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Research Article

BIOTHERAPEUTIC ACTIVITY OF *Boerhaavia diffusa* AGAINST OXIDIZED CHOLESTEROL INDUCED LIPID PEROXIDATION AND ANTI-OXIDANT STATUS IN HYPERCHOLESTEROLEMIC RATS

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Abstract

Present days, herbal medicines are widely practiced to treat cardio vascular diseases (CVD) and heart care management in most of the Asian countries. The present study was to evaluate the hypercholesterolemic and antioxidative effect of *Boerhaavia diffusa* root extract (BD) on oxidized cholesterol induced hypercholesterolemia in rats. The root extract was prepared in 70% ethanolic medium by soxhlet extraction procedure. Experiment was designed to carry out with 18 male albino rats which are equally divided into 3 groups for four week experiment. All experimental animals provided rat chow and water *ad libitum.* Group II and III received oxidized cholesterol through gastric intubation at the dose of 1mg/rat/day, where as in group III followed by BD at the dose of 1ml/rat/day for 4 weeks. Parameters like lipid peroxidation, total cholesterol and antioxidant status in plasma along with catalase (CAT), glutathione reductase (Gred) and superoxide dismutase (SOD) in liver tissue were analyzed on oxidized cholesterol supplemented rats with and/or without treatment of BD. The total cholesterol (TC), triglycerides (TG) and LDL levels in plasma of group II remained high compared to the counterparts. But in group III showed significant decrease in TC, TG and LDL levels by 34.39%, 42.13% and 48.30%, respectively. The based on findings, it seems very levelheaded to believe that this greener way of formulation of herbal medicines is not just a utilization of naturally occurring medicinal herbs but also to make it available for the betterment of the society at low cost.

Keywords: Antioxidant; *Boerhaavia diffusa*; cardio vascular diseases; hypercholesterolemia; lipid peroxidation

Introduction

The increase in CVD, through a proliferation of risk factors that are heavily influenced by lifestyle choices, is the new challenge for many developing countries. An estimated 17.3 million people died from CVDs in 2008 and World health Organization (WHO) reported that by 2030 more than 23 million people will die annually from CVDs. Over 80% of chronic disease deaths occur in low and middle income countries and occur almost equally in men and women. Cholesterol is an amphipathic lipid and as such is an essential structural component of membranes and of the outer layer of plasma lipoproteins.

The primary feedback loop for regulation of cholesterol synthesis appears to be at the site where HMG-CoA is converted to mevalonic acid by the rate-limiting enzyme HMG-CoA reductase, whereas cholesterol and other oxysterols inhibit the activity of HMG-CoA reductase. Isolation, purification, and characterization of rat hepatic HMG-CoA reductase have been well studied by (Reddy *et al*., 2012).

Some of the factors which are known to cause an overall increase in cholesterol concentration in the liver are (i) uptake of lipoproteins by receptor mediated endocytosis, (ii) non-receptor mediated intake of lipoproteins, (iii) uptake of free cholesterol from the cholesterol rich lipoproteins by cell membranes, (iv) *de novo* synthesis of cholesterol, and (v) hydrolysis of cholesterol esters by cholesteryl ester hydrolase. Under above situations, not only increased cholesterol levels inhibits its own synthesis by inhibiting HMG-CoA reductase and suppressing low density lipoprotein (LDL) receptors (Russel *et al*., 1983), but also by activating cholesterol 7-α-hydroxylase and acyl CoA: cholesterol acyltransferase which utilize free cholesterol for bile acid synthesis and formation of cholesteryl esters, respectively. LDL is transported to lysosome where the protein is degraded and the cholesterol is transferred to the intracellular cholesterol pool (Brown *et*

al., 1986). LDL is a major risk factor for cardio arterary diseases, experiments have shown that incubation of macrophages with native LDL does not result in foam cell formation, a characteristic feature of atheromatous lesions (Gerald *et al*., 2012).

