World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Combined effect on antihyperlipidemic activity of *Bauhinia variegata (linn.)* and *Salvadora oleoides (decne.)* in Triton WR-1339 induced hyperlipidemic rats

Deepak Kumar[1]*, Versha Parcha[2] and Alok Maithani[2]

[1*]Department of Pharmaceutical Chemistry, Dolphin PG Institute of Biomedical and Natural Sciences, Manduwala, Dehradun, Uttarakhand, India
[2]Department of Applied Chemistry, Sardar Bhagwan Singh PG Institute of Biomedical Sciences and Research, Balawala, Dehradun, Uttarakhand, India

Received: 17-03-2014 / Revised: 27-03-2014 / Accepted: 15-04-2014

ABSTRACT

Cardiovascular and related illnesses are one of the most common diseases prevalent in many parts of the world. An increased risk of coronary heart diseaseis primarily associated with a high serum total cholesterol and low density lipoprotein (LDL) concentration and a decrease in high density lipoprotein (HDL). The present study was designed to investigate combined effect on antihyperlipidemic activity of butanol extract of leaves of *Bauhinia variegata (Linn.)* and *Salvadora oleoides (decne.)* in Triton WR-1339 induced hyperlipidemic rats. Combined dose of butanol extract of leaves of both plant tested for their anti-hyperlipidemic activity. Combined dose of butanol extract administered at a dose of 100 mg/kg (oral) at a ratio 1:1 to the Triton WR-1339 induced hyperlipidemic rats and total cholesterol, triglycerides, HDL, LDL and VLDL level in the blood were checked. Combined dose of butanol extract showed significant reduction ($P<0.05$) in plasma cholesterol (80.30 mg/dl), triglyceride (98.64 mg/dl), LDL (29.31 mg/dl), VLDL (19.73 mg/dl) and increase in HDL level (31.26 mg/dl) as comparison with standard drug fenofibrate. From the above study it could be concluded that Combined dose of butanol extract of Bauhinia variegata (Linn.) and Salvadora oleoides (decne) not only have resulted in significant reduction in cholesterol, triglyceride, LDL, VLDL level but also increase the HDL level which is good for health.

Keywords: *Bauhinia variegata, Salvadora oleoides,* antihyperlipidemic activity, Triton WR 1339, Atherosclerosis.

INTRODUCTION

Rakta Kanchan (*Bauhinia variegata* Linn.) is a medium-sized, deciduous tree, found throughout India, ascending to an altitude of 1,300 m in the Himalayas. It is commonly known as Kanchnar in Sanskrit and Mountain Ebony in English. In Sanskrit the word Kanchnar means "A glowing beautiful lady". A freshly collected bark of the plant is greyish brown externally and cream colored internally. The internal surface, however, gradually turns red and on drying becomes brown and smooth. The external surface remains greyish brown and rough due to large number of exfoliations, transverse cracks and fissures. On drying, the bark becomes curved and channeled. The fracture is short outside and fibrous within. Leaves are 10-15 cm long, rigidly sub-coriaceous and deeply cordate. The flowers are bisexual, irregular and light magenta in color. The pods are long, hard, flat, dehiscent and 10-15 seeded. The various parts of the plant viz., flower buds, flowers, stem, stem bark, leaves, seeds and roots are practiced in various indigenous systems of medicine and popular among the various ethnic groups in India for the cure of variety of ailments. Following a large number of claims on the wide range of folk curative properties of *Bauhinia variegata*, considerable efforts have been made by the researchers to justify its efficacy as a curative agent through pharmacological investigations [1]. The flowers and dried buds are anthelmentic and are used in diarrhoea, piles and dysentery. The bark is isotonic, astringent and anthelmentic and an emulsion of the powder of bark with rice mixed with ginger is used in scrofula and cutaneous

