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EVALUATION OF ANTI HYPERLIPIDIMIC ACTIVITY OF POLYHERBAL COMBINATION OF SOME INDIAN MEDICINAL PLANTS ON WISTAR ALBINO RATS

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ABSTRACT

The present work assists the credit of the plant material for further investigation and form an important aspect of drug studies. The findings of the present study suggest it could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases and other related diseases. All the obtained extracts were dried and weighed. The percentage yield of each plant was calculated as per standard method. The weighed extract of each plant drug was stored in desiccators for further use. The yields were found to be 11.50g of petroleum ether extract with semisolid mass of brown colour, 16.02g of Methanol extract with Orange-Black colour for Achyranthes aspera leaves. The plant extracts showed no toxicity at a maximum dose of 2000 mg/kg. The methanolic extracts of selected herbal drugs could be formulated into effective hypolipidemic dosage form. This may be due to the synergistic effect of the combined extracts. Thus the results of the present investigation clearly indicated that the selected medicinal plants possess good anti hyerlipidemic activity and led to the development of new Herbal formulation possessing anti hyperlipidemic activity. The results found are encouraging for further studies on the selected plants and to identify the bioactive compounds.

Key Words: Anti Hyperlipidimic, Activity, Indian Medicinal, Plants On Wistar, Albino Rats

INTRODUCTION

One of the biggest risk factors for the prevalence and seriousness of coronary heart diseases has been identified as hyperlipidemia [1]. Age-related increases in hyperlipidemia are observed. The ratio of men to women who have hyperlipidemia is 40:37, and this degree of increased risk of vascular illnesses (MI/CVA) is directly correlated. It is now well documented that high levels of total cholesterol, particularly LDL cholesterol, contribute to the development of cardiovascular disease [2]. Worldwide, developed countries have a prevalence of hyperlipidemia that ranges between 39%, 51%, and 26%, respectively [3].

According to estimates, elevated cholesterol contributes to 29.7 million disability adjusted life years (DALYS), or 2.0% of all DALYS, and 2.6 million deaths overall (4.5% of all deaths). Men aged 40 have been observed to see a 50% reduction in heart disease within 5 years, while men aged 70 have been shown to experience an average 20% reduction in heart disease occurrence over the following 5 years when their serum cholesterol is reduced by the same 10% [4].

In the present era of drug development and discovery of newer drug molecules many plant products are evaluated on the basis of their traditional uses. One of the many plants which are being evaluated for their therapeutic efficacies is *Achyranthes aspera* which is commonly known as Latjeera (Hindi) & Rough Chaff tree (English) [5].

Boswellia serrata is mentioned in ancient Ayurvedic texts like Charaka sanhita, Sushrut sanhita, Astanga Hrdayam, etc. In Sanskrit it is described as Shallaki (it is to be consumed because of its good taste), Gajabhakshya (its leaves are eaten by elephants), Suvaha and Surabhi (having good aroma), Rasa (having good taste), Maheruna (looks very beautiful), Kunduruki

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(appears like kunduru), Vallaki and Bahusrava (having gum resin) [6].

The present work assists the credit of the plant material for further investigation and form an important aspect of drug studies. The findings of the present study suggest that it can serve as a valuable source of information and provide appropriate standards to establish the quality of these plants material in future study or application, moreover it could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases and other related diseases

MATERIAL AND METHODS

Authentication of Plant Materials

Authentication of collected plant materials were confirmed by HOD Department of Botany, Safiya Science College, Bhopal, Madhya Pradesh, India.

Extraction (By Maceration Method)

Maceration [7]

Collected plant drugs namely *Achyranthes aspera* and *Boswellia serrata* were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drugs were converted into moderately coarse powder in hand grinder. Powdered plant drugs were weighed (100 gm) and packed in (1 liter) air tight glass Bottle. The plant drugs were subjected to extraction by Pet Ether and Methanol as solvent for about 24 hrs. The liquid extracts were collected in a tarred conical flask. The solvent removed from the extract by evaporation method using hot plate. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated.

Phytochemical Analysis

Preliminary Phytochemical Screening

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the Hydroalcoholic extract of leaves of *Achyranthes aspera* and *Boswellia serrata*, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids [8].

Estimation of total phenolic content

Estimation of total phenolic content Total phenolic content of all the extracts was evaluated with Folin-Ciocalteu method. Samples containing polyphenols are reduced by the Folin-Ciocalteu reagent there by producing blue colored complex. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve. To prepare a calibration curve, aliquots of 5, 10, 15, 20 and 25µg/mL methanolic gallic acid solutions were mixed with 2.5 mL Folin– Ciocalteu reagent (diluted ten-fold) and 2.5 mL (75 g/L) sodium carbonate. After incubation at 25°C for 30 min, the quantative phenolic estimation was performed at 765 nm against reagent blank by UV Spectrophotometer 1650 Shimadzu, Japan. The calibration curve was constructed by putting the value of absorbance vs. concentration. A similar procedure was adopted for the extracts as above described in the preparation of calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per g of extract [9].

