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## EVALUATION OF ANTIHYPERLIPIDAEMIC ACTIVITY OF ARTOCARPUS HETEROPHYLLUS

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### ABSTRACT:

Plants have been one of the important sources of medicines since the beginning of human civilization. *Artocarpus Heterophyllus* Linn. Belonging to family Moraceae, commonly found in India. The plant is used as anaemia, asthma, dermatitis, expectorant etc. The antihyperlipidemic activity of alcoholic extract of bark of *Artocarpus Heterophyllus* was evaluated using high-cholesterol diet model and triton-induced hyperlipidemia model in Wistar rats. In this model, five groups (six animals per group) of animals were used. Group I-served as normal control and were given only vehicle (distilled water); Group II- received high fat diet served as hyperlipidemic control; Group III-received atorvastatin 10 mg/kg served as standard drug; Group IV-received 200 mg/kg AAHP and Group V-received 400mg/kg of AAHP. Serum cholesterol and triglycerides (TGs) level are estimated for the evaluation of antihyperlipidemic effects of extracts. The ethanolic extract has shown a significant reduction in serum cholesterol and TGs level indicating antihyperlipidemic potentials in the plant. In this high-cholesterol diet model and triton-induced hyperlipidemia model, serum and TG levels were analyzed ethanolic extract was shown antihyperlipidemic activity. Further clinical studies are compulsory to confirm the findings.

**Keywords:** *Artocarpus Heterophyllus*, Antihyperlipidemic, High-density lipoprotein, Low- density lipoprotein, Triglyceride, Total cholesterol

## INTRODUCTION

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases [1]. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death [2]. Although many efficacious lipid-lowering synthetic drugs exist, none is effective for all lipoprotein disorders, and all such agents are associated with some adverse effects. Therefore, it is a need of the day to search other materials from natural sources that are less toxic, less expensive, which can provide better safety and efficacy on a long term usage. Natural products from plants are a rich source used for centuries to cure various ailments. [3]

Plants are well known in traditional herbal medicine for their hyperlipidaemic activities, and available literature indicates that there are more than 800 plant species showing hyperlipidaemic activity. [4] *Artocarpus heterophyllus* (Jackfruit) is a plant that belongs to the family Moraceae. Jackfruit is generally grown in Sri Lanka, Bangladesh, Burma, Philippines, Indonesia, Thailand, Malaysia and Brazil being the tropics countries. Jackfruits is a seasonal fruit easily available in summer season. [5, 6] It is a large evergreen tree varies with 10- 30m tall, having long tap root and the crown is dense making it the largest tree borne fruit in the world. Jackfruit seeds are boiled and included in the diets which have 77% starch content. [7] The *Artocarpus heterophyllus* contains various chemical constituents as several flavones colouring matters, morin, dihydromorin, cynomacurin, artocarpin, isoartocarpin, cyloartocarpin, artocarpesin, oxydihydroartocarpesin, artocarpetin, norartocarpetin, cycloartinone and artocarpanone. [8, 9] The various parts of the plants have different actions such as the fruit pulp and seeds are used as cooling tonic, roots are used in diarrhoea, leaves are used to increase lactation, the ash of the leaves are used for treating wounds caused due to ulcer. The bark stem is used in anaemia, asthma, dermatitis, and expectorant. [10, 11] Based on above medicinal properties of *Artocarpus heterophyllus*, the present study was conducted to investigate the antihyperlipidemic activities of ethanol extract of *Artocarpus heterophyllus* bark.

## MATERIALS AND METHODS

**Plant Material and Animals:** The fresh bark of *Artocarpus Heterophyllus* used for the present studies. Plant collection and extraction *Artocarpus heterophyllus* were collected from the regions of Gwalior, M.P. After that the plant parts bark were coarsely powdered and subjected to successive solvent extraction using soxhlet apparatus.

Wistar rats (150 –250 g) used for the study. The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatisation to the laboratory conditions. The animals were housed three per cage in a polypropylene cage and maintained in standard laboratory conditions with free access to food and water ad libitum. The experiments were performed after approval of the protocol by the minute of Institutional Animal Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

**Chemicals, Drugs and Instruments:** All the major chemicals used in the study like Nitro blue tetrazolium (NBT), Riboflavin, EDTA, Phosphate buffer, DPPH, Triton. The weighed coarse powder was used for the extraction by successive solvent extraction by Soxhlet apparatus using various solvents.