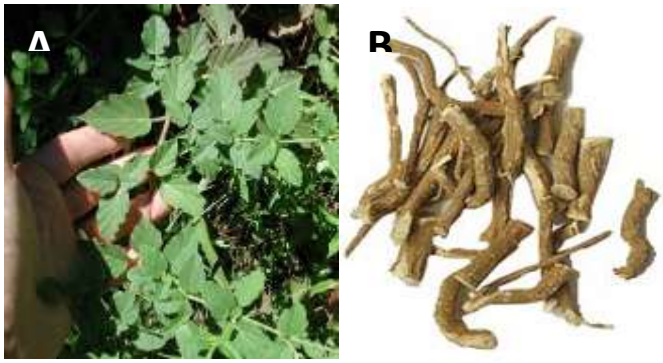


Fig. 1: Herbal perennial creeper plant of *Boerhaavia diffusa* (A) dried roots (B).

Lipid peroxidation is initiated by free radical attack on double bond associated with a polyunsaturated fatty acid (PUFA). This results in the removal of a hydrogen atom from a methylene (CH_2) group, the rate of which determines the rate of initiation, a key step in lipid peroxidation. Molecular rearrangement of the resulting unstable carbon radical results in a more stable configuration, a conjugated diene. The conjugated diene reacts very quickly with molecular oxygen, and the peroxyl radical thus formed is a crucial intermediate (Abuja *et al*., 1995)**.** Epidemiological studies have identified low density lipoproteins and high density lipoproteins as independent risk factors that modulate CVD risk.

BD is commonly known as rakta punarnava (Kokate *et al*., 2004) of family Nyctaginaceae (Bhalla *et al*., 1971) is mainly diffused perennial herbaceous creeping weed of India (known also under its traditional name punarnava). The plant root possesses diuretic, hepatoprotective and cardiotonic (Rawat *et al*., 1997), hypotensive (Devi 1986), immunomodulating (Hansen *et al*., 1995), antioxidative (Pandey *et al*., 2005), antihemorrhagic (Sathees *et al*., 2004), antispasmodic (Barthwal *et al*., 1991), antimicrobial (Borrelli *et al*., 2006), cytotoxic (Hilou *et al*., 2006), and anticancerous (Leyon *et al*., 2005) activities. Ethanolic root extract of BD showed the presence of alkaloids, flavonoids and saponins, any of which phytoconstituent may be responsible for such pharmacological activities. In this study we investigated the efficacy of BD by analyzing all the parameters in plasma, TC, TG, LDL, HDL, LDL oxidation and antioxidant enzymes CAT, Gred and SOD as well as *in vitro* oxidizability of LDL. In light of this, the present work to study the hypocholesterolemic activity of the ethanolic root extract of BD was undertaken.

Materials and Methods

Phytoextraction

Fresh root of BD were collected from Srinagar (Garhwal) and its adjoining areas. The collected plant was identified by Dr. R. L. Panoli, Taxonomist, Department of Botany and Microbiology, H.N.B. Garhwal University, Srinagar, India and the voucher of specimen (GUH- 20434) has been preserved in research laboratory for future reference. The plant root dried in shade, coarsely powdered and subjected to soxhlet extraction using 70% hydro-alcoholic solvent (70% ethanol : 30% distilled water), at 48^0C for 24 h. The final extract was allowed to evaporate resulted dark brownish solid residue.

Thermo-oxidation of cholesterol

Oxidized cholesterol for the diets was prepared by heating. Cholesterol (10 g) was dissolved in ether in a 2-L round flask, and then ether was evaporated on a rotary evaporator, resulting in a thin film. The flask was placed in a 100°C oven and heated overnight (16 hours) and remaining dried part was used as feed supplement.

Experimental organism

White male albino rats weighing 150-180 gm were used for the present study, maintained on animal house under normal condition having natural photoperiod (12 hours light/dark cycle) at temperature 25 ± 1^0C and 50-60% humidity. Maintenance and treatment of all the animals was done in accordance with the principles of Institutional Animal Ethics Committee constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. They were provided with standard pelleted rat chow and tap water *ad libitum*.

Experimental Design (Drug administration)

Animals were divided into three groups and for each group six animals were taken for four weeks experiment. Group I Normal control (1ml palm oil/rat/day) through gastric intubation, Group II hypercholesterolemic control rats were administrated 1.0 mg oxidized cholesterol/rat/day though gastric intubation and Group III hypercholesterolemic+ BD treated rats. In this group rats were administrated 1.0 mg oxidized cholesterol/rat/day is followed by the gastric intubation of root extract of BD (600 mg were solubilised in 5 ml ethanol and diluted to 100 ml in palm oil) 1 ml each/rat/day.