Estimation of total flavonoids content

The aluminum chloride colorimetric method was modified from the procedure reported by Woisky and Salatin [45]. Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 80% ethanol and then diluted to 10 to 50 µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu spectrophotometer. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 mL of Hydroalcoholic extracts and Flavonoid standard solutions (100 ppm) were reacted with aluminum chloride for determination of Flavonoid content as described [9].

Animals

Adult healthy Wistar rats of either sex of about 6-8 weeks of age, weighing 180-220 g. Female and male animals were separately kept in polypropylene cages in groups of six. The animals were given free access to water and food and were fed with standard rat pellet diet. The protocol of the experiment was approved by the Institutional Animal Ethics Committee and (IAEC NO: RKDFCP/2023/28) experiments were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Toxicity Studies

Wistar rats (200-250 g) of either sex were selected and segregated in to 8 groups of 6 animals each. Single dose of methanolic extract of polyherbal formulation, starting from the minimal dose of 50 mg/kg up to 3000 mg/kg administered orally. The drug treated animals were observed carefully for its toxicity signs and mortality. From the maximum dose, 1/5th and 1/10th of the concentration was considered as therapeutic dose for further study.

Anti Hyperlipidimic Study Triton X 100 Induced Hyperlipidimia model [10]

Triton X 100 (TR) induced hyperlipidimic model 30 Wistar rats were randomly divided into 5 groups of 6 each.

Group-I: Administered with vehicle (distilled water) & served as Normal control.

Group II: Administered (MEAA) Methanolic extraction of *Achyranthes aspera* (400 mg/kg).

Group III: Administered (MEBS) Methanolic extraction of *Boswellia serrata* (400 mg/kg).

Group IV: Administered (PHME) Poly herbal Methanolic extraction (400 mg/kg).

Group V: Administered Atorvastatin (10 mg/kg), p.o. On the 8th day, blood was collected by retero orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 15 minutes at 2500 rpm. Then serum samples were collected and analyzed for serum Total Cholesterol, Triglycerides, High Density Lipoprotein Cholesterol, Low Density Lipoprotein Cholesterol and Very Density Lipoprotein Cholesterol.

High Fat Diet (FD) induced hyperlipidimic model [11]

Preparation of Feed

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 2% , 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.

30 Wistar rats were randomly divided into 6 groups of 6 each. The chronic experimental hyperlipidimia was produced by feeding the above prepared food for 21 days.

The rats are then given test plant extracts. During these days, all the groups also received fat diet in the same dose as given earlier. The hyperlipidimic control i.e., group II animals received the hyperlipidimic diet and the vehicle. The control group animals received the normal laboratory diet and vehicle. The first group was given standard pellet diet, water and orally administered with 5% CMC.

The III, IV, V and VI group animals were injected i.p. with 10% aqueous solution of Triton 400 mg/kg body weight. After 72 hours of triton injection, the second group received a daily dose of 5% CMC (p.o). The third group was administered daily dose of MEBR 400 mg/kg, fourth group was administered a daily dose of MEAA 400 mg/kg and Group IV was administered daily dose of MEBS 400 mg/kg and Group V PHME 400 mg/kg suspended in 5% CMC, p.o., for 7 days, after inducing hyperlipidimia. VIth group was administered with the standard Atorvastatin 10 mg/kg, p.o. for once daily in the morning orally for 14 consecutive days. During these days, all the groups also received fat diet in the same dose as given earlier

Group I: Administered vehicle and served as normal control.

Group II: Administered hyperlipidimic diet.

Group III: Administered MEAA (400 mg/kg). p.o. and fed with FD

Group IV: Administered MEBS (400 mg/kg). p.o. and fed with FD

Group V: Administered PHME (400 mg/kg). p.o. and fed with FD

Group VI: Administered Atorvastatin (10 mg/kg), p.o. and fed with FD

On day 15, animals were anaesthetized with diethyl ether and blood was collected by retro orbital puncture. The blood was subjected to centrifugation for 15 min at 2500 rpm to obtain serum. The collected serum was analyzed for serum Total Cholesterol, Triglycerides, High Density Lipoprotein Cholesterol, Low Density Lipoprotein Cholesterol and Very Low Density Lipoprotein Cholesterol.

Biochemical estimations

On the 8th day, blood was collected by retero-orbital sinus puncture, under mild ether anaesthesia in both the experimental models. The collected samples were centrifuged for 15 minutes at 2500 rpm. Then serum samples were collected and analysed for serum Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C) serum blood glucose and atherogenic index (AI)[12].

