal variables. Binary logistic regression was used to predict the possibility of a student to smoke in the final year, as compared to the first year. To identify the factors associated with smoking prevalence, Odds Ratio (OR) was calculated through logistic regression analysis with Confidence Interval (CI) of 95%.

RESULTS

Questionnaires were distributed to 1800 students and 1487 responses were received. Response rate was 82.6%. Number of questionnaires analyzed was 1487 of which 689 were filled by female students and 798 were filled by male students. Among the respondents, 746 (50.2%), 221 (14.9%) and 520 (35%) belonged to Medical, Engineering, and Social Sciences groups respectively. The mean age of study population was 20.17 (±4.2) years with the male to female ratio of 1:1.15 (male 53.7% and females 46.3%).

The overall prevalence of smoking among Pakistani students was found to be 21.5% with 13%, 28.5%, and 30.6% among Medical, Engineering, and Social Sciences students respectively. The reported mean age of initiation of smoking was 18 years. However, 69.9% of students reported acquiring the habit of smoking between the ages of 16 and 29. The reported youngest age for initiation of smoking was seven years.

The effect of graduation year on smoking status was investigated through Chi square. Increasing smoking prevalence with each graduation year was observed in all three disciplines. It is important to note that there was a steep rise in prevalence among Engineering ($p < 0.001$) students followed by those of Social Sciences ($p < 0.001$). The rise in medical students was not significant statistically ($p=0.391$).

Table 1: Socio Demographic Characteristics of Study Population

Characteristics of Study Population	Group	Total No	%
Age (Years) (Mean ± Sd)	20.17 ± 4.22		
Gender	Female	689	46.3
	Male	798	53.7
Discipline	Medical	746	50.2
	Engineering	221	14.9
	Social Sciences	520	35
Years	1	220	14.8
	2	411	27.6
	3	437	29.4
	4	292	19.6
	5	127	8.5
City	Karachi	799	53.7
	Lahore	356	23.9
	Hyderabad	95	6.4
	Multan	237	15.9
Total		1487	100

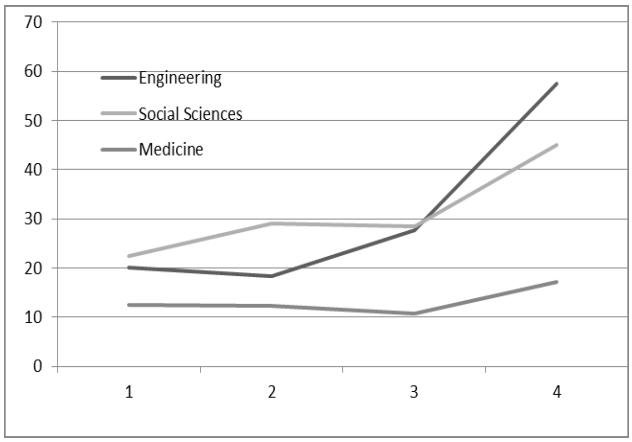

Figure 1: Relationship between graduation year and percentage of smoking (%). Graduation years are on horizontal axis.

The trend of smoking was assessed by comparing two major kinds—cigarette and shisha. Cigarette smoking was higher than shisha smoking in all three disciplines: Medical, Engineering, and Social Sciences. Both kinds of smoking were more common in males. There was an increase in both kinds of smoking with every year of graduation.

A binary logistic regression analysis was applied to assess the odds of students in the final year when compared to the first year students. Compared to Medicine disciplines [1.45 times (C.I 0.621-3.41; p=0.388)], it was significantly higher in Engineering [5.4 times (C.I1.99-14.66; p<0.01)], and Social Sciences [2.84 times (C.I 1.58- 5.10; p <0.01)].

The logistic regression model indicates that various university factors are associated with smoking status of students. Students whose teachers talk to them regarding smoking issues are 1.5 times less likely to smoke as compared to students with whom teachers never talk about it (OR 1.56, p<0.001).

Allocated place for smoking within campus (OR 0.00, p<0.001) and presence of shisha bars near campus (OR 0.00, p<0.001) were found to be significantly associated with the smoking status of students.

In Pakistan, social media is increasingly becoming the media of communication in universities between the students and the management. Regarding social media, watching promotional material or material against tobacco was significantly associated with smoking status (p<0.001). Students who had never come across material against smoking on social media were 1.72 times (p<0.001) more prone to smoking as compared to students who had come across it in the past. Moreover, 76.1% Medicine, 62.7% Social Sciences and 55.2% Engineering students reported searching for data against smoking on social media.