***Corresponding Author Address:** Dr. Deepak Kumar, Assistant Professor, Department of Pharmaceutical Chemistry, Dolphin PG institute of Biomedical and Natural Sciences, Manduwala, Dehradun, India; Email: deepsingh2304@gmail.com*

affections. The decoction of root bark is carminative and is used in dyspepsia and flatulence. An extract of its buds, flowers and bark is considered antibacterial. Besides, the flowers with sugars are used a gentle laxative. The decoction of the bark is used in diarrhea and that of its root as antifat remedy as a folklore medicine by the native people [2]. The bark is astringent, tonic and anthelmentic. It is useful in scrofula and skin diseases. It is also used for ulcers and leprosy. The flowers and buds are used as a vegetable. The flowers and buds are pickled. The decoction of buds is given in cough, piles, haematuria and menorrhagia [3]. Various medicinal properties have been reported from various parts of *Bauhinia variegata* Linn. viz antimicrobial activity [4], anti-inflammatory [5], antitumor activity [6], Nephroprotective activity [7] Hepatoprotective activity [8], anticarcinogenic [9] and antimutagenic activity [9], antioxidant [10], [12] and antihyperlipidemic activity [10], Hypoglycemic effect [11], [12].

Salvadora Linn. a small genus of evergreen trees or shrubs, distributed in tropical Africa and Asia, extending to Egypt, Mascarene Islands and China [13]. *Salvadora oleoides*, belonging to family Salvadoraceae commonly found in Western region in India. It is known as Jhal, Pilu in Hindi, Pilu in Sanskrit, Khakan in Gujrati, Kalawa, Karkol in Tamil and Diar, Godpilu, Khabbar in Marathi [13]. An evergreen plant or shrub or a small tree. Bark is grey or whitish grey, Leaves linear-lanceolate, coriaceous, Flower sessile, greenish white minute in paniculate spikes, drupes globose, usually yellow when ripe, seeds are greenish yellow 3 m in diameter. The Plant coppices fairly well and regenerates freely by root-suckers, and to some extent by natural layers. It suffers considerably from frost. A dense almost impenetrable growth is often formed by a parent stem surrounded by a ring of root-suckers, while seedling also spring up under its shade. It is suitable for growing in shelter-belts and as wind breaks in the desert tracts. The tree suffers little from grazing, but it is often iopped for camel fodder. It is susceptible to several fungi, and is attacked by the larvae of some beetles. Leaves are used as purgative and as cure of cough. Leaves and stem are used for diabetes and hypercholestremia. Root and bark are used as vasic. Fruits are used as aphrodisable. Oil and seed are used as stimulating application in painful rheumatoid, affection and after child birth [14]**.** Fruit are employed in the treatment of enlarge spleen, rheumatism and low fever. Seed contains 40-50% of greenish yellow fat. Purified seed fat can be directly used for soap making and is a potential industries substitute of coconut oil for this purpose. Fat is used for preparation of Suppositories. It is also used as a base for ointments [13]. A very few medicinal properties have been reported from various parts of *Salvadora oleoides* as it is less explored plant. Few medicinal properties have been reported viz. Hypoglycemic [15] and antihyperlipidemic activity [15], antimicrobial activity [16]. Butanol fraction from *Bauhinia variegata* and *Salvadora oleoides* was found to reduce Serum Cholesterol, Triglycerides, HDL, LDL and VLDL level significantly in Triton WR1339 induced hyperlipidemic rats [17], [18]. So it was aimed to study synergistic action (if any) of butanol fractions of both mentioned plant materials when taken in combination in different proportions. The effect of combinations was studied on fasting blood Cholesterol level and other biochemical parameters of Triton WR 1339 induced hyperlipidemic rats.

EXPERIMENTAL

Collection and Identification of Plant materials: Leaves of *Bauhinia variegata (Linn.)* were collected from locality of Dehradun (India). The plant material was deposited and authenticated by the Botanical Survey of India, Dehradun. Authenticated specimen number is Acc. No. 113245 and voucher specimen sample is preserved in Dept. of Pharmaceutical Sciences, S. B. S. P. G. I., Balawala, Dehradun for further reference.

Leaves of *Salvadora oleoides (Decne.)* were collected from Delhi (India). The plant material was deposited and authenticated by the Botanical Survey of India, Dehradun. Authenticated specimen number is Acc. No. 113246 and also authenticated by Dr. H. B. Singh, NISCAIR, New Delhi, reference no. NISCAIR/ RHMD/ Consult/ 2010-11/1675/273 and voucher specimen sample is preserved in Dept. of Pharmaceutical Sciences, S. B. S. P. G. I., Balawala, Dehradun for further reference.

