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Anti Hyperlipidemic Potential of Polyherbal Formulation in Wistar Albino Rats

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ABSTRACT

The present study aimed that evaluation of anti hyperlipidemic activity of *Momordica charantia*, stem and root of *Tinospora cordifolia*, whole plant of *Andrographis paniculata* and wood of *Pterocarpus marsupium* and leaves of *Gymnema sylvestre* by Maceration method. Individually and combined both plants extracted poly herbal extraction and screened for phytochemical study and Anti-Hyperlipidemic activity by Triton X 100 Induced Hyperlipidemia model, High Fat Diet (FD) induced hyperlipidemic model, Estimation of Serum total cholesterol (TC) CHOD- PAP, Estimation of serum triglycerides, Estimation of HDL-cholesterol, Estimation of LDL cholesterol, Phytochemical investigation reveals the presence of alkaloids, flavonoids, saponins, tannins, steroids, triterpinoids, carbohydrates and glycosides in poly herbal methanolic extraction and individual plant extraction, In acute toxicity studies no mortality was observed with either of the extracts even at the dose level of 2000 mg/kg body weight In the present study, the methanol extracts of three plants reduced the cholesterol and triglycerides in a manner similar to the reduction facilitated by atorvastatin. The hypolipidemic activities of atorvastatin and the methanol extract of individual and polyherbal extraction were evident in both synthesis and excretory phases of triton-induced hyperlipidemia in rats.

Keywords: Hyperlipidemia, Polyherbal formulation, atorvastatin, *Tinospora cordifolia*, *Andrographis paniculata*.

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INTRODUCTION

lipoprotein metabolism, including lipoprotein overproduction or deficiency and manifested by elevation of the serum cholesterol low -(LDL) cholesterol and triglyceride concentrations, and a

decrease in the high density lipoprotein (HDL) cholesterol concentration. [1] Hyperlipidemia is the major risk factor contributing to the prevalence and progression of cardiovascular complications like atherosclerosis causing angina, peripheral arterial disease, strokes and heart attacks. Allopathic anti-

hyperlipidemic drugs available in the market are having side effects like hepatotoxicity, hyperuricemia and myositis. [2]

The use of traditional medicine on holistic approach date backs between 4000 BC to 1600 BC written in Rig Veda, Until late 1980's the scientific approach to the use of holistic medicine is based on identifying the active principle responsible for their folkloric claim. However, World Health Organization suggested that scientific validation of the traditional medicine in use is warranted to support their clinical use. Since then the use of alternative system medicines are explored for the most common diseases like obesity, hypertension, diabetes mellitus, anti-HIV, which require lifelong therapy in modern medicine. The possibility of use of herbal food supplements in these situations is a welcome change globally. In the present study, The leaves of *Gymnema sylvestre* (fam. Apocynaceae), aerial parts of *Andrographis paniculata* (fam. Acanthaceae), Fresh fruits of *Momordica charantia* (Cucurbitaceae), wood of *Pterocarpus marsupium* (Fabaceae), stem and root of *Tinospora cordifolia* (Fabaceae) that have a claim for the beneficial effects in obesity was investigated to provide scientific validation. The ingredients have ancient claim for their efficacy in the management of hyperlipidemia. [3-4] One among the many contributing factors to the obesity is genetic component. [5] The World Health Organization identified malfunction of certain biochemical parameters attributing to obesity. They are disorders of very low density lipoproteins and chylomicrons, hyper triglyceridaemia disorders of low density lipoprotein, high-density lipoprotein and combined hyperlipidemia. In certain circumstances hyperlipidemia may be secondary due to hypothyroidism, alcohol dependence and / or renal insufficiency. Among the lipid profile low density lipoprotein (LDL) derived especially from very low density lipoprotein (VLDL) contribute significantly to obesity and its associated disorders. [6] The management of hyperlipidemia comprises of drug therapy with or without life style modification. In fact, currently the American Heart Association has developed a step I diet [7] indicating diet control is a major factor in the management of hyperlipidemia. Common drugs those are currently available are statins and fabric acid derivatives besides omega-3-marine triglycerides for therapy of hyperlipidemia. [8] Pharmacotherapy requires regular medical monitoring. The suggestions to use herbal food supplements sans side effects deserve considerations. One such poly herbal formulation with proved clinical efficacy [9] was identified for scientific validations through reverse pharmacology in the present study.

