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THE EVALUATION OF ANTIHYPERLIPIDEMIC PROPERTIES OF ETHANOLIC EXTRACT OF WHOLE PLANTS OF *AMMANNIA BACCIFERA (L)*

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Abstract : The rational and scientific use of plants, based on chemical and pharmacological research, is truly the only way to correctly use medicinal herbs. Furthermore, the efficiency of medicinal herbs increases when they are used within the frame of natural revitalizing treatment. The aim of the present study is to promote a potential new herbal formulation to prevent atherosclerosis at a low cost. The ethanolic extract has shown a significant reduction in serum cholesterol and TGs level indicating antihyperlipidemic potentials in the plant while aqueous extract has not shown any beneficial effect.
In this hyperlipidemia model, serum and TG levels were analyzed ethanolic extract was shown antihyperlipidemic activity. Further clinical studies are compulsory to confirm the findings.

Keywords: ***Ammannia baccifera (L);*** **Hyperlipidemia; Ethanol extract; Phytochemicals; Flavonoids and alkaloids; Total Cholesterol.**

1. INTRODUCTION

Hyperlipidemia is a secondary metabolic dysregulation associated with diabetes. Besides the cause-effect relationship with diabetes, elevated serum levels of triglycerides, cholesterol and LDL are major risk factors for the premature development of cardiovascular diseases like atherosclerosis, hypertension, coronary heart disease etc.1Increased plasma lipid levels mainly total cholesterol, triglycerides and LDL along with decrease in HDL are known to cause hyperlipidemia which is the reason for initiation and progression of atherosclerosis impasse.

The developments of the newly born chemical and pharmaceutical industry have brought about great social enthusiasm. The on-going discovery of more and more powerful new medicines, though not less toxic, seems to promise a bright future in which there is a specific pharmacological product to treat every disease. The rational and scientific use of plants, based on chemical and pharmacological research, is truly the only way to correctly use medicinal herbs.

Ammannia baccifera is widely used in traditional Chinese/Indian herbal formulations for treating human female infertility, gastroenteropathy, spinal disease, haemorrhoids, urethritis, common cold, abscess, sore, itching and other skin diseases. It has been reported to possess anticancer, antirheumatic, antidiuretic, antipyretic, antisteroidogenic, antimicrobial, rubefacient and anti-urolithic activities.

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2. METHODS:

Preparation of Extracts

Collected plants were shade-dried for 15 days and they were coarsely powdered using a pulverizer. The pulverized plant materials were taken up for extraction using hydro alcohol in the proportion of 5:95. The extraction was carried out by cold

percolation method. The extracts were then dried in a vacuum and they were stored in a desiccator and subsequently in a refrigerator.

2.1 PRELIMINARY PHYTOCHEMICAL TESTS

Preliminary phytochemical tests were done by the methods described by usual procedures mentioned in Trease and Evans (1958)[50] and also as specified in the book of Practical Pharmacognosy (Kokate, 2000)[51]. The details of the same are provided below.

Ethanolic extracts of the whole plant of *Ammannia baccifera (L)* were subjected to qualitative tests for the identification of various active constituents.

2.3 ACUTE TOXICITY STUDIES

Healthy albino rats of either sex of 2-2½-months-old of body weight 125-150 g were housed in polypropylene cages at 25±2°C with light dark cycle of 12 h in the Animal House ofthe study center are to be used for the study. It should be acclimatized for seven days. All animals are to be given with standard rat feed and water ad libitum. The experiments were performed after approval of the protocol by the minute of Institutional Animal Ethics Committee (IAEC) Vedic/CCSEA/2024/03/07.09.2024 CPCSEA 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.

Development of cholesterol diet model

High cholesterol diet consisting of Cholesterol (1%), sodium cholate (0.5%), sucrose(30%), casein(10%), butter (5%) and standard chow diet (53.5%) ad libitum, respectively, forthe initial period of 2 weeks. The composition and preparation of HCD as were described elsewhere.

2.4 EXPERIMENTAL DESIGN

Group 1: Normal control.1% CMC

Group 2: Hyperlipidemic control (Vehicle 1 ml/100g/day p.o).

Group 3: Hyperlipidemic treated with Atorvastatin (10 mg/kg, b.w./day p.o).

Group 4: Hyperlipidemic treated with EEAB (100 mg/kg, b.w./day p.o).