Group I- Normal control (NC)

Group II- Hypercholesterolemic control (HC)

Group III- hypercholesterolemic + BD treated (HBT)

Collection of Blood and Plasma

For the estimation of different parameters, overnight fasted rats in each group were anaesthetized with chloroform and blood drawn from cardiac puncture, and were collected in

heparinised tube. Plasma was separated from blood by centrifugation at 2500 rpm for 30 min.

Biochemical assays

Lipid profiles (TG, TC, LDL, HDL), Cu^{++} mediated LDL oxidation, plasma antioxidant and lipid peroxidation products (conjugated diene, lipid hydroperoxide and malondialdehyde) were evaluated in normal and hypercholesterolemic rats (Bharali *et al*., 2003).

Measurement of plasma "total antioxidant power" (FRAP)

The method of Benzie and Strain 1996 was used for measuring the ferric reducing ability of plasma, the FRAP assay, which estimate the "total antioxidant power", with minor modification. Ferric to ferrous ion reduction at low pH results in the formation of a colored ferrous-tripyridyl triazine complex. The assay was carried out in a total volume of 1.0 ml containing a suitable aliquot of plasma in 0.1 ml and 900 µl of freshly prepared FRAP reagent, prepared by mixing 10.0 ml of 22.78 mM sodium acetate buffer, pH 3.6, 1.0 ml of 20 mM ferric chloride and 1.0 ml of 10 mM 2, 4, 6-tripyridyl-s-triazine solution prepared in 40 Mm HCl. Before starting the reaction, both FRAP reagent and plasma samples were pre-incubated for 5 min at 300°C. Incubation was done for 5 min at 300°C and absorbance was recorded at 593 nm against a reagent blank in spectrophotometer. Ferrous sulphate (1mM) was used as a standard for calculating the "total antioxidant power".

Preparation of Liver homogenate and in vivo antioxidant status

At the end of the experiment, liver from each rat were promptly excised and chilled in ice cold saline. After washing with saline, liver was blotted and weighed. Each liver was cut into pieces, mixed and 10 g of wet tissue was homogenized with 90 ml of chilled 0.1 M sodium phosphate buffer, pH 7.4, containing 1.17% KCl in a waring blender. The volume of each homogenate was recorded and centrifuged at 1,000 rpm for 10 min at 4^0C. After centrifugation, a portion of each homogenate from liver thus obtained was aliquoted and stored at -20^0C. The antioxidant system was performed in liver. Lipid peroxidation was characterized by measuring the Gred level (Reitman *et al*., 1957), CAT activity and SOD (Sinha 1972) and (Kakkar *et al*., 1984) respectively.

Statistical Analyses

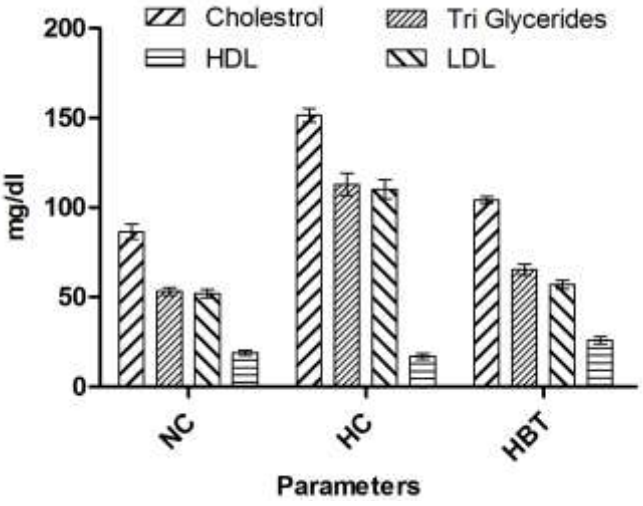
The data were subjected to statistical analyses on MS Excel, and graphs were prepared using Graph Pad Prism Ver. 5.00 for Windows (San Diego, California, USA).

