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Research Article

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

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ABSTRACT

Hyperlipidemia is the greatest risk factor of coronary heart disease. Currently available hypolipidemic drugs have been associated with number of side effects. Herbal treatment for hyperlipidemia has no side effects and is relatively cheap and locally available. The present study focus on the anti-hyperlipidemic activity of fractions of total methanol extract of leaves of *Bauhinia variegata (Linn.)* against Triton WR-1339 induced hyperlipidemia in rats. Fractions administered a dose of 100mg/kg (oral) to the Triton WR-1339 induced hyperlipidemic rats. Butanol fraction showed significant reduction ($p<0.05$) in serum cholesterol (78.86 mg/dl), triglyceride (113.52 mg/dl), LDL (28.01 mg/dl), VLDL (20.70 mg/dl) and increase in HDL level (30.15 mg/dl) in comparison with standard drug fenofibrate ($p<0.05$).

Keywords: *Bauhinia variegata (Linn.);* Triton WR-1339; Anti-hyperlipidemic; Tyloxapol; Atherosclerosis; fenofibrate.

INTRODUCTION

Hypercholesterolemia and hypertriglyceridemia, as predisposing factors to atherosclerosis, have received the most recent attention (Joseph L. G. et al, July 1973). Atherosclerosis, referred to as a "silent killer", is one of the leading causes of death in the developing countries like India. Allopathic hypolipidemic drugs are available at large in the market but the side-effects and contraindications of these drugs have marred their popularity. Recently herbal hypolipidemics have gained importance to fill the lacunae created by the allopathic drugs (Dipa A. et. al. 2010).

Bauhinia variegata (Linn.) (leguminoceae) is a medium sized tree abundant in Sub-Himalayan tract extending eastwards to Assam, Eastern, Central and South India (The Ayurvedic Pharmacopoeia, 2001). The various parts of the plants viz., leaves, flower buds, flower, stem, stem bark, seeds and roots are used in fever, as tonic, astringent, diarrhoea, dysentery, hemorrhoids, piles, edema, laxative, anthelmintic, antileprotic, in skin diseases, wound healing, antigoitrogenic, antitumor, in obesity, stomatitis, antidote for snake poisoning, dyspepsia, flatulence and as carminative (Gupta R., et. al. 2009).

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EXPERIMENTAL

Drug and chemicals

Triton WR-1339 was purchased from Fisher Scientific, Belgium. Total cholesterol, Triglyceride, HDL estimations were done using the Seimen diagnostic kit. All solvents were purchased from Rankem ltd.

Plant material

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.

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 of 112 g (Agarwal S. S and Paridhavi M., 2009). From total methanol extract, preparation of different fraction by cold

Table 1: Effect of different fractions of *Bauhinia variegata* (Linn.) on cholesterol, triglycerides, HDL level in plasma of control and experimental rats

Groups	Cholesterol (mg/ml)	Triglycerides (mg/ml)	HDL (mg/ml)
Group I: Normal	69.21±1.5	157.60±5.75	17.32±0.42
Group II: Control + Triton	186.63±1.62*	189.20±2.60*	21.00±0.6*
Group III: Pet. Ether fraction + Triton	175.23±18.20^{ns}	183.70±30.38^{ns}	20.01±1.13^{ns}
Group IV: Chloroform fraction + Triton	164.04±1.99^{ns}	154.54±078^{ns}	20.21±0.55^{ns}
Group V: Ethyl acetate fraction + Triton	135.05±2.18**	185.46±25.17^{ns}	21.48±0.49^{ns}
Group VI: Butanol fraction + Triton	78.86±2.46**	113.52±8.81**	30.15±1.87**
Group VII: Standard (fenofibrate) + Triton	65.24±1.68**	77.96±3.9**	25.87±1.6**

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

Table 2: Effect of different fractions of *Bauhinia variegata* (Linn.) on LDL and VLDL level in plasma of control and experimental rats

Groups	LDL (mg/ml)	VLDL (mg/ml)
Group I: Normal	21.64±0.67	14.23±0.41
Group II: Control + Triton	127.69±12.43*	37.97±2.11*
Group III: Pet. Ether fraction + Triton	118.57±13.55^{ns}	37.39±6.1^{ns}
Group IV: Chloroform fraction + Triton	111.75±2.5^{ns}	31.09±0.3^{ns}
Group V: Ethyl acetate fraction + Triton	77.17±4.4**	36.99±5.04^{ns}
Group VI: Butanol fraction + Triton	28.01±1.43**	20.70±1.13**
Group VII: Standard (fenofibrate) + Triton	23.77±0.63**	15.59±3.8**

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

percolation method using increasing polarity of solvents by separation technique i.e Petroleum ether (Pet. ether), Chloroform, Ethyl acetate and Butanol.