DISCUSSION

The overall smoking prevalence of 21.5% shown in the present study is comparable with WHO figures of 19.15% for adult smokers. Our results of smoking prevalence among medical students was 13%, which is low when compared to 35-56% in Turkey[6], 26.8% in China[7], and 22.4% in Italy[8], but higher than 12.2% in Puerto Rico[9], 10.3% in Japan[10], and comparable to previous data 14.4%[11] from Pakistan.

Cigarette smoking, when compared to shisha, showed a higher trend among students from all disciplines. In the last two decades, enormous work has been done to find the prevalence of shisha smoking among young adults worldwide but recent data on exact prevalence of cigarette smoking among university students are scarce and small effort is made to compare several kinds of smoking in young population.

In the present study, we tried to elicit factors linked with the universities' environment. Absence of anti-smoking literature from curriculum, lack of interaction between students and teachers regarding smoking habits, and knowledge of students regarding the presence of shisha bars near the university and in-campus dedicated area for smoking. Interestingly, non-smokers took more interest in searching and reading anti-smoking literature on social media than did the smokers.

Identifying university characteristics associated with tobacco smoking may help in focusing prevention efforts. Our results clearly demonstrate that all kinds of smoking were the most common among non-medical students, when compared with students from medical universities. This finding is consistent with previous studies in Asia and Europe[12,13]. Moreover, there was an exponential rise in the frequency of smokers as we examined successive academic years in non-medical universities, the most notable were Engineering students.

	Shisha							Cigarette						
	Yes			No				Yes			No			
	n	Row%	Column %	n	Row %	Column %	P value	n	Row %	Column %	%	Row %	Column %	P value
Dicipline														
Medical	67	9	32.5	679	91	53	0.000	84	11.3	32.1	662	88.7	54	0.000
Engineering	101	19.4	49	419	80.6	32.7		59	26.7	22.5	162	73.3	13.2	
Social Sciences	38	17.2	18.4	183	82.8	14.3		119	22.3	45.4	401	77.1	32.1	
City														
Hyderabad	10	10.5	4.9	85	89.5	6.6	0.583	9	9.5	3.4	86	90.5	7	0.025
Karachi	118	14.8	57.3	681	85.2	53.2		131	16.4	15	668	83.6	54.5	
Lahore	45	12.6	21.8	311	87.4	24.3		74	20.8	28.2	282	79.2	23.2	
Multan	33	13.9	16	204	86.1	15.9		48	20.3	18.3	189	79.7	15.4	
Gender														
Male	162	20.3	78.6	636	79.7	49.6	0.000	236	29.6	90.1	562	70.4	45.9	0.000
Female	44	6.4	21.4	645	93.6	50.4		26	3.8	9.9	663	96.2	54.1	
University Year														
1st Year	31	14.1	15	189	85.9	14.8	0.000	32	14.5	12.2	188	85.5	15.3	0.000
2nd Year	61	14.8	29.6	350	85.2	27.3		61	14.8	23.3	350	85.2	28.6	
3rd Year	45	10.3	21.8	392	89.7	30.6		72	16.5	27.5	365	83.2	29.8	
4th Year	60	20.5	29.6	232	79.5	18.2		82	28	31.3	211	72	17.2	
5th Year	9	7.1	4.4	117	92.9	9.1		15	11.9	5.7	111	88.1	9.2	

Chart 2: Factors associated with smoking among students in Pakistan

Characteristics	Smoking Status		Odds Ratio	Sig value	95% C.I. for Odds Ratio	
	Non smoker n = 1168	Smoker n = 319				
Are there any shisha bars near your university premises?						
I don't know	1140 (97.6)	4 (1.3)	0.000	0.000	0.000	0.001
No	14 (12)	179(56.1)	1.316	0.486	0.607	2.853
Yes	14 (12)	136(42.6)	1		-	-
Is there any specific allotted smoking area for students within your university premises?						
I don't know	1138 (97.4)	5 (1.6)	0.000	0.000	0.000	0.001
No	21 (1.8)	174(54.5)	0.533	0.533	0.236	1.200
Yes	3 (0.8)	140(43.9)	1		-	-
Does your university ever arrange any programme regarding smoking habits?						
No	909 (77.8)	247(77.4)	0.977	0.880	0.727	1.315
Yes	259 (22.2)	72 (22.8)	1		-	-
Do your teachers ever talk regarding smoking habits as part of your course?						
No	673 (57.6)	217 (68)	1.565	0.001	1.204	2.034
Yes	495 (42.4)	102 (32)	1		-	-
Do you ever attend any organized programme regarding shisha information outside your university?						
No	968 (82.9)	258(80.9)	0.874	0.406	0.636	1.201
Yes	200 (17.1)	61 (19.1)	1		-	-
Have you ever come across any programme on electronic media regarding hazards of smoking?						
No	466 (39.9)	133(41.7)	1.077	0.562	0.838	1.385
Yes	702 (60.1)	186(58.3)	1		-	
Have you come across any article on social media regarding hazards of smoking?						
No	339 (29)	132(41.4)	1.726	0.000	1.336	2.230
Yes	829 (71)	187(58.6)	1		-	-

