

## Research Article

# Anti-Atherosclerotic Activity of Polygonum Glabrum against High-Fat Diet Induced Atherosclerosis in Rats

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## Abstract

**Objective:** Purpose of the current study was to assess the anti-atherosclerotic activity of Ethanolic Extract of Polygonum Glabrum (EEPG) against high-fat diet induced atherosclerotic changes in male wistar rats.

**Procedure:** The Procedure used for induction of atherosclerosis was high-fat diet for 28 days. Rats were divided into five groups (n=6). Group I served as normal. Group II serves as high-fat diet-treated group. Group III serves as standard treated with high-fat diet + Orlistat (50 mg/kg, PO). Group IV serves as low dose treated with high-fat diet + EEPG (200 mg/kg, PO). Group V serves as high dose treated with high-fat diet + EEPG (400 mg/kg, PO). The following parameters, High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Total Cholesterol (TC), Very LDL (VLDL), Triglycerides (TG), Atherogenic Index (AI), Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline Phosphate (ALP) and body weight, were evaluated. Additionally oxidative stress parameters such as levels of Malondialdehyde (MDA), Reduced Glutathione (GSH) and activity of Superoxide Dismutase (SOD) and Catalase (CAT) and histopathological studies were performed.

**Results:** The results showed that EEPG at a dose of 200 mg/kg and 400 mg/kg exhibited significant decrease in, TG, TC, LDL-cholesterol (LDL-C), VLDL, AI, SGPT, SGOT, ALP and increase in HDL-Cholesterol, when compared to high-fat diet group. As well as significant decrease in, MDA content and increase in GSH, SOD and CAT activity when compared to high-fat diet group. Histopathological results are in accompaniment to above observations.

**Conclusion:** The experimental studies show that the EEPG both doses 200 mg/kg and 400 mg/kg, showed significant reduction in lipid profile and liver function parameters, improvement in pro oxidant: anti oxidant ratio and elevation in HDL Cholesterol. From the scientific study, it concluded that the plant extract showed anti-atherosclerotic activity and thus authenticates its traditional use in prescribed conditions of atherosclerosis.

**Keywords:** Atherosclerosis; Atorvastatin; High fat diet; Polygonum glabrum

## Introduction

As per World Health Organization, Cardiovascular Diseases have been listed first among the major causes of worldwide for, mortality over 15 years. WHO data also indicate that in 2011, approximately 17.3 million people died worldwide as a result of CVD, most of which (80%) recorded in westernized countries. An alarming statistic indicates that this number could reach 23.6 million in 2030, if effective interventions will not have been proposed [1]. Among the main underlying causes of CVD development is atherosclerosis. The term, from Greek origin, consists in two parts: atheros, characterized by fat accumulation accompanied by macrophages; and sclerosis, featured by fibrosis layer comprising smooth muscle cells, connective tissue and leukocyte [2].

Atherosclerosis is a multifactorial, slow and progressive disease, endpoint of a series of highly specific molecular and cellular reactions in response to endothelial aggression that results in formation of atherosclerotic plaques in the blood vessels, affecting mainly the intima of medium and large-caliber arteries [2-4]. The incidence of atherosclerosis increases exponentially in adults over 45 years and is considered a non-modifiable disorder, although some studies have found atherosclerotic plaques in young adults, suggesting that this disorder can also occur earlier [5-12]. However, actually it is established that atherosclerosis is not a simple and inevitable degenerative consequence of aging, but an inflammatory condition that can be converted into a clinical and acute event caused by the rupture of the plaque and thrombus formation [13].

**Antioxidant Function** For many years it is known that natural products, especially those rich in polyphenol compounds show significant antioxidant properties. As for the genesis of atherosclerotic disease, cholesterol oxidation is a limiting step, it is to be expected that different metabolites present in natural products can play significant vasoprotective effects.

The main natural products antioxidant mechanisms of action are through ROS concentration decreasing or production blocking and lipid chain oxidation inhibiting [14]. Antioxidant protection is linked to reduction of lipoproteins oxidative change and lipid peroxidation prevention, since it is considered an atherosclerosis key event. The most antioxidants effects of natural products are attributed to phenolic compounds and their moderate consumption have additional benefits

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in the atherosclerosis, prevention and development decreasing blood pressure and reducing platelet aggregation [14-16].

