



# AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

## Evaluation of Synergistic Effect of Green Tea and Horse Gram Powder Extract on the Treatment of Obesity Induced Rats

Dhanalaxmi Mohan Radj<sup>1\*</sup>, K. Bhaskar Reddy<sup>2</sup>

1. Faculty of Science, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India

2. Sri Venkateswara College of Pharmacy, R.V.S Nagar, Tirupati Road, Chittoor-517127, Andhra Pradesh, India.

### ABSTRACT

The present study aimed that evaluation of synergistic effect of green tea and horse gram powder extract on the treatment of obesity induced rats. Extraction of leaf of the *Camellia sinensis* and legume of *Macrotyloma uniflorum* with methanolic by Maceration method. Individually and combined both plants extracted poly herbal extraction and screened for phytochemical study performed Preliminary Phytochemical analysis of crude extracts for individual plants and poly herbal extraction and evaluated leaf of the *Camellia sinensis* and legume of *Macrotyloma uniflorum* and poly herbal extraction for Anti-Hyperlipidemic activity 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, flavanoids, 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:** *Macrotyloma uniflorum*, *Camellia sinensis*, hyperlipidemia

\*Corresponding Author Email: [dhanadlx@gmail.com](mailto:dhanadlx@gmail.com)

Received 14 October 2016, Accepted 28 October 2016

Please cite this article as: Raj DM *et al.*, Evaluation of Synergistic Effect of Green Tea and Horse Gram Powder Extract on the Treatment of Obesity Induced Rats. American Journal of PharmTech Research 2016.

## INTRODUCTION

Hyperlipidemia is a major cause of atherosclerosis and the atherosclerosis-associated conditions, such as coronary heart disease, ischemic cerebrovascular disease and peripheral vascular disease. Although the incidence of the atherosclerosis related events has declined in the united states, these condition still accounts for the majority of morbidity y and mortality among middle aged and older adults, the incidence and absolute number of annual events will increase over the next decade because of epidemic of obesity and ageing of the U.S population.<sup>1,2</sup> Dyslipidemia, including hyperlipidemia and hypercholesterolemia and low level of high density of lipoproteins cholesterol HDL are major cause of increased atherogenic risk; both genetic disorders and lifestyle diet high in calories, saturated fat, and cholesterol contribute to dyslipidemia seen in developed countries around the world.<sup>3</sup> Severe hypertriglyceridemia (i.e. Triglyceride level of >1000mg/dl) requires therapy to prevent pancreatitis.<sup>4</sup> Moderately elevated triglyceride level 150 to 400mg/dl also are concern because they often occur as part of the metabolic syndrome, which includes insulin resistance, obesity, hypertension, low HDL level and substantially increased CHD risk.<sup>5,6</sup> Medicinal plant based drug has now advantageous over modern drugs. As such are long history of use and better patient tolerance as well as public acceptance, renewable source cultivation and processing environmental friendly, local availability, plant may major source of lead generation. Several recent break through are gugulipid, taxol, artemisinin.<sup>7</sup> Medicinal plant contains so many chemical compounds which are the major source of therapeutic agents to cure human disease.<sup>8</sup> *Macrotyloma uniflorum* The seed has the ability to reduce postprandial hyperglycemia by slowing down carbohydrate digestion and reducing insulin resistance by inhibiting protein-tyrosine phosphatase 1 beta enzyme. Anti-hyperglycemic activity, anti-oxidant activity, ureolithiatic activity, anti microbial activity etc. This is the brief discussion about the plants which we are taken for the present study and we have to discuss about the disease hyper lipidemia in hyperlipidemia rodents.

Green tea extract standardised to 8.35% caffeine and 24.7% catechins has been shown to stimulate brown adipose tissue *in vivo*, with thermogenesis greater than the effect the caffeine content accounts for long term ingestion of tea catechins stopped the accumulation of body fat in mice with high fat diet induced obesity <sup>9</sup> possibly due to the activation of hepatic lipid metabolism <sup>10</sup>. This effect was also found in non obese rats <sup>11</sup>.

## MATERIALS AND METHOD

### Collection of plant and authentication

Dried leave of *Camellia sinensis* and legumes of *Macrotyloma uniflorum* was procured from Madhavachetti botanical garden, Thirupathi and was authenticated by Dr. K. Madhavachetti, Assistant Professor in Department of Botany at Sri Venkateswara University, Tirupathi.

### **Extraction by Simple Maceration**

The dried leaves of *Camellia sinensis* and The dried leaves legumes of *Macrotyloma uniflorum* 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.

### **Preliminary Phytochemical Screening**

The crude methanolic extract of *Camellia sinensis* and legumes of *Macrotyloma uniflorum* individually and poly herbal extraction were tested for its different chemical groups such as alkaloids, flavanoids, tannins, steroids, saponins ,fixed oils, gums and mucilages, tri-terpenoids, carbohydrates and glycosides, phytosterols.