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) approved the study.

Antihyperlipidemic study

Antihyperipidemic 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 used for induction of hyperlipidemia in the rats (Schurr P. E., et. al. 1972). The test and standard drugs were administered continuously for 7 days orally using infant feeding tube (Saravana K. A. et. al. 2008).

Collection of blood and experimental setup

The rats were anaesthetized and blood samples were taken. The rats were divided into 7 groups having 6 animals in each group as follows:

Normal Group I: normal diet only

Control Group II: received 1% tween 80 at a dose of 1 ml/kg b.w.

Group III: received Petroleum ether fraction in 1% tween 80 at a dose of 100 mg/kg b.w.

Group IV: received Chloroform fraction in 1% tween 80 at a dose of 100 mg/kg b.w.

Group V: received Ethyl acetate fraction in 1% tween 80 at a dose of 100 mg/kg b.w.

Group VI: received Butanol fraction in 1% tween 80 at a dose of 100 mg/kg b.w.

Group VII: received fenofibrate in 1% tween 80 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.

Estimation of blood cholesterol and lipid profile

Cholesterol, triglycerides and HDL profile were estimated using standard monograph.

LDL cholesterol was calculated as (William T. F., et. al. 1972)

LDL = Total Cholesterol - HDL - Triglycrides/5

VLDL was calculated using the formula (William T. F., et. al. 1972):

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

Results have shown in table 1 and 2, and figure 1, 2, 3, 4 and 5. Hyperlipidemia a well known risk factor for cardiovascular disease, especially atherosclerotic coronary artery disease (CAD) is one of the major causes of premature death globally and it is expected to be the most important cause of mortality in India by the year 2010 (Dhulasavant V., et. al. 2010). Earlier we have reported the total methanol extract shows significant reduction in cholesterol and triglyceride level (Kumar D., et. al. 2011)**.** Therefore in search of active principle fractionation of methanol extract was carried out using different solvents like petroleum ether, chloroform, ethyl acetate and butanol. There was elevation in plasma cholesterol, triglycerides, HDL, LDL and VLDL level in response to induction of Triton WR- 1339 as compare to normal and control group. All the results were statistically significant ($p<0.05$) and compared with normal and control group. It was observed that there is significant increase in cholesterol level from normal level 69.21 mg/dl to 186.63 mg/dl by Triton induced hyperlipidemic rats. On the treatment with all the fractions of methanol extract, petroleum ether, chloroform, ethyl acetate and butanol fractions reduced the elevated cholesterol level to 175.23 mg/dl, 164.04 mg/dl, 135.05 mg/dl and 78.86 mg/dl respectively in comparison to standard drug (fenofibrate) 65.24 mg/dl.

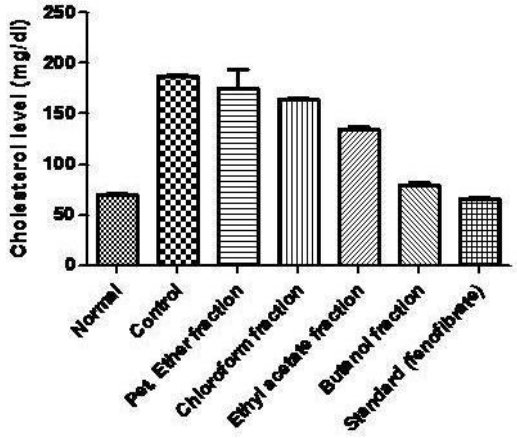

Figure 1: Showing effect of fractions on plasma cholesterol level in Triton induced hyperlipidemic rats

Triglyceride level was increased from normal level 157.60 mg/dl to 189.20 mg/dl. Petroleum ether, chloroform, ethyl acetate and butanol fractions reduced the elevated triglyceride level to 183.70 mg/dl, 154.54 mg/dl, 185.46 mg/dl and 113.52 mg/dl respectively in comparison to standard drug (fenofibrate) 77.96 mg/dl.

