Proceeding S.Z.P.G.M.I. Vol: 31(2): pp. 80-87, 2017.

Comparative Evaluation of *Nigella Sativa, Trigonella Foenum* and Clomiphene Citrate on lipid profile of rats with Letrozole induced Polycystic Ovarian Syndrome

[1]Amna Khurram ,[2] Shumaila Kanwal , [3]Saadia Shahzad Alam

[1]Department of Pharmacology Rahber Medical College, [2]Department of Pharmacology,Azra Naheed Medical College , Lahore, [3]Department of Pharmacology, Shaikh Zayed Postgraduate Medical Institute, Lahore

ABSTRACT

Polycystic ovarian syndrome (PCOS) is a heterogeneous disorder of unknown etiology affecting 5%-10% of women of reproductive age[1]. It is a disorder that affects the reproductive, endocrine and metabolic systems and it is the most common cause of anovulatory fertility[2]. Dyslipidaemias have also been found in PCO patients with hyperandrogenemia. The patients with PCOS also tend to be obese with correspondingly high serum lipid and cholesterol levels[3].

Objective: The objective of this experimental study was to evaluate the effects of *Nigella sativa, Trigonella foenum* and clomiphene citrate in correcting the lipid derangements that develop as a consequence of PCOS.

Materials and Methods: This murine experimental study was conducted on 42 female albino rats divided into six groups of seven rats each. Group 1 was maintained as healthy control. PCOS were induced in Experimental Groups (2-6) by oral administration of Letrozole 1mg/kg O.D for 21 days. Group 2 was taken as experimental control group. Groups 3, 4, 5 and 6 were treated with Clomiphene citrate (1.0 mg/kg O.D), *Nigella sativa* (200mg/kg O.D), *Trigonella foenum* (400mg/kg O.D) and a combined extract of both *Nigella sativa* and *Trigonella* in the same doses respectively for 22 days after induction of PCOS with letrozole. At the end of the experiment lipid profile i-e total cholesterol, triglycerides and HDL of all the six groups were analysed.

Results: The results showed that clomiphene, *Trigonella*, *Nigella* and combination of *Trigonella* and *Nigella* all have a positive impact on lipid profile but amongst all, the more pronounced effects are of *Nigella Sativa.* These seeds alone and in combination with *Trigonella* can remarkably increase HDL levels in rats with letrozole induced PCOS.

Keywords: *Nigella sativa, Trigonella foenum*, Clomiphene citrate, Letrozole, Polycystic Ovarian Syndrome.

INTRODUCTION

Polycystic Ovarian syndrome (PCOS), formerly known as Stein-Leventhal Syndrome is a complex endocrinal and metabolic disorder of women of reproductive age[1]. It affects the women's menstrual cycle, hormones, fertility, circulatory system and appearance. PCOS is characterized by elevated androgen levels and/or small cysts on one or both ovaries[2].This disorder can be morphological (polycystic ovaries) or biochemical (hyperandrogenism) [3].

In Pakistani women of reproductive age group, PCOs have been found in 20.7 % of women. Research suggests that 5% to 10% of females 18 to 44 years of age are affected by PCOs worldwide, making it the most common endocrine abnormality amongst women of reproductive age in whole world[4].

There are several factors that lead to PCOS. Most important of them is obesity which can be

exacerbated by poor dietary habits and sedentary life style[5]. Different reproductive and metabolic features of PCOS are thought to be reversible by slight modifications as weight loss and exercise.

Different diagnostic criteria and their corresponding therapeutic strategies are chosen by endocrinologists and gynaecologists according to the presenting complaints of PCOS patients. The latest Rotterdam diagnostic criteria for PCOS were finalized in a 2012 NIH Conference at Rotterdam. According to these if a woman of reproductive age has two findings out of following three then she is more likely to have polycystic ovarian syndrome[6].

a. Oligo or anovulation/oligo or amenorrhea
b. Hyperandrogenism
c. Polycystic ovaries

Infertility is a common presenting complaint for women with PCOS.As a syndrome, PCOS has multiple secondary consequences derived from common mechanisms. In women suffering from PCOS we can observe metabolic and hormonal imbalances leading to serious health issues. For example obesity, metabolic syndrome, lipid abnormalities and cardiovascular risks. Though not all women with PCOS are overweight or obese, the average body mass index (BMI) of women with PCOS is higher than normal, and obesity is common in women with PCOS[7]. Women of normal weight with PCOS also report difficulty in maintaining their weight.