**Preparation of Alcoholic Extract of *Artocarpus Heterophyllus* bark:** The bark was initially collected from the plant and rinsed with distilled water and shade dried and then homogenized into fine powder and stored in air tight bottles. A total of 10 g of air dried powder was weighed and was placed in 100 mL of organic solvents (methanol and ethanol) in a conical flask and then kept in a rotary shaker at 190-220 rpm for 24 h. And then it was filtered with the help of muslin cloth and centrifuged at 10 000 rpm for 5 min. The supernatant was collected and the solvent was evaporated by solvent distillation apparatus to make the final volume of one-fourth of the original volume, giving a concentration of 40 mg/mL. It was stored at 40 °C in air tight bottles for further studies. [12, 13]

**Phytochemical analysis:** Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. Phyto constituents are the contributors of pharmacological activities of a plant. The dried powdered sample is subjected to qualitative tests for identification of various plant constituents. Qualitative tests were performed for the determination and identification of secondary metabolites of plant. Secondary metabolites were responsible for therapeutic use. The ethanolic and aqueous extracts of leaves of *A. aspera* were subjected to qualitative examination for different phytoconstituents such as alkaloids, carbohydrates, flavonoids, glycosides, saponins, terpenoids, and steroids using standard methods. [14-15]

**Anti-hyperlipedemic Activity High-Cholesterol diet model:** Wistar rats weighing 150-180 gm, were divided into 5 groups of 6 animals each. Group I-served as normal control and were given only vehicle (distilled water); Group II- received high fat diet served as hyperlipedemic control; Group III-received atorvastatin 10mg/kg served as standard drug; Group IV-received 200mg/kg AAHP and Group V-received 400 mg/kg of AAHP. [16]

Method [17] with modification was used to produce high fat diet induced hyperlipidemia. Normal animal food pellets were crushed in mortar and pestle to crush into small pieces and then grinded into fine powder in mixer grinder. The other ingredients i.e. cholesterol 12%, Cholic acid 1%, sucrose 40% , and coconut oil 10% were added in the mixer grinder in an ascending order of their quantity and mixed well. This dried powder was then mixed with same quantity of water every time to make small balls of feed and later this was stored in self- sealing plastic covers in refrigerator at 2°C to 8°C. The feed for normal group was prepared similarly by grinding only the normal food pellets and then mixing with water without the other excipients. This preparation of feed was done once in three days for all the animals. The animals were administered with the high fat diet for 30 days. Check the serum blood cholesterol levels.

**Triton induced hyperlipidemic rats:** 30 Wistar rats were randomly divided into 5 groups of 6 each. The first group was given standard pellet diet, water and orally administered with 5% CMC. The II, III, IV, V group animals were injected i.p. with 10% aqueous solution of Triton 100mg /kg body weight. After 72 hours of triton injection, the second group received a daily dose of 5% CMC (p.o) for 7 days. The third group was administered with the standard Atorvastatin 10mg/kg,p.o. for 7 days, fourth and fifth group was administered a daily dose of AAHP 200 and 400 mg/kg suspended in 5% CMC, p.o., for 7 days, after inducing hyperlipidemia. Food was withdrawn 10h prior to the blood sampling. The control group animals received the vehicle in the same volume orally. [18]

**Biochemical analysis of serum:** Serum samples were analysed for total cholesterol, High density lipoproteins, Low density lipoproteins and very low density lipoproteins using standard enzymatic assay kit. [19]

#### **Estimation of lipids:**

**Total cholesterol:** Cholesterol in serum was estimated by using an Ecoline Diagnostic Kit. The absorbance of the sample and of the standard was measured against the reagent blank value at 546nm. Cholesterol level in serum was expressed as mg/dL.

**Triglycerides:** Triglycerides level in serum was estimated using Ecoline Diagnostic Kit. The absorbance of the sample and of the standard was measured against the reagent blank value at 546nm. Triglyceride level in serum was expressed as mg/dL.

**HDL cholesterol:** The cholesterol was separated from serum after precipitation of LDL cholesterol by phosphotungstic acid precipitating reagent. The supernatant after centrifugation was estimated using Ecoline Diagnostic Kits. The absorbance of the sample and of the standard was measured against the reagent blank value at 546nm. HDL cholesterol level in serum expressed as mg/dL. [20, 21]

**LDL and VLDL cholesterol:** LDL and VLDL cholesterol were calculated by using the formula: [22]

LDL cholesterol = Total cholesterol – [HDL cholesterol – Triglycerides/5].

VLDL cholesterol = Total cholesterol – HDL cholesterol – Triglycerides- LDL.