### **Experimental Animals:**

Wistar rats of either sex weighing between 160-250 g were procured from albino research & training institute for experimental purpose. Then the animals were acclimatized for 7 days under standard husbandry conditions. Room temperature -  $26 \pm 20$  C, Relative humidity - 45 - 55%, Light/ dark cycle - 12: 12 h. The animals were fed with a standard diet purchased from Amrut Laboratories & Pranav Agro Industries Ltd. Sangli, Maharashtra, India. Water was allowed *ad libitum* under strict hygienic conditions. All animal studies were performed as per the guidelines of CPCSEA and Institutional Animal Ethical Committee (IAEC). CPCSEA Approval Number: 1722/RO/ERe/S/13/CPCEA/03.

### **Anti hyperlipidemic study**

#### **Triton X 100 Induced Hyperlipidemia model**

Triton X 100 (TR) induced hyperlipidemic model forty six Wistar rats were randomly divided into 7 groups of 6 each. 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 400mg /kg body weight. After 72hours of triton injection, the second group received a daily dose of 5% CMC (p.o) for 7 days. The third group was administered daily dose of MESC 200mg/kg, fourth group was administered a daily dose of MEMU 200mg/kg and Group V and Group VI was administered daily dose of PHME 200mg/ kg and 400 mg/kg

suspended in 5%CMC, p.o., for 7 days, after inducing hyperlipidemia. Seventh group was administered with the standard Atorvastatin 10mg/kg, p.o. for 7 days. Food was withdrawn 10h prior to the blood sampling. The control group animals received the vehicle in the same volume orally.

Group 1: Administered vehicle and served as normal control.

Group 2: Administered Triton X 100 (TR) and served as hyperlipidemic control.

Group 3: Administered MECS (200mg/kg), p.o.

Group 4: Administered MEMU (200mg/kg), p.o.

Group 5: Administered PHME (200mg/kg), p.o.

Group 6: Administered PHME (400mg/kg), p.o.

Group 7: Administered Atorvastatin (10mg/kg), p.o.

On the 8thday, blood was collected by retero orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 15minutes at 2500rpm. 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. Fourty 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 21days. The rats are then given test plant extracts i.e., RNM (200 and 400mg/kg, p.o) and Atorvastatin (10mg/kg, p.o) 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. 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 400mg /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 MESC 200mg/kg, fourth group was administered a daily dose of MEMU 200mg/kg and Group V and Group VI was administered daily dose of PHME 200mg/kg and 400 mg/kg suspended in 5%CMC, p.o., for 7 days, after inducing hyperlipidemia. Seventh group was administered with the standard Atorvastatin 10mg/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 1: Administered vehicle and served as normal control.

Group 2: Administered FD and served as hyperlipidemic control.

Group 3: Administered MECS (200mg/kg), p.o. and fed with FD

Group 4: Administered MEMU (200mg/kg), p.o. and fed with FD

Group 5: Administered PHME (200mg/kg), p.o. and fed with FD

Group 6: Administered PHME (400mg/kg), p.o. and fed with FD

Group 7: Administered Atorvastatin (10mg/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 15min at 2500rpm 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 15minutes at 2500rpm. 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:<sup>12</sup>**

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): 0.02ml serum, 2.00ml reaction solution; the standard sample (S): 0.02ml standard and 2.00ml reaction solution, while for the blank sample (B): 0.02ml DW and 2.00ml reaction solution. The mixture was mixed well and incubated for 10 minutes at +20 to 25c or 5 minutes at 37c. The absorbance was read at 505/670 nm against the reagent blank

**Estimation of serum triglycerides (TG):** <sup>13</sup>

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 1ml reaction solution was added. In an another test tube (S) 0.01ml standard and 1ml reaction solution were added. The solution was mixed well and incubated at +20 to 25C for 10min. The absorbance of standard and test against reagent blank was read at 505 (500-540 nm).

**Estimation of HDL-cholesterol:** <sup>14</sup>

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 2ml 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 10min at +15 to 25c and then centrifuged for 15min at 4000rpm. Within 2hr 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 rreaction solution was added to it. In another test tube 0.1 ml DW was taken and 1ml reaction solution (B) was added. The mixtures were mixed thoroughly, incubated for 10min at 15-25 c or for 5min at 37c and measured the absorbance of the sample against reagent blank at 546nm