In general, preclinical study and clinical trials show that the natural products exert their effects on several important aspects involved in the atherosclerotic process, including serum lipid profile, endothelial function, inflammation, oxidative stress, and platelet aggregation/coagulation. So the aim of our study was to evaluate the action of Polygonum Gabrum as an add-on therapy in prevention of accumulation of atherosclerotic plaque that can contribute to heart attack, stroke, and other cardiovascular diseases against high fat diet.

## Materials and Methods

### Plant material

Aerial Parts of Polygonum Glabrum used were collected from Tirumalla Hills, Tirupati, District of Andhra Pradesh. The plant was taxonomically identified and authenticated by Dr. Madhav shetty, Department of Botany, Sri Venkateswara University, Tirupathi where the voucher specimen for the same is restored under the reference number AB-01.

### Preparation of the extracts

Aerial Parts of Polygonum Glabrum were cut into small pieces were cleaned and dried under shade at room temperature for several days and powdered [17]. The resulting powder was then used for extraction. The dried powder of barks were defatted with petroleum ether and then extracted with 70% ethanolic using soxhlet apparatus. The extract was concentrated under reduced pressure using rota flash evaporator which resulted with a yield of 9.34% w/w. The extract was stored in air tight container in refrigerator. Ethanolic Extract of Polygonum Glabrum (EEPG) was further used for pharmacological evaluation. The preliminary phytochemical screening was carried out on 70% ethanolic extract of Polygonum Glabrum for qualitative identification of type of phytoconstituents present.

### Drugs and chemicals

Atorvastatin were obtained from Sun Pharma, Vadodara, India. Total Cholesterol, Triglyceride, SGPT, SGOT and ALP kits were obtained from Span Diagnostics, Surat, India. All other chemicals used in this study were obtained commercially and were of analytical grade.

High fat diet is a hyper caloric diet and was prepared by mixing the above constituents in fixed percentage. The above mentioned percentage is for 100g diet. The feed was prepared, dried, powdered and administered every day in morning to animals with water ad libitum. Diet was administered and weight gain was observed in rats on third day, therefore confirming the development of atherosclerosis in rats. Study was continued for 28 days.

**High fat diet formula:** Casein -20%, D,L methionine-0.3%, corn starch-15%, sucrose-27.5%, cellulose powder-5%, mineral mixture-3.5%, vitamin mixture-1%, choline bitartrate-0.2%, corn oil -9.9%, lard oil-17.6% [18].

### Experimental animals

Wistar albino rats (200 g to 250 g) of either sex were maintained under controlled conditions of temperature ( $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) and humidity ( $50\% \pm 5\%$ ) and a 12-hour light-dark cycle, were used for the experiment. The animals were housed in sanitised polypropylene cages containing sterile paddy husk as bedding. They had free access to standard rat pellet diet and water ad libitum. The animals were

given a week's time to get acclimatized with the laboratory conditions. All the experimental procedures were performed according to the committee for the purpose of control and supervision of experiments on animals (CPCSEA-1015/PO/Re/S/06), Ministry of Social Justice and Empowerment, Government of India, norms and approved by the Institutional Animal Ethics Committee (IAEC).

### Acute toxicity studies

Rats were kept overnight fasting prior to drug administration. Animals received a single oral dose (2000 mg/kg bw and 4000 mg/kg bw) of ethanolic extract of barks of Polygonum Glabrum. After the administration of Polygonum Glabrum extract, food was withheld for further 3 hrs to 4 hrs. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 hrs (with special attention during the first 4 hrs) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes. Mortality, if any, was determined over a period of two weeks [19].

### Selection of dose of the extract

LD50 was done as per OECD guidelines for fixing the dose for biological evaluation. The LD50 of Polygonum Glabrum as per OECD guidelines falls under class four values with no signs of acute toxicity at 4,000 mg/kg. The biological evaluation was carried out at doses of 200 mg/ kg and 400 mg/ kg body weight [20,21].

### High Fat Diet-induced (HFD) atherosclerosis in rats

Thirty Wistar albino rats of either sex were assigned to five groups (n= 6): Group I: Rats in this group were injected with normal saline, intraperitoneally and served as control; Group II: Rats in this group were given HFD for twenty eight consecutive days; and served as normal (HFD) Group III: Rats in this were given HFD for twenty eight consecutive day and Orlistat (50 mg/ kg, po) for 28 consecutive days. Group IV: Rats in this group were given HFD and Polygonum Glabrum extract (EEPG) (200 mg/ kg, PO) for 28 consecutive days; Group V: Rats in this group were given HFD and Polygonum Glabrum extract (EEPG) (400 mg/ kg, PO) for 28 consecutive days. After the last dosing of 28<sup>th</sup> day after 24 hrs the blood sample were collected by puncturing retro-orbital plexus and serum was separated by centrifugation. Serum was analysed for all Lipid profile and Liver Function Test (LFTs) Parameters such as Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and Alkaline Phosphatase (ALP) using standard kits [22].

