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**Antihyperlipidemic activity of methanolic extract of
*Abroma augusta***

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Abstract

Hyperlipidemia is a major cause of cardiovascular diseases. Approximately 10% of the global population is affected by dyslipidemia. At present, hyperlipidemia is most commonly treated with lipid-lowering drugs, some of which are associated with serious adverse side effects. Many medicinal plants have been found to be useful to successfully manage hyperlipidemia. The present study was undertaken to study Antihyperlipidemic effect of methanol extract of *Abroma augusta* leaves. The powdered crude leaves were extracted in a Soxhlet apparatus with methanol. Extract was dried at 40°C under pressure and stored at 4°C until use. Phytochemical screening of extract was done by standard methods. Methanol extract of *Abroma augusta* was studied for acute oral toxicity according to OECD guidelines No. 423 on Wistar rats. High cholesterol diet induced hyperlipidemia model was used to study antihypercholesteremic effect. After 7 days samples from animals were collected. Serum samples were analyzed spectrophotometrically for cholesterol, triglyceride and HDL-C. Treatment with methanolic extract of *Abroma augusta* showed a marked reduction in TC, TG and LDL-c levels ($p < 0.05$). There was a marked reduction in TC:HDL-c ratio, and in the atherogenic index after the treatment of rats with 400mg/kg dose of methanol extract of *Abroma augusta*. The findings of current study proved that the methanol extract of *Abroma augusta* effectively treated hyperlipidemia in murine model may be due to presence of flavonoids, polyphenols, alkaloids found in extract. However isolation and characterization of active constituent responsible for this activity is necessary.

Key-Words: Phytochemical screening, Methanolic extract and *Abroma augusta*

Introduction

Hyperlipidemia is a major cause of cardiovascular disease including atherosclerosis and atherosclerosis-associated conditions, such as coronary heart disease (CHD), ischemic cerebro-vascular disease and peripheral vascular disease. ¹ Hyperlipidemia is the presence of high levels of cholesterol in the blood. ² It is not a disease but a metabolic derangement of lipid metabolism rank as the most firmly established and best understood risk factor for atherosclerosis and cardiovascular complications. ³ Approximately 10% of the global population is affected by dyslipidemia. ^{4, 5} Cholesterol is the essential for human body but some types of cholesterol has very adverse effect on human body and leads to heart attack and death. ^{6, 7} Dyslipidemia is one of the most common complications of Diabetes Mellitus. ⁸ The treatment of hyperlipidemia depends on the patients cholesterol profile. Many antihyperlipidemic agents like statin, fibrates, niacin, bile acids, ezetimibe etc reduce cholesterol level with different condition. ^{9, 10}

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The statin corrects the altered blood lipid profile by inhibiting the bio-synthesis of cholesterol and the fibrates acts by enhancing the clearance of triglyceride rich lipoproteins. ¹¹

At present, hyperlipidemia is most commonly treated with lipid-lowering drugs, some of which are associated with serious adverse side effects. ¹² Importance of natural products in modern medicine is increased recently. ^{13, 14} The search for new agents capable of reducing serum lipid levels has therefore become an important research focus. Plant products are generally considered to be less toxic and less prone to side effects than drugs manufactured by chemical synthesis. ¹⁵

In the past two decades, herbal hypolipidemics have gained importance to fill the lacunae created by the allopathic drugs. Many medicinal plants have been found to be useful to successfully manage hyperlipidemia; these include *Dracocephalum kotschyi*, ¹⁶ *Allium porrum*, ¹⁷ *Eclipta prostrate*, ¹⁵ *Scoparia dulcis*, ¹⁸ *Trigonella foenum-graecum* ^{19, 20} and red yeast rice. ²¹

Abroma augusta, or Devil's Cotton, is an evergreen tree native from Asia to Australia. *Abroma augusta* is

known to show antidiabetic, antihyperglycemic and antifertility potential.²²⁻²⁴ Root of the plant is used as abortifacient and anti-fertility agent. Leaves are known for treatment of uterine disorders, rheumatic pain of joints, and headache with sinusitis.²⁵ Aerial parts are used as demulcent and in treatment of gonorrhoea.²⁶ The root-bark is used as uterine tonic.²⁷ The plant finds multirole use in modern and traditional medicine; however there is dearth of knowledge regarding antihyperlipidemic potential of *Abroma Augusta*. Hence, the present study was undertaken to study antihyperlipidemic effect of methanol extract of leaves of *Abroma Augusta*.