**Statistical Analysis:** Data were statically analysed as mean + SEM and expressed as just significant P<0.05 and significant P<0.01 as the case may be using one way ANOVA followed by Dunnett’s multiple comparison test. [23, 24]

**RESULTS AND DISCUSSION**

**Extraction of Artocarpus heterophyllus Bark:** The percentage yield of the Artocarpus Heterophyllus bark was found to be 1.03%w/v.

**Table 1: Extraction of Artocarpus Heterophyllus Bark**

Plant & Part used	Method of Extraction	Solvents	Percentage Yield (%W/V)
<i>Artocarpus Heterophyllus</i> Bark	Maceration	Ethanol (95%)	1.03

**Preliminary phytochemical screening:** Alcoholic Extract of Artocarpus Heterophyllus bark (AAHB) was subjected various chemical tested as per the standard methods for the identification of the various constituents. The result if this phyto-chemical analysis is listed below.

**Table 2: Qualitative phytochemical screening of AAHB**

S. No.	Plant Constituent	Extract
1	Carbohydrate	-
2	Alkaloids	+
3	Flavonoids	+
4	Proteins and amino Acids	+
5	Glycosides	-
6	Fixed oil	+
7	Terpenoids	+
8	Volatile oil	-
9	Tannins	-

“+”Presence,“-” Absence.



**Figure 1: Qualitative phytochemical screening of AAHB**

**Anti-hyperlipedemic Activity:** Hyperlipidemia is associated with heart disease, which is the leading cause of death in the world. The results are discussed under the lipid profile in serum and the lipid profile in liver. Lipid profile in serum and liver indicates that increased phospholipids (PL), triglyceride (TG) and cholesterol levels were significantly reduced by treatment of 200 and 400 mg/kg of AAHB. LDL and VLDL levels were significantly

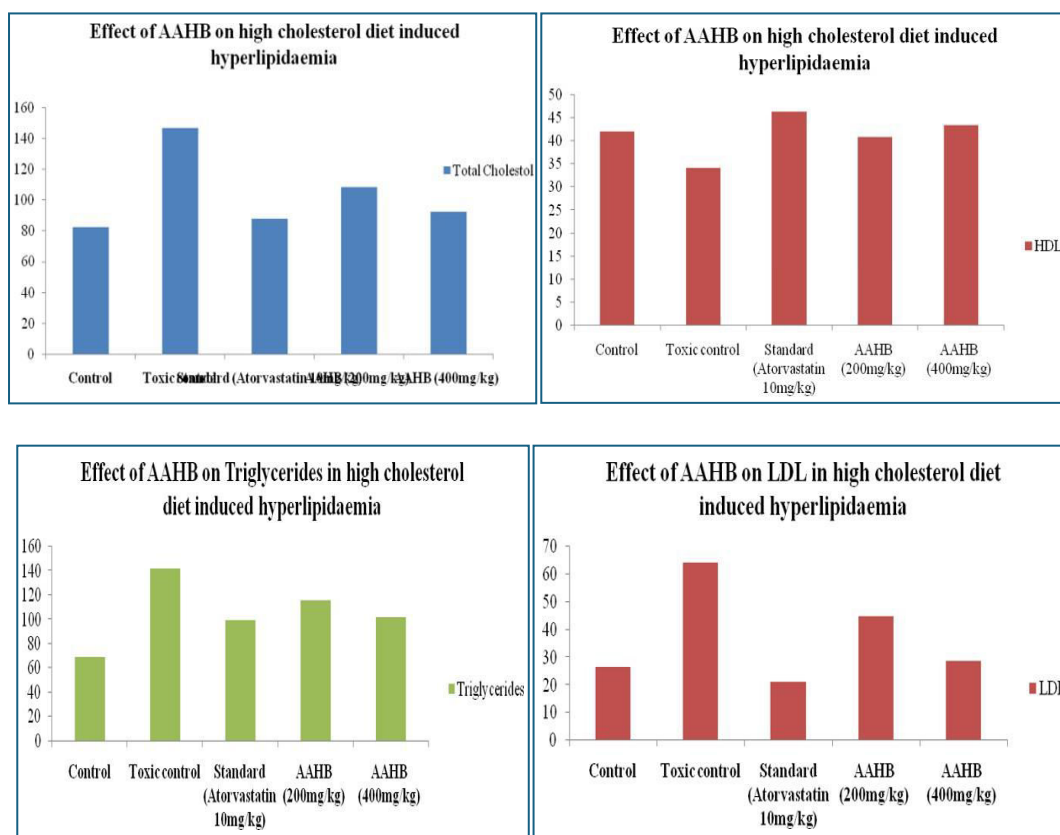
increased in triton-injected animals to control rats.