After collection of blood samples, the rats were sacrificed by light ether anesthesia and their kidneys were excised, rinsed in ice cold normal saline, followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A small 10% (w/v) portion of the kidney was homogenized in chilled Phosphate buffered saline (50 mM, pH 7.4) using a Potter Elvehjem Teflon homogenizer. The homogenate obtained was centrifuged in a cooling centrifuge at 1,000×g for 10 min at 4°C to remove nuclei and unbroken cells. The pellet was discarded and portion of supernatant was again centrifuged at 12,000×g for 20 min at 4°C obtain a post- mitochondrial supernatant which was used for enzyme analysis 91. The contents of Malondialdehyde (MDA), Reduced Glutathione (GSH), Superoxide Dismutase (SOD) and

Catalase (CAT) activity were estimated spectrophotometrically using above post-mitochondrial supernatant. Rats some parts of kidneys preserved in 10% formalin for histopathological study [23].

**Histopathological studies**

Portions of the artery from all the experimental groups were fixed in 10% formalin, dehydrated in alcohol and then embedded in paraffin. Microtome sections (5 μm thick) were prepared from each liver sample and stained with hemtoxylin-eosin (H&E) dye. The sections were examined for the pathological findings [24].

**Statistical analysis**

The experimental outcomes were expressed as Mean ± SEM for six animals in all groups. All parameters were analyzed statistically using one-way Analysis of Variance (ANOVA), followed by Bonferroni post test using Graph Pad prism 5.0 software [23]. Data were considered statistically significant at P<0.05.

**Results and Discussion**

Preliminary Phytochemical studies of Ethanolic Extract of Polygonum Glabrum (EEPG): It revealed the presence of alkaloids, carbohydrates, steroids, glycosides, saponins, flavonoids, proteins, fixed oil and resins in ethanolic root extract of Echinops echinatus (Table 1).

**Acute toxicity study of Ethanolic Extract of Polygonum Glabrum (EEPG)**

In present study, the ethanolic extract of roots of Polygonum Glabrum was tested for its acute toxicity study to find out the LD50. It was found that acute administration of the extract using 2000 mg/kg and 4000 mg/kg dose did not caused any mortality in rats, hence the LD50 of the extract is considered as 4000 mg/kg. Based on the recorded LD50 dose, 1/5<sup>th</sup> and 1/10<sup>th</sup> of the dose is considered as therapeutic dose, and therefore a dose of 200 mg/kg and 400 mg/kg is selected for the evaluation of its anti atherosclerotic activity.

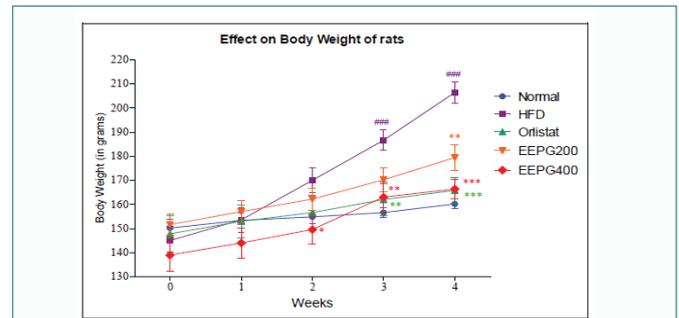
**Effect of ethanolic extract of roots of echinops echinatus on body weight of rats**

Weekly changes in the body weight of rats of each group were

**Table 1:** Preliminary phytochemical screening of polygonum glabrum extract.

S. No.	Test	Result
1	Carbohydrates test	Positive
	a. Molish's test	
2	Alkaloidal test	Positive
	a. Mayer's test	
	b. Hager's test	
3	Steroids test	Positive
	a. Libermann Buchard test	
	b. Salwoski test	
4	Glycosides test	Positive
	a. Legal test	
	b. Borntagers test	
5	Saponins test	Positive
	a. Foam test	
6	Flavonoids test	Positive
	a. Alkaline reagent test	
7	Triterpenoidal test	Negative
	Phenolics & tannins test	
8	a. Ferric chloride test	Negative
	b. Gelatin test	
	Protein & aminoacids test	
9	a. Buret's test	Positive
	b. Ninhydrin test	
	Fixed oil test	
10	a. Spot test	Positive
	Resin test	
11	a. Acetic anhydride test	Positive