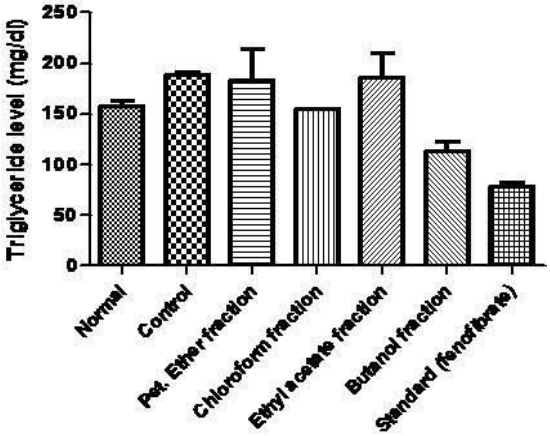

Figure 2: Showing effect of fractions on plasma triglyceride level in Triton induced hyperlipidemic rats

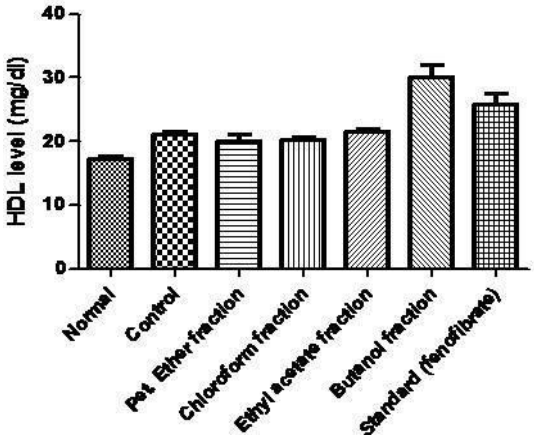

Figure 3: Showing effect of fractions on plasma HDL level in Triton induced hyperlipidemic rats

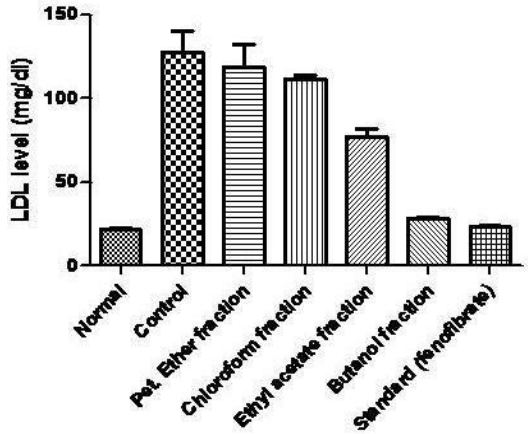

Figure 4: Showing effect of fractions on plasma LDL level in Triton induced hyperlipidemic rats

Elevated HDL level is good for health. After induction of Triton HDL level increased from normal level 17.32 mg/dl to 21.00 mg/dl. Petroleum ether, chloroform,

ethyl acetate fractions reduced the elevated HDL level reduced to 20.01 mg/dl, 20.21 mg/dl, 21.48 mg/dl respectively but butanol fraction shows increase in HDL level to 30.15 mg/dl in comparison to standard drug (fenofibrate) 25.87 mg/dl.

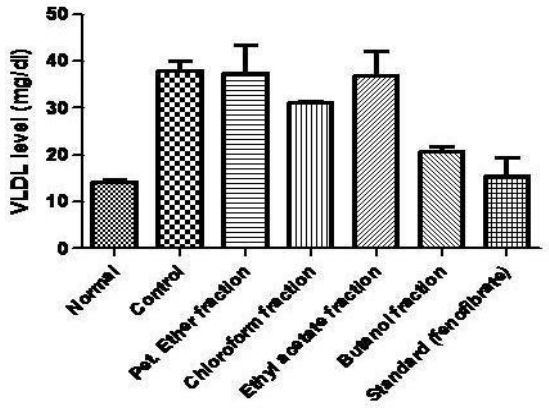

Figure 5: Showing effect of fractions on plasma VLDL level on Triton induced hyperlipidemic rats

LDL level was increased from normal level 21.64 mg/dl to 127.69 mg/dl by induction of Triton. Petroleum ether, chloroform, ethyl acetate and butanol fractions reduced the elevated LDL level to 118.57 mg/dl, 111.75 mg/dl, 77.17 mg/dl and 28.01 mg/dl respectively in comparison to standard drug (fenofibrate) 23.77 mg/dl. .