Estimation of Serum total cholesterol (TC)

This method was used for the estimation of serum cholesterol. In this method the following were pipetted into the reaction vessel using a micro pipette. Test samples (T): 0.02 ml serum, 2.00 ml reaction solution; the standard sample (S): 0.02 ml standard and 2.00 ml reaction solution, while for the blank sample (B): 0.02 ml DW and 2.00 ml reaction solution. The mixture was mixed well and incubated for 10 minutes at +20 to 25°C or 5 minutes at 37°C. The absorbance was read at 505/670 nm against the reagent blank [13]

Estimation of serum triglycerides (TG)

This method was used to estimate the serum triglycerides. For this 0.01 ml of serum was taken in a test tube (T) in which 1 ml reaction solution was added. In an another test tube (S) 0.01 ml standard and 1ml reaction solution were added. The solution was mixed well and

incubated at +20 to 25°C for 10 min. The absorbance of standard and test against reagent blank was read at 505 (500-540 nm). [14]

Estimation of HDL-cholesterol

This method was used to estimate the serum HDL cholesterol level. CHOD-PAP method (Henry 1974) was used to estimate the serum HDL cholesterol level. For this 2 ml if serum was taken in a test tube and 0.5 ml of precipitation reagent was added. The mixture was shaken thoroughly and left to stand for 10 min at +15 to 25°C and then centrifuged for 15 min at 4000 rpm. Within 2 hour after centrifugation, the clear supernatant was used for the determination of HDL-C. One ml of the supernatant was taken in a test tube (T) and 1 ml of reaction solution was added to it. In another test tube 0.1 ml DW was taken and 1 ml reaction solution (B) was added. The mixtures were mixed thoroughly, incubated for 10 min at 15-25°C or for 5 min at 37°C and measured the absorbance of the sample against reagent blank at 546 nm. [15]

Estimation of LDL cholesterol

LDL cholesterol was estimated by using Friedwald's (1972) formula as follows

$$LDL\ in\ mg\% = \frac{Total\ cholesterol - HDL - C - Triglyceride}{5}$$

Estimation of VLDL cholesterol

VLDL cholesterol was estimated by using following formula

$$VLDL\ in\ mg\ \% = \frac{Triglyceride}{5}$$

RESULTS AND DISCUSSION

Physicochemical parameters

The present work assists the credit of the plant material for further investigation and form an important aspect of drug studies. The findings of the present study suggest that it can serve as a valuable source of information and provide appropriate standards to establish the quality of these plants material in future study or application, moreover it could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases and other related diseases.

The total ash, acid insoluble ash, water soluble ash, sulphate ash, loss on drying and foreign matter of *Achyranthes aspera was* found 8.4%, 2.1%, 3.4%, 4.5%, 0.25 and 0.50 respectively. The total ash, acid insoluble ash, water soluble ash, sulphate ash, loss on drying and foreign matter of *Boswellia serrata* was found 9.1%, 1.70%, 3.10 %, 3.80%, 0.25% and 0.25% respectively.

Extraction of Plant Drugs

All the obtained extracts were dried and weighed. The percentage yield of each plant was calculated as per standard method. The weighed extract of each plant drug was stored in desiccators for further use. The yields were found to be 11.50g of petroleum ether extract with semisolid mass of brown colour, 16.02g of Methanol extract with Orange-Black colour for ***Achyranthes aspera*** leaves.

Evaluation of in Vitro Antioxidant Activity

Quantitative antioxidant activity

Estimation of total phenolic content

The total phenolic content of the *Achyranthes aspera* leaf extracts was compared with standard curve of catechol (y=0.004x-0.001, R2=0.990) and the results were expressed as the number of equivalents of catechol (µg/mg of extract). Among the two solvents used, methanol extract showed prominent total phenolic activity (77µg of catechol/mg of extract) and petroleum ether (31µg of catechol/mg of extract). The results were presented in Fig. 7.7.

The total phenolic content of *Boswellia serrata* extracts was compared with standard curve of catechol (y=0.004x-0.001, R2=0.990) and the results were expressed as the number of equivalents of catechol (µg/mg of extract). Among the two solvents used, methanol extract showed prominent total phenolic activity (78µg of catechol/mg of extract) followed petroleum ether (33µg of catechol/mg of extract). The results were presented in Fig. 7.8.

Estimation of total flavonoids content

The standard curve of catechol (y=0.027x-0.075, R2=0.987) was used to express the total flavonoid content of the *Achyranthes aspera* leaf extracts in terms of µg/mg of extract (Fig.7.9). methanol extract of *Achyranthes aspera* found to be 27µg of catechol/mg .In petroleum ether extracts, it was 15µg of catechol /mg of extract respectively.