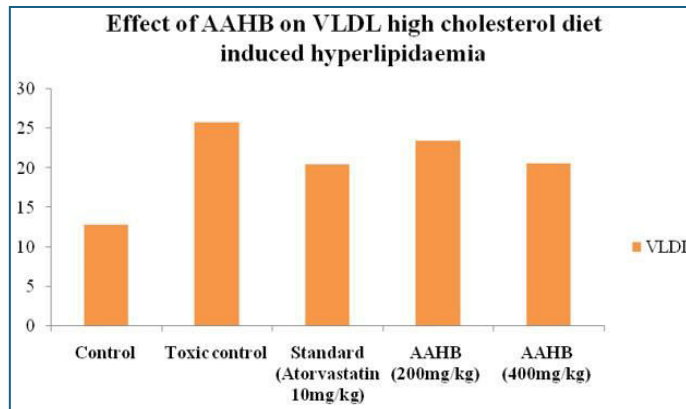
**High–Cholesterol Diet Model:** In High–Cholesterol Diet Model induced study results shows serum lipid parameters in animals were significantly reduced ( $p < 0.01$ ,) by 30 days treatment with AAHP at dose levels 200 mg/kg and 400 mg/kg, when compared with control group 400 mg/kg of AAHP group animals has shown significant ( $p < 0.001$ ) compared with control group. At this time, an increased level of HDL was also observed.

**Table 3: Effect of AAHB on Lipid Profile in high-cholesterol diet induced hyperlipidaemia**

Group	Total Cholesterol (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Control	82.1±3.79	42.12±1.34	68.89±2.12	12.79±0.42	26.39±3.55
Positive control	146.72±2.5	34.19±2.82	142.18±3.80	25.83±0.75	64.35±2.82
Atorvastatin 10mg/kg	87.92±1.76	46.34±2.02	99.1±2.35***	20.53±0.64	21.23±2.42**
200mg/kg AAHP	108.42±2.35	40.81±2.40	116.02±2.10**	23.5±0.42	44.73±2.33
400mg/kg AAHP	92.35±1.63**	43.49±2.18***	101.06±2.89	20.6±0.60	28.59±2.02**

Values were mean ±SD (n=6). Values are statistically significant at \* $P < 0.05$  and more significant at \*\* $P < 0.01$ , \*\*\* $P < 0.001$  Vs hyperlipidemic control using one way ANOVA followed by Dunnet’s test.