Material and Methods

Plant material

The leaves of *Abroma augusta* (Ulatkambal) were collected in the month of January from Jawaharlal Nehru Krishi Vishwavidyalaya Jabalpur (M.P.). The plant was identified and authenticated by Dr. A.B. Tiwari, Sr. Scientist, Department of Crop & Herbal Physiology, Jawaharlal Nehru Krishi Vishwavidyalaya Jabalpur (M.P.). Leaves of *Abroma augusta* were dried in shade, coarsely powdered and used for the preparation of extracts. The powdered crude drug was extracted in a Soxhlet apparatus with methanol. Finally extract was dried at 40°C under pressure and stored at 4°C until use. Phytochemical screening of extract was done by standard methods.²⁸

Drugs and Chemicals

Cholesterol, sodium cholate was purchased from CDH, India. Coconut oil (Parachut, India), Atrovastatin (Microlabs, India) was used in the study. All other reagent used in this study was of analytical grade.

Animals

Healthy adult male Wistar albino rats between 6 and 8 months of age and weighing about 150-250 g were used for the study. The animals were housed in polypropylene cages, maintained under standard conditions (12 h light: 12 h dark cycle; 25 ± 30°C; 35-60% humidity). All the experimental protocols were approved by Institutional Animal Ethics Committee.

Acute Toxicity Studies

Methanol extract of *Abroma augusta* was studied for acute oral toxicity as per revised OECD guidelines No. 423.²⁹ The extract was devoid of any toxicity in rats when given in doses up to 2000 mg/kg by oral route. Hence 200 and 400 mg/kg doses of extract were used for the study.

Antihyperlipidemic activity³⁰

High cholesterol diet induced hyperlipidemia Induction of hyperlipidemia

High cholesterol diet was prepared by mixing cholesterol (2%), sodium cholate (1%) and coconut oil (2%). The diet was administered for seven days.

Dose Preparation and Administration of Extracts

The extract of *Abroma augusta* was dissolved in distilled water and a dose of 200 mg/kg and 400 mg/kg was given to the animals once in a day orally along with the high cholesterol diet orally. Treatment was given daily for seven days.

Animal Grouping

The experimental animals were divided into five groups, six animals in each group

Group 1: Normal Control

Group 2: Hyperlipidemic Control

Group 3: Atrovastatin 10 mg/kg + High cholesterol diet.

Group 4: Methanolic Extract (200 mg/kg, orally) + High cholesterol diet.

Group 5: Methanolic Extract (400 mg/kg, orally) + High cholesterol diet

Blood sample collection and analysis

On the 8th day, blood was collected by retro-orbital sinus puncture, under mild ether anesthesia after 8 hr fasting and allowed to clot for 30 minutes at room temperature. Blood samples were centrifuged at 3000 rpm for 20 minutes. Serum was separated and stored at -20°C until use.

Serum samples were analyzed spectrophotometrically for Cholesterol, triglyceride and HDL-C was estimated using diagnostic kits which were procured from Lab-Care Diagnostics (India) Pvt. Ltd.- Mumbai (India). VLDL, LDL, HDL-ratio and atherogenic index were calculated by using the formula of Friedewald and colleagues.³¹

Statistical Analysis

Experimental results were mean±SEM of 6 animals. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Dunnett's test. Data were considered statistically significant only when p value < 0.05, p < 0.01.

Results and Discussion

There was significant increase in the levels of serum TC, TG, LDL-c and VLDL-c in high fat diet induced hypercholesterolemia group of animals. Treatment with methanolic extract of *Abroma augusta* showed a marked reduction in TC, TG and LDL-c levels (p < 0.05). But there was a significant rise in HDL-c levels in group IV and group V. Atrovastatin also produced significant reduction in serum TC, TG, LDL-c levels and a rise in HDL-c levels. There was a marked reduction in TC: HDL-c ratio, and in the atherogenic index after the treatment of rats with

400mg/kg dose of methanol extract of *Abroma augusta* as given in the Table No.1 & Figures 1-12.

