Proceeding S.Z.P.G.M.I. Vol: 33(3): pp. 20-25, 2019. PSZMC-717-33-3-2019

Protective Effects of Ajwa Fruit on Nicotine Induced Changes in Rat Body and Ovarian Weight with Relative Tissue Weight Index

[1]Faeza Rauf, [2]Muhammad Suhail, [2]Tayyaba Muzaffar, [2]Javaid Iqbal, [2]Hadia Zulfiqar
[1]*Department of Anatomy, Amina Inayat Medical College, Sheikhupura*
[2]*Department of Anatomy, Shaikh Zayed Medical Complex, Lahore*

ABSTRACT

Introduction: Nicotine alters ovarian function and Ajwa (Phoenixdactylifera) being an antioxidant prevents nicotine toxicity. **Aims & Objectives:** To assess the protective effects of Ajwa on nicotine induced changes on body weight, ovarian weight and relative tissue weight index in adult albino rats. **Place and duration of study:** This research was done in Anatomy Department of Federal Postgraduate Medical Institute, Lahore. The total duration of study was eight months. **Material & Methods:** Forty adult female albino rats were equally divided into four groups. First group received distilled water by gastric intubation (1ml/day), second group received nicotine injection intraperitoneally (0.1 mg/kg body weight/day), third group received Ajwa fruit extract by gastric intubation (1gm/kg body weight/day), fourth group received nicotine intraperitoneally (0.1mg/kg body weight/day) plus Ajwa fruit extract by gastric intubation (1gm/kg body weight/day). The whole treatment continued for 28 days. **Results:** One way ANOVA test revealed no significant difference (p-value>0.005) in mean body weight of animals at the end of the experiment. Mean paired ovarian weight was significantly reduced in nicotine treated group and near normal restoration of ovarian weight was seen with nicotine+Ajwa treated group**.** Relative tissue weight index was not significantly different among the groups. **Conclusion:** Ajwa improves nicotine induced ovarian wright reduction in adult albino rats, so it may be useful to improve infertility or subfertility in female smokers.

Key words: Nicotine, Phoenixdactylifera, Ajwa, body weight, ovarian weight

INTRODUCTION

WHO report 2011 states that 32.4% males & 5.7% females are tobacco smokers in Pakistan.[1] Tobacco use in any form is quite dangerous and unhealthy. Nicotine, is a natural alkaloid present in Nicotianatobacum, freely available formulations are cigarettes, topical patches, nicotine gums and nasal spray.[2] Nicotine is a clear liquid in its pure form with a characteristic odor and is soluble in water. Use of nicotine sulphate, as agricultural pesticide has progressively banned[3] due to systemic adverse effects of nicotine. After inhalation, nicotine is rapidly dispersed in the blood stream[4] and reaches the blood brain barrier within 10-20 seconds[5] and damages the central nervous system.[6,7]

Activity in visual system and prefrontal cortex is raised by nicotine. At low doses, nicotine causes stimulatory effects on nAChRs. However, higher doses or more sustained exposures cause inhibitory effects due to neuromuscular blockade.[4] Nicotine suppresses appetite and raises metabolic rate, smokers weigh 4kg less than nonsmokers.[9]

P. dactylifera L usually known as Ajwa date, is cultivated for its fruit.[10] The history of Ajwa dates goes back to 5000 BC. Arabs called it tree of life and is a symbol of richness and affection to Muslims.[11] Ajwa dates are deliciously sweet and soft with a good consistency and black color. Date fruit is highly nutritious food, a good source of fibers, some vital minerals and many vitamins. Date fruits are rich in simple sugars (65% - 80%) but low in fat and protein with no starch. In date fruits, proteins are 1-3% with their amino acid arrangement good for humans.[12] The amount of selenium is 1.48 and 2.96 µg/g. P. dactylifera fruit comprise carotenoids, lutein, β-carotene and neoxanthin.[13,14] In flesh of date 0.5- 3.9% pectin, 0.9% potassium and fluorine is also present.[15] Several other benefits of Ajwa dates are also known.[16,17] P.dactylifera is useful in treating cough, bronchitis, rheumatism, burning sensation, nephropathy and sexual incapacity.[18] It is

antiemetic, laxative, demulcent, expectorant, nutrient and is good for the heart. The P. dactylifera fruit extract possesses antioxidant,[19,20] antimutagenic,[21] analgesic[22] and antitumor activity.[23,24] Male flowers of Phoenix dactylifera or date palm pollen were regularly used as an aphrodisiac in old-style medicine. Date palm pollen suspension contains cholesterol, carotenoids, rutin and estrone which increase FSH and LH and exhibits gonadotrophic activity.[25]

As Nicotine suppresses appetite and raises metabolic rate causing reduction in the body weight of smokers, so this study was designed to assess the protective properties of Ajwa on nicotine induced changes on body weight, ovarian weight and relative tissue weight index in adult albino rats.

