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RESEARCH ARTICLE

Evaluation of Anti-hyperlipidemic Activity of Ethanolic Extract of *Elaeocarpus angustifolius* Blume Leaves in Albino Wistar Rats

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Abstract

Backgrounds: Hyperlipidemia is traditionally defined as conditions in which the concentration of cholesterol or triglyceride-carryingnlipoproteins in plasma exceeds an arbitrary normal limit. *Elaeocarpus angustifolius* Blume (*Elaeocarpus ganitrus*) is an average-sized evergreen tree with a various biological activity. The present study aimed to evaluate the antihyperlipidemic activity of ethanolic extract of *E. angustifolius* Blume leaves in albino Wistar rats. **Materials and Methodology:** The investigation was intended to scrutinize the anti-hyperlipidemic activity of extract which is ethanolic of EAB in high-fat fructose-fed hyperlipidemic rats. The EEEAB was managed at an amount level of 125, 250, and 500mg/kg p.o for 21 days in hyperlipidemic rats and atorvastatin was set as standard control. At the end of the 21 days, samples of blood were poised by the orbital retro and the profile of lipids was determined. For antioxidant activity *in vitro* of EEEAB was judged by using Hydrogen peroxide (H_2O_2) radical scavenging analysis. **Result:** The high-fat fructose diet-treated group exhibited hyperlipidemia with increased lipid concentrations along with an increase in body weight and downfall in temperature in contrast to the group regarded as control. EEEAB showed a decrease in the lipid levels ($p<0.001$, $p<0.01$, $p<0.05$) in a dose dose-dependent manner in treated rats and the HDL ratio improved after administration with EEEAB. For the *in vitro* study, the IC_{50} worth of EEEAB was calculated to be 22µg/ml. **Conclusion:** The complete experimental outcomes suggested that the pharmacological operative phytoconstituent for instance flavonoids present in the EEEAB exhibited significant antihyperlipidemic activity on hyperlipidemic rats.

Keywords: Antihyperlipidemic, Atorvastatin, *Elaeocarpus angustifolius*, Hydrogen Peroxide

1. Introduction

The atherosclerotic coronary disease continues to be the primary cause of death in both developed and developing nations despite ongoing advances in therapeutic interventional and surgical interventions[1]. Dyslipidaemia and the atherosclerosis it causes are believed to be caused by an imbalance of lipid metabolites in the afflicted organism. Hyperlipidemia, including raised levels of Total cholesterol, Triglycerides, and low-density lipoprotein as well as a drop in high-density lipoprotein cholesterol, is a major contributor to atherosclerosis and coronary heart diseases[2].

Depending on the variation in lipid and Apo lipoprotein composition as well as density, lipoprotein can be divided into 5 major types Triglycerides, Total

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cholesterol, High-density lipoproteins, Low-density lipoproteins and very low-density lipoprotein are involved in the regulation of microvascular function[3].

The usual standard profile of lipids is shown in Table 1.

Elaeocarpus angustifolius Blume (Figure 1), a sizable evergreen tree with broad leaves, fits in the Elaeocarpaceae family. It is known as Rudraksha in Hindi and is well-known for its intriguing fruit stones and therapeutic powers[4]. According to a thorough review of the literature, *E. angustifolius* Blume is a crucial source of many pharmacologically and medicinally important chemicals, including essential triterpenes, tannins like geraniin and 3, 4, and 5-trimethoxy geraniin, indolizilidine alkaloids grandisines, rudrakine, and flavonoids quercitin[5].

According to our review of the literature, no scientific research has been done to determine the effectiveness of *E. angustifolius* Blume in decreasing cholesterol. Against this backdrop, the current investigation was conducted to evaluate the antioxidant properties of *E. angustifolius* Blume *in vitro* and it's capacity to lower lipid levels in hyperlipidemic rats[6].

2. Materials and Methods

2.1 Collection and Authentication of Plant

The herb was procured from the SGRR University of Herbal Garden, School of Pharmaceutical Sciences, Dehradun, and it was recognized by the (BSI) Botanical Survey of India, Dehradun, under the identification number BSI/NRC Tech./Herb(ident.)/2022-23/110 and Account no. 903.