MATERIALS AND METHODS

Plant Material

The leaves of *Gymnema sylvestre* (fam. Apocynaceae), aerial parts of *Andrographis paniculata* (fam. Acanthaceae), Fresh fruits of *Momordica charantia*

(Cucurbitaceae), wood of *Pterocarpus marsupium* (Fabaceae), stem and root of *Tinospora cordifolia* (Fabaceae) Were collected from Thirupathi in Andhra Pradesh and authenticated from Dr. K. Madhava Chetti, Department of botany, S. V. University, Thirupathi.

Preparation of Poly Herbal Extract

The leaves of *Gymnema sylvestre* (fam. Apocynaceae), aerial parts of *Andrographis paniculata* (fam. Acanthaceae), Fresh fruits of *Momordica charantia* (Cucurbitaceae), wood of *Pterocarpus marsupium* (Fabaceae), stem and root of *Tinospora cordifolia* (Fabaceae) individually and poly herbal of both plant materials are made into powder and then gone for the Maceration with sufficient quantity of methanol for 7 days. During maceration, it was shaken twice daily. On 7th day it was filtered and the filtrate was concentrated. The remaining solvent was evaporated by heating on a water bath (50°C) to get methanolic extract and the extract was stored in desiccator.

Phytochemical Screening of Poly Herbal Extract

The poly herbal extract were subjected to qualitative test for the identification of varies active constituents viz. alkaloids, carbohydrates and glycosides, cardiac glycosides, steroids, saponins, tannins and flavonoids using standard test procedures.

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: P37/VCP/2015/10/DBP/AE12/RATS) 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 Hyperlipidemic Study

Triton X 100 Induced Hyperlipidemia model [10]

Triton X 100 (TR) induced hyperlipidemic model forty-eight Wistar rats were randomly divided into 8 groups of 6 each.

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

Group II: Administered (MEGS) methanolic extraction of *Gymnema sylvestre* (400 mg/kg).

Group III: Administered (MEPM) methanolic extraction of *Pterocarpus marsupium* (400 mg/kg).

Group IV: Administered (MEMC) methanolic extraction of *Momordica charantia* (400 mg/kg).

Group V: Administered (MEAP) methanolic extraction of *Andrographis paniculata* (400 mg/kg).

Group VI: Administered (METC) methanolic extraction of *Tinospora cordifolia* (400 mg/kg).

Group VII: Administered (PHME) Poly herbal methanolic extraction (400 mg/kg).

Group VIII: 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 hyperlipidemic model

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. Forty six Wistar rats were randomly divided into 7 groups of six each. The chronic experimental hyperlipidemia 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 hyperlipidemic control i.e., group II animals received the hyperlipidemic 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 II, III, IV, V, VI, VII 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 MECA 400 mg/kg and Group V and Group VI was administered daily dose of MEMP 400 mg/kg and PHME 400 mg/kg suspended in 5% CMC, *p.o.*, for 7 days, after inducing hyperlipidemia. Seventh 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 Triton X 100 (TR) and served as hyperlipidemic control.

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

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

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

Group VI: Administered METC (400 mg/kg). *p.o.* and fed with FD

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

Group VIII: 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).

Estimation of Serum total cholesterol (TC) CHOD-PAP

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

Estimation of serum triglycerides (TG)

GPO-PAP 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).

Estimation of HDL-cholesterol

CHOD-PAP 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 of 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.