Preparation of Plant extracts: The plant material was dried under shade and powdered. The 500g powdered material was extracted with methanol by cold percolation for 1 week. The extract was evaporated to dryness to obtain a residue [19]. From total methanol extract, preparation of different fraction by cold percolation method using increasing polarity of solvents by separation technique i.e Petroleum ether, Chloroform, Ethyl acetate and Butanol. Only butanol extracts from both plants have subjected to screened for their antihyperlipidemic effect.

Anti-hyperlipidemic activity

Test Animals: Adult albino rats of both sexes weighing 180-300 gm were procured from disease

free CPCSEA approved animal house (Reg. no. 273/CPCSEA) of S. B. S. P. G. I. Dehradun. The animals were fed with standard pellet diet. The Institutional Animal Ethics committee (IAEC) of the Institute and Department of Pharmaceutical Sciences approved the study.

Antihyperlipidemic study: Antihyperlipidemic studies were carried out and total cholesterol, triglycerides, HDL, LDL and VLDL level in the blood were checked.

Induction of hyperlipidemia: A single dose (350 mg/kg body weight i.p) of Triton WR-1339 dissolved in 0.15 N NaCl solution was used for induction of hyperlipidemia in the rats. Hyperlipidemia was confirmed 48 hrs after triton injection by determining the blood cholesterol concentration [20]

Collection of Blood and Experimental Setup:

Evaluation of Anti-hyperlipidemic activity of Combined butanol fractions of Bauhinia variegata (Linn.) and Salvadora oleoides (decne.): The combination of active butanol fractions from *Bauhunia variegata* and *Salvadora oleoides* leaves in a proportion of 1:1 was administered at a dose of 100mg/kg, p.o. in 1% v/v of tween 80 (1ml/kg) for a period of 07 days. The rats were anaesthetized with diethyl ether and blood samples were drawn from the retro orbital plexus of eye. The rats were divided into 04 groups having 06 animals in each group as follows:

1. Normal Group I – normal diet only
2. Control Group II:
3. Group III: (Combined Butanol Fraction): received combined dose of *Bauhinia variegata* and *Salvadora oleoides* (Butanol fraction) at a dose of 100 mg/kg b.w.
4. Group IV (Standard Drug): received fenofibrate at a dose of 65 mg/kg b.w.

Blood cholesterol, triglycerides, LDL, HDL and VLDL profile were estimated before starting the treatment and end of the treatment period i.e.7 days. Fenofibrate was used as standard and was administered orally (once daily for 7 days) at a dose of 65 mg/Kg body wt. Blood cholesterol, triglycerides, LDL, HDL and VLDL profile were estimated before starting the treatment and end of the treatment period i.e.7 days. [21]

Estimation of blood cholesterol and lipid profile: Total cholesterol estimation was done by using the seimen cholesterol diagnostic kit. Serum triglycerides were estimated by seimen triglycerides diagnostic kit. HDL cholesterol was estimated by seimen HDL diagnostic kit. Cholesterol, triglycerides and HDL profile were estimated using standard monograph.
LDL cholesterol was calculated as [22]
LDL = Total Cholesterol - HDL - Triglycerides/5
VLDL was calculated using the formula [22]
VLDL = Triglycerides/5

Statistical analysis: All results are expressed as the mean_±_SEM. The results were analysed for statistical significance by Dunnett test of one-way ANOVA test.

RESULT AND DISCUSSION

Earlier we have reported the antihyperlipidemic activity of Methanol extract [23], [24] as well as their fraction i.e., Petroleum ether, Chloroform, Ethyl acetate and Butanol fractions [17], [18]**.** Butanol fraction from *Bauhinia variegata* and *Salvadora oleoides* were significantly reduce fasting serum cholesterol, Triglycerides, HDL, LDL and VLDL level in Triton WR1339 induced hyperlipidemic rats. So evaluation of synergistic action of active butanol fraction of above mentioned plant materials were done on fasting blood Cholesterol level and other biochemical parameters of Triton WR 1339 induced hyperlipidemic rats. The most significant results were obtained when butanol fractions were combined in a ration of 1:1 and 100mg/kg dose was administered orally. The combination of active butanol fraction of *Bauhinia variegata* and *Salvadora oleoides* (BV+SO) in a ratio of 1:1 was screened for antihyperlipidemic activity in Triton WR 1339 induced hyperlipidemic rats. The Cholesterol, triglycerides, HDl, LDL and VLDL level was monitored before induction of Triton and after induction of Triton then measured 7^{th} day of treatment to evaluate the antihyperlipidemic potential of the combined fractions. At the end of the treatment i.e., on 7^{th} day the blood was withdrawn from the retro-orbital plexus of the animals and serum was separated which was evaluated for effect on lipid profile as Cholesterol, Triglycerides, HDL, LDL and VLDL level shown in table 1 and figure 1-5.