Group 5: Hyperlipidemic treated with EEAB (200 mg/kg, b.w./day p.o).

PARAMETERS

Serum Triglycerides (TG), total cholesterol (TC), and HDL-cholesterol (HDL-C) were estimated according to the methods of Zlatkis *et al.*,[53] Foster and Dunn [54] and Burstein *et al.*,[55] respectively.The serum levels of VLDL and LDL cholesterol were calculated using Friedewald formula

$$\text{Atherogenic index} = \frac{\text{TC} - \text{HDL-C}}{\text{HDL-C}}$$

3. RESULTS

3.1 PRELIMINARY PHYTOCHEMICAL SCREENING

The results of the physiochemical, elemental analysis and quantitative estimation of phytoconstituents followed by the pharmacological screening of various activities have been presented and discussed here below. In this study, ethanolic extract of *Ammannia baccifera*- showed positive to following phytochemical constituent's alkaloids, carbohydrates, saponins,

tannins, flavonoids, total phenolic substances, glycosides, carotenoids, fatty acids and terpenoids. The results are tabulated in Table No. 1

Table No 1: Preliminary phytochemical screening of *Ammannia baccifera* extract

Name of the Phytoconstituents	**EEAB extract**
Carbohydrates	+
Total Phenolic substances	+
Glycosides	-
Alkaloids	+
Proteins and Amino acids	+
Flavanoids	+
Tannins	+
Phytosterols	+
Terpenoids	-
Saponins	-
Fixed oil and Fats	+
Carotenoids	-

3.2 ACUTE TOXICITY STUDIES

In acute toxicity study, no death was recorded, in the 14 days of observation period in the male and female animals given 2 g/kg of the ethanolic extract of *Ammannia baccifera* orally. The animals did not show any changes in the general appearance during the observation period. Based on the results, the dose was fixed by oral route for further studies.

3.3 EFFECT OF AMMANNIA BACCIFERA EXTRACT ON CHOLESTEROL LEVEL OF RATS

The hyperlipdemia control group showed significant ($P<0.001$) increase in cholesterol level when compared to normal control group. In hyperlipdemia control group mixed withAtorvastatin (10mg/kg); *Ammannia baccifera* (100, 200 mg/ kg) showed significant ($P<0.001$) decrease when compared to hyperlipdemia control group. The cholesterol level of EEAB displayed highly appreciated decrease ($P<0.001$) when the values are looked upon the values of Atorvastatin (10mg/kg) (Table no.2, Figure: 1).

Table No 2: Effect of drugs on serum total cholesterol (mg/dl)

S.No.	Treatment	Cholesterol (mg/dl)
1	Normal Control	142.4 ± 2.0
2	HCD	209.0 ± 9.0 ###
3	ATORVAS 10mg/kg	146.6 ± 5.2 **
4	EEAB 100 mg/ kg	169.86±0.98 ## ***
5	EEAB 200 mg/ kg	155.3±0.94 ***

