



EVALUATION OF HYOLIPIDEMIC ACTIVITY OF *BETULA ALNOIDES* BARK ON TRITON WR-1339 INDUCED HYPERLIPIDEMIA IN ALBINO RATS

Dominic Amal Raj¹, Malarvili T*², Velavan S³

¹Research Scholar, P.G. and Research Department of Biochemistry, Rajah Serfoji Govt. College (Autonomous), Thanjavur-613 005, Tamil Nadu, South India.

²P.G. and Research Department of Biochemistry, Rajah Serfoji Govt. College (Autonomous), Thanjavur-613 005, Tamil Nadu, South India.

³Department of Biochemistry, Marudupandiyar College, Thanjavur, Tamil Nadu, South India.

Article Info

Received 22/10/2013

Revised 16/11/2013

Accepted 18/12/2013

Key words: Triton WR-1339, *Betula alnoides*, Coronary risk index, Hyperlipidemia, Lipid profile.

ABSTRACT

The objective of the study was to investigate the antihyperlipidemic effect of *Betula alnoides* bark in triton WR 1339 induced hyperlipidemia in rats. Triton WR 1339 (350mg kg⁻¹, i.p.) injection significantly ($p < 0.001$) increased total cholesterol, triglyceride, very low density lipoprotein (VLDL), Low density lipoprotein (LDL), phospholipids and decreased high density lipoprotein (HDL). *Betula alnoides* showed dose dependant decrease in the total cholesterol, triglyceride, very low density lipoprotein (VLDL), Low density lipoprotein (LDL) and phospholipids. *Betula alnoides* increased high density lipoprotein (HDL). Administration of triton alone increased coronary risk index, atherogenic index and LDL, *Betula alnoides* and atorvastatin treatment decrease both the indices. It is concluded that *Betula alnoides* reduces total cholesterol, triglyceride, very low density lipoprotein (VLDL), Low density lipoprotein (LDL), phospholipids, coronary risk index, atherosclerotic index and increased HDL in triton induced hyperlipidemia in rats.

INTRODUCTION

Diseases associated with high triglycerides (TG) levels (Diabetes mellitus, obesity, chronic renal disease and primary hyperlipoproteiemia) carry high risk of cardiovascular disorder [1]. Hyperlipidemia is metabolic complication of both clinical and experimental obesity [2]. Majority of the obesity patients have dyslipidemia and have 2-5 times the risk of cardiovascular disease [3]. Dyslipidemia is characterized by low level of high density lipoprotein- cholesterol (HDL-C) and high level of low density lipoprotein- cholesterol (LDL-C) and triglycerides (TG). Consumption of much fat may lead to the production

of extra VLDL, resulting in the formation of large amounts of LDL which may stick to the walls of the blood vessels if the quantity of HDL is insufficient, causing blockages for the normal flow of blood. Ultimately this leads to atherosclerotic plaque [4].

Currently available hypolipidemic drugs have been associated with a number of side effects. The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function [5]. Medicinal plants are used for various research purposes. It has been reported that traditional systems have immune potential against various diseases. More than thirteen thousand plants have been studied for various pharmacological properties. An herbal treatment for hypercholesterolemia has no side effects and is relatively cheap, locally available. The chosen medicinal plant namely as *Betula alnoides* bark L belongs to the *Betulaceae* family. A survey of literature

Corresponding Author

Dominic Amal Raj
Email: malarsai96@gmail.com



revealed that no systematic approach has been made to study antihyperlipidaemic activity of this plant. Therefore, the present study was to investigate the antihyperlipidemic properties of ethanolic extract of *Betula alnoides* bark in Triton WR 1339 induced hyperlipidemic rats.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain approximately weighing 100-125g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature $27 \pm 2^\circ$ C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use.

Chemicals

Triton WR 1339, Ethylene diamine tetra acetic acid (EDTA), sodium nitroprusside (SNP), Trichloro acetic acid (TCA) were purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

Plant materials

The mature *Betula alnoides* barks were collected in May 2012 from Kodaikanal, Dindugal district, Tamil Nadu, India. The barks were identified and authenticated by Botanist, Prof. S. Palaniappan, Department of Botany, H.H. Rajahs College (Autonomous), Pudukkottai, Tamil Nadu, India. A Voucher specimen (RJOBS/JJC/2013) has been deposited at the Herbarium, J. J. College, Pudukkottai, Tamil Nadu, India.