A dire need exists to formulate policies nationwide that specifically target students. We are making the following suggestions based on our data:

· Shisha bars near educational institutes and dedicated on-campus smoking area should be dealt with full force of law.

· Major councils responsible for curriculum, like Pakistan Medical & Dental Council and the Pakistan Engineering Council, should be directed to introduce a curriculum specifically designed to address all kinds of tobacco consumption and their hazards in each year of graduation in all kinds of universities.

· Teachers should be encouraged to discuss the pros and cons of smoking at university level and act as role models for their students.

· The World No Tobacco Day as declared by WHO on 31st May, should be observed across all educational institutes.

· Adolescents these days spend considerable hours on social media. Awareness against smoking should be promoted on social media by assigning roles to people from educational institute.

Limitations of the Study:

The major limitation of our study was its cross-sectional nature. In addition, the convenient sampling technique might not reflect the true nature of targeted population. Also, the smoking status was based on self-reporting and was not validated by any objective measurements like carbon monoxide level measurements, thus we suspect under-reporting of facts, especially by females. Previously, under-reporting by females has been reported from this part of the World because of cultural reasons[14]. The strengths of this study were its generalizability and usage of standardized interview technique. Additionally, the use of personalized approach ensured data validity and achieved high response rate.

Despite these constraints, our study has raised concerns regarding universities' environment and our findings can be utilized in policy making and law enforcement in future.

CONCLUSION

In this study, the findings are more consistent with the authors' postulated idea. A significant association of smoking status of students was observed in non-medical universities and successive academic years. Also, being male and having limited exposure to anti-smoking material on social media was associated with increased frequency of smoking. Policies targeting universities' environment, curriculum, and policies regarding tobacco can help in decreasing the number of student smokers.

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REVIEW ARTICLE

Celecoxib as an Effective Cox-2 Inhibitor for Rheumatologic Disorders: Efficacy and Safety Concerns

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ABSTRACT

Background: NSAIDs are among the most commonly used anti-inflammatory drugs by patients, the mechanism of action of NSAIDs is quite clear. It inhibits the cyclooxygenase mechanism resulting in decreased production of *arachidonic* acid as the COX-action induces its production.
Objective: To elucidate and compare celecoxib as an effective Cox-2 inhibitor for rheumatologic disorders alongwith efficacy and safety concerns.
Methodology: A systemic review was carried out and literature survey was done. In this regard, more than 50 articles were surveyed for related terms.
Results: The review concluded that besides the inhibition produced by NSAIDs on COX-1, some adverse effects arise which include mainly gastric discomfort. NSAIDs are recommended to be given along with any Proton Pump Inhibitor (omeprazole or esomeprazole). Non-selective NSAIDs which inhibit COX-2 action have less adverse effects on gastrointestinal system. Celecoxib is used for the treatment of rheumatologic disorders and is a safe NSAID in comparison to COX-1 inhibitors.
Conclusion: Celecoxib inhibits the release of glycosaminoglycan and stimulates the synthesis of proteoglycan in the body. Researchers have proved that there is no difference in the activity of traditional NSAIDs and Celecoxib, although the statement is debatable.