The role of lipids in diabetes is very crucial as any abnormality in lipid profile is associated with atherosclerosis and other macro and micro cardiovascular disorders. An increased level of triglyceride and LDL levels are the indicator of coronary risk [25]. Thus it may be understood that cure of diabetes is not only to restore normoglycemic condition but also modulatory effect on lipid profile required. Hyperlipidemia was marked by Triton WR 1339 in hyperlipidemic rats by elevated triglycerides, cholesterol, low density

lipoprotein (LDL) and high density lipoprotein (HDL), constitutes an important cardiovascular risk factors [26].

There were an elevation in plasma cholesterol, triglycerides, HDL, LDL and VLDL-C levels in response to induction of hyperlipidemia by Triton WR- 1339 as compared to normal and control group. A significant increase was observed in cholesterol level from normal level 72.33 mg/dl to 256.80 mg/dl by Triton induced hyperlipidemic rats. On the treatment with combination of BV+SO reduced the elevated cholesterol level to 80.30 mg/dl, in comparison to standard drug (fenofibrate) 70.13 mg/dl.

Triglyceride level was increased from normal level 159.70 mg/dl to 275.40 mg/dl. combination of BV+SO reduced the elevated triglyceride level to 98.64 mg/dl, in comparison to standard drug (fenofibrate) 80.88 mg/dl.

Elevated HDL level is good for health. After induction of Triton, HDL level increased from normal level 21.17 mg/dl to 24.87 mg/dl. combination of BV+SO showed increase in HDL level to 31.26 mg/dl in comparison to standard drug (fenofibrate) 30.30 mg/dl.

LDL level was increased from normal level 19.23 mg/dl to 176.80 mg/dl by induction of Triton. combination of BV+SO reduced the elevated LDL level to 29.31 mg/dl, in comparison to standard drug (fenofibrate) 23.65 mg/dl. VLDL level was increased from normal level 31.94 mg/dl to 55.10 mg/dl by induction of Triton. combination of BV+SO reduced the elevated VLDL level to 19.73 mg/dl, in comparison to standard drug (fenofibrate) 16.18 mg/dl.

All the results were statistically significant ($p<0.05$) and compared with normal and control group.

Thus BV+SO showed significant reduction in plasma cholesterol (80.30 mg/dl), triglyceride (98.64 mg/dl), LDL (29.31 mg/dl), VLDL (19.73 mg/dl) and increase in HDL level (31.26 mg/dl) as we know that HDL is good for health.

CONCLUSION

In present study combined butanol fraction (100mg/kg) showed significant ($p<0.5$) reduction in triglyceride, LDL and cholesterol levels and increased HDL level hyperlipidemic rats. Similar reduction in triglyceride, LDL and cholesterol levels and increased HDL level was observed with standard drug fenofibrate. However, the lowering effect of combined extracts on LDL and triglyceride level was superior in comparison to standard drug Fenofibrate. Thus it could be stated that combined extracts may play an important role in coronary heart disease associated with diabetes.

CONFLICT OF INTERESTS

The authors do not have any conflict of interests regarding the content of this research paper.

ACKNOWLEDGEMENT

Authors are thankful to Director and Management of SBSPGI, Balawala, Dehradun, India for providing necessary facilities and one of the authors is thankful to National Medicinal Plant Board, Department of AYUSH, Ministry of Health & Family Welfare, Government of India, for financial aid.

Please paste the tables and figures here

Effect of combined butanol extract on serum Lipid profile:

Table 1 effect of Combined Butanol Fraction of BV and SO (BV+SO) in 1:1 ratio on Cholesterol, Triglycerides, HDL LDL and VLDL level in plasma of control and experimental rats.

