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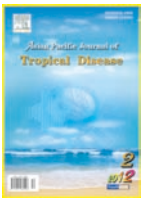

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Effect and evaluation of antihyperlipidemic activity guided isolated fraction from total methanol extract of *Bauhinia variegata* (linn.) in Triton WR-1339 induced hyperlipidemic rats

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ABSTRACT

Objective: To investigate the effect and evaluation of Anti-hyperlipidemic activity guided sub-fraction isolated from total methanolic extract of *Bauhinia variegata* (Linn.) leaves on Triton WR-1339 induced hyperlipidemic rats. **Methods:** Column chromatographic fractionation of butanol fraction of total methanol extract of leaves of *Bauhinia variegata* (Linn.) yields four sub-fractions (sub-fraction A-D). All sub-fractions tested for their anti-hyperlipidemic activity. Sub-fractions administered at a dose of 65 mg/kg (oral) to the Triton WR-1339 induced hyperlipidemic rats and total cholesterol, triglycerides, HDL, LDL and VLDL level in the blood were checked. **Results:** Sub-fraction D showed significant reduction ($P<0.05$) among four sub-fraction in comparison with standard drug fenofibrate. **Conclusions:** From the above study it could be concluded that butanol sub-fraction D of *Bauhinia variegata* (Linn.) not only have resulted in significant reduction in cholesterol, triglyceride, LDL, VLDL level but also increases the HDL level at a reduced dose level.

1. Introduction

Coronary heart diseases (CHD) are the main cause of death in western countries and Asia. Among CHDs, ischemic heart disease (IHD) leads to the highest mortality rate. The number of heart patients suffering from IHD worldwide is gradually increasing. Several epidemiological studies have demonstrated the relationship between plasma cholesterol levels and the development of IHD. Hypercholesterolemia is generally, associated with an increase in plasma concentration of LDL and VLDL. Lowering of elevated levels of LDL cholesterol can slower the progression of atherosclerotic lesions. About 70% of total cholesterol in human is synthesized de novo and the remaining is also supplied by absorption from diet (0.3-0.5 g/day in human). Several methods are presently practiced to control blood cholesterol levels. These include balance of dietary fats; bile acids sequester and use of HMG-CoA reductase inhibitors (statins). HMG-CoA reductase is the key enzyme in the cholesterol biosynthesis pathway. Inhibition of this enzyme has proven to be the most efficient therapy for managing hypercholesterolemia [1]. Although a range of synthetic drugs are available as anti-hyperlipidemic drugs, many of them do not fulfill all requirements and their numerous side effects and potential interference with drug metabolism are common. The search for compounds from nutraceutical sources for reduction of serum cholesterol and reduction of hypercholesterolemic atherosclerosis is on. Thus a survey among medicinal herbs is also still important and might provide a useful source for therapy or alternatively as simple dietry adjuncts to existing therapies [2].

Hyperlipidemia is implicated as the cause for coronary heart diseases. Though varieties of synthetic drugs are used in the treatment, still the searches are on for better medicaments especially from the plant kingdom. Many medicinal plants have been studied in this context. But most of them are seasonal or have restricted availability. One such weed, available throughout the year is *Bauhinia*

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variegata Linn. (Caesalpiniaceae) [3].

Bauhinia Linn. (Caesalpiniaceae) is a genus of shrubs or tree, very rarely climbers, distributed throughout the tropical regions of the world. About 15 species of this genus occur in India. *Bauhinia*s are chiefly propagated from seeds; vegetative propagation except inarching has not shown much success. Many useful products such as tannins, fibre, gum and oil are obtained from *Bauhinia* spp. Many species are grown as ornamental plant. *Bauhinias* are also cultivated for afforestation and the manufacture of woodwool board. *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" [4].

2. Experimental

2. 1. Drug and chemicals

Triton WR-1339 was purchased from Fisher Scientific, Belgium. Total cholesterol, Triglyceride, HDL estimations were done using the Seimen diagnostic kit. Silica gel 100-200 mesh size and solvents were purchased from Rankem ltd.

2.2. 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. 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 [5].

2.3. Fractionation by Column Chromatography

From total methanol extract, preparation of different fraction by cold percolation method using increasing polarity of solvents by separation technique i.e Petroleum ether (Pet. ether), Chloroform, Ethyl acetate and Butanol. The silica gel 100-200 mesh size was used for fractionation by column chromatography.