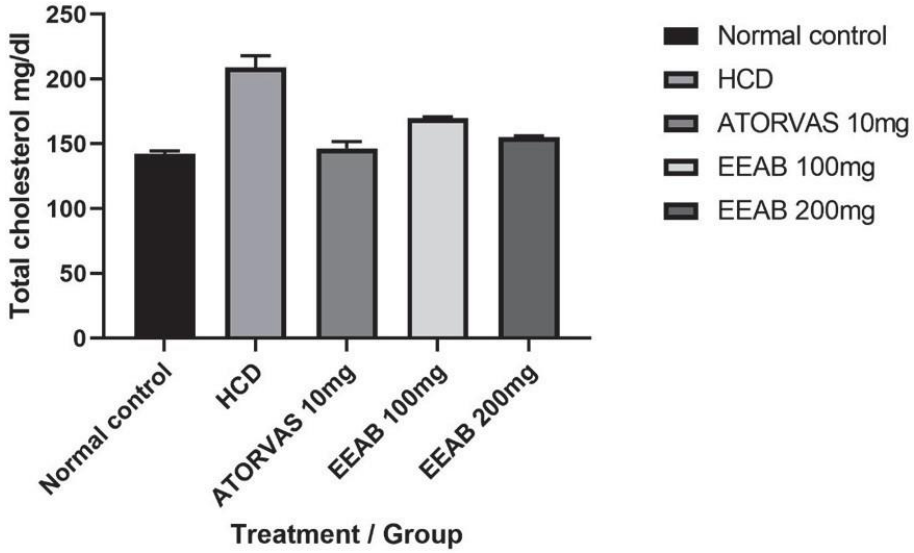

Figure: 1 EVALUATION OF TOTAL CHOLESTEROL

3.4 EFFECT OF *AMMANNIA BACCIFERA* EXTRACT ON HDL-C LEVEL OF RATS

The HDL-C levels of hyperlipidemia control group provides the reported values of the lowestorder having the significant (P<0.001) decrease when those values of extract brought in comparison to normal control group. In the hyperlipidemia control group stirred with Atorvastatin (10mg/kg); *Ammannia baccifera* (100, 200 mg/ kg) a prominent (P<0.05 and P<0.001) increase when it related to Atorvastatin treated group (Table no.3, Figure: 2).

Table No 3: Effect of drugs on serum HDL-C level (mg/dl)

S. No.	Treatment	HDL-C (mg/dl)
1	Normal Control	45.8 ± 0.991
2	HCD	38.31 ± 4.712 ##
3	ATORVAS 10mg/kg	42.76 ± 0.745 ***
4	EEAB 100 mg/ kg	44.01 ± 0.761*
5	EEAB 200 mg/ kg	45.33 ± 2.009 ***

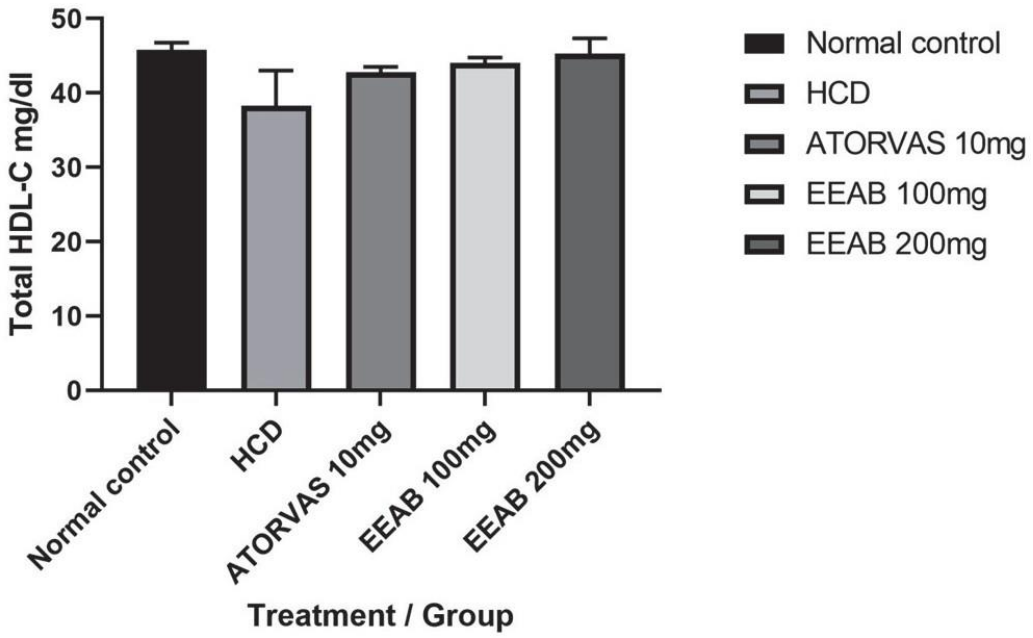

Figure: 2 Evaluation of serum HDL-C level

3.5 EFFECT OF AMMANNIA *BACCIFERA* EXTRACT ON TRIGLYCERIDE LEVEL OF RATS.

The triglyceride levels of hyperlipidemia control group provides the reported values of the lowest order having the significant ($P<0.001$) decrease when those values of extract brought in comparison to normal control group. In hyperlipidemia control group stirred with Atorvastatin (10mg/kg); *Ammannia baccifera* (100, 200 mg/ kg) a prominent ($P<0.05$ and $P<0.001$) increase when it related to Atorvastatin treated group (Table no.4, Figure:3).