VLDL level was increased from normal level 14.23 mg/dl to 37.97 mg/dl by induction of Triton. Petroleum ether, chloroform, ethyl acetate and butanol fractions reduced the elevated VLDL level to 37.39 mg/dl, 31.09 mg/dl, 36.99 mg/dl and 20.70 mg/dl respectively in comparison to standard drug (fenofibrate) 15.59 mg/dl.

The reported antihyperlipidemic activity of total methanol extract of *Bauhinia variegata (Linn.)* leaves shows the reduction in elevated cholesterol and triglyceride level (Kumar D., et. al. 2011).

Thus among all fractions butanol fraction showed significant reduction in plasma cholesterol (78.86 mg/dl), triglyceride (113.52 mg/dl), LDL (28.01 mg/dl), VLDL (20.70 mg/dl) and increase in HDL level (30.15 mg/dl) as we know that HDL is good for health.

Ample of evidence exists with respect to the fact that HDL cholesterol is inversely related to total body cholesterol and a reduction of plasma HDL cholesterol concentration may accelerate the development of atherosclerosis leading to ischaemic heart diseases, by impairing the clearing of cholesterol from the arterial wall (Dhulasavant V., et. al. 2010). Flavonoids are reported to increase HDL concentration and decrease in LDL and VLDL levels in hypercholesteremic rats (Patel D. K., et. al. 2009).

The protective effect of the leaves of *Bauhinia variegata (Linn.)* on Triton WR-1339 induced hyperlipidemia may be attributed to a decrease in cholesterol synthesis, an increase in cholesterol excretion and expression of LDL receptor and subsequent catabolism and also increase in HDL cholesterol.

CONCLUSION

From the above study it could be concluded that butanol fraction of *Bauhinia variegata (Linn.)* not only have resulted in significant reduction in cholesterol, triglyceride, LDL, VLDL level but also increases the HDL level. Studies on the isolated fractions and constituents are needed to elucidate mechanisms by which *Bauhinia variegata (Linn.)* exert protective effects on hyperlipidemia.

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REFERENCES

Agarwal S. S and Paridhavi M., (2009), Herbal Drug Technology, Universities press (INDIA) private Ltd., First edition, reprint, 323-324.

Dhulasavant V., Shinde S., Pawar M., Naikwade N. S., (2010) Antihyperlipidemic activity of *Cinnamomum tamala Nees*. on high cholesterol diet induced hyperlipidemia, International Journal of Pharm. Tech Research, 2, 4, 2517-2521.

Dipa A. I., Kirti V. P., Tejal R. G., (2010), Antihyperlipidemic activity of aqueous extract of *Terminalia chebula* & Gaumutra in high cholesterol diet fed rats, Pharma Science Monitor, 1, 1, 48-59.

Gupta R., Paarakh M. P. and Gavani U., (2009), Isolation of Phytoconstituents from the leaves of *Bauhinia variegata Linn.*, Journal of Pharmacy Research, 2, 8,1315-1316.

Joseph L. G. et al, (1973), Hyperlipidemia in coronary heart disease, The Journal of Clinical Investigation, 52, 1533-1543.

Kumar D., Parcha V., Dhulia I. and Maithani A., (2011), Evaluation of anti-hyperlipidemic activity of methanol extract of *Bauhinia variegata (Linn.)* leaves in triton WR-1339 (Tyloxapol) induced hyperlipidemic rats, Journal of Pharmacy Research, 4, 1, 58-60.

Patel D. K., Patel K. A., Patel U. K., Thounaoja M. C., Jadeja R. N., Ansarullah, Padate G. S., Salunke S. P., Devkar R. V., Ramachandra A.V., (2009), Assessment of lipid lowering effect of *Sida rhomboidea Roxb.* methanolic extract in experimentally induced hyperlipidemia, J. Young Pharma.; 1, 3, 233- 238.

Saravana K. A., Mazumder A., Saravanan V. S., (2008), Antihyperlipidemic activity of *Camellia sinensis* leaves

in Triton WR-1339 induced albino rats, Phcog. Mag., 4, 13, 60-64.

Schurr P. E., Schultz J. R. and Parkinson T. M., (1972), Triton-induced hyperlipidemia in rats as an animal model for screening hypolipidemic drugs, lipids, 7, 1, 68-74.

William T. F., Robert I. L, and Donald S. F, (1972), Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clinical Chemistry, 18, 6, 499-502.