Estimation of LDL cholesterol

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

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

Estimation of VLDL cholesterol

VLDL cholesterol was estimated by using following formula

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

Table 1: Effect of MEPM, MEMC, MEAP, METC and PHME on serum lipid parameter levels in Triton induced Hyperlipidemic rats.

S. No	Groups	Serum Lipid Parameters (mg/dl)				
		Total Cholesterol	Triglycerides	HDL-C	LDL-C	VLDL-C
1	Normal Control (Saline)	84.67 ± 1.18	64.73 ± 7.07	47.27 ± 3.62	24.46 ± 1.61	12.95 ± 1.71
2	Hyperlipidemic Control	205.7 ± 13.81	117.9 ± 5.45	34.98 ± 4.40	147.1 ± 16.1	23.58 ± 1.39*
3	MEPM (400 mg/kg)	105.5 ± 11.22	104.2 ± 7.11	38.23 ± 2.31	43.02 ± 5.12	21.11 ± 1.44
4	MEMC (400 mg/kg)	104.1 ± 11.34	103.1 ± 5.1	39.22 ± 3.12	42.11 ± 4.42	20.13 ± 1.22
5	MEAP (400 mg/kg)	101.9 ± 11.27**	99.12 ± 2.56*	41.07 ± 5.61*	41.01 ± 3.62*	19.81 ± 0.45*
6	METC (400 mg/kg)	101.8 ± 11.26**	99.13 ± 2.76*	42.07 ± 5.51*	41.04 ± 3.62*	19.71 ± 0.45*
7	PHME(400 mg/kg)	93.36 ± 14.16**	83.19 ± 2.30*	45.03 ± 3.66**	32.09 ± 12.01*	17.83 ± 0.46*
8	Atorvastatin (10 mg/kg)	91.17 ± 12.21**	84.32 ± 3.13**	45.10 ± 2.69*	32.44 ± 13.90*	16.86 ± 0.70*

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

Table 2: Effect of MEPM, MEMC, MEAP, METC and PHME serum lipid parameter levels in fat diet induced Hyperlipidemic rats.

S. No	Groups	Serum Lipid Parameters (mg/dl)				
		Total Cholesterol	Triglycerides	HDL-C	LDL-C	VLDL-C
1	Normal Control (Saline)	83.84 ± 1.32	64.07 ± 7.13	48.34 ± 5.58	22.69 ± 5.34	12.81 ± 1.27
2	Hyperlipidemic Control	187.0 ± 11.85	102.9 ± 5.28	25.05 ± 3.43	141.4 ± 13.04	20.58 ± 1.24
3	MEPM (400 mg/kg)	127.1 ± 11.22*	87.11 ± 3.21*	27.11 ± 3.21*	80.11 ± 12.12*	20.12 ± 1.05*
4	MEMC (400 mg/kg)	125.2 ± 11.41*	85.12 ± 2.12*	29.11 ± 2.21*	78.11 ± 12.21*	18.11 ± 1.09*
5	MEAP (400 mg/kg)	124.0 ± 11.23*	84.16 ± 3.56*	30.00 ± 4.36*	76.41 ± 12.34*	17.59 ± 0.81*
6	METC (400 mg/kg)	123.0 ± 11.23*	83.16 ± 3.56*	31.00 ± 4.36*	75.41 ± 12.34*	16.59 ± 0.81*
7	PHME(400 mg/kg)	98.7 ± 12.34**	72.28 ± 5.66**	36.19 ± 5.77**	50.25 ± 3.14*	15.23 ± 1.25*
8	Atorvastatin (10 mg/kg)	97.62 ± 11.64**	70.24 ± 3.20*	38.34 ± 2.4*	45.28 ± 13.24*	14.00 ± 0.47*

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

Estimation of LDL cholesterol

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

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

Estimation of VLDL cholesterol

VLDL cholesterol was estimated by using following formula

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

RESULTS AND DISCUSSION

Phytochemical analysis of the plant extract showed different phyto constituents viz. glycosides, phytosterols, triterpinoids, alkaloids and flavonoids. Several phyto constituents like glycosides, triterpinoids, Saponins, alkaloids and flavonoids are known screening of anti hyperlipidemic 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 MEPM, MEMC, MEAP, METC 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 be

associated 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 transesterification 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.

