ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 6, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

ANTIATHEROSCLEROTIC AND ANTIOXIDANT POTENTIAL OF PETROLEUM ETHER EXTRACT OF *PROSOPIS CINERARIA* POD IN HYPERCHOLESTEROLEMIC RABBITS

NOOPUR JAIPAL*, HEERA RAM

Department of Zoology, Jai Narain Vyas University, Jodhpur, Rajasthan, India. Email: noopur.jaipal@gmail.com

Received: 01 March 2019, Revised and Accepted: 26 April 2019

ABSTRACT

Objective: The present study is done to investigate the anti-atherosclerotic efficacy of petroleum ether extract of *Prosopis cineraria* pod in hypercholesterolemic diet-induced atherosclerosis in rabbits.

Methods: Atherosclerosis was induced in rabbits by feeding normal diet supplemented with oral administration of cholesterol (500 mg/kg body weight/day) which was mixed in coconut oil for 15 days. Rabbits were then administered with petroleum ether extract of *P. cineraria* pod for 45 days (Group III), and another set of the group was treated with standard drug Atorvastatin for 45 days. After the completion of the experimental period, the serum biochemistry and histological analysis of thoracic aorta was done. Along with it lipid peroxidation(LPO) and antioxidant parameters such as catalase (CAT), superoxide dismutase (SOD), and ferric reducing ability of plasma (FRAP) were estimated.

Results: There was a significant increase ($p \leq 0.001$) in serum total cholesterol (TC), low density lipoprtein (LDL) and triglycerides after cholesterol feeding when compared with the control group (Group I). Antioxidant parameters were altered too with an increased serum LPO while the reduction in CAT, SOD, and FRAP was observed. After the administration of the petroleum ether extract, significant reduction in TC, LDL-C, very low-density lipoprotein -C, and triglyceride was observed as compared to high-fat diet control rabbits. Slightly significant reduction in ($p \leq 0.05$) atherosclerotic plaque in the aorta was observed while cardiac LPO was lowered alternatively CAT, SOD, and FRAP levels increased in petroleum ether extract administered rabbits.

Conclusion: The outcomes from this study recommend that *P. cineraria* pod extract has hypocholesterolemic effect thereby controlling atherosclerosis and has potent antioxidant activity which may be responsible for improving the lipid profile.

Keywords: *Prosopis cineraria,* Atherosclerosis, Antioxidant, Hypercholesterolemia.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i6.33333

INTRODUCTION

Dietary changes have been the basis of numerous metabolic syndromes which plays a pivotal role in the development and progression of atherosclerosis, one of the progressing cardiovascular diseases. Atherosclerosis is characterized by lipid deposition and inflammation in the arterial wall resulting in a narrowing of arteries causing a situation like heart attacks [1]. Atherosclerosis is mainly influenced by hypercholesterolemia and dyslipidemia [2] that are developed through various risk factors, that is, hereditary, lifestyle, diabetes, and high-fat diet consumption accumulation of cholesterol and forming of plaque which ultimately bursts resulting in myocardial infarction [3]. A number of pharmacological agents are available in the market to manage dyslipidemia and atherosclerosis; one such advancement in combating atherosclerosis is the use of HMG-CoA inhibitors which lowers the serum cholesterol. The mostly used HMG-CoA inhibitor is statin available in the market and is widely used [4]. However, its been seen that long-term usage of statins showed adverse effects which majorly includes myalgia, diabetes mellitus, Alzheimer, dementia, and many more [5]. These issues diverted the interest to look for alternatives which are not only reliable effective but also have no adverse effects. These requirements direct focus toward herbal formulations. Studies have demonstrated that some plants and their products exhibit hypolipidemic potential [6].

Plant has been a reservoir of secondary metabolites such as flavonoids, terpenoids, alkaloids, carotenoids, and many more which are considered exceptional antioxidants, therefore, have played a cheif role in therapeutic [7]. *Prosopis cineraria* L. (Fabaceae) has been reported to have pharmacological potential. It is one of the key ingredients of "Panchkutta," one of the important Rajasthani dish. *P. cineraria* commonly called as khejari is a state tree of Rajasthan. Studies have concluded that it is not only a good source of food and fodder but also persist great medicinal value too. Its pod is locally known as Sangri, considered as a dry fruit of desert and a rich source of nutrients. *P. cineraria* plant and its various parts found to be rich in polyphenols, alkaloids, tannins, saponins, and flavonoids. Its bark is used in treating bronchitis, asthma, dysentery, etc., while the smoke of leaves is considered for treating ailments of eye and leaf paste cures boils blisters, and many more [8,9] but its pod have not been explored much on its bioactivities and phytochemical level.