2.2 Plant Material Extraction for Research

Leaves of the herb were collected, cleaned and dried in air at 25°C using an oven, mesh with a blender to form powder and covered in a closed container to avoid sunlight and moisture. Maceration can be used as an extraction method using ethanol solvent. 200gm of dried powder was kept in a maceration container, addition of 70% ethanol and it was left for 7 days. Then the mixture was clarified using Whatman filter paper[7].

2.3 Preliminary Phytochemical Screening[8]

Phytoconstituents such as alkaloids, flavonoids, saponins, steroids/terpenoids, tannins, glycosides etc.,

Table 1. Profile of lipid normal values

Parameters	Desirable	Borderline	High risk
Total Cholesterol	Range <200 mg/dl	Range 200-239 mg/dl	Range 240 mg/dl
Triglycerides	Range <150 mg /dl	Range 150-199 mg/dl	Range 200-499 mg/dl
Cholesterol HDL	Range 60 mg/dl	Range 35-45 mg/dl	Range <35 mg/dl
Cholesterol LDL	Range 60-130 mg/dl	Range 130-159 mg/dl	Range 160-189 mg/dl
Cholesterol/ HDL	Ratio 4.0	5.0	6.0

Figure 1. *Elaeocarpus angustifolius* Blume tree and leaves.

were tested for their presence and are absent in the ethanolic extract.

2.4 Procurement of Experimental Animals

Albino Wistar rats of either sex, weighing 110 and 160 gm, were acquired from the departmental animal residence at SGRR University in Dehradun. The Institutional Animal Ethical bureau (IAEC) gave its acceptance for the experimental protocol, which was carried out by CPCSEA regulations under the designation 264/CPCSEA/IAEC/2022/09.

2.5 Housing Conditions

Animals were kept in a regular laboratory environment with a series of days for 12 hours and 12 nights of hours (temperature 22–20 °C, humidity 50–15 %). The creatures were assumed a standard workshop diet and agreed on unlimited entrees to water. As instructed by the CPCSEA (Committee for Control and Supervision of Experiments on Animals), each of the protocols was carried out analogously along the Institutional Animal Ethical Committee (IAEC).

2.6 Induction of Hyperlipidemia

Animals of all groups except normal control were administrated with high fat (vanaspati ghee: coconut oil in ratio of 3:1) mixed with animal food diet and 10% fructose in water for 21 days for induction of hyperlipidemia[9-11].

2.7 Acute Toxicity Study

Extract of EAB (also called *Elaeocarpus ganitrus*) was shown to be non-toxic and did not result in any mortality in mice up to 5.0g/kg orally based on the LD50 value. The LD50 value is therefore >5.0g/kg of body weight[12].

2.8 Experimental Design

36 animals of unisexual were selected and split up into 6 groups having 6 animals per group.

- Group I: Rats were served with a normal pellet diet and distilled water.
- Group II: Rats were served a high-fat diet and a 10% fructose diet for 21 days.
- Group III: Rats were served a high-fat diet and 10% fructose + leaves extract (125 mg/kg/day) for 21 days.
- Group IV: Rats were served a high-fat diet and 10% fructose + leaves extract (250 mg/kg/day) for 21 days.
- Group V: Rats were served with 10% fructose and high-fat diet + leaves extract for 21 days (500 mg/kg/day).
- Group VI: Hyperlipidemic rats were orally administered with atorvastatin having 10 mg/kg dose.

2.9 Evaluation Parameters

- **Weight of the body:** The weight of the body (gm) of rats was noted on 0, 11 and 21 days of the study period using electronic weighing balance and changes in body weight were noted.
- **Body temperature:** The body temperatures of each rat in each group were measured on days 0, 11, and 21 after drug administration with a contact time of one minute, using a digital temperature indicator. The average reading obtained from six rats was then calculated for each group.

2.10 Collection of Blood

The blood samples were taken using a retroorbital, under anaesthesia at the end of the 21 days. The serum was then segregated by process of centrifugation after 30 minutes and for biochemical examination set aside at -20° C.

2.11 Biochemical Analysis

Levels of the serum for high-density lipoprotein, total cholesterol, triglycerides, very small-density lipoprotein and low-density lipoprotein were estimated utilizing the formula: VLDL- TG/5, which was obtained from the lipid profile kit.