Elevated triglycerides and reduced high-density lipoprotein levels, which are exacerbated by obesity, are among the components of Metabolic syndrome (MetS) and are cardiovascular risk factors. As part of MetS, lipid derangements are common to women with PCOS regardless of BMI[8].

The main goal of management of PCOS is to cure and prevent its long-term and short-term consequences which includes to regulate menstrual irregularities, restore ovulation, prevent infertility, reduce the risk of type 2 diabetes and to reduce the incidence of cardiovascular disease[9].

The first line of treatment for PCOS is life style modifications for example healthy dietary habits, exercise and weight reduction. These simple changes improve metabolic and reproductive functions. There is marked improvement in metabolic and reproductive functions by only 2-5% reduction in weight[10]. Standard medicines that are used now a days for treatment of PCOS has shown a very little role in improvement of abnormal lipid profile resulting as a consequence of PCOS.

Nowadays the term "Alternative medicine" is gaining importance. It focuses on the idea of belief that medicine which comes in the form of pills or capsules are not the only medicines that we can use and there are many plants which can be used as medicines[11].

The Nigella sativa (kalonji) and Trigonella foenum(fenugreek) are annual flowering plants. The parts of these plant most commonly used for the therapeutic purposes in the "Alternative Medicinal" systems are the seeds with considerable amount of oil having pungent and bitter taste. Phytochemical studies revealed that seeds of *Nigell*a contain nigellone, nigellicine, nigellimine, volatile oil, linoleic acid, resins, proteins, thymoquinone and others[12]. In addition many important alkaloids and steroidal saponins present in fenugreek seeds have proved to be beneficial in reduction of serum total cholesterol, triglyceride and LDL-cholesterol[13]. In the current research work we have seen the effect of *Nigella Sativa* and *Trigonella Foenum* extracts on derangements in lipid profile appearing as consequence of PCOS. We observed the effect of these seeds individually and in combination and compared them with the standard drug used nowadays i-e clomiphene citrate.

In this experimental study we used letrozole induced rat model of PCOS as it is closely related to key diagnostic features of PCOS[14].

Rat Model of PCOS: For the formation of PCOS model, rats with normal estrous cycle were treated with Letrozole 1mg/kg orally, daily for 21 days[15]. Letrozole is an oral non-steroidal aromatase inhibitor. Inhibition of aromatase prevents the conversion of androgens to estrogens and therefore, produces hyperandrogenism.

This model of PCOS fulfilled the Rotterdam criteria for PCOS which are hyperandrogenism, oligomenorrhea and polycystic ovaries.

METHODOLOGY

This experimental study was conducted in Post Graduate Medical Institute Lahore (PGMI).The total time span for this experiment was four months.

Nigella sativa seed and *Trigonella foenum* seeds were purchased from Hamdard Dawakhana Lahore. The seeds were identified and authenticated by the Department of Botany, Punjab University Lahore.

Preparation of Ethanolic extracts of Nigella and trigonella: Ethanol extracts of *Nigella sativa* and *Trigonella* seeds were made and standardized using facilities available at Applied Chemistry Research Centre, PCSIR Laboratories, Lahore. The seeds were ground to a powder using an electric grinder (National, Model MX-915, Kadoma, Osaka, Japan) for 6 minutes.

The 500 grams of prepared powder of each herbal seed was mixed with 96% ethanol (1.5L) and extracted with a Soxhlet apparatus for 16-18 hours[16]. Letrozole (Femara) and Clomiphene citrate (clomid) tablets were purchased from Fazal Din laboratories The Mall Lahore and were finely ground and mixed in distilled water

Six week-old female albino rats weighing between 100-150 gm., with 4 day regular estrous cycles were bought from University of Veterinary and Animal Sciences, Lahore. **Experimental Setup**: The rats were placed in animal house of PGMI Lahore maintaining the room temperature. Rats were given standard Laboratory diet. All rats were acclimatized for 14 days to laboratory conditions before commencement of experiment. They were divided into six groups with seven rats in each. Every rat of all these six groups was clearly and carefully numbered. Groups 1 &2 were maintained as the healthy control and experimental control respectively. The experimental groups 2-6 were administered Letrozole 1mg/kg for 21days for induction of PCOs and further drugs as detailed below. All drugs were given orally in suspension form to rats using a 1ml syringe as per experimental requirement :

Group 1: Healthy control group; was not treated with letrozole and given equal quantity of normal saline[17].