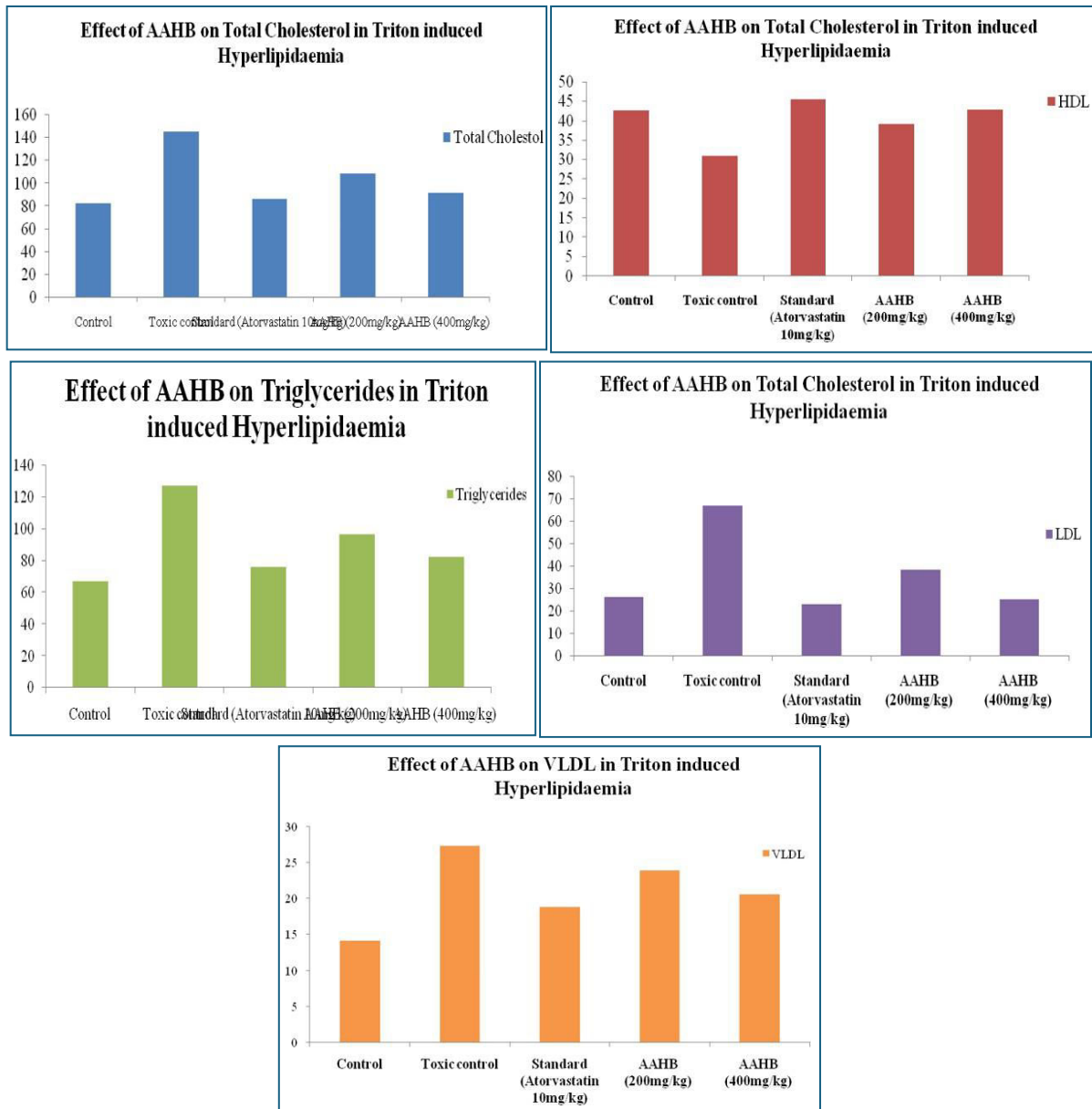
**Figure 2: Effect of AAHB on Lipid Profile in high-cholesterol diet induced hyperlipidaemia**

**Effect of Alcoholic Extract of Artocarpus Heterophyllus bark on lipid profile in Triton induced hyperlipidemia:** In triton induced study results shows serum lipid parameters in animals were significantly reduced ( $p < 0.01$ ) by seven days treatment with AAHP at dose levels 200 mg/kg and 400 mg/kg, when compared with control group 400 mg/kg of AAHP group animals has shown significant ( $p < 0.001$ ) compared with control group. At this time, an increased level of HDL was also observed.

**Table 4-Effect Of AAHB on Lipid Profile Triton Induced hyperlipidemia**

Group	Total Cholesterol (mg/dl)	HDL (mg/dl)	Triglycerid e s (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Control	82.63±0.20	42.70±0.69	67.23±0.78	14.04±0.50	26.09±0.95
Positive control	145.16±2.25	31.04±2.33	127.56±2.54	27.31±0.99	66.99±1.93
Atorvastat i n 10mg/kg	86.40±0.91** *	45.40±0.70	76.07±0.53	18.75±0.34* *	22.74±0.59
200mg/kg AAHP	108.56±0.94	39.12±0.52	96.15±0.62*	23.88±0.5	38.35±0.61
400mg/kg AAHP	91.36±1.72** *	42.79±0.91* *	82.18±0.86	20.5±0.94	25.09±0.73* *

Values were mean ± SD (n=6). Values are statistically significant at \* $P < 0.05$  and more significant at \*\* $P < 0.01$ , \*\*\* $P < 0.001$  Vs hyperlipidemic control using one way ANOVA followed by Dunnet’s test.



**Figure 3: Effect Of AAHB on Lipid Profile Triton Induced hyperlipidemia**

**CONCLUSION**

The preliminary phytochemical screening revealed the presence of sterols, flavonoids, polyphenolics and fixed oil in ethanolic extract and sterols, proteins, polyphenolics, pectin’s in the aqueous extract. Ethanolic extract showed decreased blood lipids in hyperlipidemic rats when compared to normal and standard groups. The bark extracts shows a decreased in the TC, TG, and LDL and an increase in HDL in biphasic model of the Triton induced hyperlipidaemia rats, Diet induced hyperlipidemic rats and also in Normocholesteremic rats. The present study concludes that the extracts of Artocarpus heterophyllus bark possess significant anti-hyperlipidemic activity.

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