**Estimation of LDL cholesterol:**

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

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

**Estimation of VLDL cholesterol:**

VLDL cholesterol was estimated by using following formula

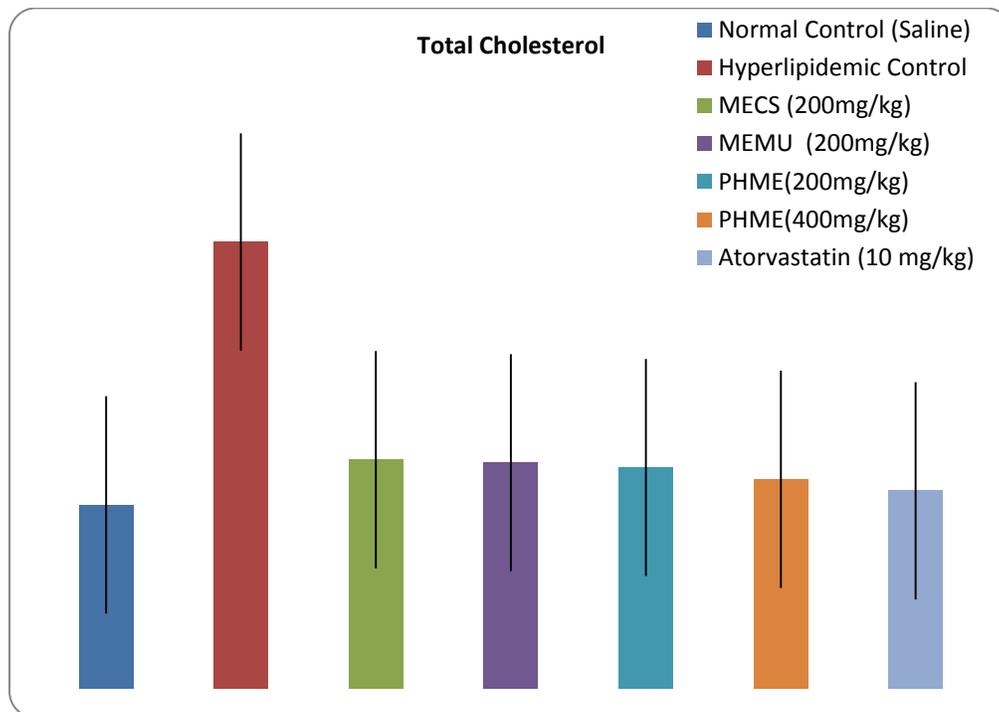
$$\text{VLDL in mg \%} = \text{Triglyceride}/5$$

**RESULTS AND DISCUSSION**

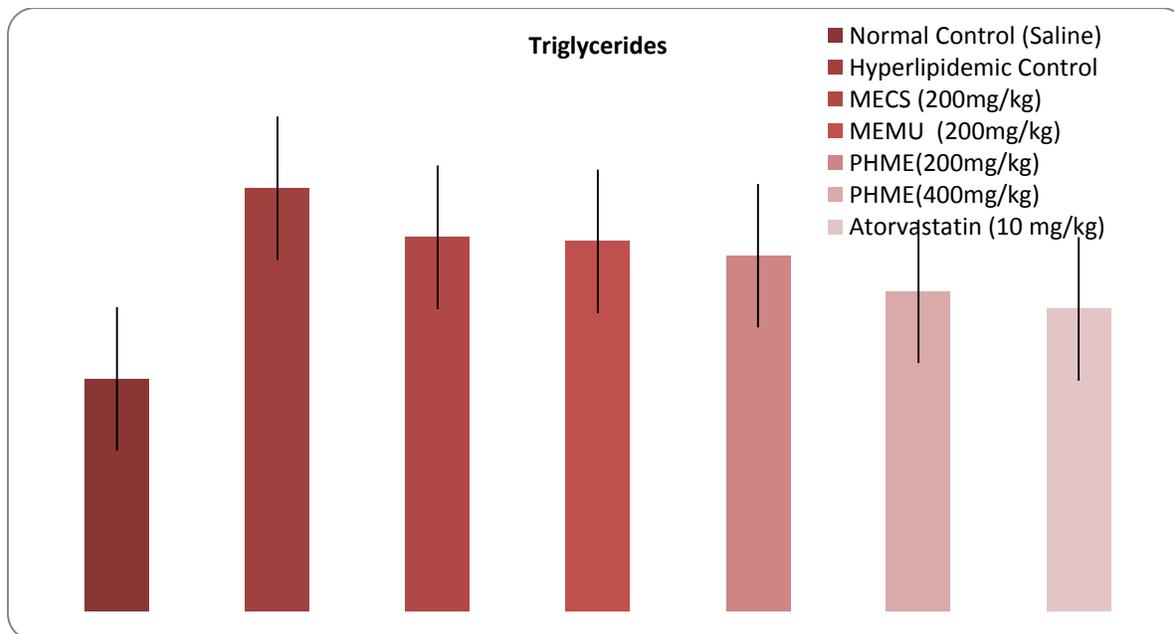
**Table 1: Effect of MECS, MEMU and PHME on serum lipid parameter levels in Triton induced Hyperlipidemic rats**

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	MECS (200mg/kg)	105.5±11.22	104.2±7.11	38.23±2.31	43.02±5.12	21.11±1.44
4	MEMU (200mg/kg)	104.1±11.34	103.1±5.1	39.22±3.12	42.11±4.42	20.13±1.22
5	PHME(200mg/kg)	101.9±11.27**	99.12±2.56*	41.07±5.61*	41.01±3.62*	19.81±0.45*
6	PHME(400mg/kg)	96.36±14.16**	89.19±2.30*	43.03±3.66**	36.09±12.01*	17.83±0.46*
7	Atorvastatin (10 mg/kg)	91.17±12.21**	84.32±3.13**	45.10±2.69*	32.44±13.90*	16.86±0.70*