Sufficient quantity of a column grade silica gel (100 - 200 mesh size) was wet-packed using chloroform solvent system. The butanol fraction was first dissolved in methanol, and then mixed with about sufficient amount of the silica gel to become slurry. The slurry was loaded onto the wet packed column and continuously eluted with the mobile phase.

From the column we could get fraction A, B, C and D, monitored by TLC (thin layer chromatography), solvent system using chloroform and methanol as a mobile phase. The column was eluted with chloroform, 5 % methanol in chloroform, 10 % methanol in chloroform and 15 % methanol in chloroform respectively.

2.4. 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 Department of Pharmaceutical Sciences approved the study.

2.5. Antihyperlipidemic study

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

2.6. 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 [6].

The quantities of individual drug (fraction) to be administered were calculated at a dose of 65 mg/kg b.w (Body weight). The drug was administered continuously for 7 days orally using infant feeding tube. The results were compared with that of the standard drug fenofibrate which was also given continuously for 7 days at a dose of 65 mg/kg b.w [7].

2.7. Collection of blood and experimental setup

The rats were anaesthetized with diethyl ether and blood samples were drawn from the retro orbital plexus of eye. The rats were divided into 7 groups having 6 animals in each group as follows:

1.Normal Group I - normal diet only
2.Control Group II
3.Group III (fraction A): received fraction 'A' at a dose of 65 mg/kg b.w.
4.Group IV (fraction B): received fraction 'B' at a dose of 65 mg/kg b.w.
5.Group V (fraction C): received fraction 'C' at a dose of 65 mg/kg b.w.
6.Group VI (fraction D): received fraction 'D' at a dose of 65 mg/kg b.w.
7.Group VII (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.

2.8. 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 [8]

LDL = Total Cholesterol − HDL − Triglycerides/5

VLDL was calculated using the formula [8]

VLDL = Triglycerides/5

2.9. 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.

3. Result

Earlier we have preliminary reported that total methanol extract at a dose of 100 mg/kg body weight shows reduction in cholesterol and triglycerides level in blood plasma [9]. After fractionation of methanol extract, we reported that the butanol fraction shows reduction in cholesterol, triglyceride, HDL, LDL and VLDL level in blood plasma [10].

From this study we fractionate the butanol fraction by using column chromatography technique. Butanol fraction yielded four sub-fraction A-D. All four sub-fraction tested against Triton WR-1339 induced hyperlipidemic rats and results are given in table 1 and 2 and figure 1-5.

There was elevation in plasma cholesterol, Triglycerides, HDL, LDL and VLDL-C level in response to induction of hyperlipidemia by Triton WR- 1339 as compare to normal and control group. It was observed that there is significant increase in cholesterol level from normal level 69.21 mg/dl to 249.90 mg/dl by Triton induced hyperlipidemic rats. On the treatment with all the sub-fractions A, B, C, D reduced the elevated cholesterol level to 190.30 mg/dl, 159.70 mg/dl, 106.80 mg/dl and 69.64 mg/dl respectively in comparison to standard drug (fenofibrate) 67.09 mg/dl.

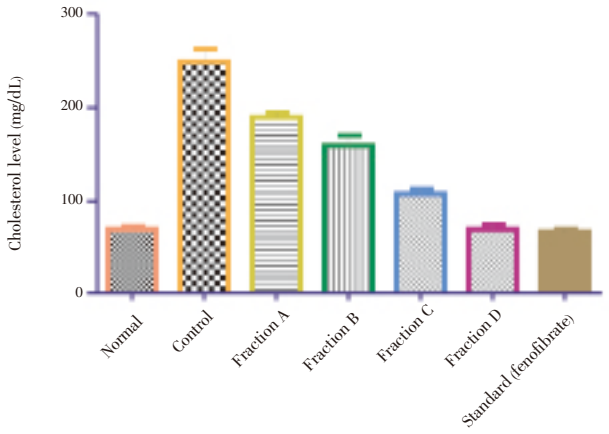

Figure 1: showing effect of Sub-fractions on plasma cholesterol level on Triton induced hyperlipidemic rats.