The total flavonoid content of the *Boswellia serrata* leaf extracts in terms of µg/mg of extract (Fig.7.10) methanol extract of *Boswellia serrata* found to be 43µg of catechol/mg . In petroleum ether extracts, it was 27µg of catechol /mg of extract respectively.

Table: 1 Solvents used for extraction of plant drugs

S. No.	Plant Drug	Solvent used	Solvent used
1.	*Achyranthes aspera*	Pet. ether	Methanol
2.	*Boswellia serrata*	Pet. ether	Methanol

Table 2: Effect of MEAA, MEBS and PHME on serum lipid parameter levels in Triton induced Hyperlipidimic rats.

S. No	Groups	Serum Lipid Parameters (mg/dl)	
		Total Cholesterol	Triglycerides
1.	Normal Control (Saline)	86.25 ± 1.16	65.66 ± 7.06
2.	MEAA (400 mg/kg)	102.7 ± 10.25**	98.99 ± 2.56*
3.	MEBS (400 mg/kg)	102.9 ± 10.29**	98.94 ± 2.76*
4.	PHME(400 mg/kg)	94.22 ± 14.16**	84.19 ± 2.30*
5.	Atorvastatin (10 mg/kg)	92.14 ± 11.31**	85.32 ± 3.13**

Values are mean ± SEM (n=6).Values are statistically significant at **P≤0.01 vs hyperlipidemic control using one way ANOVA.

Table 3: Effect of MEAA, MEBS and PHME on serum lipid parameter levels in Triton induced Hyperlipidimic rats.

S. No	Groups	Serum Lipid Parameters (mg/dl		
		HDL-C	LDL-C	VLDL-C
1.	Normal Control (Saline)	48.24 ± 3.64	25.46 ± 1.61	13.95 ± 1.72
2.	MEAA (400 mg/kg)	42.07 ± 5.61*	42.01 ± 3.62*	20.74 ± 0.49*
3.	MEBS (400 mg/kg)	43.07 ± 5.51*	42.04 ± 3.62*	20.47 ± 0.47*
4.	PHME(400 mg/kg)	46.03 ± 3.66**	33.09 ± 12.01*	18.84 ± 0.47*
5.	Atorvastatin (10 mg/kg)	46.10 ± 2.69*	33.44 ± 13.90*	17.55 ± 0.71*

Table 4: Effect of MEAA, MEBS and PHME serum lipid parameter levels in fat diet induced Hyperlipidimic rats.

S. No	Groups	Serum Lipid Parameters (mg/dl)	
		Total Cholesterol	Triglycerides
1.	Normal Control (Saline)	85.77 ± 1.18	65.21± 7.06
2.	Hyperlipidemic Control	206.74 ± 13.81	118.2 ± 5.55
3.	MEAA (400 mg/kg)	102.91 ± 11.27**	100.11 ± 2.55*
4.	MEBS (400 mg/kg)	102.88 ± 11.26**	100.13 ± 2.61*
5.	PHME(400 mg/kg)	94.44 ± 14.16**	84.21 ± 2.23*
6.	Atorvastatin (10 mg/kg)	92.19 ± 12.21**	85.33± 3.22**

Table 5: Effect of MEAA, MEBS and PHME serum lipid parameter levels in fat diet induced Hyperlipidimic rats.

S. No	Groups	Serum Lipid Parameters (mg/dl)		
		HDL-C	LDL-C	VLDL-C
1.	Normal Control (Saline)	47.27 ± 3.62	24.46 ± 1.61	12.95 ± 1.71
2.	Hyperlipidemic Control	34.98 ± 4.40	147.1 ± 16.1	23.58 ± 1.39*
3.	MEAA (400 mg/kg)	41.07 ± 5.61*	41.01 ± 3.62*	19.81 ± 0.45*
4.	MEBS (400 mg/kg)	42.07 ± 5.51*	41.04 ± 3.62*	19.71 ± 0.45*
5.	PHME(400 mg/kg)	45.03 ± 3.66**	32.09 ± 12.01*	17.83 ± 0.46*
6.	Atorvastatin (10 mg/kg)	45.10 ± 2.69*	32.44 ± 13.90*	16.86 ± 0.70*

Figure 1: Total phenolic content of Achyranthes aspera Linn.