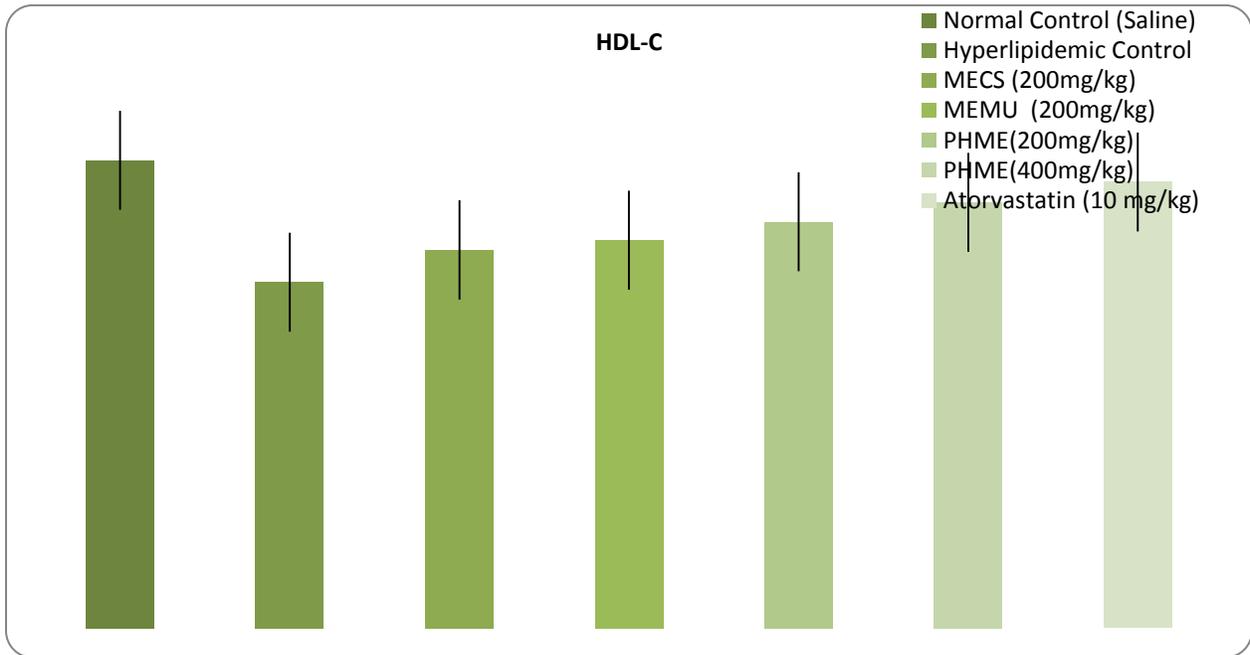
Values are mean  $\pm$  SEM (n=6). Values are statistically significant at  $**P \leq 0.01$  vs hyperlipidemic control using one way ANOVA followed by Dunnet's test



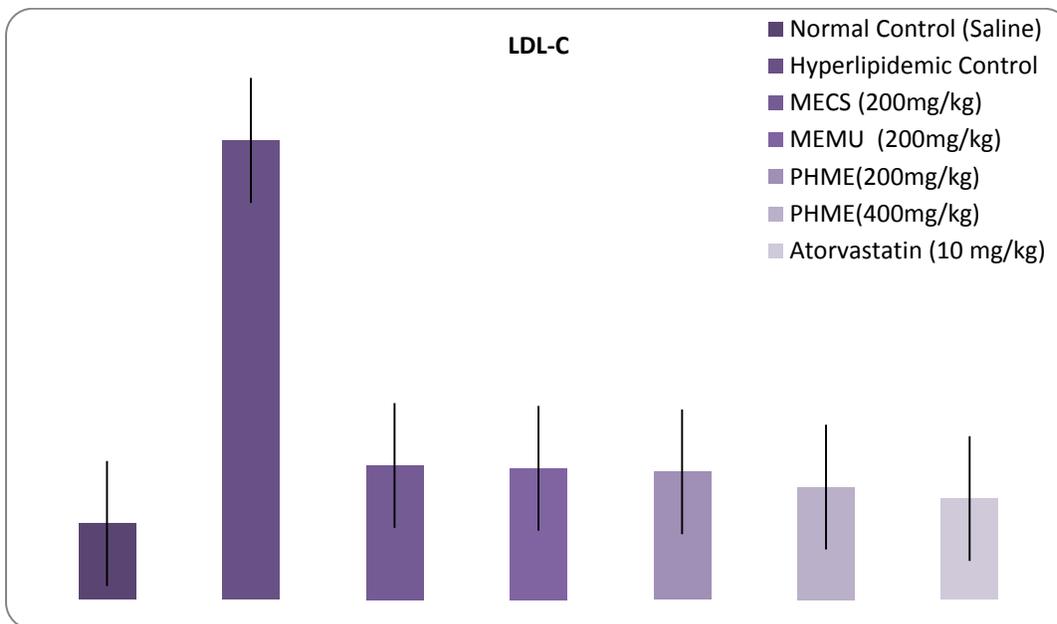
**Figure 1: Effect of MECS, MEMU and PHME on Total cholesterol in Triton induced Hyperlipidemic rats**