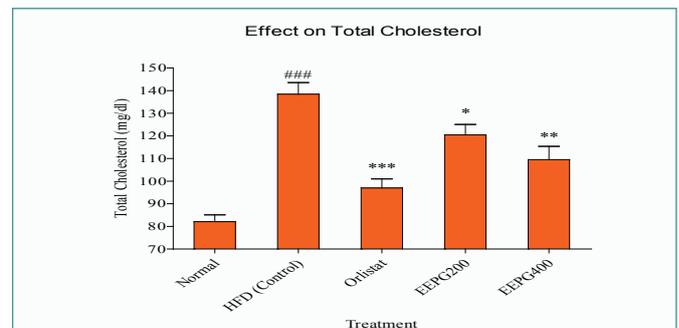
evaluated and it was found that after four weeks the weight of rats fed with HFD increased up to significant level (P<0.001) as compared to the normal rats. Orlistat, a standard drug, administered to the rats fed with HFD showed significant inhibition (P<0.001) of increase in the body weight of rats as compared to increase in the body weight of rats fed with HFD only. After four weeks of oral administration of both selected dose of EEGP (200 mg/kg and 400 mg/kg) found significant (P<0.01 and P<0.001 respectively) in the inhibition of increase in the body weight of rats fed with HFD. EEGP at dose of 400 mg/kg showed significant and similar reduction as compared to the standard Orlistat (50 mg/kg) in the body weight of rats fed along with HFD (Figure 1).



**Figure 1:** Effect of EEGP on body weight of rats. Values are expressed as mean ± SEM (n=6). Data were analyzed by two-way ANOVA followed by Bonferroni post test. ####P<0.001 as compared to Normal. \*P<0.05, \*\*P<0.01 and \*\*\*P< as compared to HFD induced atherosclerotic rats.

**Effect on lipid profile level in serum of rats**

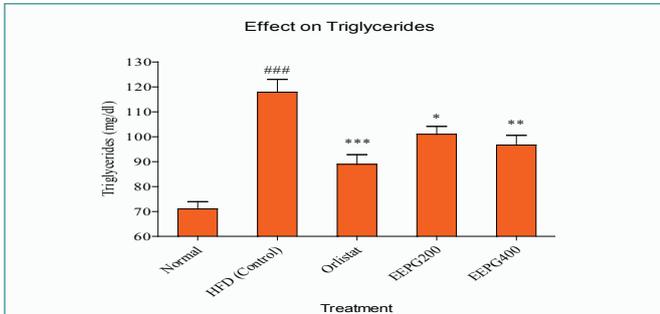
EEGP at 200 mg/kg, showed significant reduction (P<0.05), while, EEGP at 400 mg/kg produced significant (P<0.01) decrease in the total cholesterol levels and highly significant reduction of P<0.001 was observed with orlistat at 50 mg/kg as compared to rats fed with HFD only (Figure 2).



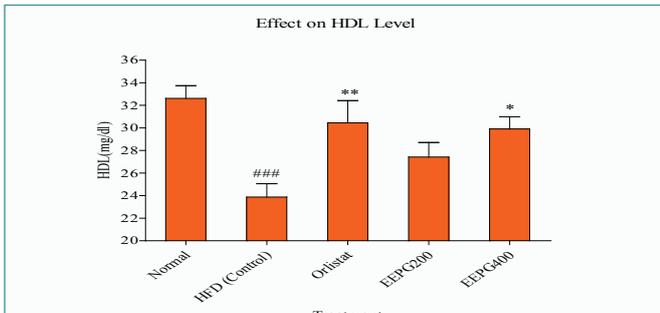
**Figure 2:** Effect of EEGP on total Cholesterol. Values are expressed as mean ± SEM (n=6). Data were analyzed by two-way ANOVA followed by Bonferroni post test. ####P<0.001 as compared to Normal. \*P<0.05, \*\*P<0.01 and \*\*\*P< as compared to HFD induced atherosclerotic rats.

Four weeks HFD feeding to the rats also increased (P<0.001) the triglycerides level (117.87 mg/dl ± 5.12 mg/dl) in the blood serum as compared to the triglycerides level of normal rats (71.05 mg/dl ± 2.89 mg/dl) and the simultaneous treatment with orlistat, EEGP200 and EEGP400 for four weeks produced significant inhibition (P<0.001, P<0.05 and P<0.01 respectively) in the increase of triglycerides levels (88.91 ± 3.89, 100.98 ± 3.16, and 96.63 ± 3.91 respectively) by HFD to the rats. EEGP at 400 mg/kg dose produced more significant decrease in the TG level as compared to EEGP at 200 mg/kg dose (Figure 3).