2.12 Assay *in vitro* Antioxidant

2.12.1 Hydrogen Peroxide Scavenging (H_2O_2) Assay

In a 50 mM buffer of phosphate having a pH value of 7.4, H_2O_2 (40mM) is produced as a solution. A spectrophotometer is used to calculate the hydrogen peroxide content based on absorbance at 230nm. After adding extract (20-60 g/mL) to hydrogen peroxide for 10 minutes, at 230 nm the absorbance was calculated in contrast to phosphate buffer a blank solution made up without hydrogen peroxide. According to the formula below, the amount of hydrogen peroxide scavenging is:

[(Ai-At)/Ai] x 100 = % scavenged (H2O2).

where, At is the test absorbance and Ai is the absorbance of the control[12].

2.13 Statistical Analysis

Graph pad Prism 9.5.0 software was utilized for the analysis of statistical data. All the outcomes were represented as mean ± SEM, analysed for ANOVA and Tukey's post hoc test was also done.

3. Results

3.1 Preliminary Phytochemical Screening

Phytoconstituents such as alkaloids, flavonoids, saponins, steroids/terpenoids, and tannins are present and glycosides are absent in the ethanolic extract of *E. angustifolius* (Table 2).

Table 2. Preliminary Phytochemical screening of *Elaeocarpus angustifolius* Blume leaves in ethanol

Phytochemical Investigation	Secondary metabolite Test	Extract (ethanolic)
Alkaloids compounds	Dragendroff"s Test, Wagner's test	+
Flavonoids compounds	Shinoda Test, Alkaline reagents	+
Glycosides compounds	Legal Test, Keller-Killani test	–
Saponins compounds	Saponin Test, Hemolytic Test	+
Steroids/ Terpenoids compounds	Shinoda Test, Salkowski's test	+
Tannins compounds	"Gold Beater" Ox Skin Test, Ferric chloride test	+

(+) present, (-) not present

3.2 Body Weight

3.2.1 Effect of Ethanolic Extract of EAB on Body Weight of Animals

The body weight of rats was observed to significantly increase as a result of an increase in body fat content after consumption of an increased fat fructose diet. Animals fed on increased Fat-fructose diet levels significantly their body weight when contrasted to the control group shown in Table 3.

The standard drug used Atorvastatin showed a significant decrease in the weight of the body whereas the ethanolic extract displayed a reasonable decrease in body weight of rats.

3.3 Body Temperature

3.3.1 Effect of Ethanolic Extract of EAB on Body Temperature of Animals

Diet-induced thermogenesis in the obese animal model will decline as sympathetic initiation of brown-coloured adipose tissue declines. The neurotransmitter NPY, which is generated throughout the brain, is the primary cause of this reduction in diet-tempted thermogenesis. Within 10 to 15 minutes of its endogenous release, NPY promotes a decrease in thermogenesis as well as an increase in food intake. By lowering the beginning of brown-coloured adipose tissue, NPY suppresses thermogenesis.

Accordingly, a substantial decrease in the body's temperature was observed in High-fat fructose diet-fed rats after being associated with control. The standard drug, i.e., Atorvastatin significantly overturned the reduction in the temperature of the body thus viewing an important increase in temperature shown in Table 4.

Table 3. Result of EEEAB on the weight of the body in hyperlipidaemic rats

S. No.	Group	Body weight		
		0 Day	11 Day	21 Day
1	Control	113.24±1.15	113.92±3.67	115.72±0.92
2	HFD-FD	131.36±1.63	136.42±0.91	144.83±1.40
3	HFD-FD + Atorvastatin (10mg/kg)	184.77±0.63	179.70±1.47	173.09±2.45
4	HFD-FD +Ethanolic extract (125mg/kg)	177.59±1.11	176.11±0.66	175.53±3.48
5	HFD-FD + Ethanolic extract (250mg/kg)	144.83±1.40	142.10±1.24	140.69±2.93
6	HFD-FD+ Ethanolic extract (500mg/kg)	177.11±0.66	174.53±3.48	171.78±1.54

Data are represented in Mean± SEM, n=6 (no. of six animals).

