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Research Article

AMELIORATIVE EFFECT OF *BAUHINIA VARIEGATA* ON HYPERCHOLESTEROLEMIA-INDUCED OXIDATIVE STRESS IN RATS

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ABSTRACT

Objective: The objective of this study was to assess the effect of the methanolic extract of *Bauhinia variegata* (L.) stem bark in albino rats.

Methods: The coarsely powdered stem bark of *B. variegata* (L.) was extracted with methanol (*BVMtE*) and was finally filtered. The experimental animals were divided into five groups for the duration of 60 days: control group (Group I); high cholesterol diet (HCD)-fed group (Group II); *BVME*- treated (100 mg/g b. wt./day) HCD group (Group III); *BVME*-treated (200 mg/g b. wt./day) HCD group (Group IV); and atorvastatin-treated (40 µg/g b. wt./day) HCD group (Group V). Animals were sacrificed and blood and tissues were removed for biochemical and histological analysis.

Results: Oral *BVMtE* administration to high cholesterol diet (HCD)-fed rats significantly normalized the serum total cholesterol, low-density lipoprotein and very low-density lipoprotein cholesterol, triglycerides (TGs), phospholipid, aspartate and alanine transaminase, and alkaline and acid phosphatase. Reduced glutathione, superoxide dismutase, as well as atherogenic index of *BVMtE*-treated HCD rats showed significant normalcy as compared to HCD-fed rats. The histoarchitecture of heart and aorta of *BVMtE*-treated HCD-fed rats depicted marked normalcy as compared to HCD rats. Phytochemical analysis showed that plant is rich in active constituents, i.e., flavonoids, steroids, terpenoids, β-sitosterol, kaempferol-3-glucoside, and tannins.

Conclusions: *B. variegata* has proved lipid lowering as well as antioxidant activity in high cholesterol diet-fed animals.

Keywords: Oxidative stress, Low-density lipoprotein, Very low-density lipoproteins, Cardiovascular diseases.

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INTRODUCTION

Many attempts have been made to explore the remedial option for hypercholesterolemic condition. *Bauhinia variegata* (Family – Fabaceae) is prescribed as antidiabetic [1,2], antibacterial [3], antioxidant [4], free radical scavenging [5,6], anti-inflammatory [7], anticarcinogenic [8], hepatoprotective [9], and antiulcer [10] agent. Although attempts were made to evaluate the hypolipidemic activity of *B. variegata* in rats [11,12], yet scientific validation is insufficient to prove its antihypercholesterolemic and antioxidant activity. Therefore, this study was undertaken to evaluate the antihypercholesterolemic and antioxidant potential of *B. variegata* in rats as well as screening of its active phytoconstituents.

METHODS

Preparation of extract and screening of phytoconstituents

B. variegata stem bark was freshly collected from the University of Rajasthan and authenticated by deposition of the specimen in Herbarium, Department of Botany, University of Rajasthan, Jaipur, India. Stem bark was shade dried and powdered for methanol extraction at 37°C ± 3°C. The filtrate was vacuum dried (yield 6.12% W/W) and treated with ethyl acetate for removal of fat portion. Lupeol was isolated with benzene and ethyl acetate elution in 3:1 ratio. The solvent was removed under reduced pressure and solid product so obtained was crystallized as colorless powder (m. p. 220°C).

Phytoconstituent screening of *B. variegata* revealed flavonoids, fixed oils, triterpenes saponins, tannins, glycosides, and polyphenols. Flavonoids such as apigenin, rutin, quercetin, apigenin 7-O-glucoside, quercitroside, rutoside, myricetin glycoside, β-isosterol, lupeol, kaempferol-3-glucoside, and fibers were isolated from *B.* variegata [13,14].

Animal model

Adult Wistar rats (*Rattus norvegicus*) of either sex weighing (150–250 g) were used in the experiment. They were housed in clean and hygienic polypropylene cages and were maintained under standard conditions: temperature (25°C ± 2°C), 12 h light and dark cycles, humidity (55%±4%), and water were provided *ad libitum*. The experiment protocols were consented by the Institutional Animal Ethics Committee and conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, registration no.1678/Go/Re/S/12/CPCSEA dated June 16, 2017.