Group 2:Experimental control rats were given Letrozole 1 mg/kg for 21 days[16] and left for self-recovery.

Group3: This group was given repetitive dose of 1.0 mg/kg O.D clomiphene citrate for 22 days, after treating with letrozole for 21 days[17].

Group 4: Ethanolic extract of 200mg/kg *Nigella sativa* orally O.D [18] for 22 days post Letrozole induction.

Group 5: Ethanolic Extract of 400mg/kg *Trigonella foenum* orally O.D[19] once daily for 22 days post Letrozole induction.

Group 6: Combined ethanolic extract of 200mg/kg *Nigella sativa* and *Trigonella foenum* 400mg/kg once daily orally for 22 days post Letrozole induction.

At the end of the experiment the rats were weighed and anesthetized with chloroform Blood samples were collected by cardiac puncture and processed for lipid profile.. Cholesterol and triglycerides were estimated with colorimetric method and HDL by precipitation method[20].

Data was entered and analyzed by using SPSS version 20.0. Comparisons between different groups was performed by using One Way ANOVA or Kruskull Wallis ANOVA as per data characteristics. Results with $p<0.05$ were considered significant.

RESULTS

Cholesterol: The cholesterol levels of different groups (Fig 1) were determined. Group 2 which was experimental control group and was not given any treatment showed highest levels, with mean value 79.7±3.4 mg/dl and the lowest was 65.4±9.3md/dl for group 4 i-e taking *Nigella Sativa*. The groups which were treated with clomiphene, *Trigonella foenum* and combined herbal extract also showed the cholesterol levels which were comparable to normal. The overall difference among groups was significant with p-value 0.002.

Triglycerides: The results of triglyceride levels (Fig 2) of different groups also showed same pattern as that for cholesterol l. The triglyceride levels were also highest for group 2 with mean value 104.6±10.8 mg/dl and lowest for group 4, the group taking *Nigella sativa* and were 78.0±13.4 mg/dl. The overall difference among groups was significant with p-value <0.001.

HDL cholesterol: The HDL cholesterol level was 25.4±2.0mg/dl for group 1, which was healthy control group and was 17.7±2.1, for group 2 which was experimental control group. For groups 2 – 6 the HDL levels were 21.0±1.9, 26.1±2.1, 18.0±2.2 and 22.7±1.8 mg/dl respectively. Group 4 i.e. the *Nigella sativa* treated group had greatest improvement in HDL levels as compared to other groups. The

difference for HDL cholesterol was also significant among groups with p-value <0.001.

DISCUSSION

Polycystic ovarian syndrome is gaining a lot of attention of many endocrinologists and gynaecologists nowadays, as it is not only the major cause of infertility but also can lead to other ailments. Derangements in lipid profile are commonly seen in women having PCOS which results in serious cardiovascular hazards.

In the current experimental study we found the beneficial effects of ethanolic extracts of *Nigella sativa* and *Trigonella foenum* on the lipid profile of rats with letrozole induced PCOS.

Total cholesterol: The total cholesterol level was 69.3±9mg/dl for group 1(healthy control group) at the end of experiment. The group 2(experimental control group i-e PCO induced and no treatment given) showed an increase of 14% in cholesterol level as compared to group 1.This increase in group 2 cholesterol levels is suggestive of lipid derangements as a consequence of PCOS.

While comparing the cholesterol levels of other experimental groups with those of group 2, we observed a decrease of 13%, 18%, 13.2% and 15% for the groups 3, 4, 5, and 6 respectively. The group 3 was clomiphene treated group showed 13%decrease in cholesterol level as compared to group 2. It showed that clomiphene has role in correction of lipid abnormalities which developed due to PCOS.

The highest fall in cholesterol level as compared to group 2 was observed in *Nigella sativa* treated group and in the group which was treated with both combined extract of *Nigella sativa* and *Trigonella foenum* as mentioned above. From these calculations it is obvious that *Nigella sativa* can correct the lipid abnormalities which developed due to PCOS.

