Tropical Journal of Pharmaceutical Research September 2015; 14 (9): 1621-1628
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v14i9.11

Original Research Article

Hypolipidemic Activity of *Prosopis cineraria* L (Druce) Fruit Extract and Molecular Modeling Study with Farnesoid X Receptor (FXR)

Pankaj G Jain* and Sanjay J Surana
R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist. -Dhule- 425405, (MS) India

For correspondence:* ***Email:* *pgjain@yahoo.com;* ***Tel:*** *+91-9823668845*

Received: 27 December 2014 *Revised accepted: 20 June 2015*

Abstract

Purpose: *To investigate the hypolipidemic potential of the 70 % ethanol fruit extract of Prosopis cineraria (Fabaceae) (Et. PCF) in triton-induced hyperlipidemia in rats.*
Methods: *Et-PCF was obtained by pulverizing whole dried fruits and extracting with 70 % ethanol. Adult Sprague Dawley rats were divided into six groups of six rats each. The groups were namely normal control, hyperlipidaemic control, standard drug-treated (simvastatin 4 mg/kg), and three Et-PCF (200, 400 and 600 mg/kg, respectively)-treated groups. Apart from normal control, all other groups received a single dose of triton (200 mg/kg, i.p.) exactly 30 min after a dose of the standard drug and Et-PCF for the induction of hyperlipidemia. Twenty four hours after triton injection, hyperlipidemia was confirmed by collecting blood samples from all the rats and testing for serum lipid profile. Antioxidant activity, in the form of inhibition of lipid peroxidation, was determined along with chromatographic analysis. Moreover, molecular docking study of β-sitosterol (active constitute of PCF) was performed with Farnesoid X receptor.*
Results: *Triton-induced hyperlipidemia group showed significant increase in total cholesterol, low density lipoprotein cholesterol (LDL), very low density lipoprotein cholesterol (VLDL) , triglyceride, atherogenic index and decreased high density lipoprotein cholesterol (HDL), compared to normal control group. Et-PCF treated groups showed reduction in serum cholesterol, triglyceride, VLDL and LDL levels compared to triton treated control group. Extract at the dose of 200 mg/kg significantly reduce serum cholesterol ($p < 0.01$) and serum LDL ($p < 0.01$). At the dose level of 400 mg/kg and 600 mg/kg extract is effective to significantly reduce serum cholesterol ($p < 0.05$), triglyceride ($p < 0.05$), VLDL ($p < 0.05$), LDL ($p < 0.05$) and atherogenic index ($p < 0.05$) and these results are almost equivalent to those of standard drug simvastatin. Furthermore, antioxidant activity, i.e., IC_{50} of Et-PCF was 58.33 µg/ml. Molecular docking score of β-sitosterol for Farnesoid X receptor was -8.32 kcal/mol, suggesting excellent binding conformation of Et-PCF to receptor molecules.*
Conclusion: *The findings suggest that Prosopis cineraria may be beneficial for preventing hyperlipidaemic complications by its anti-hyperlipidemic and antioxidant activities.*

Keywords: *Prosopis cineraria, Anti-hyperlipidemic activity, Simvastatin, Triton, Docking studies, Farnesoid X receptor*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Hypercholesterolemia is an excessive high plasma cholesterol level and a strong risk factor for negative cardiovascular events such as obesity, diabetes and hypertension [1,2]. Atherogenesis is a multifactor process that

includes oxidative modification of LDL which further leads to atherosclerosis [3].

Research, in recent years, has focused on dietary antioxidants from plant-derived sources to normalize elevated levels of cholesterol atherogenous fractions, mainly LDL, and of glucose in an attempt to reduce the cardiovascular risk [4,5]. Hyperlipidemia is a clinical condition characterized by elevation in blood lipid levels. Increase in blood lipid levels stimulates atherosclerosis and leads to hardening of arteries. This hampers normal blood circulation and results in fatal cardiovascular disease (CVD) [6].

A logical strategy to prevent or treat atherosclerosis and reduce the incidence of cardiovascular disease events is to target hyperlipidaemia and oxidative stress by diet and/or drug intervention. Therefore, there is an urgent need to have drugs with lipid lowering and antioxidant activities with no side effects. Previously conducted studies demonstrated natural products derived from different parts of various plants possess significant hypolipidemic potential [7-10].