Table 4. Effect of EEEAB on the temperature of the body in hyperlipidemic rats

S. No.	Group	Body Temperature		
		0 Day	11 Day	21 Day
1	Control	39.00±0.57	39.50±0.29	39.65±0.33
2	HFD-FD	45.81±0.40	43.8±0.57	40.51±0.56
3	HFD-FD + Atorvastatin(10mg/kg)	39.01±0.47	42.4±0.47	45.81±0.40
4	HFD-FD + Ethanolic extract (125mg/kg)	43.73±0.32	44.03±0.42	44.60±0.89
5	HFD-FD + Ethanolic extract (250mg/kg)	41.09±0.39	42.3±0.46	43.28±0.73
6	HFD-FD + Ethanolic extract (500mg/kg)	38.06±0.50	39.38±0.53	41.60±1.35

Information is uttered in Mean± SEM, where n=6 (six animals in number).

3.4 Biochemical Analysis

3.4.1 Outcome of Ethanolic Extract of Elaeocarpus angustifolius Blume Leaves on Lipid Profile in Hyperlipidemic Rats

Administration of HFD-FD significantly ($p<0.001$, $p<0.01$) increases the lipid level of TC, TGs, LDL, and VLDL sideways with low heights of HDL in comparison to the controller group (Figure 2). However, treatment through standard medicine caused a decrease in lipid level lengthways with modest elevation in HDL.

The EEEAB (125mg, 250mg, 500mg) pointedly ($p<0.05$, $p<0.01$, $p<0.001$) reduced the lipid level and increased the HDL level as compared to the ailment regulator group. The result of EEEAB (500mg) was analysed and designated better than the outcome of EEEAB (125mg and 250mg) shown in Table 5.

3.5 Antioxidant Potential of *Elaeocarpus angustifolius* Blume

3.5.1 Antioxidant Potential of Extract of Ethanol in Elaeocarpus angustifolius Blume by H_2O_2 Radical Scavenging Assay

Ethanolic extract of *Elaeocarpus angustifolius* Blume showed good antioxidant activity by assay hydrogen

Table 5. Effect of EEEAB on lipid levels

Group (mg/kg dose)	Level of Cholesterol (mg/dl)	Level of Triglyceride (mg/dl)	Level of VLDL (mg/dl)	Level of LDL (mg/dl)	Level of HDL (mg/dl)
Control	81.54±2.99	74.04±2.97	14.81±0.59	23.25±3.56	48.58±1.327
HFD-FD	120.9±2.82 a***	132.3±2.57 a***	26.47±0.51 a**	71.51±2.16 a***	41.12±1.58 a***
HFD-FD+ Atorvastatin (10mg/kg)	84.79±2.63 b***	92.63±2.94 a***, b***	18.53±0.58 a***, b***	26.70±2.86 b***	48.41±0.91 b***
HFD-FD+ Ethanolic extract (125mg/kg)	109.7±2.82 a***, b**, c***	121.9±3.79 a***, b*, c***, d***	24.39±0.75 a*, b***, c***	60.50±3.29 a***, b*, c***	41.04±1.63 a***, b**, c***
HFD-FD+ Ethanolic extract (250mg/kg)	101.9±2.73 a***, b***, c***, d***	107.4±1.84 a***, b***, c***, d***	21.47±0.36 a***, b***, c**	53.04±1.61 a***, b***, c***, d***	40.70±1.41 a***, b***, c***, d***
HFD-FD+ Ethanolic extract (500mg/kg)	88.55±2.68 b***, c***, d***	97.15±2.65 a***, b***, c**, d*	19.43±0.53 a***, b***, c**, d**	32.77±2.96 a*, b***, c***, d***	46.48±1.25 a***, b***, c**, d***

Values are denoted as Mean ± SEM (n=6); statistics were interpreted by ANOVA followed by Tukey's post hoc test (Graph pad Prism 9.5.0 software was used.

Significant values are expressed as ***p<0.001, **p<0.01, *p<0.05

a = Difference in significance comparison with the control group.

b= Difference in significance comparison with the hyperlipidaemic control group.

c= Difference in significance comparison drug with standard group.

d= Difference in significance comparison with drug-treated tested groups.