Table 1

Effect of different sub-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±1.49	157.60±5.75	17.32±0.42
Group II: Control + Triton	249.90±12.13*	280.60±3.57*	22.05±0.84*
Group III: Fraction A + Triton	190.30±4.80**	186.80±12.72**	19.80±1.22ns
Group IV: Fraction B + Triton	159.70±10.02**	166.21±1.89**	20.28±0.73ns
Group V: Fraction C + Triton	106.80±4.66**	140.80±8.28**	22.76±1.84ns
Group VI: Fraction D + Triton	69.64±4.34**	109.80±3.64**	30.88±1.41**
Group VII: Standard (fenofibrate) + Triton	67.09±1.42**	79.76±3.91**	28.75±1.63**

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 sub-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	20.37±1.28	31.60±1.20
Group II: Control + Triton	172.60±13.61*	56.12±0.71*
Group III: Fraction A + Triton	133.10±5.74**	37.36±2.54**
Group IV: Fraction B + Triton	99.29±11.44**	33.25±0.38**
Group V: Fraction C + Triton	55.83±6.34**	28.17±1.66**
Group VI: Fraction D + Triton	17.76±5.20**	21.91±0.73**
Group VII: Standard (fenofibrate) + Triton	22.39±3.22**	15.95±0.78**

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).

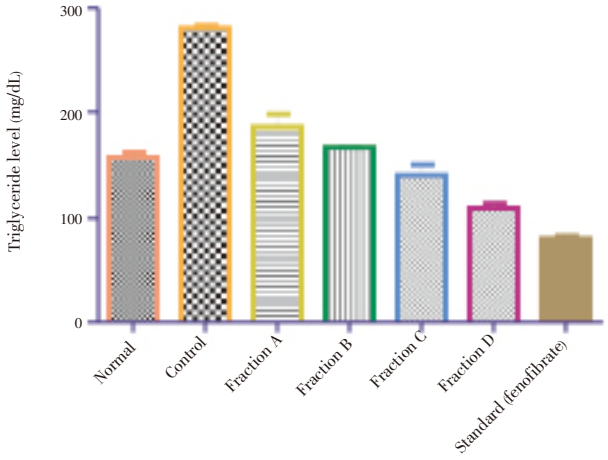

Figure 2: showing effect of Sub-fractions on plasma triglycerides level on Triton induced hyperlipidemic rats.

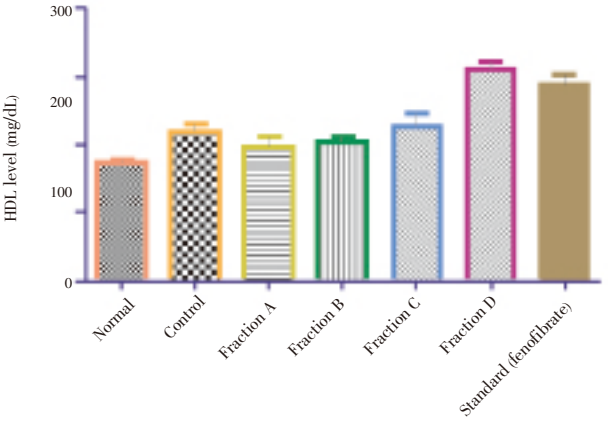

Figure 3: showing effect of Sub-fractions on plasma HDL level on Triton induced hyperlipidemic rats.

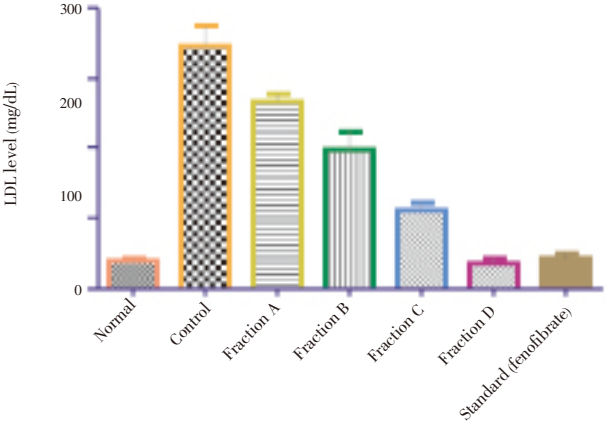

Figure 4: showing effect of Sub-fractions on plasma LDL level on Triton induced hyperlipidemic rats.