Groups	Cholesterol	Triglycerides	HDL	LDL	VLDL
Normal	**72.33±0.95**	**159.70±5.77**	**21.17±0.28**	**19.23±0.78**	**31.94±1.15**
Control+ Triton	**256.80±12.15**	**275.40±3.60**	**24.87±0.78**	**176.80±12.93**	**55.10±0.72**
BV + SO + Triton	**80.30±4.32**	**98.64±3.19**	**31.26±1.42**	**29.31±6.00**	**19.73±0.64**
Fenofibrate+ Triton	**70.13±1.40**	**80.88±3.98**	**30.30±1.61**	**23.65±3.15**	**16.18±0.80**

Value are in mean±SEM, No. of animals in each group N=6, *Statistically significant different from normal group ($p^*<0.05$), **Statistically significant different from Group II ($p^{**}<0.05$).

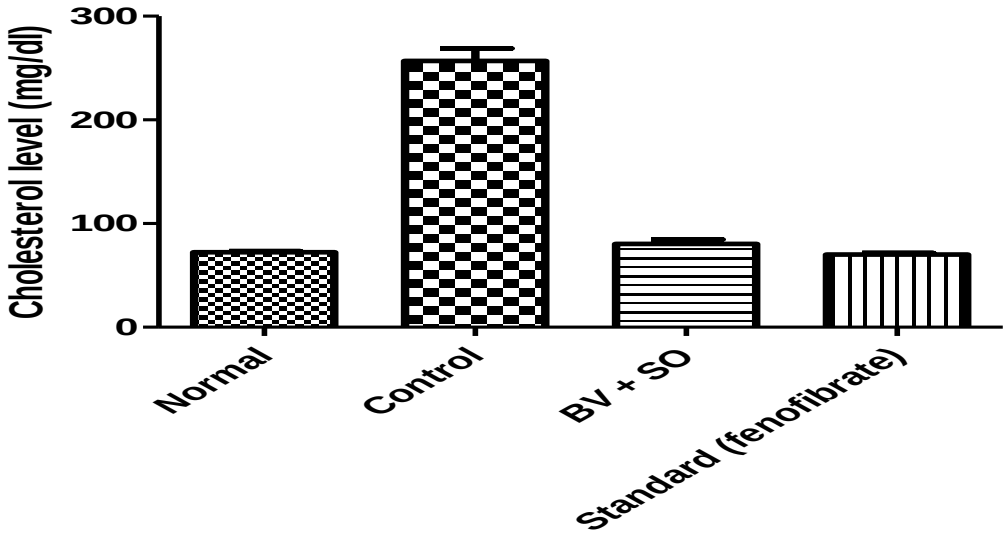

Figure 1 effect of Combination of Butanol fraction of BV and SO on plasma Cholesterol level on Triton induced hyperlipidemic rats.