Prosopis cineraria, a plant belonging to the family Fabaceae), is known as 'Khejri' in many parts of India. It is used as a therapeutic agent in greater parts of India, Burma and Sri Lanka [11]. Literature suggests that, the bark is used in the treatment of asthma, bronchitis, dysentery, leucoderma, leprosy, muscle tremors and piles [12,13]. Several bioactive compounds such as flavonoids, alkaloids, phenolic contents, free amino acids, spicigerin, prosogerin A, B, C and D, β-sitosterol and vitamins have been isolated from different parts of the plant [14,15]. The presence of these bioactive compounds suggest *Prosopis cineraria* may possess hypolipidemic and antioxidant potentials like other previously studied medicinal plants [7-10]. Fruits of *Prosopis cineraria* are an important constituent of the diet of tribal people from Satpuda region of Maharashtra, India and these people are free from cardiovascular complaints.

Hence, we hypothesized that oral treatment with *Prosopis cineraria* fruits extract probably possesses hypolipidemic effect in triton-induced hyperlipidemia. As the antioxidant from plant derived sources help to reduce elevated levels of cholesterol [4,5], we also investigated the antioxidant potential of Et-PCF. Docking study of β-sitosterol for FXR was undertaken by in-silico to evaluate binding conformation of Et-PCF to receptor molecules [16].

EXPERIMENTAL

Animals

Sprague Dawley rats (180-200 g) of either sex were obtained from college animal house of R.C. Patel Institute of Pharmaceutical Education and Research (RCPIPER), Shirpur, India. They were housed under standard laboratory conditions and were fed commercial rat feed (Lipton India Ltd., Mumbai, India) and previously boiled water allowed to cool, *ad libitum*. Purpose of using boiled water was to maintain strict hygienic conditions and avoid chances of infection. The experimental protocol was approved by IAEC and CPCSEA, Department of forest and environment, Government of India, New Delhi (RCPIPER/IAEC/2012-18/20 with reg no. 651/02/C/CPCSEA).

Chemicals

Triton WR 1339 (Loba- chemie Ltd, India), cholesterol, triglyceride, HDL kits (BEACON Diagnostic Pvt. Ltd, Gujarat, India), ethanol procured from college central store (RCPIPER) were used in this study. All the solvents used were analytical reagent AR and high performance liquid chromatography (HPLC) grade.

Preparation of *Prosopis cineraria* extract

The plant material was collected between 8.00-10.00 AM in the month of July-2013 at Satpuda region, Maharastra, India. Authentication was made by Professor Dr. L. K. Kshirsagar, Taxonomist, Department of Botany, S.S.V.P.S's L. K. Dr. Ghogre Science College, Dhule, North Maharashtra University, Jalgaon. A voucher specimen (RCPIPER/Pharmacology/25-2013) has been kept in the Department of Pharmacology of Institute for further reference. Fruits were separated, cleaned, washed and air-dried. The dried fruits were pulverized by passing through 40 mesh sieve and extracted with 70 % ethanol using a Soxhlet extractor. The extract was concentrated in vacuum evaporator below 40 °C.

Phytochemical investigation and chromatographic analysis of extract

The ethanol extract was subjected to various tests for various phytochemical constituents as previously described by Kokate [17]. High performance liquid chromatography (HPLC) analyses was carried out on the extract using Shimadzu class LC-10AT HPLC system, equipted with Rheodyne 7725i injector fitted with

a 20 µl loop, column oven, and a photodiode array (PDA) detector, using Column Exsil ODS C-18 column (250 mm × 4.6 mm). The results were analyzed by chromquest version 3.0 software on Pentium computer (Hewlett Packard). Samples were eluted in Acetonitrile:water (90:10) as the mobile phase. Flow rate was constantly kept at 0.3 ml/min. and detection was carried out at 210 nm.

Acute oral toxicity study

Albino female mice weighing 25-30 g were used for acute oral toxicity study. Acute toxicity study was carried out as per Organization for Economic Co-operation and Development (OECD) Guideline 425 "Up & Down" method [18]. The test drug was found to be safe up to the dose of 5000 mg/kg body weight hence 1/10th of this dose was taken as an effective dose (500 mg/kg). The doses selected were 200, 400 and 600 mg/kg.