Result and Discussion

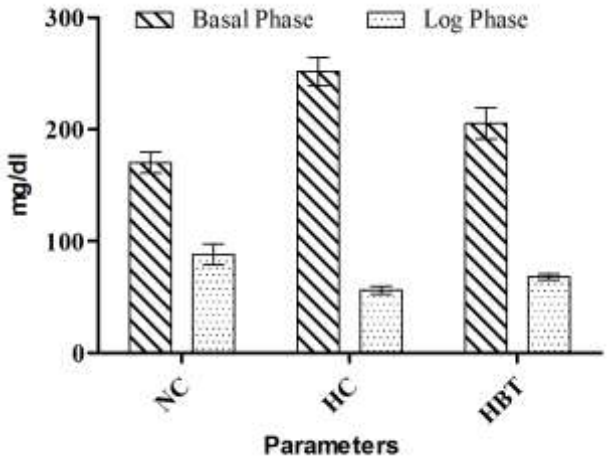
The experiment was carried out for 4 weeks and at the end of the experiment blood samples and organ were collected by scarifying the animals for detailed study of several parameters.

Serum lipid profile level and LDL oxidation level

After 4 weeks of treatment cholesterol, triglycerides and LDL level in the BD treated rat group were reduced by 34.39%, 42.13% and 48.30 respectively as compare to Group II (hypercholesterolemic rats). HDL was significantly improved by 52.79% after treatment of BD in Group III compare to hypercholesterolemic rats (Fig 2A). And Cu^{++} mediated LDL oxidation in basal phase in BD treated rat group shows significant increase compare to Group II rats whereas in lag phase group III shows decreasing result compare to hypercholesterolemic rats (Fig 2B).



A



B

Fig. 2: Variations in the TC, TG, LDL and HDL level (A) and basal phase and log phase LDL oxidation (B) in plasma during oxidized cholesterol induced hypercholesterolemia with and/or without BD administration.

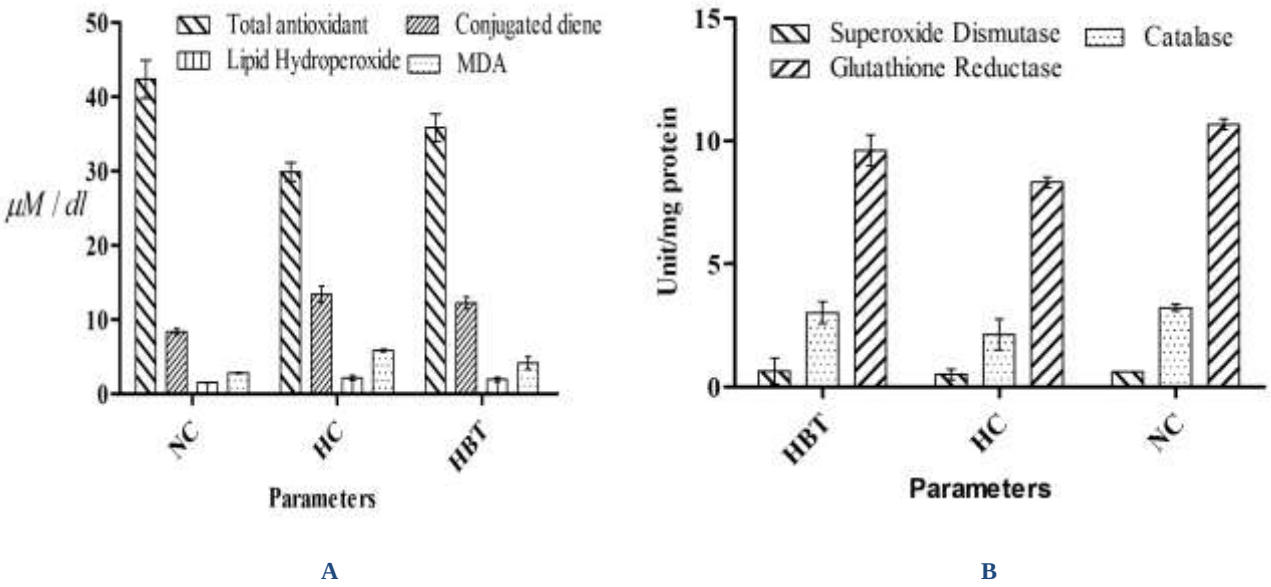


Fig. 3: Variations in the total antioxidant, conjugated diene, lipid hydroperoxide and MDA level in plasma (A) and superoxide dismutase, catalase and glutathione reductase level in liver (B) during oxidized cholesterol induced hypercholesterolemia with and/or without BD administration.