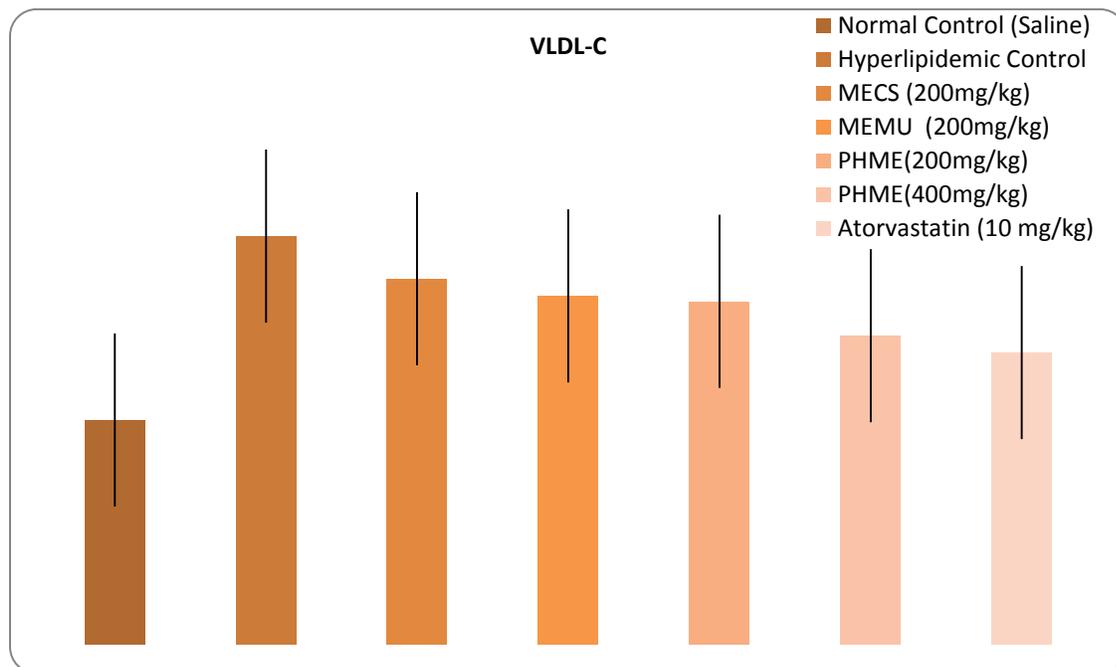
**Figure 2: Effect of MECS, MEMU and PHME on triglycerides in Triton induced Hyperlipidemic rats**