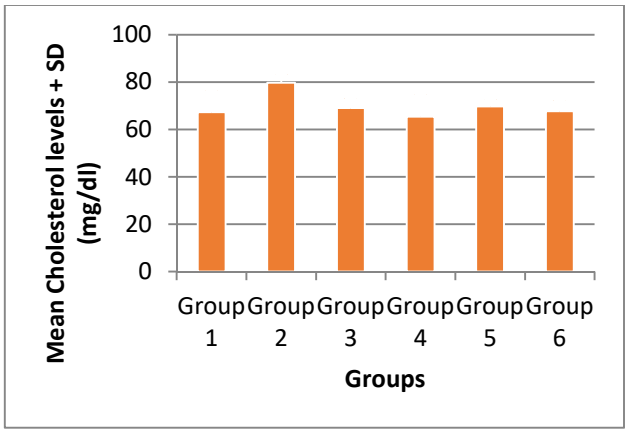

Figure 1: Cholesterol levels

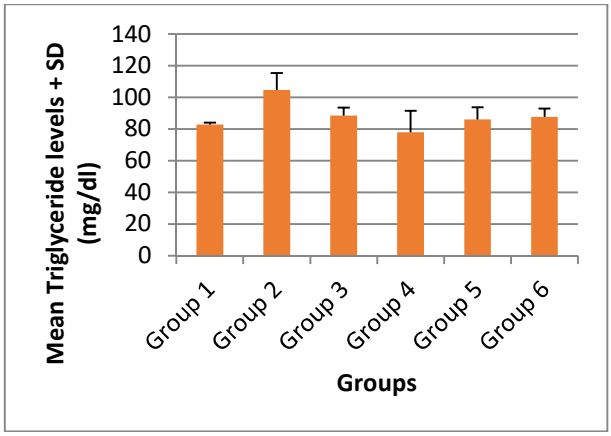

Figure 2: Triglyceride levels

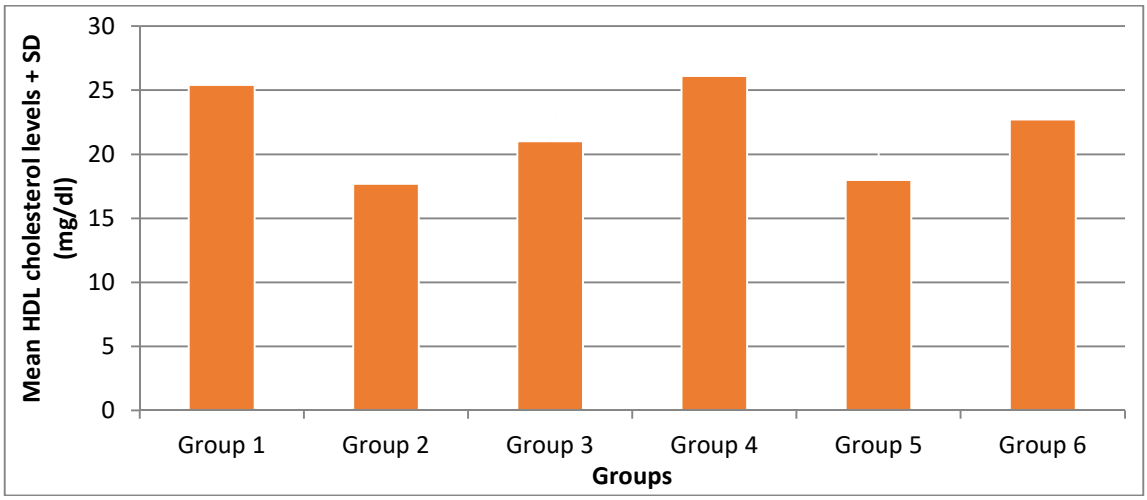

Figure 3: HDL Cholesterol levels

Nigella sativa role in lipid profile correction had been demonstrated in previous studies[21]. It was concluded that *Nigella. sativa* was able to regulate cholesterol synthesis through regulation of HMG-CoA reductase, an effect mediated by TQ (Thymoquinone) and other *N. sativa* constituents[22].

Beneficial effect of combination of *Nigella sativa* and *Trigonella foenum* with glibenclamide on serum triglyceride, cholesterol and HDL in diabetic patients also favors our results[23].

The group which was treated with *Trigonella foenum* alone also showed a decrease of 13.2% in cholesterol levels as compared to group 2. This is fortified by the previous study in which hypocholesterolaemic effect of fenugreek seeds in women with diabetes mellitus type 2 was observed[24].

Serum triglyceride levels: The triglyceride level was highest for group 2 which was PCO induced but not given any treatment. The group 1 mean triglyceride value was 82.7± 1.4. The % age increase in group 2 levels was 21.2%.