MATERIAL AND METHODS

This experiment was conducted in the Anatomy Department of Shaikh Zayed Postgraduate Medical Institute, Lahore in collaboration with Department of Anatomy, Punjab PGMI, Lahore. 40 adult healthy female albino rats aged 3-4 month (average body weight 200-250 gm) were kept at 23±2ºC and a 12 hour light and dark cycle was maintained. They were nourished on normal food and given water ad libitum. They were acclimatized for two weeks before the experiment. Ajwa Dates were purchased from Madinah Al-Munawarah and their extract was prepared by following method.[26]

3000 gram of date fruits were separated from pits by hand and 1 liter distilled water was added to this crudely crushed date fruit (3:1). This was left for 2 days in refrigerator (4°C) with occasional stirring. This aqueous extract of Ajwa contains Phenolic content (1.752 mg Gallic Acid Equivalent /g of extract) and Flavonoid Content (0.1239mg Quercetin equivalent/g of extract)quantified by Chemistry Department, PCSIR, Lahore. Nicotine synthesis grade *(*99% pure*)* loose liquid Fluka, was purchased from Sigma-Aldrich chemical company (USA).

The albino rats were randomly divided into four groups by lottery method, each containing ten animals. First group(healthy control)received distilled water by gastric intubation (1ml/day), second group(diseased control)received nicotine injection intraperitoneally (0.1 mg/kg body weight/day), third group (healthy control with improved diet) received Ajwa fruit extract by gastric intubation (1gm/kg body weight/day), and the fourth group (experimental)received nicotine intraperitoneally (0.1mg/kg body weight/day) plus Ajwa fruit extract by gastric intubation (1gm/kg body weight/day). The whole treatment continued for 28 days.

On 30th day, 48 hours after giving last dose, these rats were weighed properly. Rats were humanely sacrificed by using intraperitoneal administration of morphine at a dose of 0.3-0.5mg/kg and the ovaries were dissected out, weighed and kept in 10% neutral buffered formaldehyde solution for 48 hours.

Statistical analysis:

Data was entered and analyzed by using Statistical Package for Social Sciences (SPSS), version 20. The data for body weight of rats and ovarian weight was reported as mean ± S.D and comparison among groups for these parameters was performed by using ANOVA (one way). For post hoc analysis, Tukey's test was used where required. P-value ≤ 0.05 was considered significant.

RESULTS

All animals were weighed at the start and end of experiment. The mean body weight of rats at start of experiment for healthy control group was 201.7±1.7g and that of nicotine treated group, Ajwa treated group and nicotine plus Ajwa treated group were 203.8±2.7, 201.8±1.8 and 202.3±2.8 respectively. (Fig-1)

One way ANOVA test was applied to compare the initial body weight and final body weight among groups. It was found that at the start of the experiment, the mean body weight of animals in all groups were not significantly different. (p-value>0.05)

At the end of experiment, the mean body weight of animals of healthy control group was 212.2± 4.0 gram, while the mean body weight for animals of nicotine treated group 208.6±7.5g, for Ajwa treated group and nicotine plus Ajwa treated experimental group was recorded as 212.5±4.6 and 208.8±2.5 grams respectively. One way ANOVA test revealed no significant difference (p-value>0.005) at the end of the experiment in mean body weight of animals. Bar chart shows comparison of body weight of rats of healthy control group A &diseased control group B, healthy control with improved diet group C & experimental group D. (Fig-1)

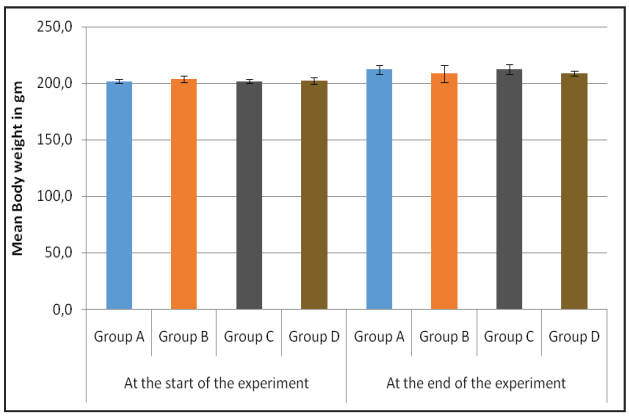

Fig-1: Bar chart showing comparison of Body weight (in gm) among groups.