Development of atherosclerotic disease is a complicated process involving accumulation of lipid-containing particles in the walls of coronary arteries & other major arteries within the body. Lowering high cholesterol levels significantly reduce the risk of heart attacks, strokes, and death. Normally hepatocytes initiate synthesis of triglycerides and cholesterol during states of increased free fatty acid flux to the liver but due to anti-hyperlipidemic drug, there may be inability of hepatocytes to increase cholesterol synthesis and decrease hepatocytes cholesterol concentration by increase in the catabolic conversion of cholesterol to bile acids in liver. High cholesterol diet increased serum cholesterol and LDL-C level significantly.^{32, 33} A rise in LDL may cause deposition of cholesterol in arteries and aorta and hence it is a direct risk factor for coronary heart disease.³⁴ Studies show that both LDL and VLDL have a positive role in atherogenesis.³⁵

Plant based pharmaceuticals are of the important gift provided by nature to mankind. Plants serve to be an excellent source of lead molecules for discovery of new drugs. A number of phytoconstitutes like alkaloids, glycosides, tannins, phenolics etc. are known

to demonstrate antihypercholesteremic potential.³⁵⁻⁴¹ A number of plants have shown protective effect on hypercholesteremia. *T. chebula* and its combination with gaumutra significantly decreases the cholesterol, triglyceride, VLDL-C, LDL-C, atherogenic index and a significantly increase in HDL-C in serum.⁴² *Bauhinia purpurea* extracts significantly reduced atherogenic index, TC: HDL-c and LDL: HDL-c ratios. The antihyperlipidemic activity may be due to the presence of polyphenolic compounds flavonoids, tannins and proanthocyanidines.⁴³

Abroma augusta was found to be a rich source of flavonoids, tannins, alkaloids. Extract at a dose of 400 mg/kg b.w., significantly reduced the elevated serum cholesterol, LDL, atherogenic index and significantly increased the HDL-C. The *Abroma augusta* extract had significant effect on triglyceride and on VLDL. The finding of current study proves that the methanol extract of *Abroma augusta* effectively treated hyperlipidemia in murine model. This activity may be due to presence of flavonoids, polyphenols, alkaloids found in extract. However isolation and characterization of active constituent responsible for this activity is necessary.

Table 1: Effect of *Abroma augusta* extract on various parameters in High cholesterol diet rats

Parameters	Normal	Hyperlipidemic control	Standard (Atorvastatin)	Extract 200mg/kg	Extract 400mg/kg
Cholesterol (mg/dl)	114.16 ±3.86	254.83±2.22	163.83±2.92**	215.16±3.18** ψ ψ	184.66±2.73** ψ ψ
Triglyceride (mg/dl)	101.33±2.58	186.83±2.63	134.66±3.07**	173.83±3.25* ψ	145.66±3.50**
HDL-C (mg/dl)	56.33±2.73	36±2.60	75.16±2.31**	64.5±2.16** ψ	71.16±3.18**
LDL (mg/dl)	53.66±1.96	166.33±1.63	75.66±2.80**	108.83±2.63** ψ ψ	94.5±2.34** ψ ψ
VLDL (mg/dl)	24.83±1.94	75±3.74	34.33±2.58**	55.33±2.33** ψ ψ	44.5±3.08**
Atherogenic Index	0.257±0.008	0.715±0.002	0.253±0.008**	0.430±0.027** ψ ψ	0.311±0.0028** ψ
Cardiac risk ratio TC:HDL-C	2.04±0.026	7.05±0.023	2.16±0.018**	3.33±0.095** ψ ψ	2.57±0.023** ψ ψ

Each value is mean ± S.E.M. (n = 6) .Data was analyzed by One-way ANOVA followed by Dunnett's test.

*Significantly compared to Hyperlipidemic control group ($p < 0.05$). **Significantly compared to Hyperlipidemic control group ($p < 0.01$).