Assessment of hypolipidemic activity

Sprague Dawley rats were selected for the experimental model. The rats were fed with the standard diet and water *ad libitum* before the experiment. Room temperature was maintained between 20 and 30 ^{o}C and relative humidity of 40 – 60 %. The weight of the selected rats was between 180 and 200 g. 36 rats were divided into 6 groups of 6 rats per group. According to the body weight of individual rat, 200 mg/kg of triton was injected intraperitoneally [19].

Group 1 - Normal saline 1ml orally/animal.

Group 2 - Triton WR 1339 (200 mg/kg)

Group 3 - Triton WR 1339 (200 mg/kg) + Simvastatin 4 mg/kg orally

Group 4 - Triton WR 1339 (200 mg/kg) + Et-PCF 200 mg/kg orally

Group 5 - Triton WR 1339 (200 mg/kg) + Et-PCF 400 mg/kg orally

Group 6 - Triton WR 1339 (200 mg/kg) + Et-PCF 600 mg/kg orally

Blood samples were collected from normal rats and taken for analysis. For the treatment groups, the extract/simvastatin was given orally 30 min prior to triton injection. 3 ml of blood sample was collected from all the rats by making retro-orbital puncture both before triton injection (0 h) and after triton injection (24 h) [7]. Blood serum was separated by centrifugation at 3000 rpm and analyzed for lipid profile.

Determination of LDL cholesterol and atherogenic index

LDL cholesterol (LDLC) was calculated as in Eq 1 [20].

LDLC = TC – HDLC – VLDL …….. (1)

where TC, HDLC and VLDL are total cholesterol, HDL cholesterol and VLDL cholesterol, respectively.

Atherogenic index (AI) was computed as in Eq 2 [21].

AI = {(TC – HDLC)/HDLC} ……. (2)

Evaluation of antioxidant activity

Anti-lipid peroxidation

The mixture containing 0.5 ml of rat liver homogenate, 1 ml of 0.15 ml potassium chloride (KCl) and 0.5 ml of different concentrations (20 %, 40 %, 60 %, 80 %, 100 %) of drug extract were prepared. Lipid peroxidation was initiated by adding 100 µl of 1 mM ferric chloride. The reaction mixture was incubated for 30 min at 37 °C. After incubation 2 ml of ice-cold 0.25 N Hydrochloric acid (HCl) containing 15 % trichloroacetic acid (TCA) and 0.38 % thiobarbituric acid (TBA) and 0.2 ml of 0.05 % butylated hydroxytoluene (BHT) was added in the reaction mixture. This reaction mixture was heated for 60 min at 80 °C, cooled and centrifuged at 5000 g for 15 min. The absorbance of the supernatant was measured spectrophotometrically (Microplate reader power wave XS2, BIOTEK, USA) at 532 nm against a blank, which contained all reagents except liver homogenate and drug. Identical experiments were performed to determine the normal (without drug and ferric chloride) and induced (without drug) lipid peroxidation levels in the tissue. Percent anti-lipid peroxidation effect (ALP) was calculated as in Eq 3 [8].

ALP (%) = {(Af – As)/Af – An}100 …….. (3)

where Af, As and An are the absorbance of ferric chloride, test sample and normal sample, respectively.

Reducing power assay

The reducing power of the ethanolic extract of *Prosopis cineraria* was determined on the ability

of antioxidants to form colored complex with potassium ferricyanide. Various concentrations of the extract (10, 20, 40, 60 and 80 µg/mL) were mixed with 2.5 mL phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide (1 %). The mixture was incubated at 50 °C for 20 min. 2.5 mL TCA (10 %) was added to it and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of water and 0.5 mL of $FeCl_3$ (0.1 %) were added to it, and the absorbance was measured spectrophotometrically at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power [22].

Molecular docking study

The molecular docking tool, GLIDE (Schordinger Inc, USA, 2009), was used for ligand docking studies into the FXR binding pocket. The crystal structures of FXR was obtained from protein data bank (PDB ID: 1OSH) [23]. The protein preparation was carried out using 'protein preparation wizard' in Maestro 9.0 in two steps, preparation and refinement. After ensuring chemical correctness, water molecules in the crystal structures were deleted and hydrogens were added, where they were missing. Using OPLS 2005 force field energy of crystal structure was minimized [24]. This is forcefield in Schordinger 9.0, which is used to optimize the geometry of protein crystal structure and beta sitosterol. After optimization, we docked beta sitosterol into the cavity of FXR to check the binding mode.