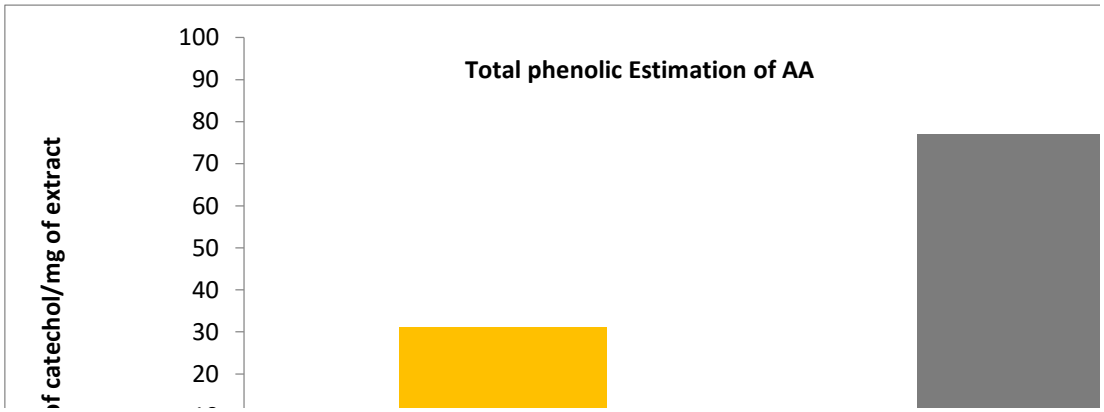


Figure 2: Total phenolic content of Boswellia serrate.

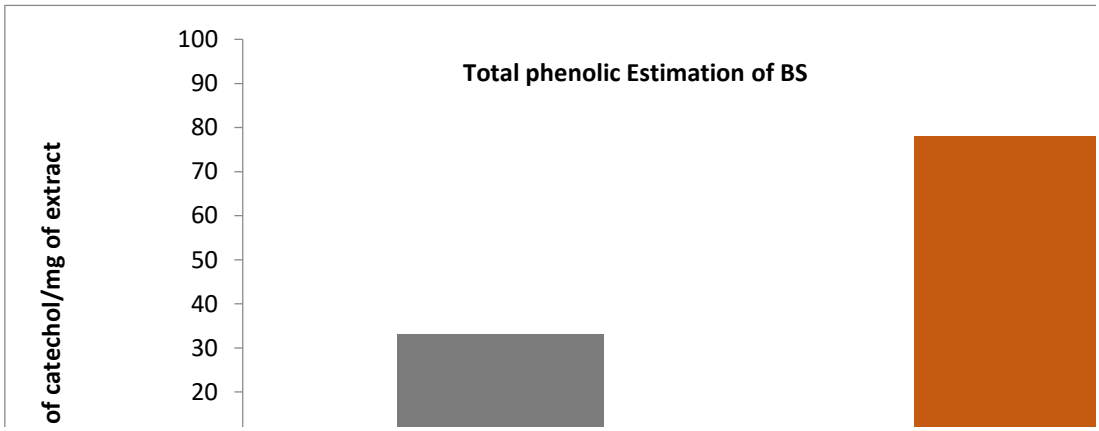


Figure 3: Total flavonoid content of Achyranthes aspera Linn.

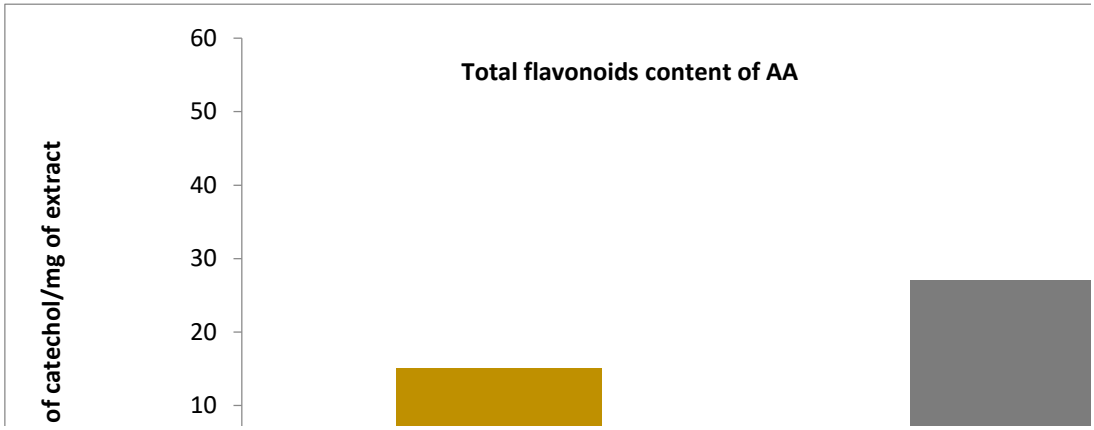


Figure 4: Total phenolic content of Boswellia serrate.