**Figure 3: Effect of MECS, MEMU and PHME on HDL-C in Triton induced Hyperlipidemic rats**



**Figure 4: Effect of MECS, MEMU and PHME on LDL-C in Triton induced Hyperlipidemic rats**

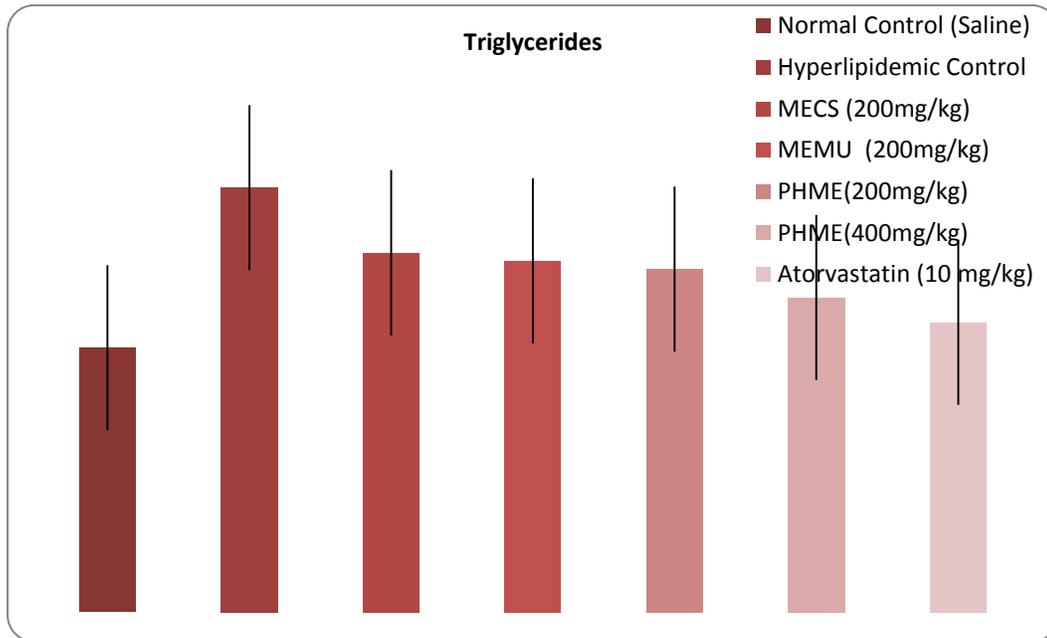


**Figure 5: Effect of MECS, MEMU and PHME on VLDL-C in Triton induced Hyperlipidemic rats**

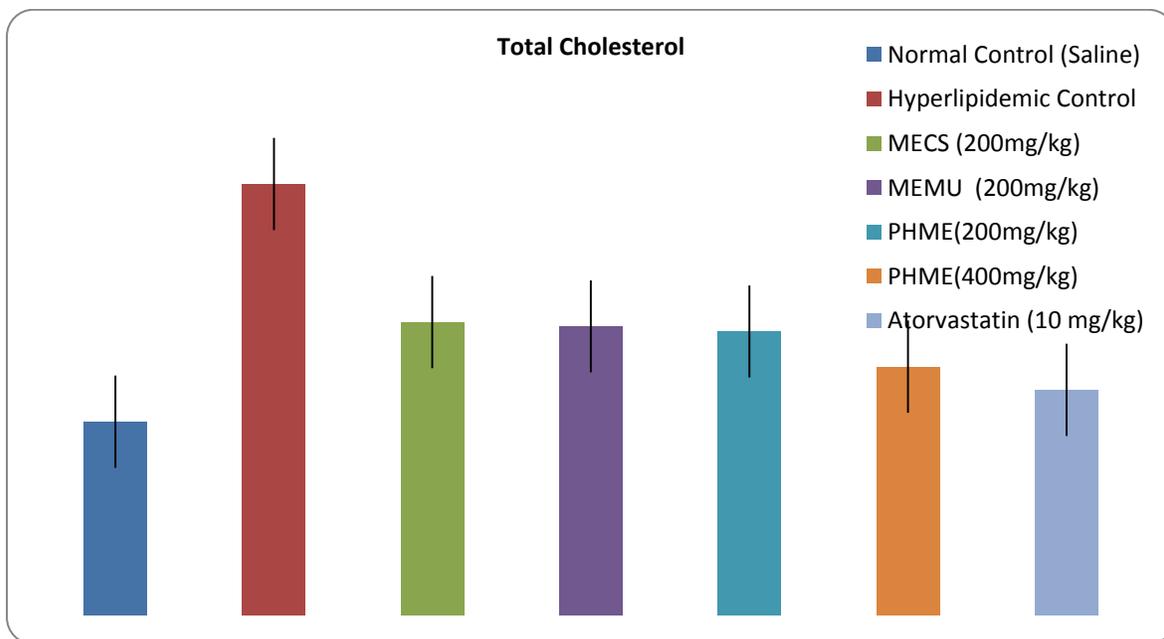
**Table 2: Effect of MECS, MEMU and PHME serum lipid parameter levels in fat diet induced Hyperlipidemic rats**

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	MECS (200mg/kg)	127.1±11.22*	87.11±3.21*	27.11±3.21*	80.11±12.12*	20.12±1.05*
4	MEMU (200mg/kg)	125.2±11.41*	85.12±2.12*	29.11±2.21*	78.11±12.21*	18.11±1.09*
5	PHME(200mg/kg)	123.0±11.23*	83.16±3.56*	31.00±4.36*	75.41±12.34*	16.59±0.81*
6	PHME(400mg/kg)	107.7±12.34**	76.28±5.66**	36.19±5.77**	56.25± 3.14*	15.23±1.25*
7	Atorvastatin (10 mg/kg)	97.62±11.64**	70.24±3.20*	38.34±2.4*	45.28±13.24*	14.00±0.47*

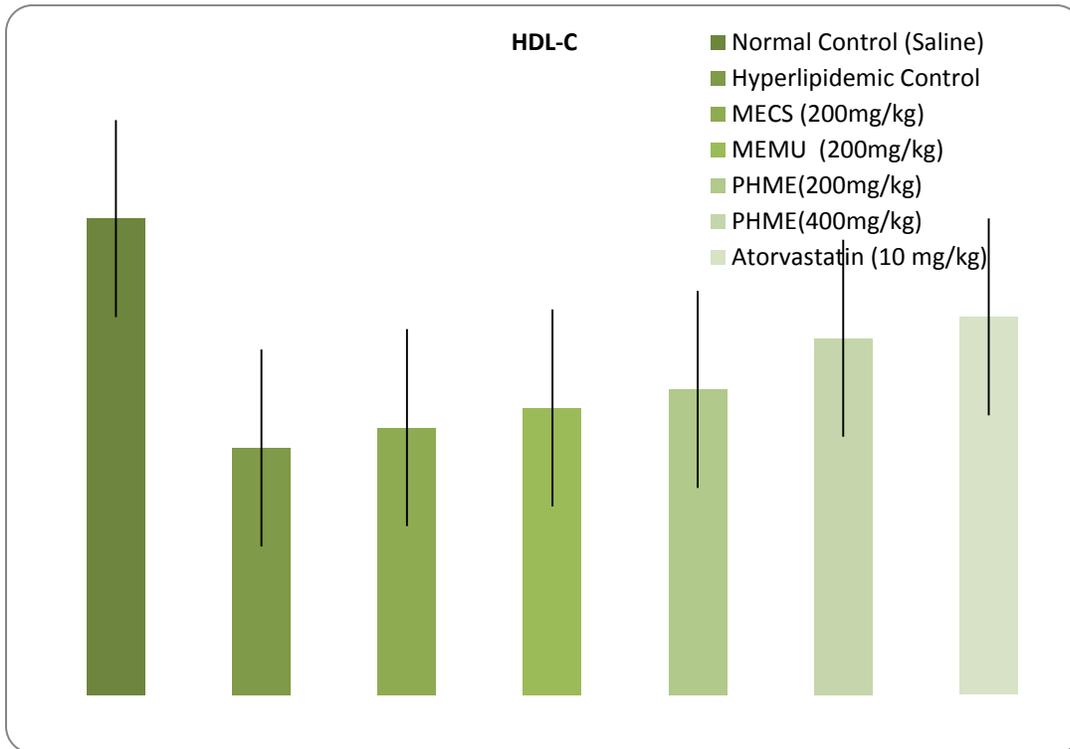
Values are mean ± SEM (n=6). Values are statistically significant at \*\*P<0.01 vs hyperlipidemic control using one way ANOVA followed by Dunnet's test