Grids were defined centering them on the ligand in the crystal structure using the default box size. The ligands were built using maestro build panel and prepared by Ligprep 2.2 module which produce the low energy conformer of ligands using OPLS 2005 force field. The low energy conformation of the ligands was selected and was docked into the grid generated from protein structures using standard precision (SP) docking mode. The final evaluation is done with glide score (docking score) and single best pose is generated as the output for particular ligand.

Statistical analysis

The data are presented as mean ± standard error of mean (SEM). All data were analyzed using one-way analysis of variance (ANOVA) with Dunnet's post hoc test by Graph Pad Prism 5. Differences were considered significant at $p < 0.05$.

RESULTS

The ethanol extract tested positive for flavonoids, tannins, saponins, sterols and phenolic compounds. HPLC analysis also confirmed the presence of these phytoconstituents. The HPLC chromatogram of the extract is shown in Fig 1. The HPLC finger print for standard β-sitosterol showed a single peak while the extract showed six peaks at the wavelength of 210 nm.

***In vivo* hypolipidemic effect of Et-PCF on lipid profile**

The effect of Et-PCF on serum lipid profile in control and treatment groups at different doses are shown in Table 1. The acute injection of TWR caused a significant increase in lipid levels when compared with Group I rats. Group VI showed a significant ($p < 0.01$) reduction in cholesterol (26.67 %), triglyceride (26.52 %), LDL (54.93 %) and VLDL (26.60 %) levels, where as significant increase ($p < 0.05$) was observed in HDL (11.53 %) after treatment of Et-PCF when compared to Group II.

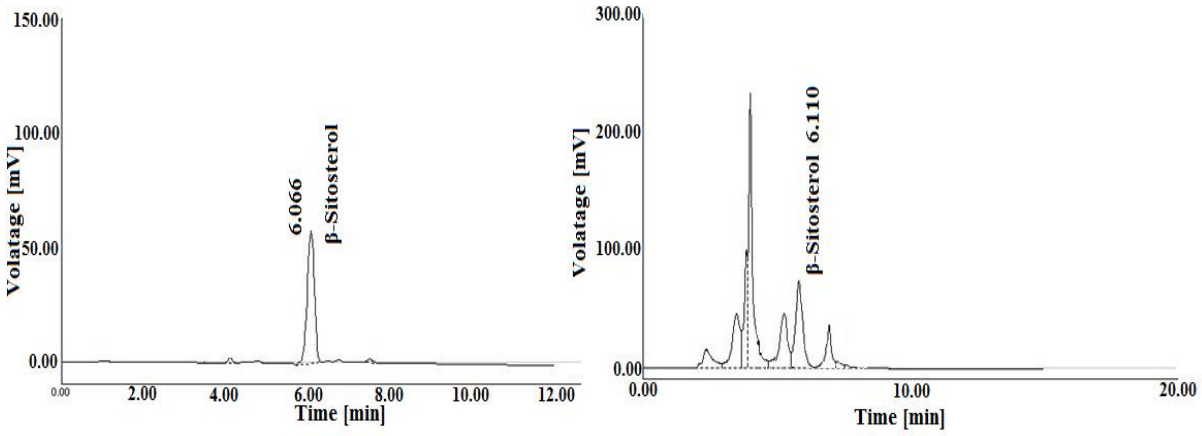

Fig. 1: HPLC fingerprint of ethanolic extract of *Prosopis cineraria*

The AI was also significantly ($p < 0.01$) decreased (53.34 %) in Group VI after treatment. In group IV at the minimum dose of 200 mg/kg Et-PCF significantly reduce both serum cholesterol ($p < 0.01$) and serum LDL ($p < 0.01$) as compared to group II. We observed a dose-dependent reduction of lipid profile and maximum antihyperlipidemic effect ($p < 0.01$) was seen in Group V (400 mg/kg of Et-PCF) and VI (600 mg/kg of Et-PCF) when compared to Group II. This effect of reduction in lipid profile and maximum antihyperlipidemic effect is almost equivalent to group III who received standard drug simvastatin ($p < 0.01$).