After four weeks of HFD to the rats, the HDL level decreased ( $23.87 \text{ mg/dl} \pm 1.19 \text{ mg/dl}$ ,  $P < 0.001$ ) as compared to the normal rats ( $32.62 \text{ mg/dl} \pm 1.12 \text{ mg/dl}$ ). Simultaneous administration of orlistat, EEPG200 and EEPG400 for four weeks, lesser reduction in the HDL values ( $30.45 \pm 1.97$ ,  $27.42 \pm 1.29$ , and  $29.91 \text{ mg/dl} \pm 1.07 \text{ mg/dl}$ ) was recorded as compared to group II rats (Figure 4).



**Figure 3:** Effect of EEPG on Triglyceride levels. Values are expressed as mean  $\pm$  SEM (n=6). Data were analyzed by two-way ANOVA followed by Bonferroni post test. #### $P < 0.001$  as compared to Normal. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  as compared to HFD induced atherosclerotic rats.



**Figure 4:** Effect of EEPG on HDL levels in serum of rats. Values are expressed as mean  $\pm$  SEM (n=6). Data were analyzed by two-way ANOVA followed by Bonferroni post test. #### $P < 0.001$  as compared to Normal. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  as compared to HFD induced atherosclerotic rats.

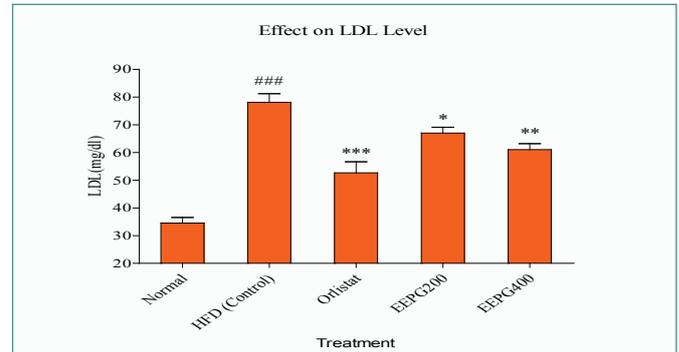
LDL level was significantly ( $78.09 \text{ mg/dl} \pm 3.12 \text{ mg/dl}$ ,  $P < 0.001$ ) increased in group II rats after four weeks feeding with HFD as compared to the LDL level recorded in normal rats ( $34.54 \text{ mg/dl} \pm 2.01 \text{ mg/dl}$ ). After four weeks treatment with orlistat, EEPG200 and EEPG400 to the HFD rats, there was significant ( $P < 0.001$ ,  $P < 0.05$  and  $P < 0.01$  respectively) reduction in the level of LDL value ( $52.67 \pm 3.96$ ,  $66.98 \pm 2.12$ , and  $61.02 \text{ mg/dl} \pm 2.14 \text{ mg/dl}$  respectively) (Figure 5).

VLDL level was significantly ( $27.59 \text{ mg/dl} \pm 2.88 \text{ mg/dl}$ ,  $P < 0.01$ ) increased in group II rats after four weeks feeding with HFD as compared to the VLDL level recorded in normal rats ( $15.39 \text{ mg/dl} \pm 1.97 \text{ mg/dl}$ ). After four weeks treatment with orlistat, EEPG200 and EEPG400 to the HFD rats, there was significant ( $P < 0.01$ ,  $P < 0.05$  and  $P < 0.01$  respectively) reduction in the level of VLDL value ( $16.29 \pm 1.87$ ,  $19.24 \pm 1.08$  and  $18.06 \text{ mg/dl} \pm 1.55 \text{ mg/dl}$  respectively) (Figure 6).