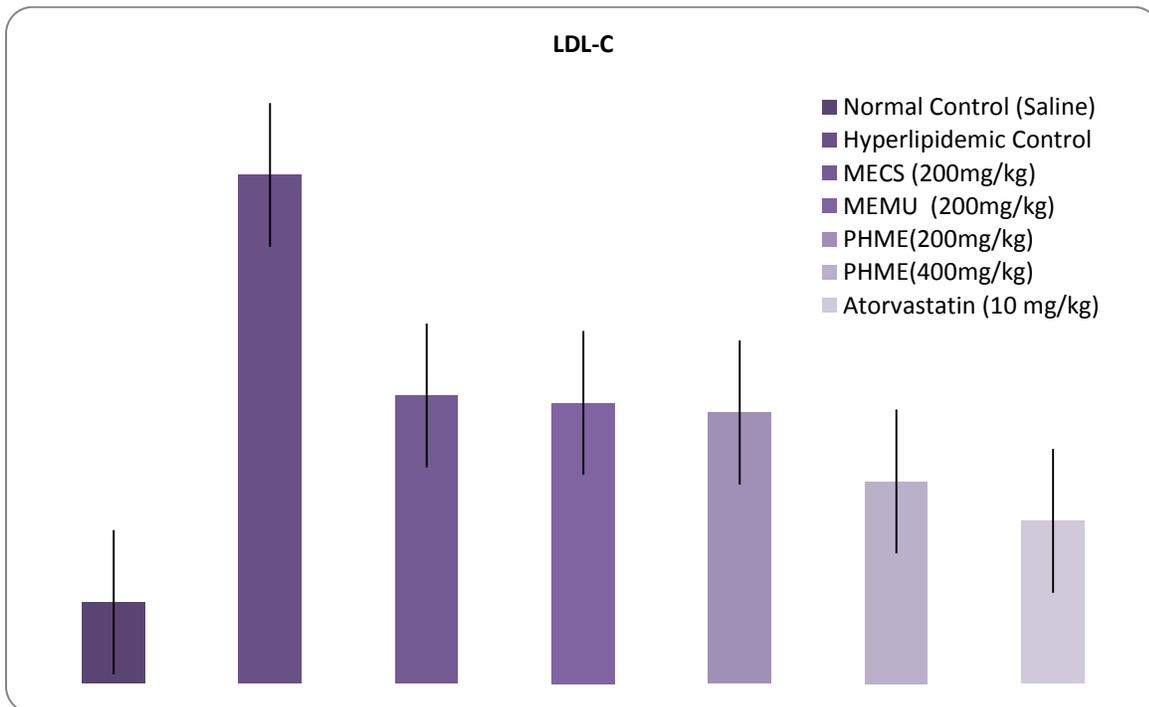
**Figure 6: Effect of MECS, MEMU and PHME Triglyceride levels in fat diet induced Hyperlipidemic rats**



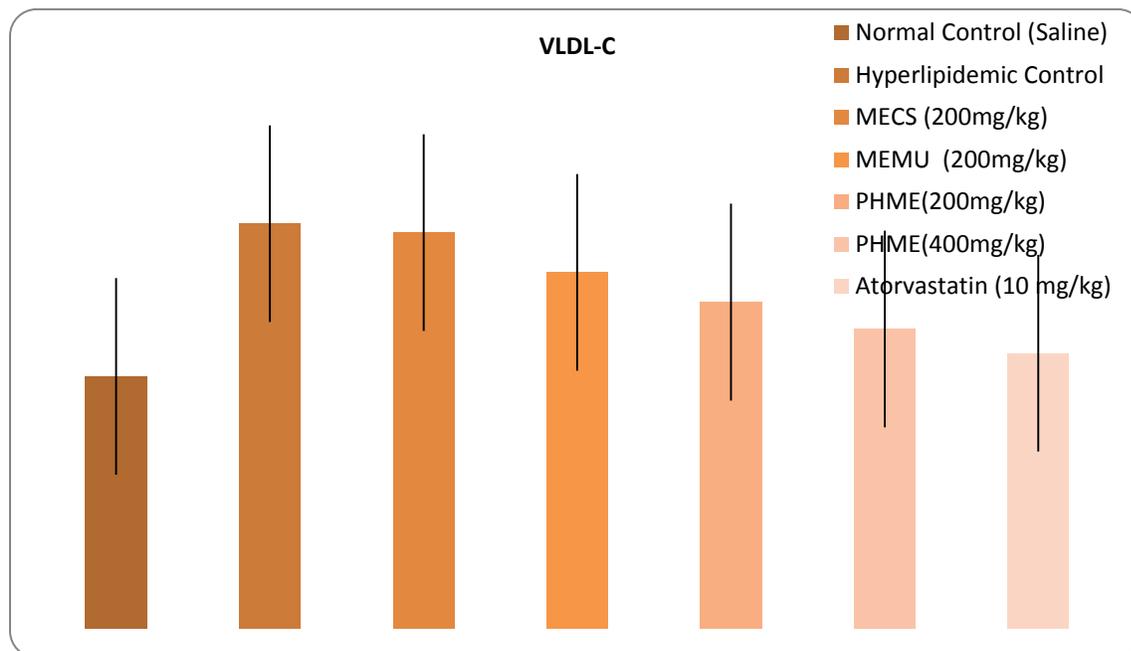
**Figure 7: Effect of MECS, MEMU and PHME Total cholesterol levels in fat diet induced Hyperlipidemic rats**