This study aims at investigation its antiatherosclerotic activity and at the same times exploring its antioxidant activities in the petroleum ether extract of *P. cineraria* pod, using high-fat diet-induced atherosclerotic rabbits as a working model. Atherosclerotic plaques in aorta; lumen volume; cardiac lipid peroxidation (LPO); circulating total cholesterol (TC), high-density lipoprotein -cholesterol (HDL-C), low-density lipoprotein -cholesterol (LDL-C), triglycerides (TG), and very low-density lipoprotein -cholesterol (VLDL-C) levels; atherogenic index (AI); and organ (heart, aorta, kidney, and liver) weight were considered as key parameters. Planimetric studies of the aortal wall and histopathological studies of heart, aorta, kidney, and liver were studied as supporting parameters to correlate with altered conditions.

MATERIALS AND METHODS

Preparation of plant extract

The selected plant *P. cineraria* was identified by the experts of Botany Department Jai Narain Vyas University, Jodhpur. The required part of this plant, i.e. pod of *P. cineraria* was procured from the local registered vendor. Pod of *P. cineraria* was dried and ground to powder and later its

extract was prepared in petroleum ether using Soxhlet apparatus for 18 h. Later the extract was distilled to remove the excess of petroleum ether and then dried off. A powder form extract was obtained and was stored in desiccation for further use. Standard drug statin (atorvostatin) was purchased from a local medical store.

Experimental animals

New Zealand white male rabbits were taken, weighing around 1.25–1.50, were used as a working model. They acclimatized for 10 days and were kept under controlled conditions housed in cages with 12 h of light and controlled temperature (23±2°c) with humidity at 40–60%. Food was supplemented with green leafy and seasonal vegetables and water. The animal studies were done with safety taking ethical clearance from the Institutional Animal Ethics Committee (IAEC), Department of Zoology, Jai Narain Vyas University, Jodhpur, Registration No. 1646/GO/Ere/S//12CPCSEA. IAEC of our University followed all the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India, New Delhi.

Experimentation

Induction of hypercholesterolemia

Hypercholesterolemia was induced by giving an oral dose of 500 mg cholesterol powder mixed with 5 ml coconut oil/kg for 15 days alongside high-fat diet.

Atorvastatin dose regime

Atorvastatin (Atorlip-10, Cipla) was used as a standard hypocholesterolemic drug. It was orally administrated at the dose of 0.25 mg/kg. B.wt./day dissolved in 5 ml of distilled water.

Preparation of plant drug

Petroleum ether extract (500 mg/kg B.Wt./day, p.o) of *P. cineraria* was then given to the experimental animals by mixing it in 5 ml distilled water. The dose of the extract was determined by LD_{50} test.

Experiment design

Animals were divided into four groups (n=5) and experimentation period was of 60 days

- Group I: Intact control (distilled water-5 ml/animal/day, p.o)
- Group II: Hypercholesterolemic control
- Group III: High-fat diet and cholesterol powder (15 days) + petroleum ether extract (45 days)
- Group IV: High-fat diet and cholesterol powder (15 days)+ Atorvastatin (45 days)

Collection of blood sample and planimetric studies

After completion of 60 days, overnight fasting animal was sacrificed under ether anesthesia. Blood samples were collected by cardiac puncture method and kept in Ethylenediaminetetraacetic acid coated test tubes and normal tubes for biochemical and hematological assessments. Planimetric studies of aorta wall and atherosclerotic plaque were performed using camera lucida and measured layers (intima, media, and adventitia), lumen volume, and atherosclerotic plaque area, as routinely done in our laboratory.

Assessment of serum lipid profile

Serum was separated by centrifugation and stored at –20°c. Serum TC, TG, and HDL-C were analyzed using Biochemistry Analyzer RX-50 and commercial diagnostic kits (Siemens Healthcare Diagnostic, USA) and LDL-C, VLDL-C, and AI were calculated using Friedewald's formula [10].