Preparation of alcoholic extract

The bark of *Betula alnoides* were first washed well and dust was removed from the bark. Barks were washed several times with distilled water to remove the traces of impurities from the bark. The barks were dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Betula alnoides* bark extract (BABE) was stored in refrigerator until used. Doses such as 250, 500 and 750mg/Kg. were chosen for this study.

Induction of hyperlipidemia

A single dose (350 mg/kg body weight i.p) of Triton WR-1339 dissolved in saline solution was used for induction of hyperlipidemia in the rats. Hyperlipidemia was confirmed 48 hrs after triton injection by determining the blood cholesterol concentration [6]. The results were

compared with that of the standard drug atorvastatin at a dose of 10mg/kg b.w [7].

Experimental design and protocol

Overnight fasted rats were divided into six groups containing six rats in each group. Group I control received intraperitoneal administration of normal saline and distilled water orally. Animals of group II to VI were treated with intraperitoneal injection of triton WR-1339 to induce hyperlipidemia. Group III, IV and V received increasing doses i.e. 250, 500 and 750 mg kg⁻¹ respectively and Group VI rats received atorvastatin (ATR) which was administered continuously for 7 days orally using infant feeding tube.

Collection of blood and preparation of plasma sample

At the end of the experimental period, the animals were anaesthetized using chloroform vapour prior to dissection. Blood was collected by cardiac puncture into plasma separator tubes containing anticoagulant. The blood was allowed to standing at room temperature for 30 minutes and then refrigerated for another 30 minutes. The resultant clear part was centrifuged at 3000rpm for 10 minutes, and then the plasma (supernatant) was isolated and refrigerated until required for analysis.

Biochemical analysis

The total cholesterol was estimated by the method of Allain et al. [8]. Triglyceride was estimated by the method of Werner et al. [9]. HDL cholesterol was separated by adding phosphotungstic acid and magnesium chloride to the fresh samples to precipitate other lipoproteins and the HDL cholesterol was estimated by the method of Allain et al. [8]. The concentration of LDL cholesterol was calculated by using the Friedewald et al. [10] formula and VLDL cholesterol was calculated by dividing the triglycerides value (in mg/dl) by 5. The phospholipids were estimated by the method of Zilversmit *et al.*, [11] and liberated phosphorous was estimated by using Fiske and Subbarow method [12]. Atherogenic Index [13] and Coronary Risk Index [14] were calculated by the following formula;

$$AI = LDL-C/HDL-C$$

$$CRI = Total\ cholesterol/HDL - Cholesterol$$

Statistical Analysis

Values were expressed as mean \pm SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons. The results were statistically analyzed by Graphpad Instat Software (Graphpad Software, San Diego, CA, USA) version 3 and $p < 0.01$ was considered to be significant.

RESULTS

The effect of BABE on levels of plasma lipid



parameters in triton induced hyperlipidaemia is reported in Tables 1 and 2. In comparison with control group, Triton WR-1339 caused an increase in TG, LDL, and VLDL caused marked fall in HDL-cholesterol plasma concentrations measured. The administration of 250, 500 and 750 mg/kg doses of BABE in Triton injected rats decrease the plasma TC, Phospholipids, TG, LDL VLDL and increased the HDL cholesterol with dose dependent manner. The reduction of TG was 12.49%, 29.16%, 37.50%, for 250, 500 and 750mg/Kg. body weight respectively. The reduction of TC was 17.38%, 26.08%, 66.68%, for 250, 500 and 750mg/Kg. body weight respectively. The reduction of LDL was 23.62%, 46.42%, 62.07%, for 250, 500 and 750mg/Kg. body weight respectively. The reduction of Phospholipids was 7.28%, 32.29%, 33.33%, for 250, 500 and 750mg/Kg. body weight respectively. The reduction of VLDL was 12.52%, 29.17%, 37.75%, for 250, 500 and 750mg/Kg. body weight respectively. The increment of HDL was 9.98%, 39.94%,