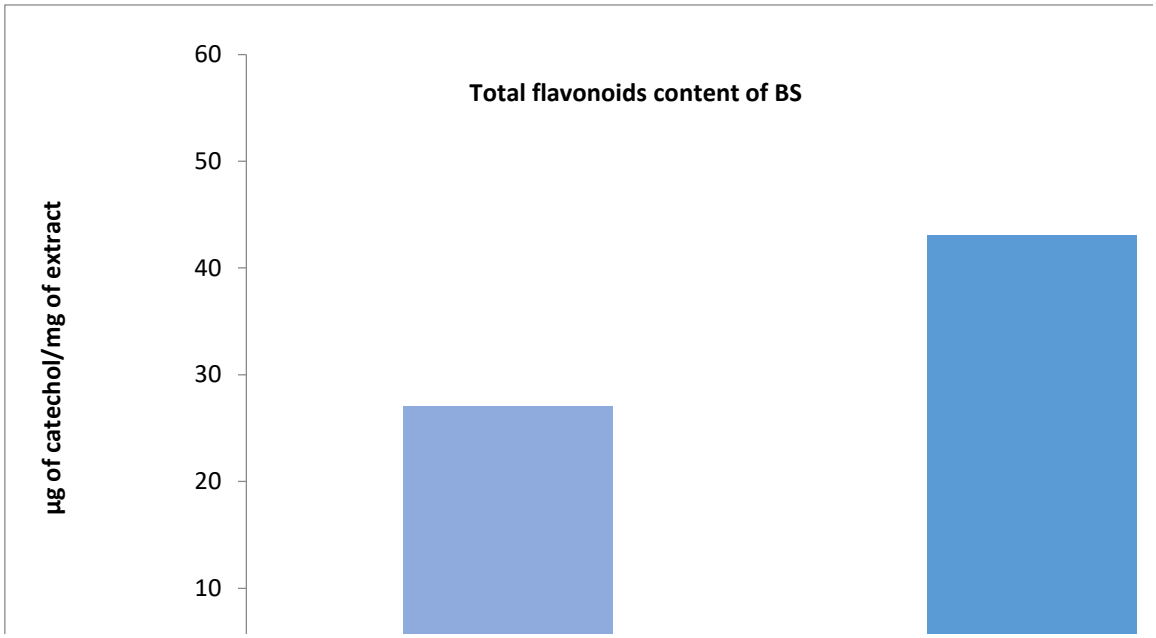


Figure 5: Effect of MEAA, MEBS and PHME on serum total cholesterol and triglycerides parameter levels in Triton induced Hyperlipidimic rats.

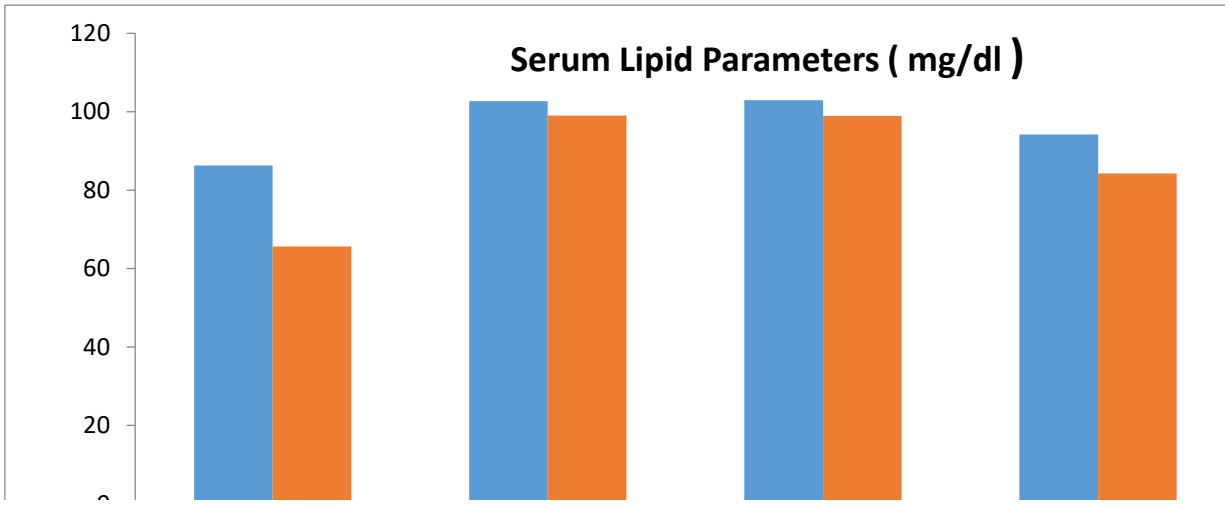


Figure 6: Effect of MEAA, MEBS and PHME on serum HDL, LDL and VLDL parameter levels in Triton induced Hyperlipidimic rats.