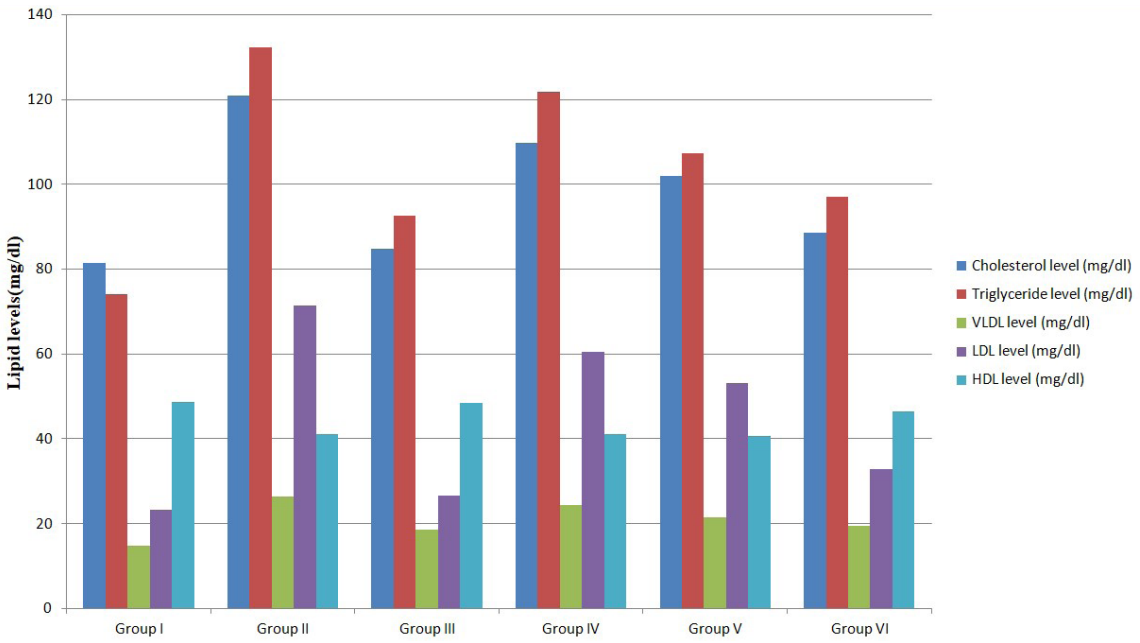

Figure 2. Effect of EEEAB on lipid levels.

peroxide with IC_{50} value 22µg/ml (Figure 3). Ascorbic acid was used as a reference standard showed the IC_{50} value 19µg/ml with hydrogen peroxide assay shown in Table 6.

Table 6. Effect of Antioxidant activity of EEEAB by H_2O_2 radical activity scavenging

S. No	Compounds	Conc. (µg/ml)	Inhibition %	Value IC_{50} (µg/ml)
I	EEEAB	10 20 30 40 50	20.05±0.863 48.47±0.901 57.87±0.823 64.30±0.063 70.95±0.238	22
II	Ascorbic acid	10 20 30 40 50	32.97±0.273 52.57±0.402 60.67±0.828 68.71±0.240 73.25±0.113	19

Data are expressed in mean ±SEM

4. Discussion

Despite constant improvements in healing interventional and therapies operating for the healing of coronary disease known as atherosclerotic, the latter continues to be the foremost reason for death in both the developed and developing worlds. Atherosclerosis and dyslipidemia are thought to occur from an imbalance of lipid metabolites in the diseased organism. The appearance and progression of CHDs) coronary heart diseases (and atherosclerosis are significantly influenced by hyperlipidaemia, which is primarily categorized by growth in whole), Low-Density Lipoprotein (LDL) cholesterol (TC), triglycerides (TG), cholesterol together with a decrease in High-Density Lipoprotein (HDL) and cholesterol.

Elaeocarpus angustifolius Blume, a sizable evergreen tree with broad leaves, is a member of the Elaeocarpaceae family. It is recognized as Rudraksha in Hindi and is well-known for its intriguing fruit stones and therapeutic powers. According to a thorough review of the literature, *E. angustifolius* Blume is a crucial source of many pharmacologically and medicinally important chemicals, including essential triterpenes, tannins like geraniin and 3, 4, and 5-trimethoxy geraniin, indolizilidine alkaloids grandisines, rudrakine, and flavonoids quercitin.

According to our review of the literature, no scientific research has been done to determine the effectiveness of *E. angustifolius* Blume in decreasing cholesterol. Against this backdrop, the current examination was conducted to assess the antioxidant properties of *E. angustifolius* Blume *in vitro* and it's capacity to lower lipid levels in hyperlipidemic rats.