Antioxidant activity

In the present study, the ethanol extract showed potent inhibition of lipid peroxidation induced by Iron/ADP/Ascorbate complex in rat liver homogenate, IC_{50} value was 58.33 µg/ml. It showed dose dependent inhibition of lipid peroxidation. Standard ascorbic acid showed IC_{50} of 50.92 µg/mL (Fig 2).

The measurement of reductive ability was done by Fe^{3+}-Fe^{2+} transformation in the presence of ethanol extract and standard antioxidant, ascorbic acid [25]. The reducing power is associated with antioxidant activity. As shown in Fig. 3, the ethanolic extract showed a reducing power comparable with the standard at the highest concentration i.e. at 80 µg/ml.

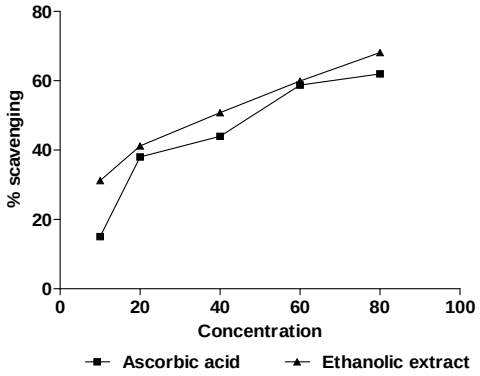

Fig 2: Effect of *Prosopis cineraria* on lipid peroxidation activity in rat liver homogenate

Table 1: Effect of the ethanolic extract of *Prosopis cineraria* on the lipid profile of triton induced hyperlipidemia

Group (mg/kg dose)	Cholesterol level (mg/dl)	Triglyceride level (mg/dl)	VLDL level (mg/dl)	LDL level (mg/dl)	HDL level (mg/dl)	Atherogenic index
Normal saline 1ml/animal	81.54±2.99	74.04±2.97	14.81±0.59	23.25±3.56	48.58±1.327	0.68±0.08
Triton only 200	120.9±2.82###	132.3±2.57###	26.47±0.51###	71.51±2.16###	41.12±1.58##	1.95±0.09##
Simvastatin 4+ TWR	84.79±2.63**	92.63±2.94**	18.53±0.58**	26.70±2.86**	48.41±0.914**	0.75±0.06**
Et-PCF 200 + TWR	109.7±2.82*	121.9±3.79	24.39±0.75	60.50±3.29*	41.04±1.63	1.69±0.13
Et-PCF 400 +TWR	101.9±2.73**	107.4±1.84**	21.47±0.36**	53.04±1.61**	40.70±1.41	1.50±0.04**
Et-PCF 600+TWR	88.55±2.68**	97.15±2.65**	19.43±0.53**	32.77±2.96**	46.48±1.25*	0.91±0.07**

*Values are expressed as mean ± SEM (n = 6); data were analyzed by one-way ANOVA followed by Dunnet's test; # denotes compared to normal control group, *p < 0.01 = very significant; **p < 0.05=significant, compared with triton-treated rats (Group II)*