The mean paired ovarian weight and relative tissue weight index in all groups were observed. It was noted that the paired ovarian weights for the four groups A, B, C and D were 0.119±0.010, 0.102±0.011, 0.121±0.019 and 0.120±0.011g respectively.

One way ANOVA test was applied to compare the paired ovarian weight and relative tissue weight index among groups. It was found that the mean paired ovarian weight was statistically significantly different, p-value = 0.007. (Table-1)

The mean values of relative tissue weight index for group A, group B, group C and group D were 0.051±0.009, 0.045±0.007, 0.0530±0.013, 0.055±0.007 respectively (Table-1). Relative tissue weight index was not significantly different among the groups, p-value = 0.101. (Table-1)

Parameters	Group A Mean ±SD	Group B Mean ±SD	Group C Mean ±SD	Group D Mean ±SD	p-value
Paired ovarian weight (gm)	0.119±0.010	0.102±0.011	0.121±0.019	0.120±0.011	0.007*
Relative tissue weight index	0.051±0.009	0.045±0.007	0.053±0.013	0.055±0.007	0.101

Table-1: Comparison of paired ovarian weight and relative tissue weight index among groups:

Based on oneway ANOVA
*p value ≤ 0.05 is considered statistically significant

The difference between paired ovarian weights of healthy control group A and nicotine treated group B was significant with p-value 0.030*. The difference of nicotine treated group B was significant from Ajwa treated group C and Ajwa plus nicotine treated group D with p-values 0.013* and 0.020*. The difference of healthy control group A was insignificant from Ajwa treated group C and ajwa plus nicotine treated group D with p-values 0.986 and 0.998. The difference of Ajwa treated group C from Ajwa plus nicotine treated group D was insignificant with p-value 0.998. For multiple comparisons, post hoc Tukey test was used which showed that paired ovarian weight in nicotine treated group B was significantly lower as compared to remaining all groups. However, there was no significant difference in the paired ovarian weight among healthy control group A, Ajwa treated group C and Ajwa plus nicotine treated group D (Fig-2). Bar chart showing comparison of relative tissue weight index (Fig-3) shows non-significant p-value > 0.05.

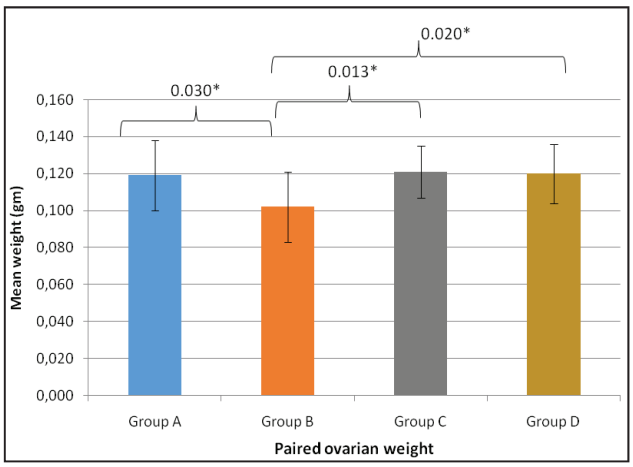

Fig-2: Bar chart showing comparison of paired ovarian weight (in gm) among groups.

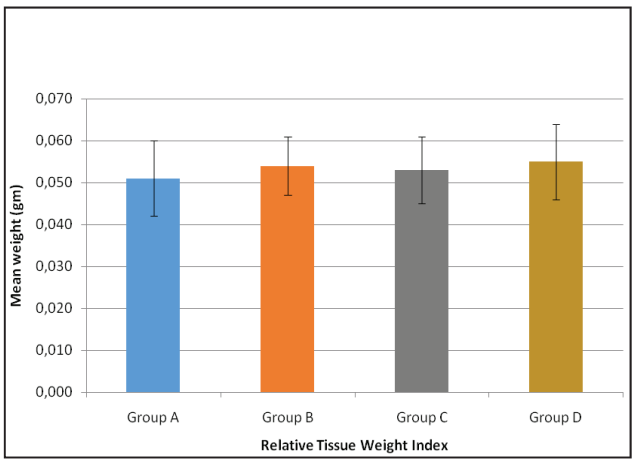

Fig-3: Bar chart showing comparison of relative tissue weight index among groups
Non-significant p-value > 0.05