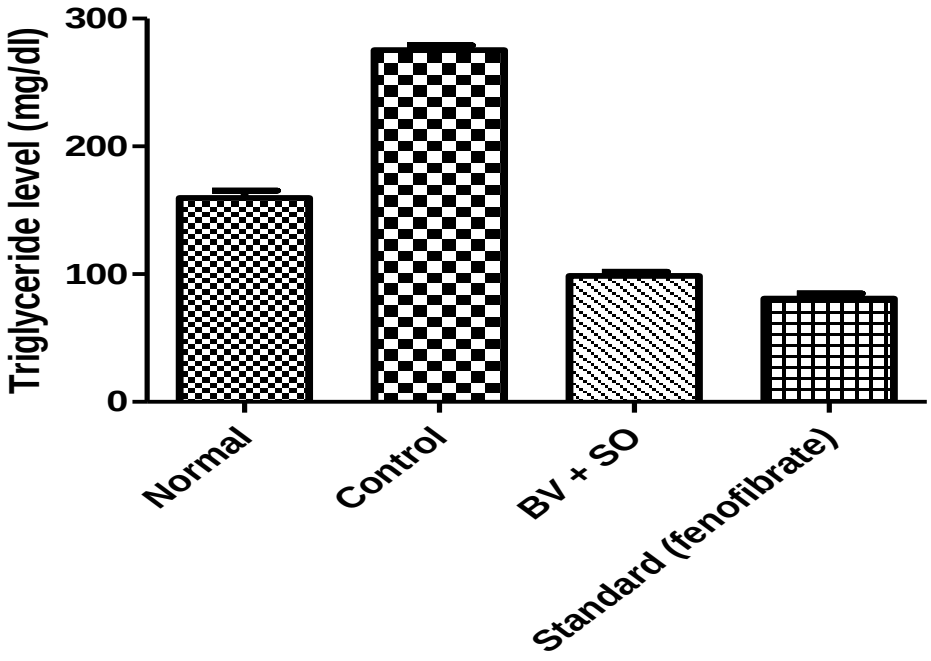

Figure 2 effect of Combination of Butanol fraction of BV and SO on plasma Triglycerides level on Triton induced hyperlipidemic rats.

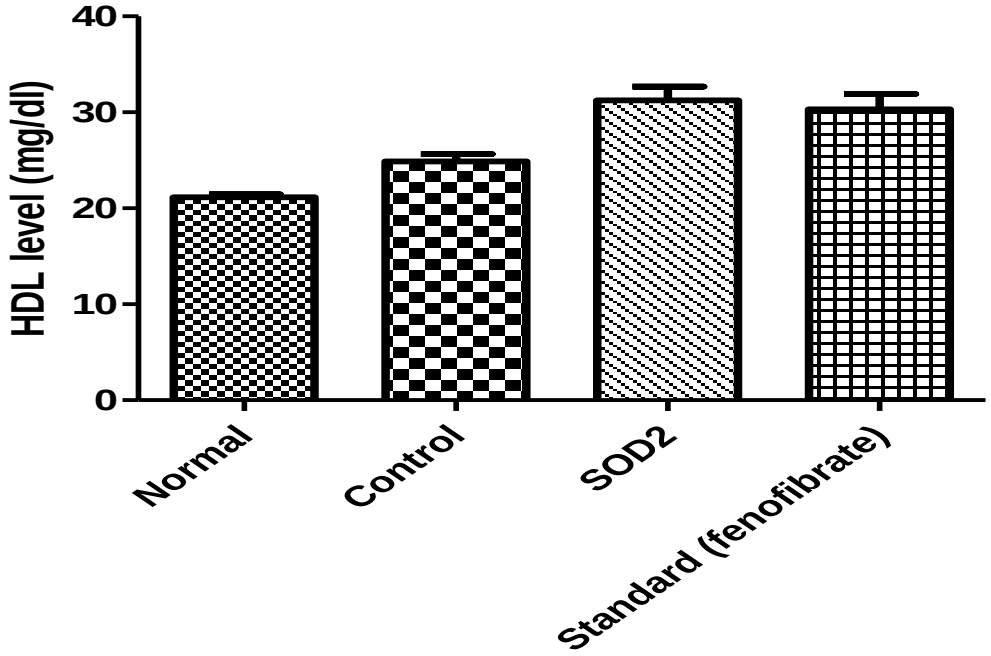

Figure 3 effect of Combination of Butanol fraction of BV and SO on plasma HDL level on Triton induced hyperlipidemic rats.