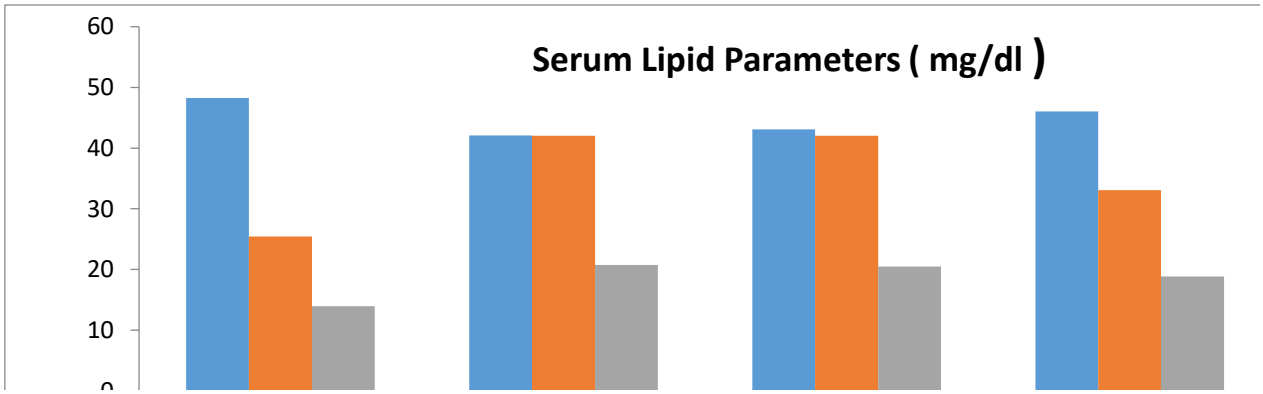


Figure 7: Effect of MEAA, MEBS and PHME on serum total cholesterol and triglycerides parameter levels in Triton induced Hyperlipidimic rats.

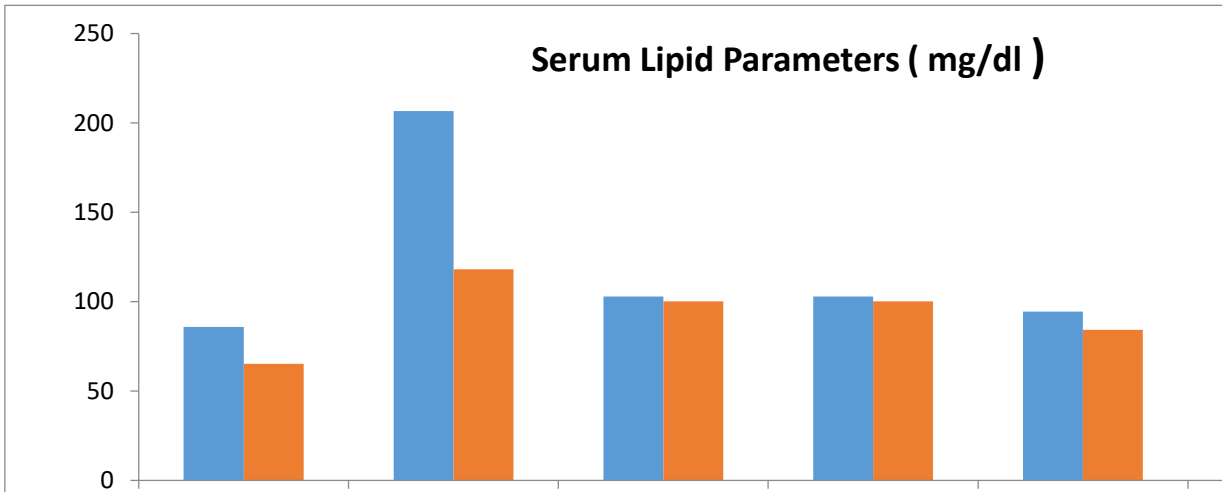
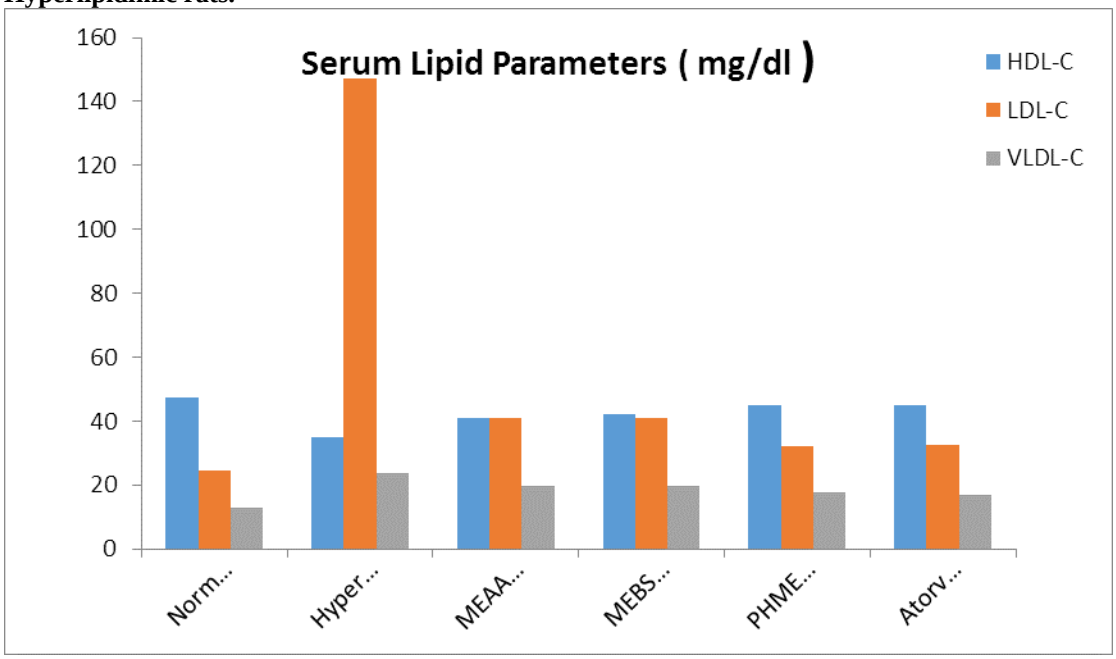