59.98%, for 250, 500 and 750mg/Kg. body weight respectively. Supplementation of *Betula alnoides* extract at the doses of 500mg and 750mg/kg body weight were found to be significant on Triton induced hyperlipidemic rats. Atherogenic Index (AI) and Coronary Risk Index (CRI) were increased in Triton induced hyperlipidemic rats. Supplementation of *Betula alnoides* extract at the doses of 250, 500mg and 750mg/kg body weight were found to decrease AI and CRI on dose dependent manner.

The atorvastatin significantly reduced the level of TC, Phospholipids, TG, VLDL and LDL-cholesterol levels ($P<0.05$) and caused increase in HDL-cholesterol level significantly ($P<0.05$) compared to triton control group. The reduction was 23.04% for TC, 31.71% for Phospholipids, 29.16% for TG, 33.15% for LDL and 29.17% for VLDL. The increment was 50% for HDL-C. Administration of atorvastatin reduced the atherogenic Index and coronary risk index in triton induced hyperlipidemic rats.

Table 1. Effect of ethanolic extract of *Betula alnoides* bark on Triglycerides, Cholesterol, HDL-cholesterol and Phospholipids of control and experimental rats

Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL-Cholesterol (mg/dl)	Phospholipids (mg/dl)
Group-I (Normal)	77.78±6.06	48.48±3.78	36.96±2.88	90.43±7.05
Group-II (Induction)	133.33±10.39 ^a	69.70±5.43 ^a	21.74±1.69 ^a	166.95±13.02 ^a
Group-III (250mg/kg)	116.67±9.10 ^b	57.58±4.49 ^b	23.91±1.86	154.78±12.07
Group-IV (500mg/kg)	94.44±7.36 ^b	51.52±4.01 ^b	30.43±2.36 ^b	113.03±8.81 ^b
Group-V (750mg/kg)	83.33±6.49 ^b	46.48±3.62 ^b	34.78±2.71 ^b	111.30±8.83 ^b
Group-VI (Atorvastatin 10mg/kg)	94.44±7.36 ^b	53.61±4.72 ^b	32.61±2.54 ^b	114±8.89 ^b

Each value is expressed as mean ± SD for six rats in each group. ^aAs compared with group I ($p<0.01$), ^bAs compared with group II ($p<0.01$).

Table 2. Effect of ethanolic extract of *Betula alnoides* bark on VLDL, LDL- Cholesterol, CRI and AI of control and experimental rats

Groups	VLDL (mg/dl)	LDL (mg/dl)	CRI	AI
Group-I (Normal)	15.56±1.21	27.08±2.11	1.31±0.08	0.73±0.04
Group-II (Induction)	26.67±2.08 ^a	74.63±5.82 ^a	3.20 ±0.21	3.43±0.23
Group-III (250mg/kg)	23.33±1.81	57±4.44 ^b	2.40±0.16	2.38±0.16
Group-IV (500mg/kg)	18.89±1.47 ^b	39.98±3.11 ^b	1.69±0.11	1.31±0.08
Group-V (750mg/kg)	16.6±1.29 ^b	28.30±2.20 ^b	1.33±0.09	0.81±0.05
Group-VI (Atorvastatin 10mg/kg)	18.89±1.47 ^b	46.89±3.65 ^b	1.64±0.12	1.43±0.09

Each value is expressed as mean ± SD for six rats in each group. ^aAs compared with group I ($p<0.01$), ^bAs compared with group II ($p<0.01$).

DISCUSSION

In the present study, the antihyperlipidaemic effects of 250–750 mg/kg of BABE in normal and experimental models of hyperlipidaemia and its possible mechanism(s) of action were investigated. Triton WR-1339 has been widely used for a number of different aims particularly, in rats it has been used for screening natural or chemical hypolipidemic drugs and also studying lipid metabolism and for investigating metabolic interrelationship between plasma lipoproteins [15, 16].