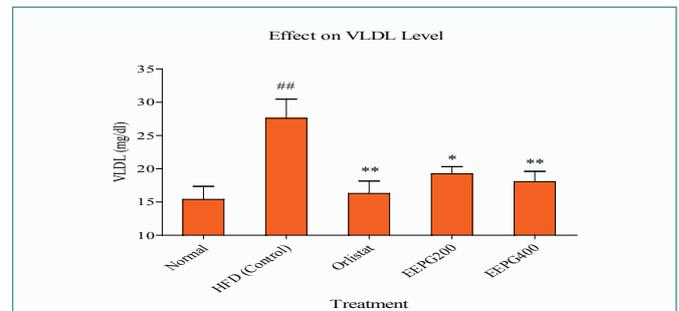
**Effect on liver function test parameters in serum of rats**

SGOT level was significantly ( $39.98 \text{ mg/dl} \pm 3.87 \text{ mg/dl}$ ,  $P < 0.001$ ) increased in group II rats after four weeks feeding with HFD as compared to the SGOT level recorded in normal rats ( $17.34 \text{ mg/dl} \pm 3.67 \text{ mg/dl}$ ). After four weeks treatment with orlistat, EEPG200 and EEPG400 to the HFD rats, there was significant ( $P < 0.01$ ,  $P < 0.05$  and

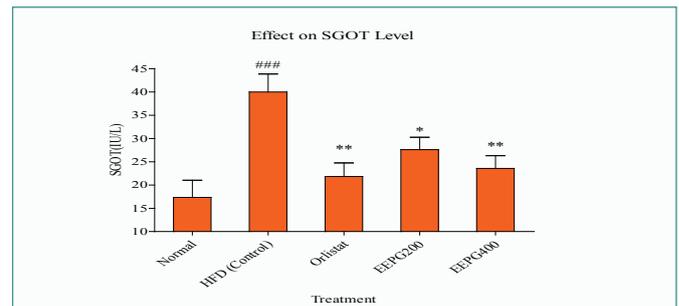
$P < 0.01$  respectively) reduction in the level of SGOT value ( $21.84 \pm 2.91$ ,  $27.59 \pm 2.66$ , and  $23.56 \text{ mg/dl} \pm 2.75 \text{ mg/dl}$  respectively) (Figure 7).



**Figure 5:** Effect of EEPG on LDL levels in serum of rats. Values are expressed as mean  $\pm$  SEM (n=6). Data were analyzed by two-way ANOVA followed by Bonferroni post test. #### $P < 0.001$  as compared to Normal. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  as compared to HFD induced atherosclerotic rats.

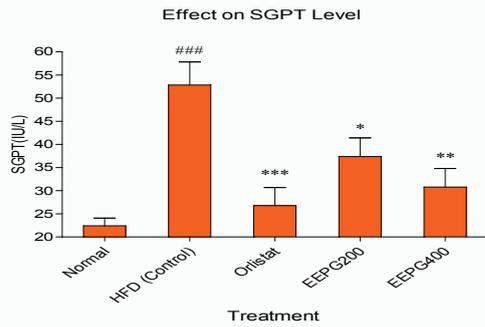


**Figure 6:** Effect of EEPG on VLDL levels in serum of rats. Values are expressed as mean  $\pm$  SEM (n=6). Data were analyzed by two-way ANOVA followed by Bonferroni post test. #### $P < 0.001$  as compared to Normal. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  as compared to HFD induced atherosclerotic rats.



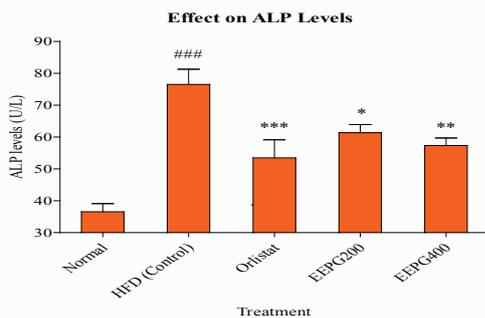
**Figure 7:** Effect of EEPG on SGOT levels in serum of rats. Values are expressed as mean  $\pm$  SEM (n=6). Data were analyzed by two-way ANOVA followed by Bonferroni post test. #### $P < 0.001$  as compared to Normal. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  as compared to HFD induced atherosclerotic rats.

SGPT level was significantly ( $52.85 \pm 4.98 \text{ mg/dl}$ ,  $P < 0.001$ ) increased in group II rats after four weeks feeding with HFD as compared to the SGPT level recorded in normal rats ( $22.42 \text{ mg/dl} \pm 1.65 \text{ mg/dl}$ ). After four weeks treatment with orlistat, EEPG200 and EEPG400 to the HFD rats, there was significant ( $P < 0.001$ ,  $P < 0.05$  and  $P < 0.01$  respectively) reduction in the level of SGPT value ( $26.78 \pm 3.92$ ,  $37.39 \pm 4.03$ , and  $30.78 \text{ mg/dl} \pm 4.02 \text{ mg/dl}$  respectively) (Figure 8).