Acute toxicity studies

The acute toxicity of *B. variegata* extract was evaluated using the method of Lorke [15]. Rats were administered different doses of *B. variegata* extract, ranging from 1000 to 3000 mg/kg b. wt. for 21 days and were examined for mortality. The LD_{50} value of BS was found to be 2200 mg/kg (data not shown). Two doses, i.e., 100 and 300 mg/kg b. wt./day were used in subsequent experiments.

The five experimental groups (n = 6 in each) used in the present study were as follows:

- Group I: Vehicle-treated control group kept on a normal diet
- Group II: Hypercholesterolemic (HCD) group
- Group III: *BVME*-treated (100 mg/g b. wt./day) HCD group
- Group IV: *BVME*-treated (200 mg/g b. wt./day) HCD group
- Group V: Atorvastatin-treated (40 µg/g b. wt./day) HCD group.

After animals were sacrificed, blood was collected by cardiac puncture and allowed to stand for 30 min. Later, centrifugation was

carried out at 2500 rpm for 20 min for serum separation for total cholesterol, triglycerides (TGs), HDL, LDL, VLDL, atherogenic index, and phospholipids (PL) estimation.

The superoxide dismutase (SOD), reduced glutathione (GSH), and lipid peroxidation (LPO) were conducted in liver, heart, and aorta.

Statistical analysis

All the observed data were expressed as mean ± standard error mean. The data were analyzed by GraphPad Prism version 7.0. Statistical significance testing for the comparisons was made by one-way ANOVA, followed by *post hoc* test. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$, and $^{ns}p>0.05$ compared with HCD group.

RESULTS

The present study was conducted to explore the effect of the methanolic extract of *B.* variegata (L.) stem bark on high cholesterol-fed rats and screen the phytoconstituents present in the plant by high-performance liquid chromatography (HPLC) analysis. The plant material was collected and extracted in methanol and further subjected to HPLC analysis. The result obtained was a flavonoid compound quercetin at a wavelength of 365 nm that was compared with the standard at a peak of 365 nm. The results obtained by HPLC analysis have been shown in Fig. 1a and b.

Table 1 and Fig. 2 display the results of total cholesterol, TGs, and PL and Table 2 and Fig.3 show the results of HDL, LDL, and VLDL. Furthermore, atherogenic index was calculated with the percentage protection of *BVME*. Table 3 shows the calculation of atherogenic index. It has been found that the rats fed on hypercholesterolemic diet (HCD) for 60 days resulted with increased levels of total cholesterol (TC), TGs, LDL, VLDL, and PL ($p<0.05$) in serum and simultaneous decrease in the level of HDL ($p<0.05$) in serum. Comparative to the rats fed on HCD and treated with doses of *BVME*, the results obtained were significantly different. There was a noteworthy change in the lipid profile which was comparable to the standard group. There was a consequential reduction in the levels of TC, TG, LDL, VLDL, and PL ($p<0.05$) in serum of treated group with *BVME* of dose level of 200 mg/kg b. wt and results of a dose level of 100 mg/kg b. wt. were found to be nonsignificant and increment in the levels of HDL ($p<0.05$) in treated Group 4 and were nonsignificant in Group 3.

The oxidative stress parameters were analyzed in liver, heart, and aorta. Table 4 shows the results of the levels of SOD (Fig. 4), GSH (Fig. 5), and LPO (Fig. 6) in the tissues of liver, heart, and aorta. The levels of SOD and GSH ($p<0.05$) were significantly high in treated and standard group (Groups 4 and 5), whereas it was observed lower levels in case of HCD group and Group 3. The level of LPO ($p<0.05$) was measured by the TBARS method and it was found that there was an increment in the HCD group and Group 3, and there was a significant decrement in Groups 4 and 5.

The hepatic toxicity tests were performed in the serum. The levels of aspartate transaminase (AST) and alanine transferase (ALT) were increased in Group 2 and there was a decreased level of same in Groups 4 and 5. Similarly, the level of alkaline phosphatase (ALP) was also observed and there was increased level in Groups 2 and 3, decrement in Groups 4 and 5. Table 5 and Fig. 7 depict the results.

DISCUSSION

Due to side effects of synthetic drugs, herbal medicines have been main source of primary health care in all over the world. The medicinal plants play a major role in hypolipidemic activity [16]. Hence, this study was conducted to assess the hypolipidemic activity of *B.* variegata (L.) and to screen its active principles. The results of phytochemical analysis of the methanolic extract of *BVME* showed the presence of quercetin a flavonoid by HPLC analysis. It is reported that flavonoids are found to inhibit HMG-CoA reductase activity [17] and quercetin, a flavonoid has been proven to improve dyslipidemia, decrease oxidative stress through stimulation of lipolysis activity, and upregulate the adipocytes genes expression which increases the lipids beta-oxidation [18,19]. It may be concluded that the cholesterol-lowering effect of *B. variegata* (L.) bark extract may be due to inhibition of HMG-CoA reductase activity.