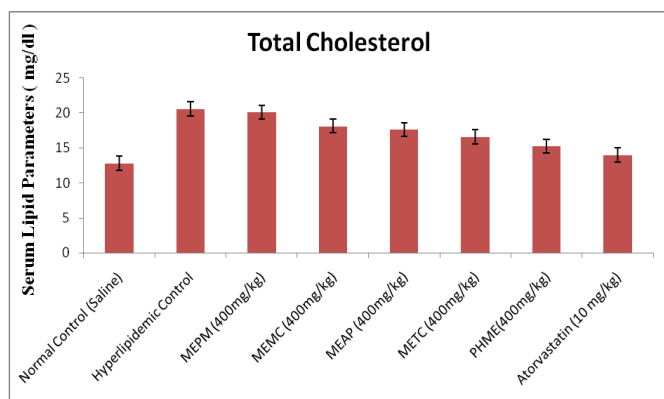


Fig. 1: Effect of MEPM, MEMC, MEAP, METC and PHME on Total cholesterol in Triton induced Hyperlipidemic rats.

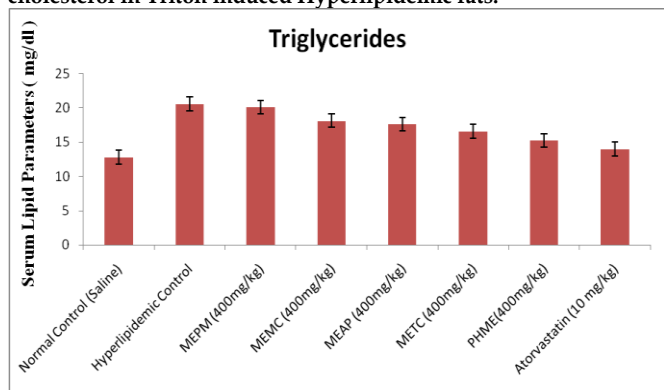


Fig. 2: Effect of MEPM, MEMC, MEAP, METC and PHME on Total cholesterol in Triton induced Hyperlipidemic rats.

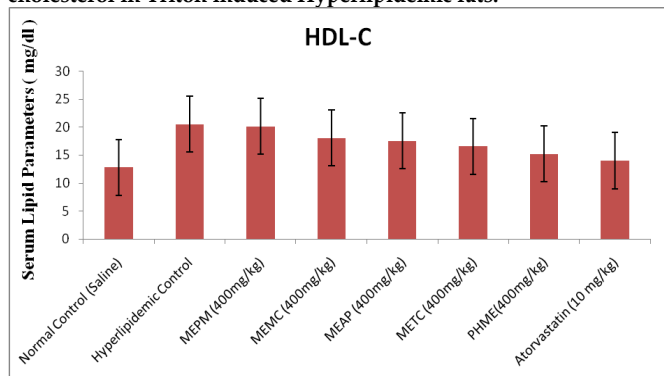


Fig. 3: Effect of MEPM, MEMC, MEAP, METC and PHME on Total cholesterol in Triton induced Hyperlipidemic rats.

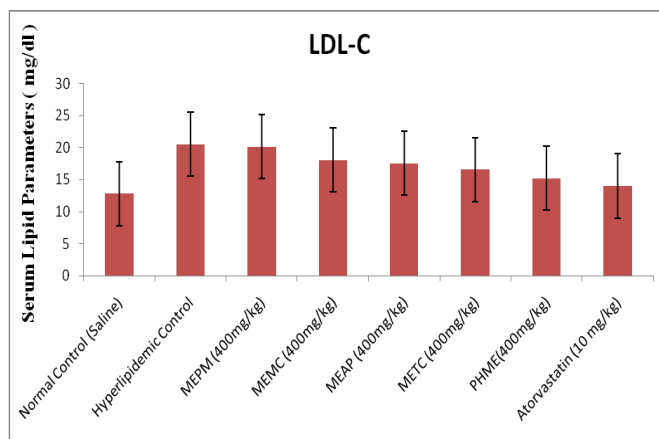


Fig. 4: Effect of MEPM, MEMC, MEAP, METC and PHME on Total cholesterol in Triton induced Hyperlipidemic rats.