Figure 8: Effect of MEAA, MEBS and PHME on serum HDL, LDL and VLDL parameter levels in fat diet induced Hyperlipidimic rats.



Animal Studies

Phytochemical analysis of the plant extract showed different phyto constituents viz. glycosides, alkaloids and flavonoids. Several phyto constituents like glycosides, triterpinoids, Saponins, alkaloids and flavonoids are known screening of anti hyperlipidimic agents.

Triton physically alters very low density lipoprotein cholesterol rendering them refractive to the action of lipolytic enzymes of blood and tissues, preventing or delaying their removal from blood and tissues.

Hence the anti hyperlipidemic effect of MEAA, MEBS and PHME could be due to an increased catabolism of cholesterol into bile acids. Administration of PHME (400 mg/kg, p.o) for 14 days in fat diet model successfully prevented the elevation of serum Total Cholesterol, Triglycerides, Low Density Lipoproteins Cholesterol (LDL-C), Very Low Density Lipoproteins Cholesterol (VLDL-C), and decrease of serum High Density Lipoprotein Cholesterol (HDL-C) in Fat diet model rats respectively.

It has been well established that nutrition plays an important role in the etiology of hyperlipidemia and atherosclerosis. Fat diet model is used as a chronic model for induction of hyperlipidemia.

In this study we chose fat diet which contains the common ingredients in our daily food. Diet containing saturated fatty acids increases the activity of HMG CoA reductase, the rate determining enzyme in cholesterol biosynthesis; this may be due to higher availability of acetyl CoA, which stimulated the cholesterol genesis rate. Moreover, this could beassociated with a down regulation in LDL receptors by the cholesterol and saturated fatty acids in the diet, which could also explain the elevation of serum LDL-C levels either by changing hepatic LDLR (LDL receptor) activity, the LDL-C production rate or both. LCAT enzyme is involved in the trans esterification of cholesterol, the maturation of HDL-C and the flux of cholesterol from cell membranes into HDL. The activity of the enzyme tends to decrease in diet-induced hypercholesterolemia.

The possible mechanism of RNM may involve increase of HDL-C, which is attributed to the mobilization of cholesterol from peripheral cells to the liver by the action of Lecithin Cholesterol O-acyltransferase (LCAT). The increased HDL-C facilitates the transport of TG or cholesterol from serum to liver by a pathway termed ‘reverse cholesterol transport’ where it is catabolised and excreted out of the body.

Low Density Lipoproteins Cholesterol (VLDL-C) and decrease of serum High Density Lipoprotein Cholesterol (HDL-C) in Triton model rats respectively. Results are shown in Table 2 and 3.

The plant extracts showed no toxicity at a maximum dose of 2000 mg/kg. The Methanolic extracts of selected herbal drugs could be formulated into effective hyperlipidimic dosage form. This may be due to the synergistic effect of the combined extracts. Thus the results of the present investigation clearly indicated that the selected medicinal plants possess good anti hyperlipidimic activity and led to the development of new Herbal formulation possessing anti hyperlipidimic activity. The

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

CONCLUSION

The plant extracts showed no toxicity at a maximum dose of 2000 mg/kg. The methanolic extracts of selected herbal drugs could be formulated into effective hypolipidemic dosage form. This may be due to the synergistic effect of the combined extracts. Thus the results of the present investigation clearly indicated that the selected medicinal plants possess good anti hyerlipidemic activity and led to the development of new Herbal formulation possessing anti hyperlipidemic activity. The results found are encouraging for further studies on the selected plants and to identify the bioactive compounds.

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