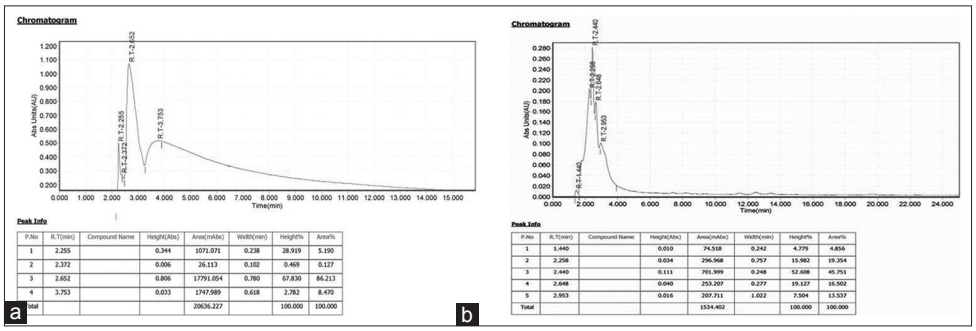

Fig. 1: (a) High-performance liquid chromatography chromatogram of standard for the comparison of test sample of the methanolic bark extract of *Bauhinia variegata*. (b): High-performance liquid chromatography chromatogram of test sample of the methanolic bark extract of *Bauhinia variegate*

Table 1: Effect of *Bauhinia variegata* (L.) was extracted with methanol on total cholesterol, triglycerides, phospholipids, high-density lipoprotein, low-density lipoprotein, and very low-density lipoprotein levels in hyperlipidemic rats

Groups	TC (mg/dL)	TG (mg/dL)	Phospholipids (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
I	114.35±4.28^{b}	144.76±2.55^{b}	110.05±4.03^{c}	46.19±2.22^{b}	44.39±2.05^{b}	28.95±0.51^{b}
II	136.61±3.50	159.72±3.82	131.91±3.12	35.25±1.89	54.90±2.28	31.94±0.76
III	121.46±2.39^{a}	144.09±2.71^{b}	116.47±2.53^{b}	43.56±2.62^{a}	46.45±1.65^{a}	29.50±0.32^{a}
IV	113.32±5.57^{b}	142.54±3.74^{b}	114.82±3.38^{b}	47.35±2.41^{b}	44.33±1.49^{b}	28.51±0.75^{b}
V	104.56±4.32^{c}	137.15±2.24^{c}	107.15±2.52^{c}	51.34±1.12^{c}	40.73±1.29^{c}	27.43±0.45^{c}

The data are expressed as mean±SEM; *n*=6 in each group. Control group (I); HCD group (II); HCD+BVME 100 mg/kg body weight (III); HCD+BVME 200 mg/kg body weight (IV); and HCD+atorvastatin 40 mg/kg body weight (V). Statistical significance testing for the comparisons was made by ANOVA, followed by *post hoc* test. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$, and $^{ns}p>0.05$ compared with HCD group. HCD: High cholesterol diet, BVME: *Bauhinia variegata* (L.) was extracted with methanol, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very LDL, SEM: Standard error of mean, TC: Total cholesterol, TG: Triglyceride