Key words: Celecoxib, NSAIDs, COX-1, COX-2, Rheumatologic disorders

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سیلیکوکزب جوڑوں کے درد کا ایک پر اثر علاج: اثر پذیری اور تحفظ کا جائزہ

خلاصہ:

پس منظر: این ایس ایڈز (نون اسٹیرائیڈل اینٹی انفلا میٹری ڈرگز) مریضوں کو دی جانے والی عام دواؤں میں سے ہے۔اس دوا کے اثرات کا طریقہ کار بے حد واضح ہے۔ یہ سائیکلوآکسی جینیس (cyclooxygenase) طریقہ کار کو روکتا ہے جس کے نتیجے میں ایرا کیڈونک ایسڈ (arachidonic acid) بننا کم ہو جاتا ہے۔

مقصد: سیلیکوکزب کی افادیت اور اس کے اثرات کا تقابلی جائزہ لیا گیا کہ یہ جوڑوں کے درد کی ایک پر اثر سی او ایکس ٹو انہیبیٹر ہے اور اس کی تاثیر اور حفاظتی مسائل کیا ہیں۔

طریقہ کار: باضابطہ جائزے اور مقالاتی سروے کے حساب سے متعلقہ لفظ کے لیے پچاس سے زائد آرٹیکلز زیر غور آئے ۔

نتائج: جائزہ سے یہ بات سامنے آئی کہ این ایس ایڈ کے سی او ایکس ون پر اثرات کے ساتھ ساتھ کچھ ناموافق اثرات بھی سامنے آئے ہیں جن میں سب سے اہم نظام ہضم کی خرابی ہے۔این ایس ایڈز کو کسی پروٹون پمپ انہیبیٹر جیسے اومپرازول یا ایسومپرازول کے ساتھ دینے پر زور دیا جاتا ہے۔ایسی غیر منتخب این ایس ایڈز، جو سی او ایک ٹو کے اثرات کو روکتی ہوں، ان کے نظام ہضم پر مختلف اثرات ہوتے ہیں۔سیلیکوکزب جوڑوں کے درد کی ایک موثر دوا ہے اور یہ سی او ایکس ون انہیبیٹر کے مقابلے میں ایک محفوظ این ایس ایڈ ہے۔

حاصل مطالعہ: سیلیکوکزب گلائیکوسیمینو گلائیکن کے اخراج کو روکتی ہے اور جسم میں پروٹو گلائیکن بننے کے عمل کو تیز کرتی ہے۔تحقیق کاروں نے یہ بات ثابت کر دی ہے کہ روایتی این ایس ایڈز اور سیلیکوکزب کی سرگرمیوں میں کوئی فرق نہیں، اگرچہ یہ بیان قابل بحث ہے۔

INTRODUCTION

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are typically used in the treatment of Osteoarthritis (OA) and Rheumatoid Arthritis (RA) for symptomatic relief. Two regular kinds of joint pain are osteoarthritis and rheumatoid joint pain. Osteoarthritis (OA) is an agonizing, degenerative joint ailment that regularly includes the knees, hips, neck, back (lower region), or some hand joints. An average of 25 percent of patients considered it as serious agony (7 or higher on a scale

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of 0 to 10 points). There are in excess of 100 unique types of joint pain and related infections. The most widely recognized forms incorporate osteoarthritis and rheumatoid arthritis, psoriatic joint inflammation, gout, and fibromyalgia. The efficacy of all the NSAIDs is considered to be similar and all have one thing in common, that is Gastrointestinal (GI) side effects. NSAIDs induce gastric intolerability and toxicity. This effect has been minimized by the use of Proton Pump Inhibitors (PPI) alongwith the NSAIDs[1]. The mechanism of action of NSAIDs includes inhibition of Cyclooxygenase (COX) which is the key enzyme for *arachidonic* acid metabolism[2]. NSAIDs involving non-selective and COX-2 selective NSAIDs are drugs of choice for inflammation and pain related with OA and RA[3].

COX has been classified into two isoforms that are COX-1 and COX-2. COX-1is an isozyme which is accountable for the production of prostaglandins which results in maintaining the internal body environment including maintenance of GI mucosal layer. COX-2 is cytokine-induced isozyme producing PGs which regulate pain and inflammation. Up to certain limits, NSAIDs inhibit both COX-1 and COX-2. The gastrointestinal effect is due to inhibition of COX-1 action[4].

Rheumatoid arthritis is characterized as a systemic auto-immune disorder, which involves joint inflammation for years. NSAIDs have been the drug of choice by the physicians but the use of NSAIDs induces life threatening gastroduodenal perforations, ulcers, and bleeds[5].

Cyclooxygenase (COX) and Cyclooxygenase (COX)-2 have the same function but contain different genes and have small differences in their sequences[6]. Celecoxib is a potent drug for treatment of rheumatologic disorders, which include osteoarthritis, rheumatoid arthritis, and ankylosing spondylosis. The safety and efficacy profile of the drug varies in different races/ethnicities[7]. Celecoxib is the first COX-2 selective inhibitor which was introduced into clinical practice. The purpose was to provide anti-inflammatory/analgesic activity resembling the activity of non-selective NSAIDs but free from upper GI toxicity which was because of inhibition of COX-1[8].