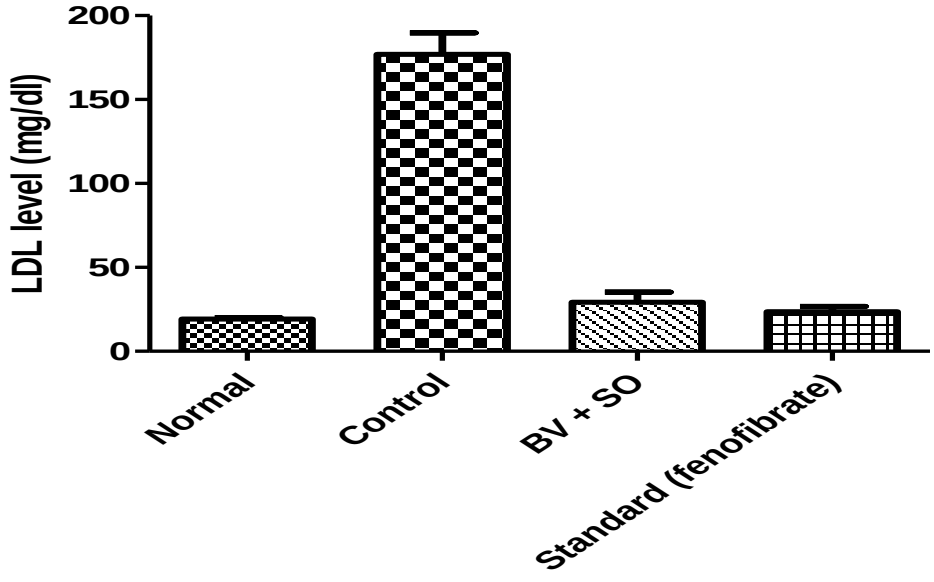

Figure 4 effect of Combination of Butanol fraction of BV and SO on plasma LDL level on Triton induced hyperlipidemic rats.

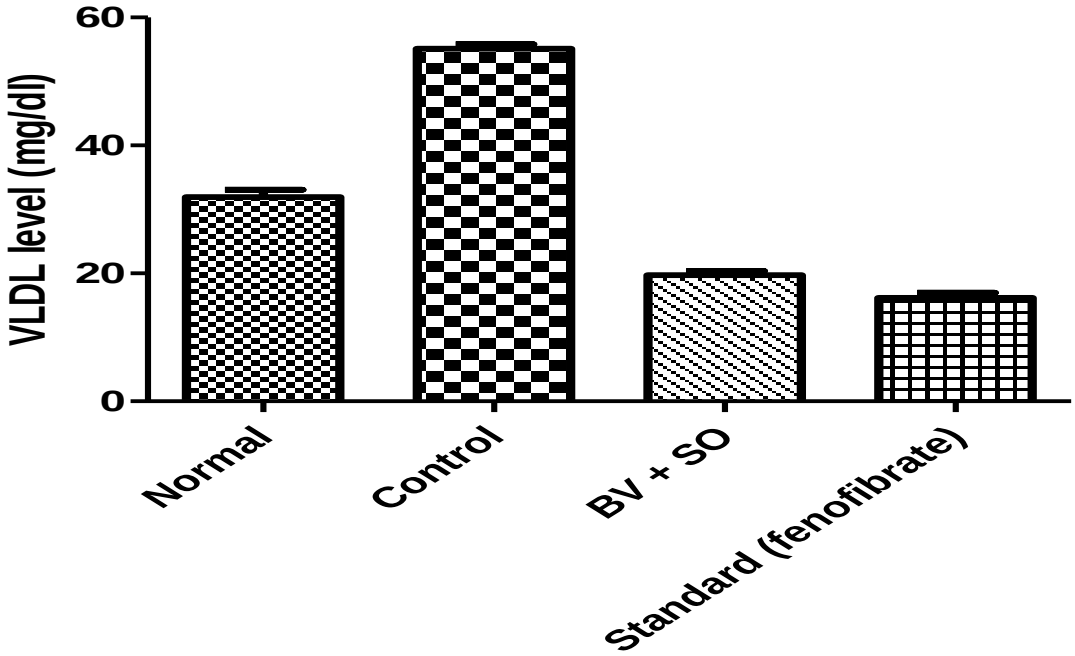

Figure 5 effect of Combination of Butanol fraction of BV and SO on plasma VLDL level on Triton induced hyperlipidemic rats.