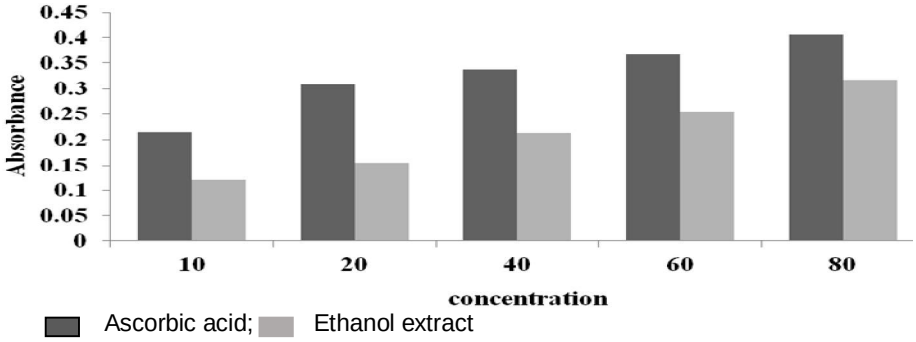

Fig 3: Effect of *Prosopis cineraria* on in-vitro reducing power assay

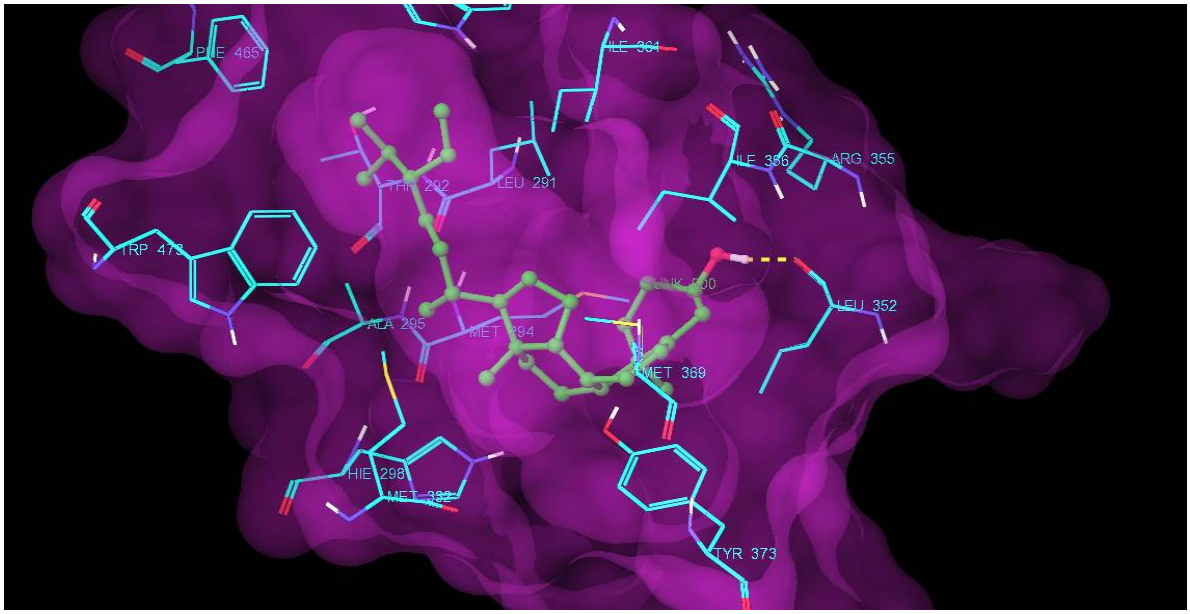

Fig 4: Binding mode of **β-sitosterol** in the FXR binding pocket (PDB: 1OSH) where **β-sitosterol** shows H-bond interaction between hydrogen of hydroxyl group and oxygen of LEU-352 amino acid

Molecular docking data

β-Sitostirol was subjected for molecular docking by calculating the minimum energy to activate the target receptor FXR, an important regulatory macromolecule whose activation promotes hypolipidemic activity. The protein structure file (PDB ID: 1OSH) was taken from PDB (www.rcsb.org/pdb) and showed the Binding mode of β-sitosterol in the FXR binding pocket in Fig 4.

DISCUSSION

In the present study, the effect of the ethanolic extract of *Prosopis cineraria* on experimentally-induced hyperlipidemia was evaluated in rats. In acute oral toxicity study, 70 % ethanolic extract of *Prosopis cineraria* up to the oral dose of 5000 mg/kg body weight did not produce signs of toxicity or mortality in rats. Hence 1/10th of this dose was selected as an effective evaluation dose (500 mg/kg). Triton WR-1339 is a non-ionic detergent and has been widely used to induce an acute hyperlipidemia in experimental animals [26]. The possible mechanism of action of triton is to block the clearance of triglyceride-rich lipoproteins by inhibiting the lipoprotein lipase [27]. In the present study, the highest levels of plasma triglycerides and total cholesterol were reached after 24h triton administration compared to normal control group. Also, there were increase levels of VLDL and LDL along with rise in atherogenic index as compared to normal control group.