ψ Significantly compared to Standard group ($p < 0.05$). ψψ Significantly compared to Standard group ($p < 0.01$).

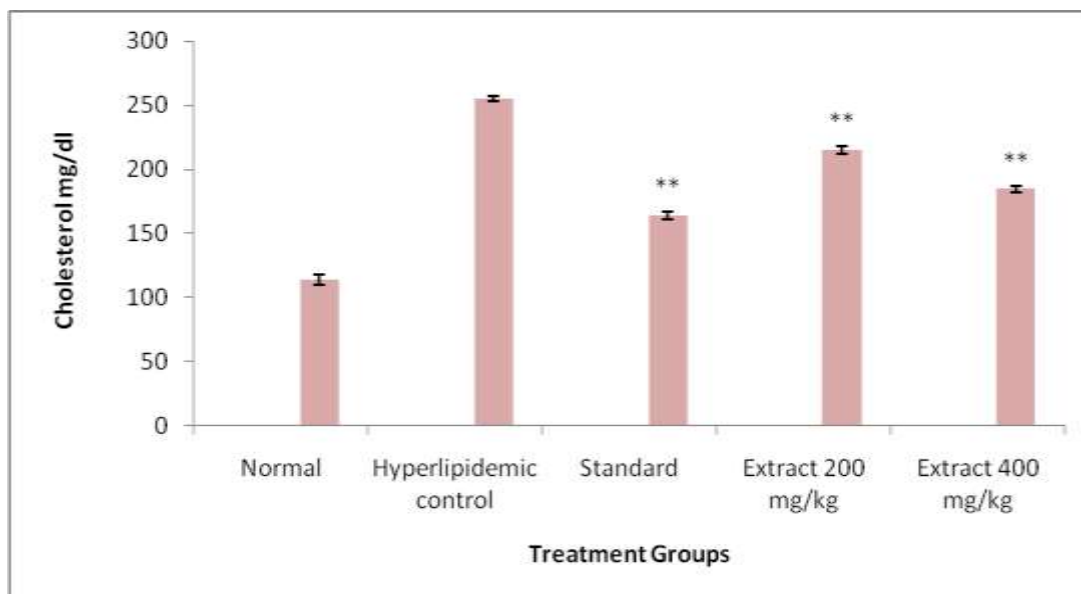


Fig. 1: Effect of *Abroma augusta* extract on cholesterol in High cholesterol diet rats.

Each value is mean \pm S.E.M. (n = 6). Data was analyzed by One-way ANOVA followed by Dunnett's test. *Significantly compared to Hyperlipidemic control group ($p < 0.05$). **Significantly compared to Hyperlipidemic control group ($p < 0.01$).