METHODOLOGY

This examination was led to clarify and look at the celecoxib as a viable Cox-2 inhibitor for rheumatologic disorders alongside viability and wellbeing concerns. An audit was done and writing overview was performed. In such manner, more than 50 articles were reviewed for related terms and data insertion.

DISCUSSION

Mechanism of Action

Feebly, celecoxib dose is the inhibitor of the release of glycosaminoglycan and stimulates the synthesis of proteoglycan in healthy humans' articular cartilages when it is combined with mononuclear cell's peripheral blood of RA individuals or IL-1â and TNF-a. The mechanism of action of the drug is reverse of IL-1â and TNF-a showing that the drug can show its effect directly on the activated cartilages. COX-2 involvement in regulation of cartilage is supported by the difference in the modes of action of NSAID and COX-2. The non-selective COX inhibitors including diclofenac and naproxen have no effect on the synthesis of proteoglycan[9]. There is no such proof of differences in efficacy between COX-2 inhibitors and traditional NSAIDs after comparison of both the drugs. There may be differences in impotency or efficacy. COX-2 may be more potent but the statement requires further research to be proven[10].

Celecoxib is a selective COX-2 inhibitor which delays the healing of the lining of stomach walls during gastric ulcers by inhibiting the production of prostaglandins[11]. Celecoxib is recognized as the first cyclooxygenase (COX-2) selective inhibitor in the history of clinical practice. Celecoxib is used as anti-inflammatory/ analgesic like other NSAIDs but differs due to lack of upper gastrointestinal toxicity. In randomized controlled trials, celecoxib shows to be more effective than paracetamol for the management of osteoarthritis, rheumatoid arthritis, or ankylosing spondylitis at the usual dosages of 200 or 400 mg in a day. In general, it is well tolerated with minimum upper GI toxicity which occurs commonly with NSAIDs[12].

In a recent study, evidence shows that an increased risk of CV disease not consistent with celecoxib and risk is expected to be same and small like non selective NSAIDs. At the time of celecoxib recommendation, risk benefits ratio must be calculated for individual patients[13].

Clinical Fates of Celecoxib

Celecoxib effects on cartilage: PGE2 has both catabolic and anabolic influence on cartilage of OA but its function is not defined correctly[14-15]. NSAIDs by inhibition of PGE2 could influence cartilage potentially.

Proteoglycan turnover: Celecoxib inhibits release of glycosaminoglycan dose dependently and triggers synthesis of proteoglycan cartilage explants in OA[16]. Celecoxib can also affect activated cartilage and decrease proteoglycan production stimulated by IL-1â

and TNF-á. Moreover, in OA cartilage explants, celecoxib stimulates proteoglycan synthesis and maintains newly produced proteoglycans[17-18]. Significantly, celecoxib normalizes *in vitro* proteoglycan content in degenerated cartilage. It is advisable that celecoxib could retard or even overturn the destructive progression in early stages of OA[19].

Prostaglandin E2-induced catabolism: Celecoxib produces their effect on proteoglycan turnover by inhibiting production of PGEs because PGEs take part in OA cartilage metabolism highly significantly[20]. Celecoxib effectively inhibits PGEs and COX-2 in OA cartilage. PGE2 increases IL-1β/TNF-α- which stimulates release of proteoglycan, as a result decreases proteoglycan in cartilage transplant[21-25].

Role of Celecoxib in Synoviocytes

Pro-inflammatory mediators and signal transduction Inhibition: Celecoxib upturned IL-1β-provoked expression of PGE_2 and synovial fibroblasts COX-2 protein. Besides, in OA patients, NF-κB in synovial fibroblasts were inhibited by celecoxib stimulated by IL-1β[26]. NF-κB is responsible for various inflammatory intermediators and one of the main causes of synovial hyperplasia, synovitis, and suppression of synovial program cell death in rheumatism. It is comprehensible that celecoxib could decrease appearance of different inflammatory mediators by suppressing management of NF-κB[27].

Proteolysis: Celecoxib, yet no further specific COX-2 suppressor, increases MMP-1 and MMP-13 protein activation in IL-1β promoted synoviocytes[26-28]. This observation could not explain the inhibition of MMP-1 by celecoxib in synoviocytes rheumatoid joint pain[29]. This difference may be due to various quantities utilized as celecoxib exerts stimulatory effect at small quantities (0.5-1μM) and shows inhibition at large quantities (5-10μM)[21].