Assessment of Antioxidant parameters

Serum LPO was determined by measuring the thiobarbituric acid reactive substances and was expressed in terms of malondialdehyde (MDA) content, following the method of Ohkawa *et al*. [11] while for measurement of catalase (CAT) and superoxide dismutase activity was carried out by Aebi's and Marklund's method, respectively [12].

Histopathology of aorta

The ascending aorta shows cut (2–3 cm length) and excised from the heart of each animal of each group. Then were kept on 10% formalin fixative, after processing the aorta was ultrasectioned (5-6 μm thickness) and stained in hematoxylin and eosin stain for histopathological observations.

Statistical analysis

The biochemical parameters were expressed in Mean±SEM (standard error of the mean). One-way analysis of variance was evaluated followed by Turkey's multiple comparison test using GraphPad Prism 7.0 software. Graphical representation was done using MS Excel 2013.

RESULTS

Effect on lipid profile

Animals receiving high-fat diet for 15 days showed a significant increase ($p \leq 0.001$) in total serum cholesterol, HDL-C, LDL-C, VLDL-C, and triglyceride level compared to the control group. Administration of the petroleum ether extract of *P. cineraria* pod to the atherosclerotic animal for 45 days showed significant reduction ($p \leq 0.001$) in TC, LDL-C, VLDL-C, and TG compared to the hypercholesterolemic group (Table 1).

Effect on aortic plaque formation

Fatty plaque with foam cells was seen in the aorta of hypercholesterolemic rabbits within the intima region. When treated with petroleum ether extract of *P. cineraria* pod, there was slightly significant $p \leq 0.05$ regression of aortic plaque, and somewhat normal histoarchitecture was observed compared to control and standard group (Figs. 1 and 2).

Effect on antioxidant parameters

The formation of MDA was high ($p \leq 0.001$) which is the indication of LPO, was seen in the hypercholesterolemic group as compared to the control group. While slight reductions of MDA were observed in petroleum ether extract treated group. Besides, this significant increase ($p \leq 0.001$) in serum SOD and Catalase was seen in extract treated group when compared with the hypercholesterolemic group (Figs. 2-4).

DISCUSSION

The resemblance shown by rabbits to atherosclerosis in humans to a higher extent made it the best model to study lipid metabolism and atherosclerosis; therefore, New Zealand white male rabbits were used for the present investigation [13]. Atherosclerosis has accelerated the rate of mortality and morbidity [14]. Many therapies which are available currently for atherosclerosis treatments including statins

Table 1: Serum lipid profile of cholesterol-fed rabbits treated with petroleum ether *Prosopis cineraria* extract (Mean±SEM)

Treatment group	TC (mg/dl)	HDL-Cholesterol (mg/dl)	LDL-Cholesterol (mg/dl)	VLDL- Cholesterol (mg/dl)	TG (mg/dl)
Group I (intact control)	63.5±3.11	21.03±2.9	20.69±1.57	17.78±1.06	69.87±2.05
Group II (hypercholesterolemic)	1216.5±49.3^{c}	26.54±8.56^{c}	1022±66.41^{c}	167.15±8.55^{c}	835.7±25.2^{c}
Group III (petroleum ether extract)	145.3±11.72^{a,g}	20.15±2.5^{b,e}	21.65±2.1^{a,g}	24.77±1.5^{c,g}	94.5±11.57^{b,g}
Group IV (atorvastatin)	80.80±4.9^{a,g}	19.77±1.75^{a,e}	46.24±7.8^{c,g}	18.74±4.42^{a,g}	74.13±3.01^{b,g}

Values expressed as mean±SEM (n=5). Group II–IV compared with Group I, where $P \leq 0.05$=a, $P \leq 0.01$=b, $P \leq 0.001$=c, and non-significant=d. Groups III and IV compared with Group II, where $P \leq 0.05$=e, $P \leq 0.01$=f, $P \leq 0.001$=g, and non-significant=h. SEM: Standard error of the mean, TC: Total cholesterol, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, TGs: Triglycerides