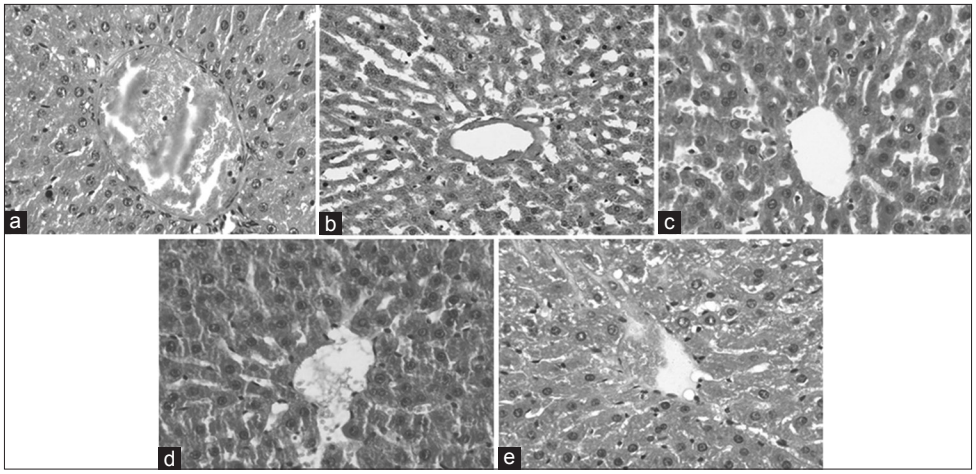

Fig. 2: Histopathological study of liver. (a) Control rat (×400), showing histoarchitecture of central vein and normal hepatocytes surrounding it. (b) Rat fed with high-fat diet (×400), showing significant damage with fatty infiltration and tissue degeneration. (c) Rat fed with high-fed diet and treated with *B. variegata* (100 mg/kg b. wt) (×400), showing mild reduction of fatty change with normal hepatocytes. (d) Rat fed with high-fed diet and treated with *B. variegata* (200 mg/kg b. wt) (×400), showing significant reduction of fatty infiltration with restoration of normal liver histological appearance. (e) Rat fed with high-fat diet and treated with atorvastatin (40 µg/kg b. wt) (×400), showing normal histological appearance of hepatocytes and the central vein.

Table 2: Effect of *Bauhinia variegata* (L.) was extracted with methanol on oxidative stress parameters in liver tissue of treated rats

Groups	SOD (U/min/mg protein)	GSH (U/g tissue)	LPO (nmol/gtissue)
I	5.37±0.26^{c}	5.14±0.07^{c}	5.68±0.27^{b}
II	3.81±0.24	3.56±0.16	7.23±0.33
III	4.92±0.30^{a}	4.73±0.23^{b}	6.10±0.29^{a}
IV	5.64±0.23^{c}	5.06±0.32^{c}	5.57±0.24^{c}
V	5.71±0.19^{c}	5.34±0.24^{c}	4.93±0.23^{c}

The data are expressed as mean±SEM.; *n*=6 in each group. Control group (I); HCD group (II); HCD+BVME 100 mg/kg body weight (III); HCD+BVME 200 mg/kg body weight (IV); and HCD+atorvastatin 40 mg/kg body weight (V). Statistical significance testing for the comparisons was made by ANOVA, followed by *post hoc* test. ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001, and ^{ns}p>0.05 compared with HCD group. HCD: High cholesterol diet, BVME: *Bauhinia variegata* (L.) was extracted with methanol, SEM: Standard error of mean, SOD: Superoxide dismutase, GSH: Glutathione reduced, LPO: Lipid peroxidation

Table 4: Effect of *Bauhinia variegata* (L.) was extracted with methanol on liver toxicity in hyperlipidemic rats

Groups	AST (U/l)	ALT (U/l)	Alkaline/acid phosphatase (U/l)
I	84.13±2.47^{c}	28.11±0.83^{c}	147.82±2.47^{b}
II	103.90±3.18	37.98±1.91	165.35±2.05
III	91.41±2.01^{a}	32.95±0.99^{a}	153.51±1.57^{a}
IV	87.92±2.88^{b}	31.29±0.68^{b}	151.08±2.30^{b}
V	79.94±3.3^{c}	23.68±0.94^{c}	130.33±4.61^{c}

The data are expressed as mean±SEM; *n*=6 in each group. Control group (I); HCD group (II); HCD+BVME 100 mg/kg body weight (III); HCD+BVME 200 mg/kg body weight (IV); and HCD+atorvastatin 40 mg/kg body weight (V). Statistical significance testing for the comparisons was made by ANOVA, followed by *post hoc* test. ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001, and ^{ns}p>0.05 compared with HCD group. HCD: High cholesterol diet, BVME: *Bauhinia variegata* (L.) was extracted with methanol, SEM: Standard error of mean, AST: Aspartate transaminase, ALT: Alanine transferase

In this study, the Wistar rats were fed with hypercholesterolemic diet to induce the condition of hyperlipidemia. There were significant changes in the experimental groups. The lipid profile decreased in the *BVME*-treated groups whereas increased in the high cholesterol-fed group. Shinde *et al*., 2013, also observed similar results with

Table 5: Effect of Bauhinia variegata (L.) was extracted with methanol on atherogenic index in hyperlipidemic rats