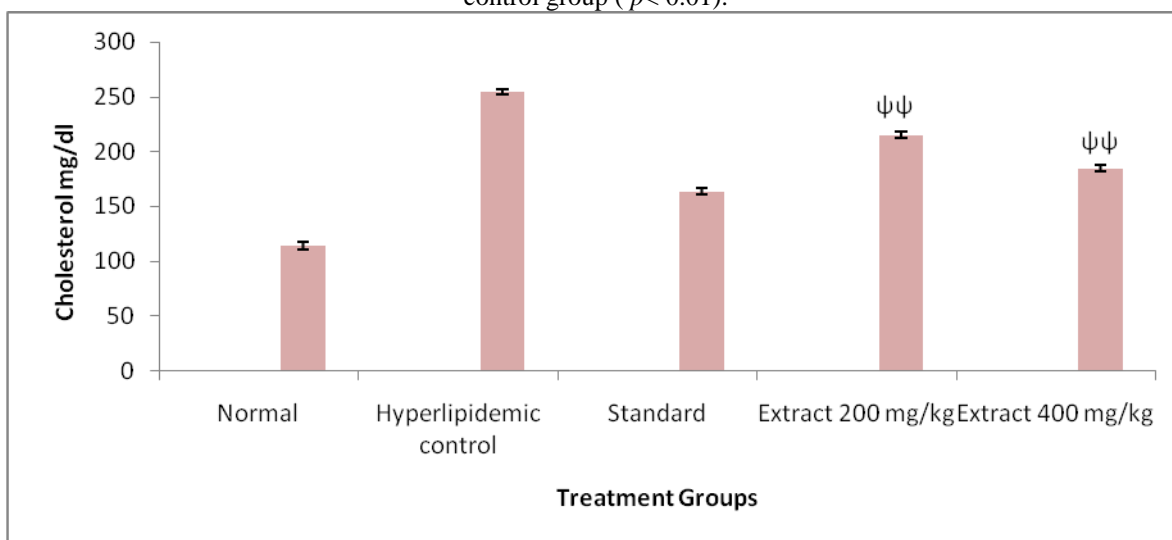


Fig. 2: Effect of *Abroma augusta* extract on cholesterol in High cholesterol diet rats.

Each value is mean \pm S.E.M. (n = 6). Data was analyzed by One-way ANOVA followed by Dunnett's test. ψ Significantly compared to Standard group ($p < 0.05$). $\psi\psi$ Significantly compared to Standard group ($p < 0.01$).

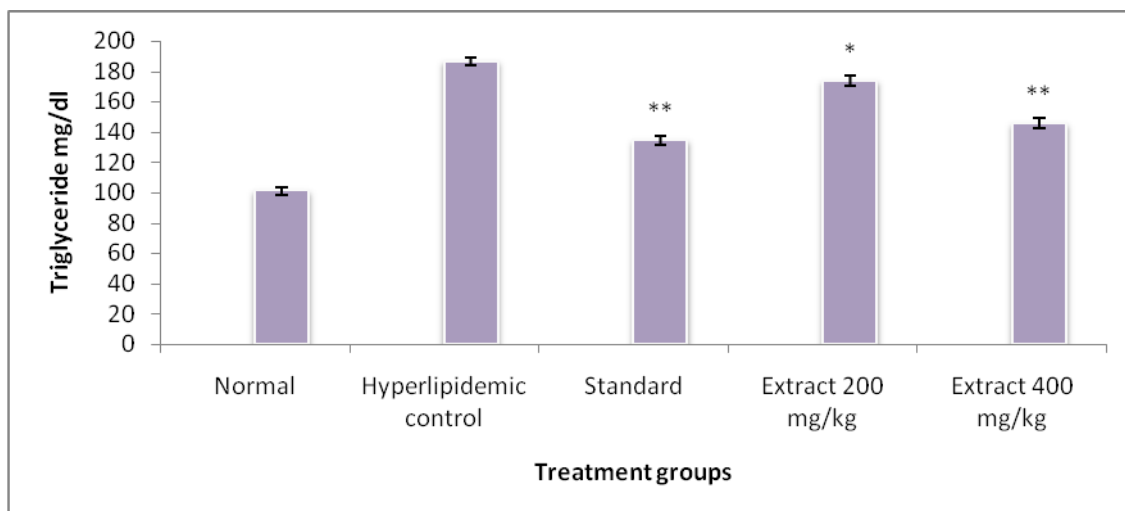


Fig. 3 Effect of *Abroma augusta* extract on Triglyceride in High cholesterol diet rats.

Each value is mean \pm S.E.M. (n = 6) .Data was analyzed by One-way ANOVA followed by Dunnett's test. *Significantly compared to Hyperlipidemic control group ($p < 0.05$). **Significantly compared to Hyperlipidemic control group ($p < 0.01$).

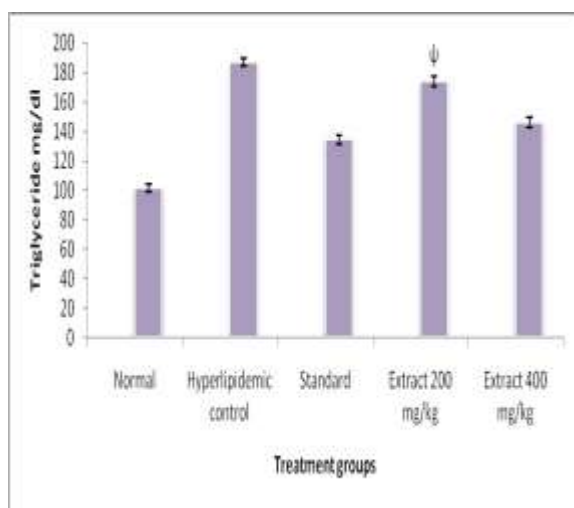


Fig. 4: Effect of *Abroma augusta* extract on Triglyceride in High cholesterol diet rats.