**Figure 8: Effect of MECS, MEMU and PHME HDL-C in fat diet induced Hyperlipidemic rats**



**Figure 9: Effect of MECS, MEMU and PHME LDL-C in fat diet induced Hyperlipidemic rats**



**Figure 10: Effect of MECS, MEMU and PHME VLDL-C in fat diet induced Hyperlipidemic rats**

Phytochemical analysis of the plant extract showed different phytoconstituents viz. glycosides, phytosterols, triterpinoids, alkaloids and flavonoids. Several phytoconstituents 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 antihyperlipidemic effect of MECS, MEMU, PHME could be due to an increased catabolism of cholesterol into bile acids. Administration of PHME (200 & 400mg/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 hyperlipidimias and atherosclerosis. Fat diet model is used as a chronic model for induction of hyperlipidemia. In our 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 cholesterologenesis 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 (LDLreceptor) 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. Antihyperlipidemic activity was observed with Atorvastatin (10mg/kg p.o.), and the PHME (200mg/kg and 400mg/kg) showed better activity than MEMU, MECS (200mg/kg), to have anti-hyperlipidemic properties. Treatment with PHME (200 & 400mg/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.

## CONCLUSION

Plants have played a significant role in human health care since the ancient times. Traditional plants exerts great role in discovery of new drugs, however, the exact mechanism responsible for activities is currently unclear. Therefore, further investigations need to be carried out to isolate and identify specific compounds present in the plant extract responsible for these activities and exact mechanism. It was concluded that individual extraction shows the activities but in combination of both plants it shows synergistic effect so poly herbal extraction is useful more when compared with given in individual plants.

## REFERENCES

1. Robert W, Mahley, Thomas P, Bersot Goodman & Gilman's. The Pharmacological Basis Of Therapeutics , 11th Edn, McGraw-Hill medical publication, 2006: 977-978.
2. Stone NJ, Robinson J, Lichtenstein AH, Merz NB, Blum CB. Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines, American heart association,2013
3. Kasper D L, Braunwald E, Fauci A S, Hauser, Longo, Jameson. Harrison's principles of internal medicine.16th Edn; Mc Graw Hill:2008, 2288

4. Rang HP, Dale MM, Ritter JM, and Flower RJ. Rang And Dale's Pharmacology, 6 th Edn, 324.
5. Nicki RC, Brian RW, Stuart HR. Davidson's Principle & Practice of Medicine, 21st Edn: 2010, 577-579.
6. Walker R, Edward C, Clinical Pharmacy and Therapeutics,3rd Edn:, 2003, p.353
7. Mishra LC., Scientific Basis For Ayurvedic Therapies, CRC press LLC,2004.4
8. Nadkarni KN, Nadkarni AK, the Indian Materia Medica, Popular Prakashan, Vol; 1,561.
9. Dulloo A, J. Seydoux L. Girardier P. Chantre, Vandermander, Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. Int. J. Obesity 2000, 2(252-8): 56.
10. Tokimitsu I. Effects of tea catechins on lipid metabolism and body fat accumulation. Biofac 2004, 22(1-4): 141-3.
11. Ito Y, T. Ichikawa, Y. Morohoshi, T. Nakamura, Saegusa and K. Ishihara. Effect of tea catechins on body fat accumulation in rats fed a normal diet. Biomed Res., 2008, 291: 27-32.
12. Cholesterol CHOD-PAP method, end point. Robonik diagnostics Navi Mumbai. 2010.
13. Triglycerides GPO-Trinder,,s method, end point. Robonik diagnostics Navi Mumbai. 2010.
14. HDL-cholesterol Phosphotungstic acid method, end point. Robonik diagnostics Navi Mumbai. 2010.

***AJPTR is***

- Peer-reviewed
- bimonthly
- Rapid publication

Submit your manuscript at: [editor@ajptr.com](mailto:editor@ajptr.com)