Table No 4: Effect of drugs on serum TG level (mg/dl)

S.No.	Treatment	TG (mg/dl)
1	Normal Control	65.58±2.11
2	HCD	125.58±2.11$^{\#\#}$
3	ATORVAS 10mg/kg	84.97±3.39***
4	EEAB 100 mg/ kg	105.78±2.08*
5	EEAB 200 mg/ kg	95.97±1.43***

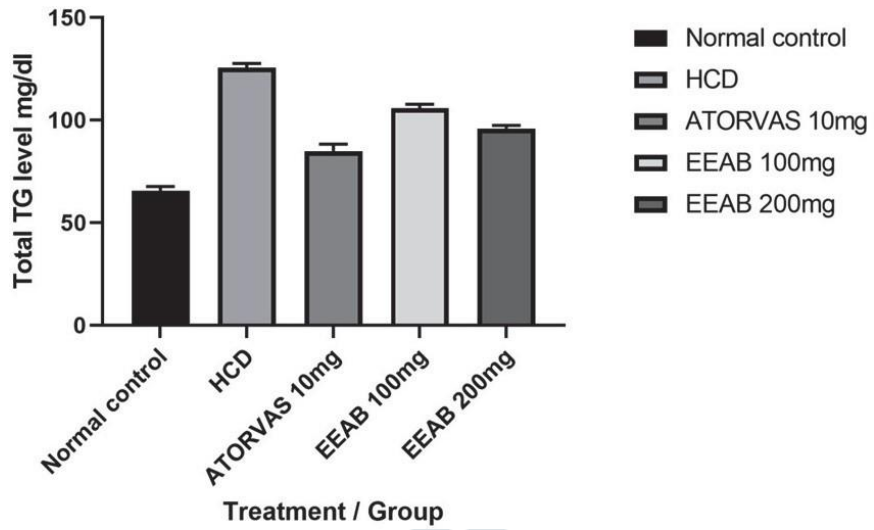

Figure: 3 Evaluation of serum TG level

4. DISCUSSION

Preliminary phytochemical screening of ethanolic extracts of the whole plant of *Ammannia baccifera* was done. The result showed the presence of the following phytochemical constituents such as totalphenolic substances, glycosides, alkaloids, flavanoids, and tannins (table: 1). the acute toxicity study does not show any deviation from the normal behaviour. It is found that the extract is non-toxic and well tolerated. From this, LD 50 is determined from that the effective oral dose for the anti-hyperlipidemic study was selected. So, the present study aimed to evaluate the anti-hyperlipidemic activity of the Cammannia baccifera extract on HCD-induced hyperlipidemia in Wistar rats. Atorvastatin at a dose level of 10mg/kg was used as the standard drug.

The in vivo antihyperlipidemic activity was evaluated on high cholesterol diet induced hyperlipidemic rats. high cholesterol diet , was used successfully to induced hyperlipidemia in previous studies. It causes these effects by activating HMG- CoA and inhibiting lipoprotein lipase activity). high cholesterol diet has been utilized in the hyperlipidemic model due to its convenience, reproducibility, and lack of undesirable underlying pathological conditions.

The hyperlipdemia control group maintained a significant ($P<0.001$) raise in cholesterol level when compared to normal control group. In hyperlipdemia control group treated with Atorvastatin (10mg/kg); *Ammannia baccifera* (100, 200 mg/ kg) displayed a significant ($P<0.001$) decrease when compared to hyperlipdemia control group. This indicates that ethanolic extract of *Ammannia baccifera* (EEAB) significantly reduces the serum cholesterol level.

5. CONCLUSION

Plant materials are used throughout the developed and developing world as home remedies, in over-the-counter drug products, and as raw material for the pharmaceutical industry, and they represent a substantial proportion of the global drug market. Certain herbs have become popular over the years, but the public, medical practitioners and the media still have a poor understanding of herbal medicine. Evidence is emerging on the dangers of herbs. As in most situations, the truth lies hidden under the media hype, poorly understood science, and exaggerated claims. Lack of experience, information, and education about herbs make consumers, physicians, and other orthodox health care provider's easy victims of market exploitation and herbal myths. There is no rational reason behind the tendency to equate "natural" with "harmlessness. Hence it is going to be concluded that the potential benefits of the extracts of *Ammannia baccifera* has been demonstrated well in advance and can be used further to demonstrate the antihyperlipidemic as well as controlling of both triglyceride levels and reducing the risk of factors of cholesterol inducers. The aforementioned results of the research suggest that the *Ammannia baccifera* found to have the potential antihyperlipidemic action.

The results found are encouraging for further studies on the selected plants and to identify the bioactive compounds.

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