Each value is mean \pm S.E.M. (n = 6) .Data was analyzed by One-way ANOVA followed by Dunnett's test. ψ Significantly compared to Standard group ($p < 0.05$). ψψ Significantly compared to Standard group ($p < 0.01$).

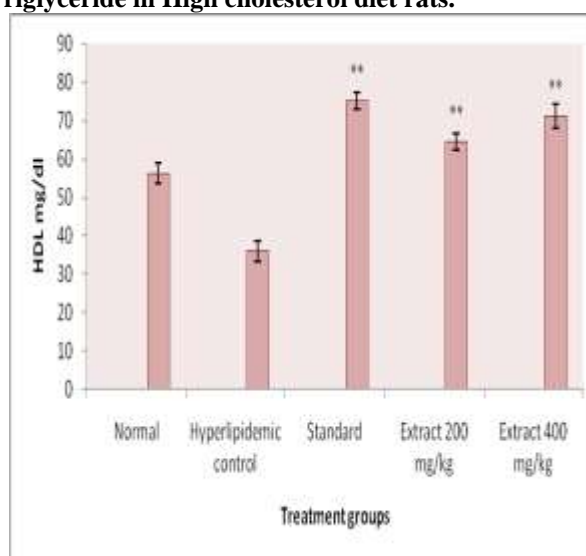


Fig. 5 Effect of *Abroma augusta* extract on HDL-C in High cholesterol diet rats.

Each value is mean \pm S.E.M. (n = 6) .Data was analyzed by One-way ANOVA followed by Dunnett's test. *Significantly compared to Hyperlipidemic control group ($p < 0.05$). **Significantly compared to Hyperlipidemic control group ($p < 0.01$).

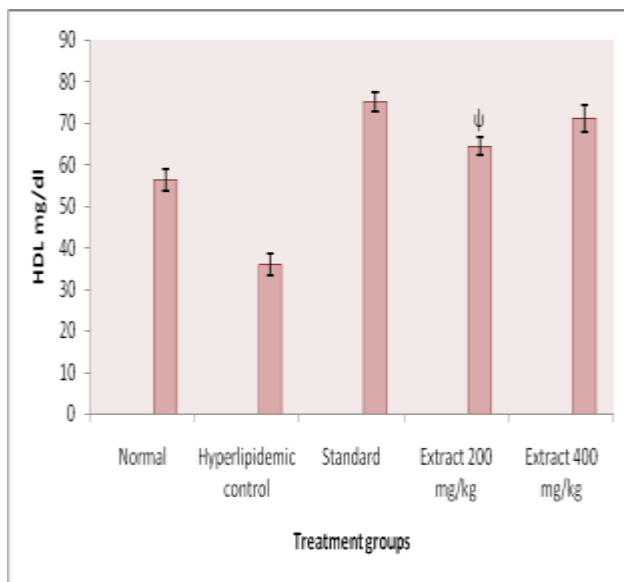


Fig. 6: Effect of *Abroma augusta* extract on HDL-C in High cholesterol diet rats.

Each value is mean ± S.E.M. (n = 6) .Data was analyzed by One-way ANOVA followed by Dunnett’s test. ψ Significantly compared to Standard group (p<0.05).