**Figure 8:** Effect of EEPPG on SGPT levels in serum of rats. Values are expressed as mean  $\pm$  SEM (n=6). Data were analyzed by two-way ANOVA followed by Bonferroni post test. ###P<0.001 as compared to Normal. \*P<0.05, \*\*P<0.01 and \*\*\*P< as compared to HFD induced atherosclerotic rats.

ALP level was significantly ( $78.52 \text{ mg/dl} \pm 2.77 \text{ mg/dl}$ ,  $P<0.01$ ) increased in group II rats after four weeks feeding with HFD as compared to the ALP level recorded in normal rats ( $36.56 \text{ mg/dl} \pm 1.56 \text{ mg/dl}$ ). After four weeks treatment with orlistat, EEPPG200 and EEPPG400 to the HFD rats, there was significant ( $P<0.01$ ,  $P<0.05$  and  $P<0.01$  respectively) reduction in the level of ALP value ( $54.47 \pm 2.67$ ,  $67.34 \pm 2.38$ , and  $64.38 \pm 2.54 \text{ mg/dl}$  respectively) (Figure 9).



**Figure 9:** Effect of EEPPG on ALP levels in serum of rats.

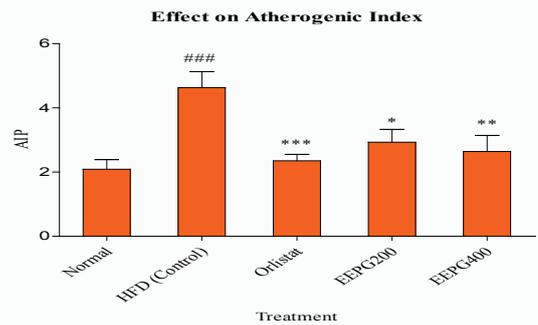
**Effect on Atherogenic Index (AI) of rats**

AI was significantly ( $4.63 \pm 0.50$ ,  $P<0.001$ ) increased in group II rats after four weeks feeding with HFD as compared to the AIP recorded in normal rats ( $2.09 \pm 0.30$ ). After four weeks treatment with orlistat, EEPPG200 and EEPPG400 to the HFD rats, there was significant ( $P<0.001$ ,  $P<0.05$  and  $P<0.01$  respectively) reduction in the AI value ( $2.35 \pm 0.20$ ,  $2.93 \pm 0.40$ , and  $2.64 \pm 0.50$  respectively) (Figure 10).

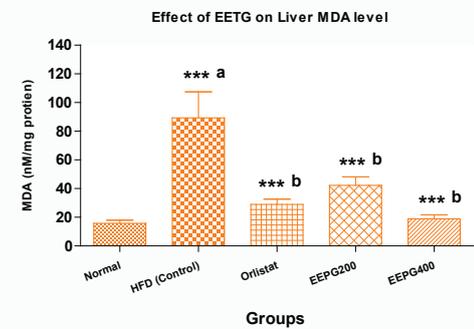
**Effects of EEPPG on oxidative stress parameters in different groups**

Oxidative stress parameters of liver were measured. A significant ( $P<0.001$ ) increase in MDA while declines in GSH levels ( $P<0.01$ ) content were found in control as compared to normal. Treatments with EEPPG (200 mg/kg and 400 mg/kg) and Orlistat (50 mg/kg) exhibited significant decrease in MDA levels while significant elevation in GSH level as compared to control (Figures 11 and 12).

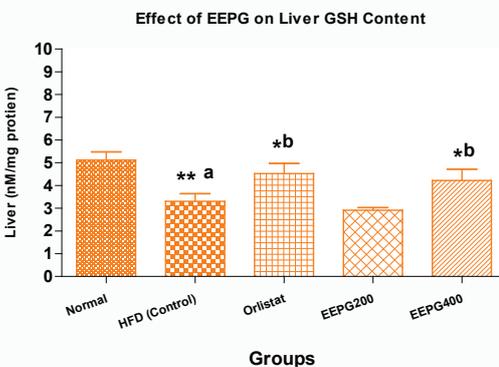
Oxidative stress parameters of liver were measured. A significant ( $P<0.001$ ) decrease SOD ( $P<0.001$ ) and CAT ( $P<0.001$ ) activities were found in control as compared to normal. Treatments with EEPPG (200 mg/kg and 400 mg/kg) and Orlistat (50 mg/kg) exhibited significant increase in SOD and CAT activity as compared to control (Figures 13 and 14).