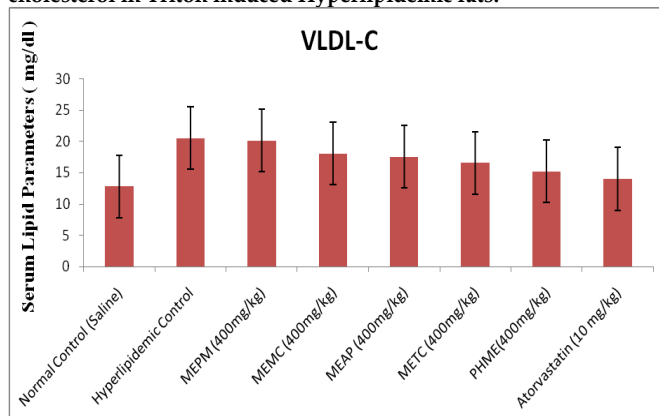


Fig. 5: Effect of MEPM, MEMC, MEAP, METC and PHME on Total cholesterol in Triton induced Hyperlipidemic rats.

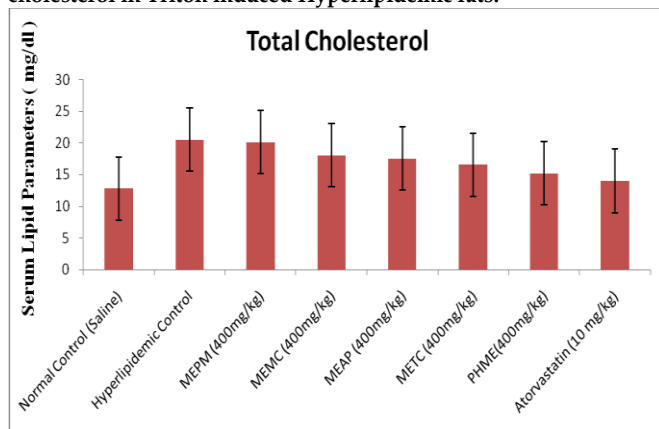


Fig. 6: Effect of MEPM, MEMC, MEAP, METC and PHME on Total cholesterol levels in fat diet induced Hyperlipidemic rats.

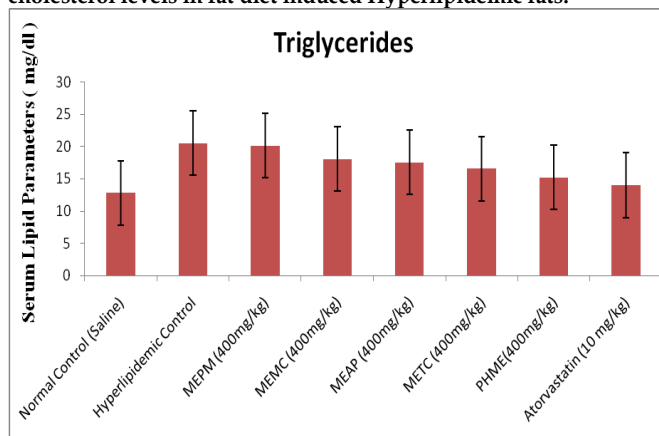


Fig. 7: Effect of MEPM, MEMC, MEAP, METC and PHME on Triglyceride levels in fat diet induced Hyperlipidemic rats.