REFERENCE

1. Mali RG et al. Plant Review Rakta Kanchan (Bauhinia variegata): Chemistry, Traditional and Medicinal uses- a review, Pharmacognosy Reviews 2007; 1, 2: 314-319.
2. Kaushik P, Dhiman AK. Medicinal plants and raw material of India 2000; 226-227.
3. Annonymous. Wealth of India, National Institute of Science and Communication, CSIR, New Delhi 1988; 2: 56-57
4. Sharma RN and Saxena VK. Invitro antimicrobial efficacy of leaves extratcs of Bauhinia variegata, Asian Journal of Chemistry 1996; 8, 4: 211-212.
5. Yadava RN, Reddy VMS. Anti-Inflammatory Activity of a Novel Flavonol Glycoside from the Bauhinia Variegata Linn, Natural Product Research 2003; 17, 3: 165–169.
6. Rajkapoor B et al. Antitumour activity of *Bauhinia variegata* on Dalton's ascitic lymphoma, Journal of Ethnopharmacology 2003; 89: 107–109.
7. Pani SR et al. Nephroprotective effect of Bauhinia variegata (Linn.) whole stem extract against cisplatin-induced nephropathy in rats, Indian Journal of Pharmacology 2011; 43, 2: 200-202.
8. Bodakhe SH and Ram A. Hepatoprotective Properties of Bauhinia variegata bark extract, Yakugaku Zasshi, The Pharmaceutical Society of Japan 2007; 127, 9: 1503-1507.
9. Agrawal RC, Pandey S. Evaluation of Anticarcinogenic and Antimutagenic Potential of *Bauhinia variegata* extract in Swiss Albino Mice, Asian Pacific Journal Cancer Prevention 2009; 10: 913-916.
10. Rajani GP, Ashok P. In vitro antioxidant and antihyperlipidemic activities of Bauhinia variegata Linn, Indian J Pharmacol 2009; 41, 5: 227-232.
11. Koti BC et al. Effect of Bauhinia variegata bark extract on blood glucose level on normal and alloxanised induced diabetic rats, Journal of Natural Remedies 2009; 9,1: 27-34.
12. Khot PR et al. Antidibetic and Antioxidant Effect of ethanolic extract of *Bauhinia varigata* leaves in streptozotocin induced diabetic rats, Inventi Rapid: Ethnopharmacology 2012; 1,: 1.
13. Annonymous. The Wealth of India, National Institute of Science and Communication and Information Resources (CSIR), Dr. Krishna marg, K. S., Pusa, New Delhi, India 1999; IX: 193-194
14. Chopra RN et al. Glossary of Indian medicinal plants, National Institute of Science and Communication, CSIR, New Delhi 2002; 219.
15. Yadav JP et al. Hypoglycemic and hypolipidemic activity of ethanolic extract of Salvadora oleoides in normal and alloxan-induced diabetic rats, Indian Journal of Pharmacology 2008; 40, 1: 23-27.
16. Kumar S et al. Antimicrobial activity of *Salvadora oleoides* Decne. against some microorganisms, Journal of Medicinal Plants Research 2012; 6, 14: 2754-2760.
17. Kumar D et al. Effect and evaluation of antihyperlipidemic activity of fractions of total methanol extract of *Bauhinia variegata (Linn.)* leaves on Triton WR-1339 (Tyloxapol) induced hyperlipidemic rats, International Journal of Research in Pharmaceutical Sciences 2011; 2, 4: 493-497.
18. Kumar D et al. Effect and evaluation of antihyperlipidemic activity of fractions of total methanol extract of *Salvadora oleoides (decne.)* leaves on Triton WR-1339 (Tyloxapol) induced hyperlipidemic rats, International Journal of Research in Pharmaceutical Sciences 2011; 2, 3: 507-510.
19. Agarwal SS, and Paridhavi M. Herbal Drug Technology, Universities press (INDIA) Private Ltd., First edition, reprint 2009; 323-324.

20. Schurr PE et al. Triton-induced hyperlipidemia in rats as an animal model for screening hypolipidemic drugs, lipids 1972; 7, 1: 68-74.
21. Saravana KA et al. Antihyperlipidemic activity of *Camellia sinensis* leaves in Triton WR-1339 induced albino rats, Pharmacognosy Magazine 2008; 4, 13: 60-64.
22. William TF et al. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clinical Chemistry 1972; 18, 6: 499-502.
23. Kumar D et al. Evaluation of antihyperlipidemic activity of methanol extract of *Bauhinia variegata (Linn.)* leaves in Triton WR-1339 (Tyloxapol) induced hyperlipidemic rats, Journal of Pharmacy Research 2011; 4, 1: 58-60.
24. Kumar D et al. Evaluation of antihyperlipidemic activity of methanol extract of *Salvadora oleoides (Linn.)* leaves in Triton WR-1339 (Tyloxapol) induced hyperlipidemic rats, Journal of Pharmacy Research 2011; 4, 2: 512-513.
25. Patel DK et al. Assessment of Lipid Lowering Effect Of *Sida rhomboidea* Roxb methanolic extract in experimentally induced hyperlipidemia , J Young Pharma. 2009; 1, 3: 233- 238.
26. Okoli CO et al. Evaluation of antidiabetic potentials of *Phyllanthus niruri* in alloxan diabetic rats. Afr J Biotechnol 2010; 9: 248-259.