DISCUSSION

Rising trend of smoking within the females of reproductive age in Pakistan is a cause of genuine concern. This study was aimed to determine ameliorative effect of Ajwa against nicotine induced toxicity in female rats. Regarding general toxicity parameters, body weight of albino rats in nicotine treated group B and Ajwa plus nicotine treated group D was less but not statistically significant than healthy control group A and Ajwa treated group C. These finding coincides with results of Iranloye & Bolarinwa[27] and Patil et al.[28] where no significant difference was observed between the growth rate in the control and nicotine treated rats. Decline in body weight in nicotine treated group B was also observed by Saeed et al.[29] Prolonged use of nicotine decreases food intake and in turn body weight. Activation of hypothalamic nicotinic acetylcholine receptors cause activation of pro-opiomelanocortin neurons, which subsequently stimulate melanocortin 4 receptors, responsible for nicotinic-induced decreased food intake in mice.[30] Nicotine induced changes in whole body metabolism contribute to its weight adjusting effects.[31]

Sexual differences in nicotine modulation of body weight and food intake has been noted. Larger fat deposits in female produces a higher fat consumption in females than males and decrease body weight. So nicotine increases lipolysis as well as fat utilization.[32,33]

Slight increase in body weight of adult albino rat after Ajwa fruit extract administration in group C was observed though not significant statistically. Ali and Abdu,[26] also observed increase in body weight of rats in experimental animals of Ajwa treated group in their research. Moshfegh et al[34] reported increase in body mass of Balb mice and ovary mass index after date palm pollen suspension. Ajwa fruit extract causes weight gain as it contains essential nutrients in high percentage as carbohydrate, protein, fatty acids, salts & minerals, vitamins and dietary fibers.[13]

Mean paired ovarian weight in nicotine treated group B was significantly lower as compared to remaining groups especially Ajwa treated group C. Nicotine intake inhibits gonadotrophin release from pituitary gland resulting in atrophy of gonadal structure and function as observed by Audi et al.[35] These findings coincide with work done by Saeed et al[29] Tuttle et al,[35] Iranloye, Bolarinwa,[27] Audi et al,[31] Syna et al,[36] Dechanet et al.[37] and Patilet al.[28] Reduced ovarian weight indicates inhibition of ovulation and estradiol production due to nicotine administration in rats that decreases the number and size of Graafian follicles, corpora lutea and increases the atretic follicles in the ovary.[27] Syna noted that reduced ovarian weight is due to significant retardation in the follicular growth, apoptotic cell death and necrosis in the granulosa cells.[36]

Paired ovarian weight among healthy control group A, Ajwa treated group C and Ajwa plus nicotine treated group D were comparable, with no significant difference. These results coincide with increase in weight in Ajwa treated group by Ali and Abdu.[26] Gain in weight of rabbit testicles on administration of palm pollen grains extract was also showed by Faleh and Sawad.[38] In Ajwa plus nicotine treated group D, mean paired ovarian weight was slightly increased, these results coincides with results of Saeed et al,[29] they also observed increase in weight of paired testis in nicotine plus pit powder treated mice. Date extract increases the levels of luteinizing hormone and follicle stimulating hormone in rats, so can restore the paired ovarian weight in nicotine plus Ajwa treated group D. Plasma estrogen and testosterone levels are also found to be raised in animals having grounded date pits in their feed, indicating improved fertility.[39] This study showed, for the first time, protective effect of Ajwa against nicotine induced toxicity in female rats.

CONCLUSION

Significant destructive impact of nicotine on reproduction are obvious. The present study results indicate that Ajwa improves nicotine induced ovarian weight reduction in adult albino rats. If a female smoker takes Ajwa regulary, it may be useful to maintain her body weight and can improve infertility or subfertility induced by nicotine to some extent.

REFERENCES

1. Khan J. Tobacco Epidemic in Pakistan. Journal of Postgraduate Medical Institute (Peshawar-Pakistan). 2012; 26(3).
2. Mahar I, Bargot RC, Davoli MA et al. Developmental hippocampal neuroplasticity in a model of nicotine replacement therapy during pregnancy and breast feeding. PLOS ONE. 2012; 7: 37219.
3. APIB. Banned Pesticides. 2014. http://megapib.nic.in/Int_pest_bannedPest.htm.