Triton is known to induce hyperlipidaemia in two phases:

Phase I is believed to be due to increased hepatic cholesterol biosynthesis through triton's interference with the tissue uptake of plasma lipids [17, 18] while Phase II involves triton's interference with cholesterol excretion and metabolism [19, 20]. In addition, triton physically increases VLDL-c level by rendering them refractive to the metabolic action of plasma and tissue lipolytic enzymes, thereby preventing or delaying their plasma clearance [21]. Thus, the overall effect of triton WR-1339 is accelerated



hepatic triglyceride and cholesterol biosynthesis which result in elevated plasma triglyceride, total cholesterol, LDL-C and VLDL-C while causing the reverse in the plasma level of HDL-C [16].

Our present studies show that BABE at a dose of 500 and 750 mg/kg significantly lowered triglycerides, cholesterol and phospholipids levels. The large increase in phospholipids, cholesterol and triglycerides due to Triton WR-1339 injection results mostly from an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism [22]. The reduction of total cholesterol by the BABE was associated with a decrease in LDL fraction, which is the target of several hypolipidemic drugs. This result suggests that cholesterol-lowering activity of the herb extract can result from the rapid catabolism of LDL cholesterol through its hepatic receptors for final elimination in the form of bile acids [23].

It is well known that HDL-Cholesterol levels have a protective role in Coronary artery disease. Similarly increased level of plasma LDL-cholesterol results in increased risk for the development of atherosclerosis [24]. The increased level of HDL- cholesterol and decreased cholesterol level along with its LDL fraction which is evident from the results could be due to an increased cholesterol excretion and decreased cholesterol absorption through gastro intestinal tract. Betula has high levels of

phenolic compounds such as flavonoids, myricetin, quercetin derivatives, chlorogenic acid, hydroxyl cinnamic acids and condensed tannins [25] which could be attributed to the hypolipidaemic activity of *B. alnoides*. Saponin, Flavonoids and polyphenolic compounds reported to possess hypolipidemic property [26].

CONCLUSION

BABE supplementation resulted in significant attenuation in the level of LDL and HDL in plasma towards the control level, which again strengthens the hypolipidemic effect of this extract. The antihyperlipidemic activity of BABE at doses of 500 and 750 mg/kg body weight against Triton WR-1339 induced hyperlipidemia showed significant activity when compared to atorvastatin treated groups. The result strongly suggests that the hypolipidemic activity of BABE could be attributed to the presence of the valuable saponins and flavonoids in the extract. The results are encouraging enough for further studies aimed at understanding the mechanism of action and to identify the bioactive compounds. Since ayurvedic/herbal medicines are needed to be used in higher doses and for relatively longer periods for permanent effect, studies are on currently to ascertain the efficacy of BABE in high fructose diet induced hyperlipidemia.

REFERENCES

1. Lamarche B, St-Pierre AC, Ruel IL, Cantin B, Dagenais GR and Despres JP. (2001). A prospective, population-based study of low density lipoprotein particle size as a risk factor for ischemic heart disease in men. *Can J Cardiol*, 17, 859-865.
2. Angelo A and Saula Vigili de Kreutzenberg. (2005). Mechanisms of endothelial dysfunction in obesity- Review. *Clinica Chimica Acta*, 360, 9-26.
3. Ebrahim K and Naderali. (2009). Obesity and cardiovascular dysfunction: A role for resveratrol. *Obesity Research & Clinical Practice*, 3, 45-52.
4. Pascot A, Lemieux I, Prud'homme D, Tremblay A, Nadeau A, Couillard C, Bergeron J, Lamarche B and Despres JP. (2001). Reduced HDL particle size as an additional feature of the atherogenic dyslipidemia of abdominal obesity. *J Lipid Res*, 42, 2007-2014.
5. Speight M T and Avery S. (1987). Drug treatment Principles and Practice of clinical Pharmacology and therapeutics, 3, 599.
6. Schurr PE, Schultz JR and Parkinson TM. (1972). Triton-induced hyperlipidemia in rats as an animal model for screening hypolipidemic drugs. *Lipids*, 7(1), 68-74.
7. Saravana KA, Mazumder A, Saravanan VS. (1974). Antihyperlipidemic activity of *Camellia sinensis* leaves in Triton WR-1339 induced albino rats. *Phcog. Mag*, 4(13), 60-64.
8. Allain CC, Poon LS, Chan CSG, Richmond W and Fu PC. (1974). Enzymatic determination of total serum cholesterol. *Clin Chem*, 20, 470-475.
9. Werner M, Gabrielson DG and Eastman G. (1981). Ultramicro determination of serum triglycerides by bioluminescent assay. *Clin Chem*, 27, 268-271.
10. Friedewald WT, Levy RI and Fredrickson DS. (1972). Estimation of concentration of lowdensity lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. *Clin Chem* 18, 439-502.
11. Zilversmit DB and Davis AK. (1950). Micro determination of plasma phospholipids by TCA precipitaton. *J.Lab.Clin.Med*, 35, 155-159.
12. Fiske CH and Subbarow. (1925). The colorimetric determination of phosphorus. *J.Biol.Chem*, 66, 375-400.
13. Abbott RD, Wilson PW, Kanne, WB and Castelli WP. (1988). High density lipoprotein-cholesterol, total cholesterol screening and myocardial infarction. The Framingham Study. *Arteriosclerosis*, 8, 207-211.