Elevated level of blood cholesterol especially LDL-c is a known major risk factor for CHD whereas HDL-c is cardio protective [6]. Treatment with the extract significantly decreased the levels of total cholesterol and LDL-c compared to the Triton treated (hyperlipdemic) group. Extract treated group also showed remarkable reduction in triglyceride and VLDL cholesterol compared to triton treated group. Hypolipidemic effect of extract increases with increase in dose levels. At the highest dose levels of 400 mg/dl and 600 mg/dl hypolipidemic effect of extract is almost equivalent to standard drug simvastatin. Atherogenic index indicates the deposition of foam cells or plaque or fatty infiltration or lipids in the heart, coronaries, aorta, liver and kidneys. The higher the atherogenic index, the higher the risk of oxidative damage to these organs [28]. This atherogenic index was significantly reduced in the *Prosopis cineraria* treated groups compared to triton control group. The reduction in atherogenic index at the highest dose of extract is almost equal to standard drug simvastatin. This may be attributable to plant sterols that inhibit the absorption of dietary cholesterol and reduce atherogenic index. But the resulting decrease in serum cholesterol has been slight [9]. Although *Prosopis cineraria* have been shown to contain β-sitosterol using the HPLC, β-sitosterol is a plant sterol with a structure similar to that of cholesterol, except for the substitution of an ethyl group at C24 of its side chain. The cholesterol lowering effect may be due to this inhibition in reabsorption of cholesterol from endogenous sources in association with a simultaneous increase in its

excretion into feces in the form of neutral steroids. Since the results of the study indicated that extract has beneficial effect on lipid profile.

The findings of the present study also showed that the fruit extract of *Prosopis cineraria* possesses strong free radical scavenging and antioxidant activity. Polyphenols other than vitamin E have been known to exert powerful antioxidant effect in vitro. They inhibit lipid peroxidation by acting as chain-breaking peroxyl-radical scavengers, and can protect LDL from oxidation. Phytochemical investigation revealed the presence of polyphenols, thus the antioxidant effects could be due to presence of these phenolic components in the extract [10].

Further docking study was carried out for the target compounds into FXR using GLIDE (Schordinger Inc., USA). The crystal structure of the enzyme with Fexaramine (PDB: 1OSH) was obtained from protein data bank PDB. Our compounds were modeled by positioning them in the co-crystallized ligand Fexaramine binding site in accordance with the published crystal structures.

The entire complex was then subjected to alternate cycles of minimization and dynamics. The intent was to get a satisfactory structure for the complex that was consistent with the published crystal structure. From the comparative docking study of our compounds with Fexaramine lead compounds we could observe how our compounds might bind to the FXR binding site, based on the knowledge of the structure of similar active sites. We redocked Fexaramine into the active site of the enzyme and then we replaced with our compounds in order to compare the binding mode of both co-crystallized ligand and the test compound. These docking studies have revealed that the beta-sitosterol shows H-bonding with Leu-352 amino acid backbone with glide score of -8.32 kcal/mol. These interactions underscore the importance of oxygen as hydrogen bond acceptor for binding and the subsequent agonistic capacity. The results of this virtual screening support the postulation that the active compounds possibly binds on the same enzyme target where Fexaramine binds.

CONCLUSION

The fruits of *Prosopis cineraria* can be used in the treatment of hyperlipidemia which is one of the major causes of cardiovascular disease, and may serve as a substitute to currently used hypolipidemic drugs in the therapy of hyperlipidemia. However, long-term studies are required to determine the adverse and beneficial effects of the isolated phytoconstituent.