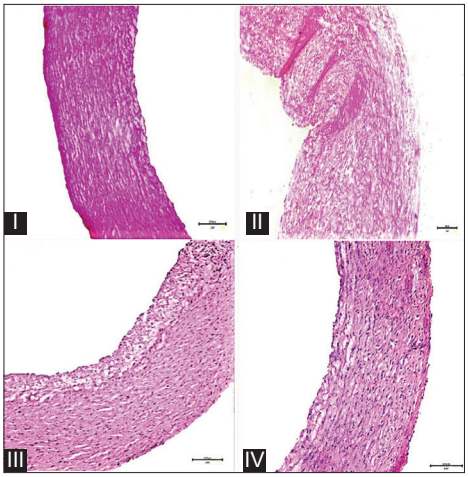

Fig. 1 (I-IV): Photomicrographs (200 × HE) of thoracic aorta of treated rabbits. (I) Intact control: Normal aortic wall consisting of tunica adventitia, tunica media, and tunica intima. (II) Hypercholesterolemic control: Aorta showing endothelium with the formation of atherosclerotic plaque, thickening of intima with excessive foam cells. (III) Petroleum ether extract treated: Reduction in plaque, slightly thickened intima showing low degree of the atherosclerotic lesion. (IV) Statin (atorvastatin): Restored histoarchitechture of the thoracic aorta

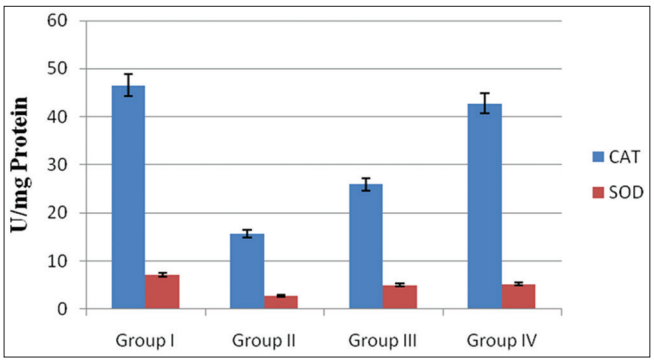

Fig. 2: Graphical presentation of effect of *Prosopis cineraria* pod (sangri) whole grain extract on superoxide dismutase and catalase activity of various treatment groups in U/mg protein (Mean of 5 values ± standard error of the mean)

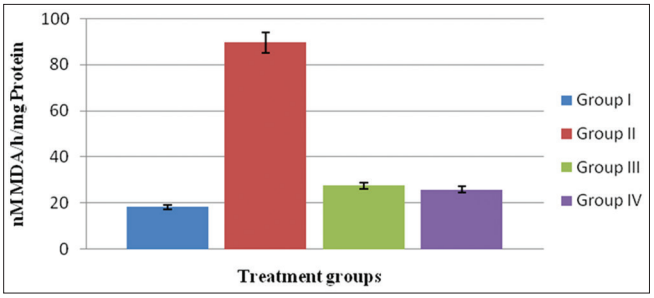

Fig. 3: Graphical presentation of effect of *Prosopis cineraria* pod (sangri) whole grain extract on LPO Lipid peroxidation in nano moles malondialdehyde/mg protein in serum (Mean of 5 values ± standard error of the mean)

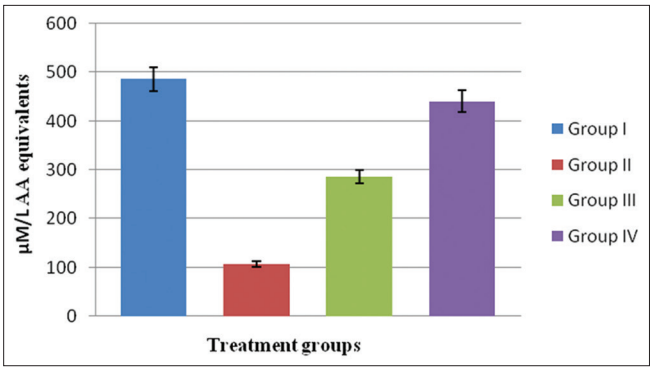

Fig. 4: Graphical presentation of the effect of *Prosopis cineraria* pod (sangri) whole grain extract on ferric reducing ability of plasma: Total antioxidant activity of various treatment groups in µM/L AA equivalents (Mean of 5 values ± standard error of the mean)