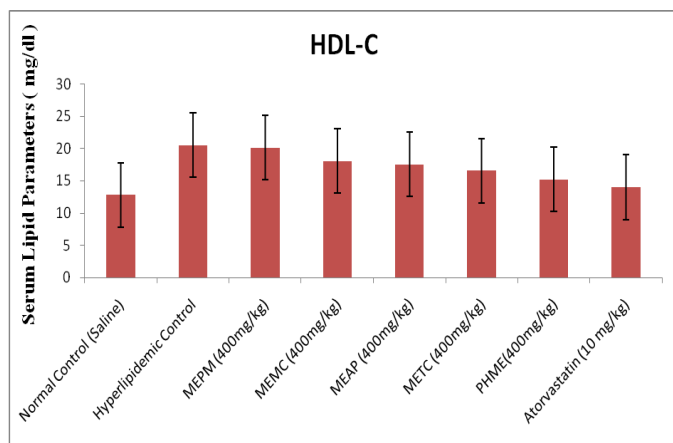


Fig. 8: Effect of MEPM, MEMC, MEAP, METC and PHME in fat diet induced Hyperlipidemic rats.

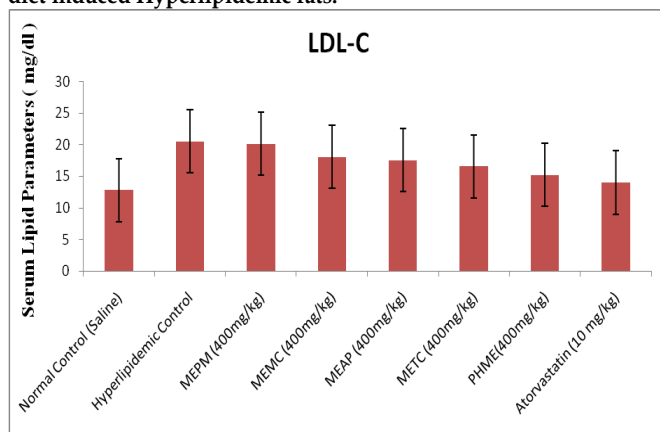


Fig. 9: Effect of MEPM, MEMC, MEAP, METC and PHME in fat diet induced Hyperlipidemic rats.

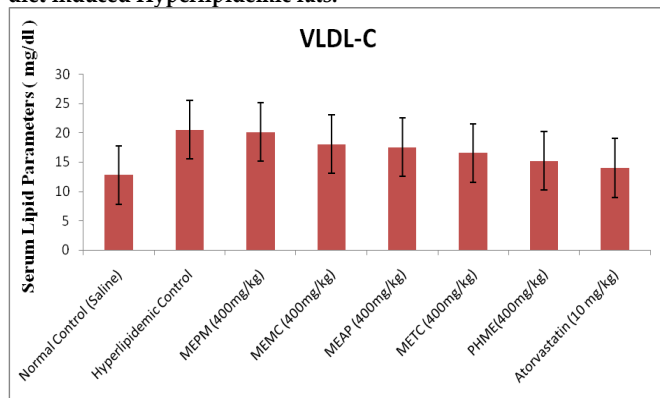


Fig. 10: Effect of MEPM, MEMC, MEAP, METC and PHME in fat diet induced Hyperlipidemic rats.

Anti hyperlipidemic activity was observed with Atorvastatin (10 mg/kg *p.o.*), and the PHME (400 mg/kg) showed better activity than MEPM, MEMC, MEAP, METC (400 mg/kg), to have anti-hyperlipidemic properties. Treatment with PHME (400 mg/kg, *p.o.*) for 7 days 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 Triton model rats respectively. Results are shown in Table 1 and 2 and figure 1-10.

Preliminary phytochemical investigations showed the presence of bioactive compounds like glycosides, sterols, terpenoids and phenolic compounds in selected plants namely, *Momordica charantia*, *Tinospora cordifolia*, *Andrographis paniculata*, *Pterocarpus marsupium* and *Gymnema sylvestre*. 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 hyperlipidemic 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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