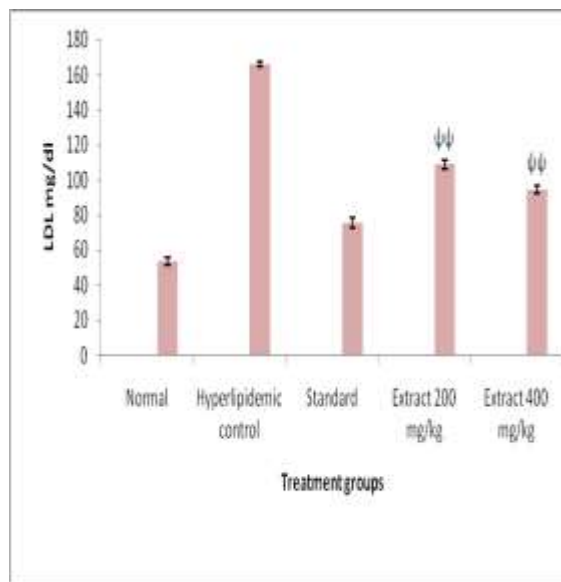


Fig. 8: Effect of *Abroma augusta* extract on LDL in High cholesterol diet rats.

Each value is mean ± S.E.M. (n = 6) .Data was analyzed by One-way ANOVA followed by Dunnett’s test. ψ Significantly compared to Standard group (p<0.05). ψψ Significantly compared to Standard group (p<0.01).

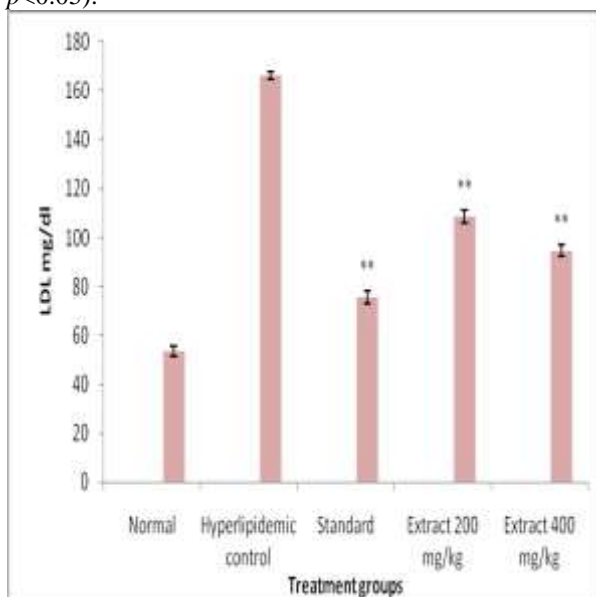


Fig. 7: Effect of *Abroma augusta* extract on LDL in High cholesterol diet rats.

Each value is mean ± S.E.M. (n = 6) .Data was analyzed by One-way ANOVA followed by Dunnett’s test. *Significantly compared to Hyperlipidemic control group (p<0.05). **Significantly compared to Hyperlipidemic control group (p< 0.01).

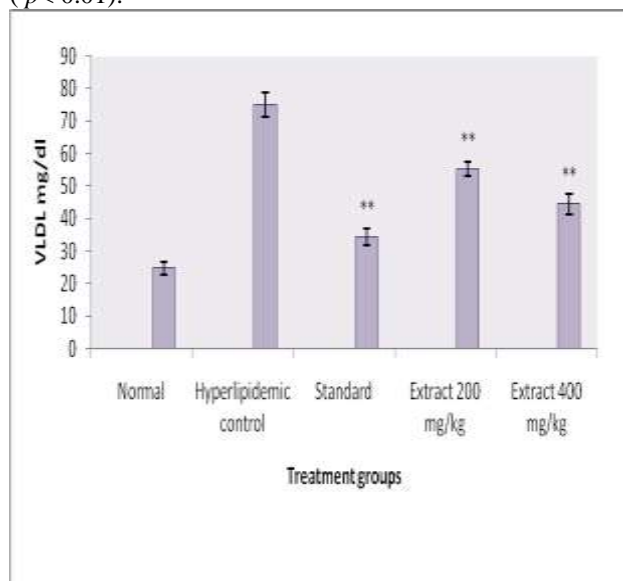


Fig. 9: Effect of *Abroma augusta* extract on VLDL in High cholesterol diet rats.

Each value is mean ± S.E.M. (n = 6) .Data was analyzed by One-way ANOVA followed by Dunnett’s test. *Significantly compared to Hyperlipidemic control group (p<0.05). **Significantly compared to Hyperlipidemic control group (p< 0.01).