which are HMG-CoA reductase inhibitors, bile acid sequestrants, fibrates, and niacin have shown improvement in lipid profile [15] but at the same time are the reason behind associated side effects. Hypercholesterolemia leads to atheromatous changes in the aorta and lead to a condition like atherosclerosis [16]. The atherosclerotic lesions are mainly lipid deposits derived from plasma LDL, modified by oxidative processes which result in escalation in uptake by the scavenger receptor of macrophages leading to foam cell formation [17]. Targeting HMG-Co A reductase enzyme, a rate-limiting enzyme in the mevalonate pathway results into the inhibition of cholesterol [18] and statins helps in inhibiting HMG-Co A Reductase, but long term usage caused adverse effects [19]. Secondary metabolites such as flavonoids, terpenoids, and phenolic compounds have found to be helpful in preventing the binding of HMG-CoA to HMG-CoA reductase alone or along with plant extracts [20]. *Prosopis cineraria*, since ancient time is being used as a therapeutic and found to be a rich in phytocompounds. Various studies have been done on *P. cineraria* and explored that its different parts have different ability to cure wide variety of ailments. *P. cineraria* bark has shown antiatherosclerotic efficacy [21]. In a study petroleum extract of guar gum reportedly reduced the cholesterol and blood sugar in hypercholesterolemic rabbits [22]. The present study aimed to explore the efficiency of petroleum ether extract of *P. cineraria* pod. Petroleum ether extract of *P. cineraria* pod showed slightly significant regression in the plaque formation when treated on rabbits which were quite comparable to the study done by Ram and Purohit on the antiatherosclerotic activity of bark of *P. cineraria* and Acacia Senegal [21,23]. In addition, petroleum ether extract of *P. cineraria* pod showed a significant reduction in total serum cholesterol and TG. Oxidative stress is also responsible for the pathogenesis of cardiovascular diseases. It is necessary to have a balance between oxidative stress and antioxidants. In the present study in hypercholesterolemic rabbits, a significant increase in MDA formation was observed which indicates oxidative stress in hypercholesterolemic condition implicating development and progression of atherosclerosis [24]. CAT and superoxide dismutase (SOD) are the antioxidant defense system which helps in the degradation of reactive oxygen species (ROS) [25]. Hypercholesterolemia leads to a reduction in SOD and CAT levels which leads to the generation of hydroxyl and peroxyl radicals resulting in the proliferation of LPO [26]. FRAP levels too were reduced in hypercholesterolemic rabbits which got increased after the administration of petroleum ether extract. Petroleum ether extract has been seen to improve the CAT and SOD levels also might be due to the presence of phytocompounds which might be having free radical scavenging activity. *P. cineraria* has found to decrease LPO levels [27], similar results were seen results were seen in this study with petroleum ether extract, reducing the levels of LPO which demonstrates that the extract has scavenging role of ROS.

CONCLUSION

This present study concluded that petroleum ether extract of *P. cineraria* pod has the capability to reduce serum cholesterol when fed to hypercholesterolemic rabbits, also showed regression in

atheromatous plague in the aorta. It was also observed that it can improve antioxidant enzyme activity. Therefore, it can be considered as an alternative therapeutic agent as compared to standard drugs to cure cardiovascular diseases without any side effects.

ACKNOWLEDGMENT

We would like to acknowledge Head, Department of Zoology, Jai Narain Vyas University, Jodhpur, for providing all the necessary amenities and UGC, New Delhi, India, for financial support to Noopur Jaipal (Ref No. F1.17.1/2017-18/RGNF-2017-18-SC-RAJ-38181 SA-III/website).

AUTHOR'S CONTRIBUTIONS

The complete research work suggested and mentored by Dr. Heera Ram. All the experimental work was conducted by Noopur Jaipal. Authors drafted and approved the final draft.

CONFLICTS OF INTEREST

The author declares no conflict of interest.