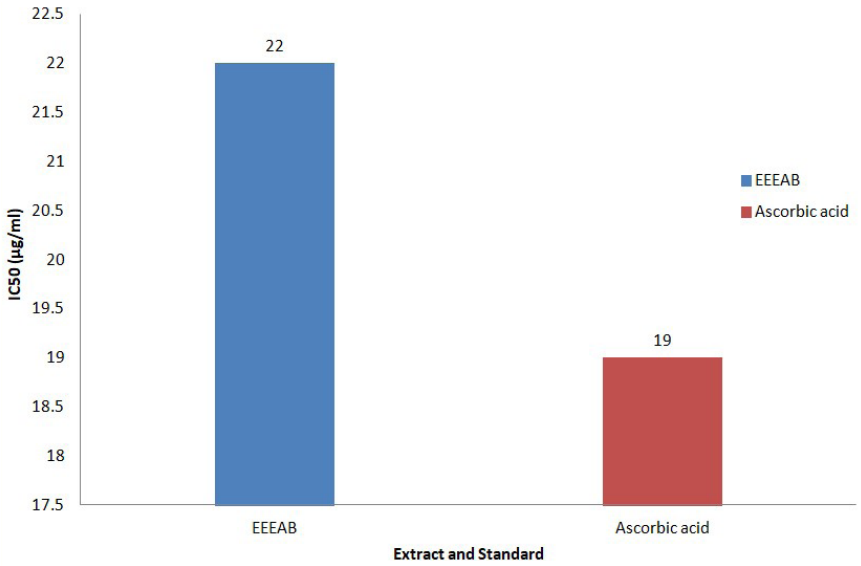

Figure 3. Antioxidant activity of EEEAB.

The outcomes of the current investigation revealed that the considerable hypolipidemic action of the EEEAB may be attributed to its biologically active phytoconstituents, including alkaloids, flavonoids, saponins, steroids, and tannins. Specifically, the existence of flavonoids in the extract may be responsible for its anti-hypercholesterolemic effects since they reduce hepatocytes' production of apo-B and levels of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA).

The purpose of the study was to determine whether *Elaeocarpus angustifolius* Blume ethanolic extract had any anti-hyperlipidemic effects on hyperlipidemic rats' food a high-heavy, fructose food. Rats with hyperlipidemia received amounts of 125, 250, and 500 mg/kg p.o. of the EEEAB for 21 days, with atorvastatin serving as the usual control. Once associated with the control group, the high-fat, fructose food-treated assembly showed signs of hyperlipidemia, including elevated lipid concentrations, increased body weight, and decreased body temperature and HDL. In treated rats, EEEAB demonstrated a dose-dependent reduction in the levels of TC, Tgs, LDL, and VLDL ($p<0.001$, $p<0.01$, $p<0.05$).

After administering EEEAB, the HDL ratio increased. EEEAB 500 mg was found to have a better effect than EEEAB 125mg and 250mg.

By using a hydrogen peroxide assay, the ethanolic extract of *E. angustifolius* demonstrated strong antioxidant activity with an IC_{50} value of 22 g/ml.

As a result, it may be thought that the extract which is ethanolic of *E. angustifolius* has strong anti-hyperlipidemic and antioxidant effects.

5. Conclusion

The goal of the experimental investigation was to regulate whether the ethanolic extract of *E. angustifolius* leaves had any antihyperlipidemic effects on albino Wistar rats. Alkaloids, flavonoids, saponins, steroids, and tannins, which are active phytoconstituents, may be to blame for the EEEAB's notable hypolipidemic action. Specifically, the existence of flavonoids in the herb extract may be responsible for its antihypercholesterolemic effects since they reduce hepatocytes' production of apo-B and levels of (HMG CoA) 3-hydroxy-3-methylglutaryl coenzyme A.

For this study, the high-fat fructose Diet (HFD-FD) was used to induce hyperlipidemia in rats.

The following findings were revealed in this study.

- The EEEAB was taken at an amount level of 125, 250, and 500mg/kg p.o for 21 days to hyperlipidemic rats and showed a decrease in the levels of TC, TGs, LDL, VLDL in a dose-dependent manner in treated rats. HDL ratio improved after administration with EEEAB. The result of EEEAB 500 mg was detected to be better than the result of EEEAB 125mg, 250 mg.

- EAB ethanolic extract has good antioxidant potential as it showed a significant effect in *in vitro* testing.

6. Acknowledgement

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