Apoptosis: Lately, it has been demonstrated that celecoxib dose inhibits multiplication and provokes programmed cell death in synovial fibroblasts received from OA tolerant patients[30,31]. Noticeably, different other COX-2 particular blockers, like nimesulide and rofecoxib, could not prompt apoptosis of synovial fibroblasts, demonstrating that celecoxib in a COX-2-freepathway promotes apoptosis[30]. Celecoxib could appear in tumour cells to balance apoptosis pathvia restraining contra-apoptotic proteins, raising Ca^{2+} strength and modifying NF-kB signaling[32]. It is noticeable that celecoxib diminished synovial hyperplasia and possibly back off the synovititis-interceded OA ailment development[33,34].

Adverse Effects

A survey was performed by the Adverse Event Reporting System (AERS) department of US Food and Drug Administration (FDA) to identify the cases and reports of renal failure associated with the use of celecoxib. Data collected by AERS and published case reports show that the effects of celecoxib's consumption on renal system were as likely as other usual or conventional non-selective NSAIDs. Celecoxib is not prescribed for use in patients suffering from any renal impairment or renal disease[35].

Celecoxib Comparison with Other Drugs: Safety, Tolerability, and Efficacy

Gastroduodenal ulcers and other related severe difficulties such as perforation blood loss and gastric outlet hindrance can be caused by conventional NSAID therapy[36-43]. Evidence suggests that NSAID-stimulated ulcers and problems are mainly due to NSAID-induced inhibition of mucosal PG synthesis, principally mediated by COX-1 action[44,45]. Gastroduodenal mucosal protection can be mediated by prostaglandins through numerous interconnected mechanics[46,47]. NSAIDs in animal modeling, stimulated gastrointestinal toxicant which segregated to COX-1 activity inhibition[48,49]. Various studies are conducted to elucidate the safety and efficacy of celecoxib in various clinical settings. In 2016, Xu et al. demonstrated the celecoxib fate in a meta-analysis for osteoarthritis. Various other randomized controlled trials were carried out to determine the tolerability of celecoxib in upper GI in comparison to naproxen and placebo. Although physicians declare that the new consumers of celecoxib show upper gastrointestinal signs, ibuprofen, and naproxen were additionally estimated in a protected population in the US. Besides NSAIDs tolerance, celecoxib and non-selective GI tolerance has been reported in 21 randomized clinical trials in elderly patients[50-53].

Naproxen: A study suggests that the anti-inflammatory and analgesic activity of celecoxib is more established than naproxen, with a considerably lesser prevalence of gastroduodenal ulcers as compared to naproxen, and not considerably different from placebo[54].

Diclofenac: In a study, celecoxib has shown constant anti-inflammatory and analgesic action parallel to diclofenac, with less occurrence of gastrointestinal adverse events such as upper GI ulceration and drug tolerability better than diclofenac[55]. In another study, the efficacy of celecoxib was determined utilizing dosages of 200 mg daily and 200 mg twice a day, similar to diclofenac 75 mg twice a day. Celecoxib

200 mg twice per day and diclofenac decrease a few factors related to inflammation more efficiently as compared to celecoxib 200 mg once daily. Treatment with celecoxib was well tolerated exhibiting fewer recurrent G.I. adverse outcomes than diclofenac[56].

Acetaminophen: In a study, the efficacy of celecoxib *was found to be greater than* acetaminophen *and* placebo, whereas, adverse events and tolerability were similar[57].

Rofecoxib: Literature suggests that the possibility of myocardial infarction and other severe cardiovascular problems can be increased by both specific and non-specific COX blockers, but the effects vary between individual drugs. Available evidence shows that a cardiovascular risk related to rofecoxib is higher than cardiovascular possibility related to celecoxib. A few nonsteroidal anti-inflammatory drugs such as diclofenac, indomethacin, and meloxicam may have cardiovascular risks parallel to rofecoxib. Multiple evidences support that the usual doses of celecoxib provide cardiovascular safety in treating arthritis as compared to any other selective COX-2 blockers or NSAIDs[58].

CONCLUSION

The review concluded that NSAIDs and Celecoxib which are generally used for the treatment of Rheumatologic Disorders, have to be used with caution and care. The side or adverse effects of COX-1 and COX-2 inhibitors are numerous and abundantly described in literature. Combined therapy is usually recommended that includes an NSAID along with proton pump inhibitor or any suitable drugs combinations.

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