14. Adeneyea AA and Olagunjub JA. (2009). Preliminary hypoglycemic and hypolipidemic activities of the aqueous seed extract of *carica papaya* in Wistar rats. *Biol. Med*, 1, 10.
15. Kellner A, Correl WJ and Ladd AT. (1951). Sustained hyperlipidaemia induced in rabbits by means of intravenously injected surface active agents. *Journal of Experimental Medicine*, 93, 373-385.
16. Banerjee A, Vaghasiya R, Shrivastava N, Padh H and Nivsarkar M. (2006). Antihyperlipidaemic effect of *Carica papaya* L. in Sprague Dawley rats. *Nigerian Journal of Natural Products and Medicine*, 10, 2006, 69-72.
17. Holmes WL. (1964). Drugs affecting lipids synthesis. In: Paolett, R. (Ed.), *Lipid Pharmacology*. Academic Press, *New York*, 3, 131-184.
18. Tamasi G, Borsy J and Pathy A. (1968). Comparison of the antilipidaemic effect of nicotinic acid (NA) and 4-methylpyrazole-5-carboxylic acid (MPC) in rats. *Biochemical Pharmacology*, 17, 1789-1794.
19. Garrattini S, Paoletti P and Paoletti R. (2008). The effect of triton and diphenylethylacetic acid on cholesterol and fatty acid biosynthesis in isolated perfused liver. *Experientia*, 15, 33-37.
20. Vogel HG and Vogel WH. (2002). Anti-atherosclerotic activity: triton-induced hyperlipidaemia. In: *Drug Discovery and Evaluation—Pharmacological Assays*. Springer-Verlag, Heidelberg, 1, 606.
21. Friedman M and Byers SO. (1957). Mechanism underlying hypercholesterolaemia induced by triton WR-1339. *American Journal of Physiology*, 190, 1957, 439-445.
22. Otway S and Robinson D.S. The effect of the nonionic detergent (Triton) on the removal of triglyceride fatty acids from the blood of the rats. *J.of.Physiol*, 190, 309-319.
23. Khanna AK, Rizvi F and Chander R. (2002). Lipid lowering activity of *Phyllanthus niruri* in hyperlipidemic rats. *J.of.Ethnopharmacol*, 82, 19-22.
24. Wilson PW, Abbott RD and Castelli WP. (1988). High density lipoprotein cholesterol and mortality, The Framingham heart study. *Arteriosclerosis*, 8,737-740.
25. Ghimire BK, Tamang JP, Yu CY, Jung SJ and Chung IM. (2012). Antioxidant, antimicrobial activity and inhibition of alpha glucosidase activity by *Betula alnoides* bark extract and their relationship with polyphenolic compounds concentration. *Immuno pharmacology and immune toxicology*, 1-08.
26. Mukherjee PK. (2003). Plant products with hypocholesterolemic Potentials. *Advances in food and nutrition research*, 47, 277-287.