On comparing the values of other experimental groups we observed 15%, 25%, 17% and 16.5% decrease for groups 3, 4, 5, and 6 respectively as compared to group 2. The overall difference among groups was significant with p-value <0.001.

The difference for triglyceride had same pattern of significance as that for cholesterol levels. The group 2 had significantly higher than all groups showing PCOS induced changes in triglyceride levels.

The clomiphene citrate and *Trigonella foenum* treated groups had almost equal drop in triglyceride levels when compared to group 2. Here again we found that the group taking *Nigella sativa* showed more significant reversal of triglyceride levels toward normal. This result is supported by a previous study in which protective effects of *Nigella sativa* on metabolic syndrome in menopausal women was observed[25]. The lipid lowering action of *Nigella Sativa* is due to its different components acting synergistically for example Thymoquinone, migellamine, soluble fibre (mucilage) and high contents of polyunsaturated fatty acids (PUFAs)[26].

The hypolipidemic properties shown by *Trigonella* seeds are due to Diosgenin which is a biologically active steroid sapogenin present in fenugreek. Diosgenin has been proposed to be effective against a variety of pathologies, including diabetes, hyperlipidemia, cancer, cardiovascular disease, oxidative stress, and inflammation[27].

HDL cholesterol level: The HDL cholesterol (Fig 3) level of group 1was 25.4±2.0mg/dl and group 2 had 30.3% drop in HDL levels. The other experimental group's i-e 3, 4, 5 and 6 showed 15%, 32%, 1% and 22% and increase in HDL levels when compared to group 2. The difference for HDL cholesterol was a significant among groups with p-value <0.001

These results showed that clomiphene, *Trigonella foenum*, *Nigella sativa* and combination of *Trigonella foenum* and *Nigella sativa* all have role in increasing the HDL, but amongst all, the more pronounced effects are of *Nigella sativa*[21]. Again the reason behind Nigella's positive impact on HDL is its key ingredient Thymoquinone[27]. This herb alone and in combination with *Trigonella foenum* can remarkably increase HDL levels.

CONCLUSION

In conclusion both *Nigella sativa* and *Trigonella foenum* improved lipid derangements that developed during PCOS producing hypocholestrolemic and hypotriglyceridemic effects. However on comparing results we concluded that *Nigella sativa* showed more pronounced effects as it markedly increase the HDL of rats with letrozole induced PCOS. The beneficial effect of *Nigella sativa* were seen when it was used alone as well as in combination with *Trigonella foenum*.

REFERENCES

1. Speroff L, Fritz MA. Clinical gynecologic endocrinology and infertility. 7th ed. Philadelphia: Williams & Wilkins, 2011.
2. Lin LH, Baracat MC, Maciel GA, Soares JM, Jr Baracat EC. Androgen receptor gene polymorphism and polycystic ovary syndrome. Int J Gynaecol Obstet 2013; 120(2): 115–8.
3. Wild RA, Painter PC, Coulson PB, Carruth KB, Ranney GB. 1985 Lipoprotein lipid concentrations and cardiovascular risk in women with polycystic ovary syndrome. J Clin Endocrinol Metab . 61:946–951
4. Liou Tang J, Hsieh C, Lee C, Hsu C, Hsu M. Clinical and biochemical presentation of polycystic ovary syndrome among obese and non-obese women. Fertil Steril 2009; 92(6): 960-1065.
5. Baqai Z, Khanam M, Parveen S. Prevalence of PCOS in infertile patients. Medical Channel 2010; 16:3.
6. Aziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril 2009; 91: 456-88.
7. Trent M, Austin SB, Rich M, Gordon CM. Overweight status of adolescent girls with polycystic ovary syndrome: Body mass index as mediator of quality of life. Ambulatory Pediatr. 2005; 5(2):107-11.
8. Angioni S, Elaine P, Francesca M, Gian BM. Anna MF. Diagnosis of Metabolic Disorders in Women with Polycystic Ovary Syndrome. CME Review Article. Obstet. Gynecol. Surg. 2008; 63(12):796-802.
9. Ching HL, Burke V, Stuckey BG. Quality of life and psychological morbidity in women with polycystic ovary syndrome: Body mass index, age and the provision of patient information are significant modifiers. Clin Endocrinol (Oxf). 2007; 66(3):373-79.
10. Wright CE, Zborowski JV, Talbott EO, McHugh-Pemu K, Youk A. Dietary intake, physical activity, and obesity in women with polycystic ovary syndrome. Int J Obes Relat Metab Disord. 2004; 28(8):1026-32.
11. Hassan BA. Medicinal plants (importance and uses). Pharmaceut Anal Acta 2012; 3:10.
12. Kocyigit Y, Atamer Y, Uysal E. The effect of dietary supplementation of Nigella sativa on serum lipid profile in rats. Saudi Med J 2009; 30(7):893-6
13. Moosa AM, Rashid M, Asadi AZ, Ara N, Mjoibuddin M, Ferdaus A. Hypolipidemic effects of fenugreek seed powder. Bangladesh J Pharmacol 2006; 1: 64-67
14. Kafali H, Iriadam M, Ozardal I, Demir N. Letrozole-Induced Polycystic Ovaries in the Rat: A New Model for Cystic Ovarian Disease. Med Res. 2004; 35: 103–8
15. Oakley O, Lin P, Bridges P, Ko Ch. Animal models for the study of polycystic ovarian syndrome. Endocrinol Metabol. 2011; 26(3):193-202.
16. Luque de Castro MD, García-Ayuso LE. Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. Analytica Chimica Acta. 1998;369(1–2):1–10
17. Sasikala, S.L. and Shamila, S. (2009). A novel ayurvedic medicine-Ashokarishtam in the treatment of Letrozole induced PCOS in rat. Journal of Cell and Tissue Research, 9(2): 1903-1907
18. Parhizkar S, Latiff LA, Rahman SA, Dollah MA, Parichehr H. Assessing estrogenic