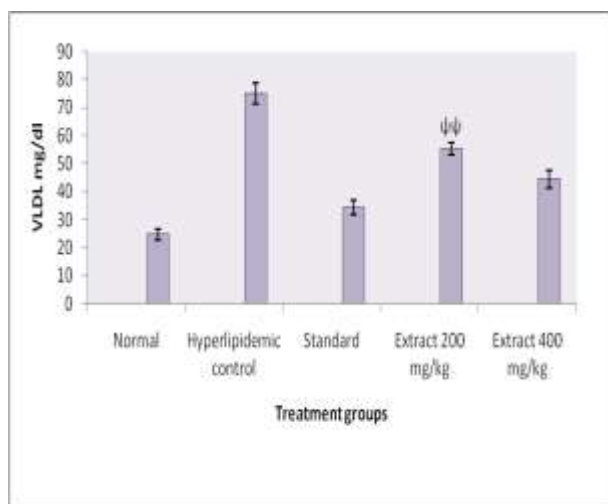


Fig. 10: Effect of *Abroma augusta* extract on VLDL in High cholesterol diet rats. Each value is mean \pm S.E.M. (n = 6) .Data was analyzed by One-way ANOVA followed by Dunnett's test. ψ Significantly compared to Standard group ($p < 0.05$). $\psi\psi$ Significantly compared to Standard group ($p < 0.01$).

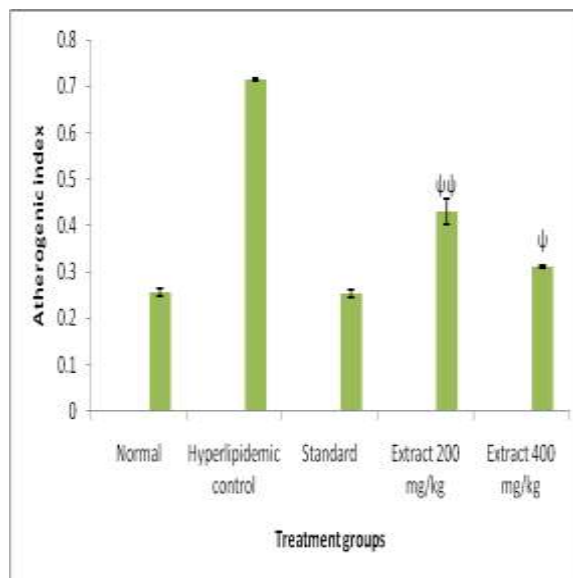


Fig. 12: Effect of *Abroma augusta* extract on Atherogenic Index in High cholesterol diet rats. Each value is mean \pm S.E.M. (n = 6) .Data was analyzed by One-way ANOVA followed by Dunnett's test. ψ Significantly compared to Standard group ($p < 0.05$). $\psi\psi$ Significantly compared to Standard group ($p < 0.01$).

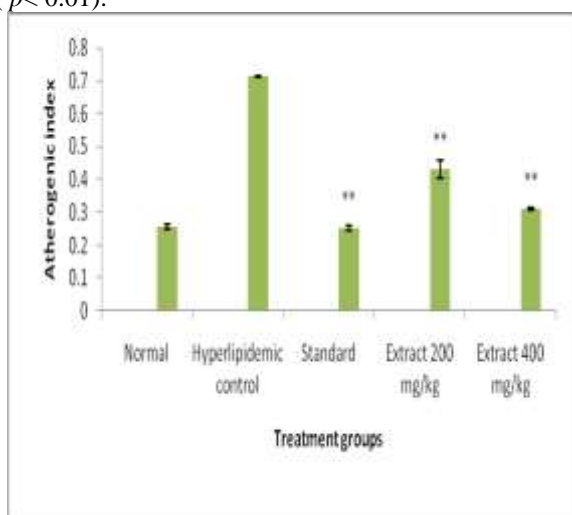


Fig. 11: Effect of *Abroma augusta* extract on Atherogenic Index in High cholesterol diet rats. Each value is mean \pm S.E.M. (n = 6) .Data was analyzed by One-way ANOVA followed by Dunnett's test. *Significantly compared to Hyperlipidemic control group ($p < 0.05$). **Significantly compared to Hyperlipidemic control group ($p < 0.01$).

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