4. Agarwal and Said TM. Oxidative stress, DNA damage and apoptosis in male infertility–a clinical approach. BJU Intern. 2005;95:503-507.
5. Andersson C, Wennstrom P and Gry OJ. Nicotine alkaloids in Solanaceous food plants. 2003. Nordic Council of Ministers, Copenhagen. Tema Nord: 531.
6. Balfour D, Benowitz N, Fagerstrom K, Kunze M, Keil U. Diagnosis and treatment of nicotine dependence with emphasis on nicotine replacement therapy. A status report. Eur Heart J. 2000; 21(6): 438-445.
7. Tyndale RF and Sellers EM. Variable cyp2a6-mediated nicotine metabolism alters smoking behavior and risk. J Drug Metab Dispos. 2001; 29 (4):48-52.
8. Dong Y, Zhang T, Li W, Doyon W and Dani JA. Route of nicotine administration influences in vivo dopamine neuron activity: Habituation, needle injection and cannula infusion. J Mol Neurosci. 2010; 40(1-2):164-171.
9. Proctor HDC, Kulasekaran A, Malmqvist and Richter A. Determination of nicotine absorption from multiple tobacco products and nicotine gum. Nico Tobac Res. 2013; 15(1):255-61.
10. Al-Daihan S, Bhat RS. Antibacterial properties of different cultivars of phoenix dactylifera L and their corresponding protein content. Afri J Biotech. 2012; 3(10): 4751-4757.
11. AbdAllaMM and Abd El- Kawy A M. Karyotype analysis for date palm (Phoenix dactylifera L) compared with tissue culture derived plants. New York Sci J. 2010; 3(11): 165-170. http;//www.sciencepub.net.
12. Alman HA and Mahmoud RM. Ecol Food and Nutr**.** 1994; 32:261–270.
13. Al Shahib W, Marshall RJ. The fruit of date palm: its possible use as best food for future? Int J Food SciNutr. 2003; 54(4):247-59.
14. Bilgari F, Alkarkhi AFM and Easa AM. Antioxidant activity and phenolic content of various date palm (Phoenix dactylifera) fruits from Iran. Food Chem. 2008; 107:16.
15. Juhaimi FA, Ghafoor K and Ozcan MM. Physical and chemical properties, antioxidant activity, total phenol and mineral profile of seeds of seven different date fruit (Phoenix dactyliferaL) varieties. Int J Food SciNutr. 2012; 63(1):84-89.
16. Ragab AR, Elkablawy M A, Sheik BY, and Baraka H N. Antioxidant and tissue protective studies on Ajwa extract: Dates from Al Madinah Al Monwarah, Saudi Arabia. J Environ Anal Toxicol. 2013; 3 (1): 163.
17. Al-Qarawi A A, Mousa H M, Ali B H, Abdel-Rahman H, and El-mougy SA. Protective effect of extracts from date (Phoenix dactylifera L.) on the carbon tetrachloride-induced hepatotoxicity in rats. IJARVM. 2004; 2(3):176-180.
18. Selvam ABD. Inventory of vegetable crude drug samples housed in botanical survey. Pharmacogn Rev. 2008; 2; 61-94.
19. Hassan NS, Amon ZH, Nor AL, Mokhtarrudin N and Essa NM. Nutritional composition and in vitro evaluation of antioxidant properties of various dates extracts (Phoenix dactylifera L.) from Libya. Asian J Clin Nutr. 2010; 208-214.
20. Saha S, Islam Md K, AnisuzzmanMd, Hassan MDM, Hossain F and Talukdar C. Evaluation of antioxidant, analgesic and antidiarrheal activity of phoenix paludosaroxb leaves. Inter J of Basic Med SCI and Phar. 2012; 2(2):46-52.
21. Sultana B, Fatima B and Mushtaq M. In vitro synergism of anti mutagenic and antioxidant activities of Phoenix dactylifera fruit. Food Sci Biotech. 2014; 23(3):881-887.
22. Lima AL, Parial R, Das M, Das A K. Phytochemical and Pharmacological studies of Ethanolic extract from the leaf of mangrove plant Phoenix paludosaRoxb. Malaysi J of Pharm Sci. 2010; 8(2):59-69.
23. Ishurda O, and John FK. The anti-cancer activity of polysaccharide prepared from Libyan dates (Phoenix dactylifera L). CarbohydPolym. 2005; 59: 531-535.
24. Khan F, Ahmad F, Pushparaj PN, Abuzenadah A, Kumosani T, Barbour E, AlQahtani M, Gauthaman K et al. Ajwa date extract (Phoenix dactylifera L.) extract inhibits human breast adenocarcinoma (MCF 7) cells invitro by apoptosis and cell cycle arrest. PLoS ONE. 2016; 11(7): e0158963.
25. Bahmanpour S, Talaei T, Vojdani Z, Panjehshahin MR, Poostpasand A, Zareei S, Ghaeminia M. Therapeutic effect of Phoenix dactylifera pollen on sperm parameters and reproductive system of adult male rats. IJMS. 2006; 31:8-12.
26. Ali A and Abdu S. Antioxidant protection against pathological mycotoxins alterations on proximal tubules in rat kidney. Func Foods in Heals & Diseas. 2011; 4: 118-134.
27. Iranloye BO, Bolarinwa AF. Effect of nicotine administration on weight and histology of some vital visceral organs in female albino rats. Niger J Physiol Sci. 2009; 24(1):7-12.
28. Patil SR, Ravindra, Patil SB, Londonkar R, Patil SR. Nicotine induced ovarian and uterine