	Groups				
	I	II	III	IV	V
Atherogenic index (units)	1.62± 0.21^{c}	2.94± 0.26	1.95± 0.17^{b}	1.66± 0.25^{c}	1.04± 0.08^{c}
Percentage protection	_	_	33.67%	43.54%	64.63%

The data are expressed as mean±SEM; *n*=6 in each group. Control group (I); HCD group (II); HCD+BVME 100 mg/kg b.wt (III); HCD+BVME 200 mg/kg b.wt (IV); and HCD+atorvastatin 40 mg/kg b.wt (V). Statistical significance testing for the comparisons was made by ANOVA, followed by *post hoc* test. ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001, and ^{ns}p>0.05 compared with HCD group. HCD: High cholesterol diet, BVME: *Bauhinia variegata* (L.) was extracted with methanol, SEM: Standard error of mean

improvement in the lipid profile by administering *Psidium guajava* (L.) leaf extract to hyperlipidemic rats [20]. These results influenced the lipid metabolism and reverted to the changes significantly and it also suggests that cholesterol-lowering activity is associated with a decrease of its LDL fraction which can be a result from the rapid catabolism of LDL-C through its hepatic receptors for final elimination in the form of bile acids. It was also found that the administration of *BVME* in the animals that were fed with HCD increased the level of HDL which is considered to be good cholesterol as compared to the HCD group which lowered the levels of HDL. HDL facilitates the translocation of cholesterol from the peripheral tissue such as arterial walls to liver for catabolism. Hence, it is widely accepted that reduction HDL is a risk factor for developing cardiovascular diseases. It is also considered that increase in HDL may slow down the atherosclerotic process. Increased levels of HDL may be due to the increase in the activity of lecithin cholesterol acyltransferase, which plays a key role in incorporating the free cholesterol into HDL and transferring back to VLDLs or intermediate density lipoproteins, which are taken back by the liver cells.

Oxidative stress is a systemic manifestation which is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses such as glutathione [21]. Production of reactive oxygen species such as free radicals and peroxides is a particularly destructive aspect of oxidative stress. Oxidative stress is thought to be involved in the development of atherosclerosis [22] and heart failure [23]. Antioxidant inhibits the oxidation of these reactive

oxygen species. As *B. variegata* (L.) is a potent antioxidant [5,6]; hence, the study was also assessed for its antioxidant activity. The oxidative stress parameters such as SOD, GSH, and LPO were performed. This study suggests that the dose levels higher of *BVME* possess antioxidative activity and the flavonoids present in the extract may be responsible for increasing the levels of SOD and GSH by scavenging the reactive oxygen species. The LPO was assessed by TBARS method. The pathogenesis of several diseases is caused due to free radical-induced LPO or oxidative stress [24,25]. Hypercholesterolemia induces not only atherosclerosis but also produces a lot of free radicals in blood and tissues [24,26]. The combined effect of decreased LPO and increase in antioxidant enzyme activities in the hepatic tissue, cardiac, and aorta would definitely lower the oxidative stress. Similar observations were obtained by Sharma H *et al*., 2018 [27].

Hyperlipidemia is one of the reasons for the cause of hepatopathy. The queer sign of hepatic damage is leakage of important cellular enzymes such as AST, ALT, and ALP into the serum [28]. Hence, in the present study, these cellular enzymes were as the biomarkers for the hepatic damage. There was a significant change in the levels of AST and ALT. This shows liver toxicity in Groups 2 and 3 and no toxicity in treated groups.

CONCLUSIONS

With this current study and findings, it can be concluded that methanolic stem bark extract of *B. variegata* (L.) contains quercetin, a flavonoid that is potent hypolipidemic agent and an antioxidant. It was helpful in decreasing the levels of total cholesterol, TGs, LDL, and VLDL whereas increasing the levels of HDL. It also had a beneficial effect on antioxidative activity by increasing the levels of SOD and GSH and reducing the levels of LPO. Furthermore, there was not any liver toxicity in the treatment of hyperlipidemia with *BVME*.

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AUTHORS' CONTRIBUTIONS

- Ms. Rucha Lakhne has conducted the whole study as well as research article writing
- Dr. Rajnish Gupta guided him in conducting research, data interpretation, and research paper writing
- Dr. R. S. Gupta designed the study and validated the results of research data.

CONFLICTS OF INTEREST

None.

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