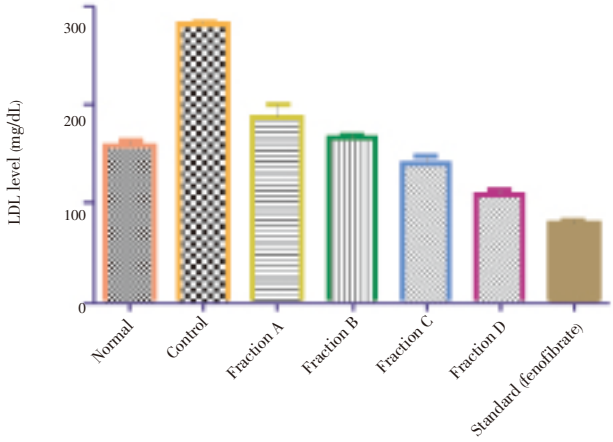

Figure 5: showing effect of Sub-fractions on plasma VLDL level on Triton induced hyperlipidemic rats.

Triglyceride level was increased from normal level 157.60 mg/dl to 280.60 mg/dl. Sub-fraction A, B, C & D reduced the elevated triglyceride level to 186.80 mg/dl, 166.21 mg/dl, 140.80 mg/dl and 109.80 mg/dl respectively in comparison to standard drug (fenofibrate) 79.76 mg/dl.

Elevated HDL level is good for health. After induction of Triton, HDL level increased from normal level 17.32 mg/dl to 22.05 mg/dl. Fraction A, B, C reduced the elevated HDL level reduced to 19.80 mg/dl, 20.28 mg/dl, 22.76 mg/dl respectively but butanol fraction shows increase in HDL level to 30.88 mg/dl in comparison to standard drug (fenofibrate) 28.75 mg/dl.

LDL level was increased from normal level 20.37 mg/dl to 172.60 mg/dl by induction of Triton. Sub-fraction A, B, C & D reduced the elevated LDL level to 133.10 mg/dl, 99.29 mg/dl, 55.83 mg/dl and 17.76 mg/dl respectively in comparison to standard drug (fenofibrate) 22.39 mg/dl.

VLDL level was increased from normal level 31.60 mg/dl to 56.12 mg/dl by induction of Triton. Sub-fraction A, B, C & D reduced the elevated VLDL level to 37.36 mg/dl, 33.25 mg/dl, 28.17 mg/dl and 21.96 mg/dl respectively in comparison to standard drug (fenofibrate) 15.95 mg/dl.

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

Thus among all fractions sub-fraction D showed significant reduction in plasma cholesterol (69.64 mg/dl), triglyceride (109.80 mg/dl), LDL (17.76 mg/dl), VLDL (21.96 mg/dl) and increase in HDL level (30.88 mg/dl) as we know that HDL is good for health.

5. Discussion

Triton WR-1339 acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extra hepatic tissues, resulting in increased blood lipid concentration [6]. The biphasic nature of triton - induced hyperlipidemia is helpful in understanding the mode of action of hypolipidemia agents. Drugs interfering

with lipid biosynthesis or uptake will be active in the synthesis phase and metabolism will be active in the excretory phase [11]. In present study the activity guided sub-fractions of total methanol extract shows a significant reduction against elevated blood lipid profile. The standard drug finofibrate are used for hypercholesterolemia. Finofibrate is fibric acid derivative acts on elevated cholesterol and triglyceride level by activating lipoprotein lipase and also modulation and catabolism of VLDL with clearance of LDL cholesterol. Finofibrate also increases the level of good cholesterol level that is HDL cholesterol. The effect of sub-fractions on elevated blood lipid level significantly reduces with increase in HDL level.

4. Conclusion

From the above study it could be concluded that butanol sub-fraction D of *Bauhinia variegata* (Linn.) not only have resulted in significant reduction in cholesterol, triglyceride, LDL, VLDL level but also increases the HDL level at a reduced dose level.

Further studies on the isolated fractions and constituents are needed to isolate compound responsible for activity and elucidate the mechanism by which *Bauhinia variegata* (Linn.) exert protective effects on hyperlipidemia.

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