activity of *Nigella sativa* in ovariectomized rats using vaginal cornification assay. Afr J Pharm Pharmacol 2011;5 (2):137-42.
19. Sreeja S, Anju V S, Sreeja S. In vitro estrogenic activities of fenugreek *Trigonella foenum graecum* seeds. Indian Journal of Medical Research. 2010 Jun; 131(6): 814-819.
20. Friedewald TW, Donald S, Fredrickson S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18(6):499-502.
21. Benhaddou-Andaloussi A, Settaf A, Cherrah Y, Haddad PS. The petroleum ether extract of *Nigella sativa* exerts lipid lowering action in the rats. J Ethnopharmacol. 2004; 94 (2-3):251.
22. Ismail M, Al-Naqeep G, Chan KW: Nigella sativa thymoquinone rich fraction greatly improves plasma antioxidant capacity and expression of antioxidant genes in hypercholesterolemic rats. Free Radic Biol Med 2010, 48:664–672
23. Ashfaque RM, Shaheen S, Abdul RM. Effect of combination of *Nigella sativa* and *Trigonella foenum-graecum* with glibenclamide on serum triglyceride, HDL and creatinine levels in type-2 diabetes mellitus patients. Pak J Pharmacol 2012;2: 91-6
24. Abu Saleh, M. Moosa. Effects of fenugreek on serum lipid profile in hypercholesteremic type 2 diabetes patients. Bangladesh J Pharmacol.2006; 1:64-67
25. Ibrahim RM, Hamdan NS, Mahmud R, Imam MU, Saini SM, Rashid SN, Latiff LA, Ismail M. Protective effects of *Nigella sativa* on metabolic syndrome in menopausal women: Advanced Pharmaceutical Bulletin, 2014;4(1):29-33.
26. Tasawar Z, Siraj Z, Ahmad N, Lashari MH: The effects of Nigella sativa (Kalonji) on lipid profile in patients with stable coronary artery disease in Multan, Pakistan. Pak J Nutr 2011, 10:162–167.
27. Son IS, Kim JH, Sohn HY, Son KH, Kim JS, Kwon CS. Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (Dioscorea spp.), on high-cholesterol fed rats. Biosci Biotechnol Biochem 2007;71:3063–71.

The Authors:
Dr Amna Khurram
Assistant Professor
Department of Pharmacology
Rahber Medical College Lahore.

Dr.Shumaila Kanwal
Assistant Professor
Azra Naheed Medical College,Lahore.

Dr.Saadia Shahzad Alam
Professor, Head of Department of Pharmacology and Therapeutics,
Shaikh Khalifa Bin Zayed Al-Nahyan Medical and Dental College, Shaikh Zayed Post Graduate Medical Institute, Shaikh Zayed Medical Complex, Lahore
Email: saadia.pharma@gmail.com

Corresponding author:
Dr Amna Khurram
Assistant Professor
Department of Pharmacology
Rahber Medical College Lahore.
amnakhurram03@gmail.com