REFERENCES

1. Urrutia JK, Tampis RL, Mercado J, Baygan AM. Survival analysis for the risk of developing heart attack. Int J Pharm Pharm Sci 2016;8:6-10.
2. Rao SP, Mohanta G, Manna P. An overview of statins as hypolipidemic drugs. Int J Pharm Sci Drug Res 2011;3:178-83.
3. Wong AP, Mohamed AL, Niedzwiecki A. Atherosclerosis and the cholesterol theory: A reappraisal. World J Cardiovasc Dis 2016;6:391-409.
4. Blum A. HMG-CoA reductase inhibitors (statins), inflammation, and endothelial progenitor cells-new mechanistic insights of atherosclerosis. Biofactors 2014;40:295-302.
5. Ramkumar S, Raghunath A, Raghunath S. Statin therapy: Review of safety and potential side effects. Acta Cardiol Sin 2016;32:631-9.
6. Jain PG, Surana SJ. Hypolipidemic activity of *Prosopis cineraria* L (Druce) fruit extract and molecular modeling study with farnesoid X receptor (FXR). Trop J Pharm Res 2015;14:1621-8.
7. Rengarajan S, Melanathuru V, Munuswamy D. Comparative studies of antioxidant activity from the petals of selected Indian medicinal plants. Int J Pharm Pharm Sci 2015;7:29-34.
8. Khokar A, Menghani E. Antimicrobial activity of *Prosopis cineraria* (Leaf) methanol and chloroform extract against selected bacterial species. Int J Pharm BioSci 2015;6:222-9.
9. Robertson S, Narayanan N. Isolation and characterization of secondary metabolite from *Prosopis cineraria* (L.) druce for anticancer activity. World J Pharm Pharm Sci 2014;3:876-84.
10. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
11. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
12. Weydert CJ, Cullen JJ. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc 2010;5:51-66.
13. Fan J, Kitajima S, Watanabe T, Xu J, Zhang J, Liu E, *et al.* Rabbit models for the study of human atherosclerosis: From pathophysiological mechanisms to translational medicine. Pharmacol Ther 2015;146:104-19.
14. Kajal A, Kishore L, Kaur N, Gollen R, Singh R. Therapeutic agents for the management of atherosclerosis from herbal sources. Beni Suef Univ J Basic Appl Sci 2016;5:156-69.
15. Jamkhande PG, Chandak PG, Dhawale SC, Barde SR, Tidke PS, Sakhare RS, *et al.* Therapeutic approaches to drug targets in atherosclerosis. Saudi Pharm J 2014;22:179-90.
16. Vettor R, Serra R. Management of hypercholesterolemia, appropriateness of therapeutic approaches and new drugs in patients with high cardiovascular risk. Ital J Med 2018;12:203-12.
17. Shashkin P, Dragulev B, Ley K. Macrophage differentiation to foam cells. Curr Pharm Des 2005;11:3061-72.
18. Jensi VD, Gopu PA. Potent inhibitors of HMG-CoA reductase phytoconstituents from *Terminalia arjuna* (Roxb. Ex Dc.) wight and arn. An *in silico* approach through molecular docking studies. Eur J Biomed Pharm Sci 2018;5:384-90.
19. Mach F, Ray KK, Wiklund O, Corsini A, Catapano AL, Bruckert E, *et al.* Adverse effects of statin therapy: Perception vs. The evidence-focus on glucose homeostasis, cognitive, renal and hepatic function, haemorrhagic stroke and cataract. Eur Heart J 2018;39:2526-39.
20. Chadha R, Purohit K. Wheat bran extract mimics statin like action in hyperlipidemic rabbits: An experimental study. Asian J Pharm Clin Res 2018;11:402-5.
21. Ram H, Purohit A. Hypolipidemic and antiatherosclerotic effects of *Prosopis cineraria* bark extract in experimentally induced hyperlipidemic rabbits. Asian J Pharm Clin Res 2012;5:106-9.
22. Joshi LD, Singhal PC, Gupta RK. Changes in serum cholesterol and blood sugar levels in feeding with guar and its solvent extracts. Curr Sci 1982;51:21-5.
23. Ram H, Jatwa R, Purohit A. Antiatherosclerotic and cardioprotective potential of acacia senegal seeds in diet-induced atherosclerosis in rabbits. Biochem Res Int 2014;2014:1-6.
24. Anwer T, Sharma M, Khan G, Alam MF, Alam N, Ali MS, *et al*. Preventive role of *Withania somnifera* on hyperlipidemia and cardiac oxidative stress in streptozotocin induced Type 2 diabetic rats. Trop J Pharm Res 2017;16:119-25.
25. Higashi Y, Noma K, Yoshizumi M, Kihara Y. Endothelial function and oxidative stress in cardiovascular diseases. Circ J 2009;73:411-8.
26. Olorunnisola OS, Bradley G, Afolayan AJ. Protective effect of *T. violacea* rhizome extract against hypercholesterolemia-induced oxidative stress in Wistar rats. Molecules 2012;17:6033-45.
27. Liu Y, Singh D, Nair MG. Pods of khejri (*Prosopis cineraria*) consumed as a vegetable showed functional food properties. J Funct Foods 2012;4:116-21.