Impact of BD on plasma total antioxidants and lipid peroxidation products

After 4 weeks of treatment with BD total antioxidant power of plasma in Group III rats notably improved by 19.97% compare to hypercholesterolemic rats of Group II. And the lipid peroxidation products (conjugated diene, lipid hydroperoxide and MDA) of plasma gave decreasing results in BD treated rat group compare to Group II by 8.66%, 12.50%, and 29.0% respectively (Fig 3A).

Liver enzymatic status of Boerhaavia diffusa treated rats

The liver enzymes in BD treated rat group shows remarkable increase in CAT, SOD and Gred by 42.45%, 27.32% and 15.62% correspondingly compare to hypercholesterolemic rats after 4 weeks of treatment (Fig 3B).

Discussion

Results from the present study indicate that hypercholesterolemic rats experience an exaggerated oxidative stress when compared with hypocholesterolemic and normal rats. The oxidized cholesterol induced extensive proatherogenic changes, that occurred in rats, were reflected on a variety of parameters, *viz*., plasma cholesterol and plasma lipid peroxidation products including LDL, plasma total antioxidants and HDL-associated activities, MDA release and antioxidant enzymes in rat liver. Supplementation of rats with BD for 4 weeks significantly reduced the overall oxidative burden and effectively ameliorated the above altered parameters, thus, indicating a strong hypolipidemic/antiatherogenic and antioxidant effect of BD.

High cholesterol increases the risk of myocardial infarction in both men and women. After 4 week BD treatment of these hypercholesterolemic rats resulted significant reduction in plasma total cholesterol, triglyceride and LDL, which were reversed back to about the normal control rats whereas HDL level shows notable increase by 52.79% in BD treatment of respective control values of hypocholesterolemic rats, similar findings reported by Khan *et al*., 2011 and Mahesh *et al*., 2012. These results indicate a strong protective effect of BD, which may help lower the risk of myocardial infarction.

Our data show that due to sustained high cholesterol diet in hypercholesterolemic rats, oxidation of lipid is considerably enhanced. Conjugated diene (which measure the initial phase of lipid peroxidation), lipid hydroperoxide (intermediate product of lipid peroxidation) and MDA (which measure the degradation phase of lipid peroxidation) in plasma are significantly increased in hypercholesterolemic rats but after the treatment of BD results indicate significant decrease in plasma lipid peroxidation products with a concomitant and significant increase in plasma total antioxidants in rats (Pareta *et al*., 2011; Devki *et al*., 2004).

It is now established that oxidation of LDL constitutes a key event in inflammation and atherogenesis (Thapliyal *et al*., 2012). The inhibitory effect of HDL on LDL oxidation was suggested to be related to metal ion chelation, or to peroxidase like activity. The Cu^{++} mediated LDL oxidation at basal phase shows notable decrease and at lag phase shows increase in plasma after treatment. The levels of reactive oxygen species (ROS) are controlled by antioxidant enzymes, SOD, CAT and Gred. These enzymes are

important in defending the body against free radicals as well as toxic substances by converting them to a form that can be readily excreted. Therefore, any changes in these enzymes could be potentially detrimental to the host by altering these defense mechanisms. Normal cellular metabolism involves the production of ROS, low levels of ROS are vital for proper cell functioning, while excessive *in vivo* generation of these products can adversely affect cell functioning (Pari *et al*., 2004 and Kuldeep *et al*., 2011). Treatment of hypercholesterolemic rats with BD for 4 weeks significantly improved the integrity of erythrocytes membrane as shown by improved protection against lipid peroxidation as well as reversal of CAT, SOD and Gred activities to near normal accordingly in liver tissues (Chauhan *et al*., 2011 and Salman *et al*., 2013). As mentioned earlier, that since certain experiments such as mechanism(s) of lipid lowering action of BD, the extent of its protective effect on enzymatic and nonenzymatic antioxidant defense system including lipid peroxidation in various tissues and plasma. It is believed that the given information will generate a real clinical assessment and estimation of the effectiveness of at least the BD in the treatment of some cardiovascular diseases.

Conclusion

The result of the present study by analyzing several parameters *viz.*, TC, TG, LDL, HDL, lipid peroxidation and liver enzymatic status make known that ethanolic root extract of *Boerhaavia diffusa* serves as an economical natural herb with antioxidative and hypercholesterolemic properties. However, a detailed study needs to be undertaken to confirm the mode of action.

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