changes in albino mice. Indian J Physiol Pharmacol. 1998; 42(4):503-8.
29. Saeed K, Tahir M, Lone KP. Effect of phoenix dactylifera (date palm) pit powder on nicotine induced spermatotoxicity in adult albino rats. JPMA. 2015; 65-43.
30. Mineur YS, Abizaid A, Rao Y, Salas R, DiLeone RJ, Gündisch D, Diano S, De Biasi M, Horvath TL, Gao XB, Picciotto MR. Nicotine decreases food intake through activation of POMC neurons. Science. 2011;332(6035):1330-2.
31. Audi SS, Abrahan ME and Broker AS. Effect of cigarette smoke on body weight, food intake and reproductive organs in adult albino rats. Ind J Exp Biol. 2006; 44:562-5.
32. Ijomone MO, Olaibi OK and Nwoha PU. Effects of chronic nicotine administration on body weight, food intake and nitric oxide concentration in female and male rats. Pathophys. 2014; 21(3):185-190
33. Bishop C, Parker GC and Coscina DV. Systemic nicotine alters whole-body fat utilization in female rats. Physiol. Behav. 2004;80:563-567
34. Moshfegh F, Baharara J, Namvar F, Zafar-Balanezhad S, Amini E, Jafferzadeh L et al. Effect of date palm pollen on fertility and development of reproductive system in female balb/c mice. JMP. 2016; 5(1):23-28.
35. Tuttle AM, Stampfli M, Foster WG. Cigarette smoke causes follicle loss in mice ovaries at concentrations representative of human exposure. Hum Reprod. 2009; 24(6): 1452-9.
36. Syna PS, Afroz SK, Usha I. Histological analysis of effects of smokeless tobacco on the ovaries of the nonpregnant female swiss albino rats. Int J SciEng Res. 2014; 5:4.
37. Dechanet C, Anahorny, Mathieu DJC, Quantin X, Refytmann L, Hamamah S, Hedon B, Dechaud H, et al. Effect of cigarette smoking on reproduction. J of Human Repro. 2010;17:76-95
38. Faleh B, Sawad AA. Effect of palm pollen grains extracts (Phoenix dactylifera L) on spermatogenic activity of male rabbits. Basrah J for Date Palm Resear. 2006; 1-10.
39. Dates: Production, Processing, Food, and Medicinal Values. Medicinal and Aromatic Plants – Industrial Profiles. Edited by Manickavasagan A, Mohamed Essa M, Sukumar E. USA: CRC Press. 2012. pp. xviii + 415. ISBN 978-1-4398-4945-3.

The Authors:

Dr. Faeza Rauf
Senior demonstrator
Department of Anatomy,
Amina Inayat Medical College, Sheikhupura

Prof. Dr. Muhammad Suhail
Head of Anatomy Department,
FPGMI, Shaikh Zayed Medical Complex, Lahore.

Dr. Tayyaba Muzaffar
Assistant Professor Anatomy,
FPGMI, Shaikh Zayed Medical Complex, Lahore

Dr. Javaid Iqbal
Associate Professor Anatomy,
FPGMI, Shaikh Zayed Medical Complex, Lahore

Dr. Hadia Zulfiqar
P.G. Trainee,
Department of Anatomy,
FPGMI, Shaikh Zayed Medical Complex, Lahore

Corresponding Author:

Dr. Faeza Rauf
Senior demonstrator Anatomy,
Amina Inayat Medical College, Sheikhupura
Email: rauf.faeza@gmail.com