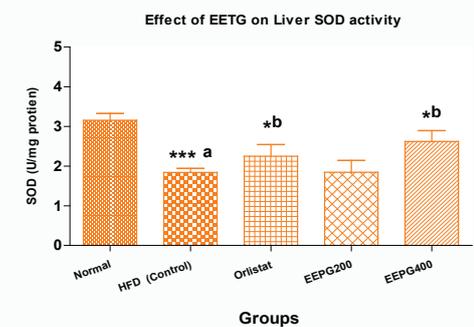
**Figure 10:** Effect of EEPPG on Atherogenic Index (AI) plasma of Rats. Values are expressed as mean  $\pm$  SEM (n=6). Data were analyzed by two-way ANOVA followed by Bonferroni post test. ###P<0.001 as compared to Normal. \*P<0.05, \*\*P<0.01 and \*\*\*P< as compared to HFD induced atherosclerotic rats.



**Figure 11:** Effect of EEPPG on Liver MDA level.



**Figure 12:** Effect of EEPPG on liver GSH content.



**Figure 13:** Effect of EEPPG on Liver SOD activity.

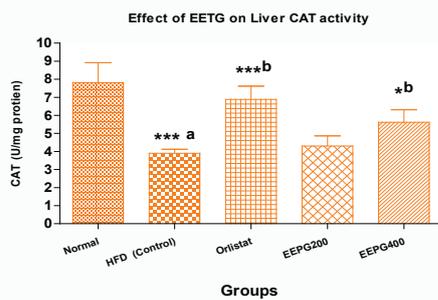


Figure 14: Effect of EEGP on Liver CAT activity.

### Histopathological study

It revealed that alteration in collagen and calcium content, mild mineralization and focal rupture of intima and media of aorta were noticed in high fat diet treated groups as compared to the control (Figure 15). These parameters were significantly reverted to near normalcy on treatment with orlistat and EEGP (200 mg/kg and 400 mg/kg) in a dose-dependent manner.

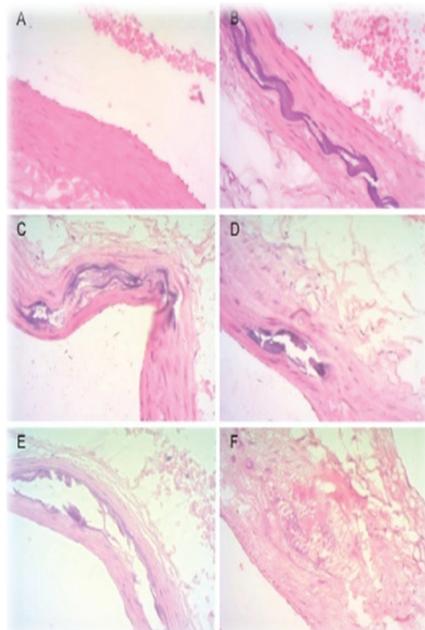


Figure 15: Histopathology of experimental rats.

- A. No changes (Group I).  
 B. Multifocal severe fragmentation of elastic and collagen fibres in media with marked mineralization (Group II).  
 C. Moderate multifocal splitting of media with marked peripheral mineralization (Group III).  
 D. Moderate multifocal marked splitting of tunica media with vacuolation and mineralization (Group IV).  
 E. Mild diffuse thickening and fragmentation of media with mild mineralization; focal rupture of intima and media (Group V).  
 F. Mild multifocal fragmentation of elastic and collagen fibres in media with mineralization; focal rupture of intima and media (Group VI).

### Conclusion

Photochemical screening of the extract shows the presence of chemical constituents like Alkaloids, steroids, fixed oils, cardio tonic aglycones, flavonoids, saponins carbohydrate, proteins, resins. Acute toxicity tests were performed according to the OECD guide line no.423, LD50 value was found to be 4000 mg/kg. Anti atherosclerotic

activity was performed by using the high fat diet induced method. In our study significant increase in plasma HDL-cholesterol with a significant decrease in other lipid profiles were observed.

It can be concluded from the present data that the level of total serum cholesterol, triglyceride and MDA which are actually raised in atherogenic diet, can be lowered significantly with Polygonum Glabrum. Total proteins and Antioxidant parameters SOD, CAT, GSH which are actually lowered in atherogenic diet can be raised significantly with Polygonum Glabrum.

Antiatherosclerotic activity of Polygonum Glabrum well supported by improvement in lipid profile and stress parameters additionally supplement of improvement in atherogenic index and histological examination of artery tissue. From this we can conclude that the extract of Polygonum Glabrum Showed the good anti atherosclerotic activity against high fatty diet induced animal model.

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