REFERENCES

1. *WHO Monica project, Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas A, Pajak A. Myocardial infarction and coronary deaths in the World Health Organisation Project. Circulation 1994; 90: 583-612.*
2. *Bennani-Kabchi N, Fdhil H, Cherrah Y, Kehel L, el Bouavadi F, Amarti A, Saidi M, Marquie G. Effects of Olea europea var. oleaster leaves in hypercholesterolemic insulin-resistant sand rats. Therapie 1999; 54(6): 717-723.*
3. *Berliner JA, Heinecke JW. The role of oxidized lipoproteins in atherogenesis. Free Radical Biol. Med 1996; 20: 707-727.*
4. *Gingliano D. Dietary antioxidants for cardiovascular prevention. Nutr Metab Cardiovasc. Dis 2000; 10: 38-44.*
5. *Anderson JK, Teuber SS, Gobeille A, Cremin P, Waterhouse AL, Steinberg FM. Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. J Nutr 2000; 131: 2837-2842.*
6. *Rohilla A, Dagar N, Rohilla S, Dahiya A, Kushnoor A. Hyperlipidemia –a deadly pathological condition. Int J Curr Pharm Res 2012; 4: 15-18.*
7. *Hemalatha S, Wahi AK, Singh PN, Chansouria JPN. Hypolipidemic Activity of Aqueous Extract of Withania coagulans Dunal in Albino Rats. Phytother Res 2006; 20: 614–617.*
8. *Umamaheswari M, Asokkumar K, Rathidevi R, Sivashanmugam AT, Subhadradevi V, Ravi TK. Antiulcer and in vitro antioxidant activities of Jasminum grandiflorum L. J Ethnopharmacol 2007; 110: 464-470.*
9. *Jain PG, Patil SD, Haswani NG, Girase MV, Surana SJ. Hypolipidemic activity of Moringa oleifera Lam., Moringaceae, on high fat diet induced hyperlipidemia in albino rats. Revista Brasileira de Farmacognosia 2010; 20(6): 969-973.*
10. *Pilaipark C, Panya K, Yupin S, Srichan P, Noppawan PM, Laddawal PN, Piyanee R, Supath S and Klaiupsorn SP. The in vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of Moringa oleifera Lam. Leaves. J. Ethnopharmacol 2008; 116: 439–446.*
11. *Burkart A. A monograph of genus Prosopis (Leguminous). J Ar Arb 1976; 57: 219-49, 450-525.*
12. *Shalini. Vedic Leguminous Plants, (Shalini, Ed), Classical publishing co, 1997; pp. 57-80.*
13. *Toky OP. Medicinal values of Prosopis cineraria in arid and semiarid India. Society of Chemical Industry, I, 1999; pp 1-10.*
14. *Purohit SD, Ramawat KG, Arya HC. Phenolics, peroxidase and phenolase as related to gall formation in some arid zone plants. Curr Sci 1979; 48: 714-716.*

15. Rhoades DF. Herbivores, their interaction with secondary plant metabolites. Acad. Press Inc. London, 1979; pp 3-54.
16. Nahla AH and Farag WT. Design, synthesis and docking studies of new furobenzopyranones and pyranobenzopyranones as photo-reagent towards DNA and as antimicrobial agents. Eur. J. Med. Chem 2010; 45: 317–325.
17. Kokate CK. Practical Pharmacognosy. Vallabh Prakashan, New Delhi, India, 1998; 107–111.
18. Guidance document on acute oral toxicity testing. Series on testing and assessment N0 24.OECD environment, health and safety publications 2001.Accessed from http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2001)4&doclanguage=en on 18 June 2013.
19. Kourounakis AP, Victoratos P, Peroulis N, Stefanou, Yiangou M, Hadjipetrou L. Experimental Hyperlipidemic and the Effect of NSAIDs. Exper Mol Pathol 2002; 73: 135–138.
20. Friedewald WT, Levy RI, Frederickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. Clin chem. 1972; 18: 499-502.
21. Muruganandan S, Srivinasan K, Gupta S, Gupta PK, Lal J. Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats. J Ethnopharmacol 2005; 97: 497-501.
22. Oyaizu M. Studies on products of browing reaction prepared from glucosamine: Jap J Nutri 1986; 44: 307-315.
23. http://www.rcsb.org/pdb/explore.do?structureId=1OSH. Accessed on 23rd October 2013.
24. Zhong H, Tran LM, Stang JL. Induced-fit docking studies of the active and inactive states of protein tyrosine kinases. J. Mol. Graph. Mod 2009; 28: 336–346.
25. Shimazaki H. Antioxidants. In: Niki E, Shimazaki, H, Mino, M (eds). Free radicals and biological defense: Japanese Science Societies Press, Tokyo, 1994; pp 45-57.
26. Bertges LC, Mourão Jr CA, Souza JB, Cardoso VA. Hyperlipidemia induced by triton WR 1339 (Tyloxapol) in wistar rats. Rev Bras Cien Med Saūde 2011; 1: 32-34.
27. Otway S, Robinson DS. The use of a non-ionic detergent (triton WR 1339) to determine rates of triglyceride entry into the circulation of the rat under different physiological conditions. J.Physiol 1967; 190: 321-332.
28. Saluja MP, Kapil RS, Popli SP. Studies in medicinal plants: part VI. Chemical constituents of Moringa oleifera Lam. (hybrid variety) and isolation of 4-hydroxymellein. Indian J Chem B 1978; 16B: 1044-1045.