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Pharmacological Evaluation of "Anti-Atherosclerotic Activity of Polygonum Glabrum in Animal Model"

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ABSTRACT

Objective: To investigate the anti atherosclerotic activity of ethanol extract of ***Polygonum galbrum*** in male Wistar rats.

Material & method: - In this model of atherosclerosis, 30 adult male wistar rats were evenly divided into 5 groups. Group-1 and Group-2 served as untreated and model controls respectively, while Group-3, 4 and 5 were the treatments groups which were simultaneously treated with standard, 200 and 400 mg/kg extract respectively along with High Fat Diet. On last day, blood samples for biochemical parameters, were obtained under inhaled diether anaesthesia.

Results: - HFD caused atherosclerosis as evidenced by marked elevation in Cholesterol, Triglycerides, LDL, VLDL and decrease in HDL levels. Co-administration of extract with HFD decreased rise Cholesterol, Triglycerides, LDL, VLDL and increase in HDL levels.

.**Conclusion:** - It was observed that the ethanol extract of ***Polygonum galbrum*** conferred Anti- atherosclerotic activity by biochemical observation against HFD induced atherosclerosis in rats. In the near future could constitute a lead to discovery of a novel drug for treatment of drug induced atherosclerosis.

INTRODUCTION

Introduction to Herbal Plants:

Herbal plant is a valuable plant that you can use some or almost every part of it for many treatments. Some people use its part such as dried leaves, roots, flowers, etc for curing diseases. Some use its chemical substance such as its extract oil for therapy. Furthermore, you can also use herbs as cooking recipes. Consequently, herbs have many benefits but the main purpose of using them is to maintain good health.

There are many kinds of herbal plants and each part of herbal plant used is different. Herbal plants can be used for various purposes that depend on your demand. You can use them for relief healing such as Aloe and some kinds of herbs and can be grown for a garden full of their sweet smelling. Crafting with herbs can make a satisfactory and beautiful home. Artemisia is a beautiful herb that is easily grown in the garden or Giver King is a lovely herb that has a fresh herbal scent and dried floral such as sunflower, roses or anything else are suitable for your crafting.

Herbs are natural in the kitchen such as lemon, pepper, chili or anything else. Many excellent cosmetics come from herbs. Almost all herbs are used for improvement human health. Herbs have long been known for therapy. You can make excellent skin tonic and fragrance in the soap and hair conditioner from herbs. Some kinds of herbs have been used in the control of acne and eczema. You can use some kinds of herbs for relieving headache and as a stimulant and tonic.

There are several reasons why herbal plants become popular plants. One of the main reasons, of course, is that each herbal plant has various properties. For example, Lavender has long been known for its classic fragrance. Although Lavender is a fragrant flower, it is also an important medicinal herb. The oil from flowers can be used to protect cloths and store linens from moths. It can be used as a scent in air fresheners. The oil can be applied as a stimulant, tonic, and headache relief and for relief of intestinal gas. Disinfecting wounds can be cured by using the oil from Lavender. In addition, Lavender oil can relieve neuralgic pains, sprains and sore joints.

Moreover, Aloe Vera is known as a medicinal plant because it has many useful parts to treat many diseases. For example, the clear gel has a dramatic ability to heal wounds, ulcers and burns by putting a protective coating on the affected areas and speeding up the healing rate. As a food supplement, Aloe is said to facilitate digestion, aid in blood and lymphatic circulation. It helps cleanse the digestive tract by exerting a soothing, balancing effect

because it has three anti inflammatory fatty acids. And for another thing, Aloe has a moisturizing effect on the skin. It is a common remedy for sun burn and skin irritation. It can relieve itching due to insect bites.[1,2,3]

Cardiovascular disease (CVD) is the leading cause of death in the world and accounts for well over one million deaths each year in the United States. Of the more than two million deaths in the United States in 1998, CVD was listed as the primary or contributing cause in 70% of cases.1 According to the Centres of Disease Control and Prevention (CDC) and the National Health and Nutrition Examination Survey III, the probability at birth of dying from CVD is 47%, compared to 22% from cancer, 2% from diabetes, and less than 1% from human immunodeficiency virus (HIV) disease. The largest proportion of this high mortality is attributed to coronary artery disease (CAD) or coronary heart disease (CHD), which was the primary contributing cause of death in 459,841 Americans in 1998.[4]

CVD includes hypertension, coronary artery disease (CAD), congestive heart failure (CHF), congenital cardio-vascular defects, and stroke. The prevalence of these entities in the United States surpasses 60 million cases. Although these diseases are associated with a high mortality, the associated morbidity affects all walks of life and has a great impact on the quality of life of affected individuals. This chapter presents a brief overview of common cardio-vascular conditions and their implications for the practice of dental medicine.

INTRODUCTION TO ATHEROSCLEROSIS: ATHEROSCLEROSIS:

Atherosclerosis can affect arteries in the heart, brain, arms, legs pelvis and intestines leading to disease of those organs. There are 4 types of atherosclerosis which include as follows:

Coronary artery disease (CAD):

When plaque build-up in the coronary arteries, supply of oxygen rich blood to heart is reduced leading to chest pain and ultimately heart attack. [2]

Carotid artery disease or cerebrovascular disease:

When plaque builds up in carotid arteries, the supply of oxygen rich blood to the brain is reduced leading to a stroke. [5]

Peripheral Arterial Disease (PAD):

When plaque builds up in arteries supplying blood to leg, arms and pelvis, the oxygen rich blood supply to these parts is restricted leading to numbness, pain and dangerous infections.[2]

Abdominal Angina and a Bowel Infraction:

Atherosclerosis leads to narrowing of arteries supplying blood to the intestines causing abdominal pain and is called abdominal angina. Complete or sudden blockage of blood supply to intestines leads to bowel infection.

In service cause, atherosclerosis could also lead to narrowing of arteries of kidney leading to renal artery stenosis. Millions of Americans are diagnosed to be suffering from atherosclerosis and millions more have the diseases but are unaware it. Atherosclerosis accounts for about 75 percent of all deaths from cardiovascular diseases. Men, African –Americans and all individuals over 65 years of age have the highest risk of developing advanced atherosclerosis. [5,6]

CAUSES AND RISK FACTORS OF ATHEROSCLEROSIS:

Following is a list of causes of atherosclerosis

- High cholesterol
- Polycystic ovary syndrome (PCOS)
- Atheroma
- Atherosclerosis
- Primary- atherosclerosis
- Verners syndrome- atherosclerosis
- Familial hypercholesterolemia
- Xanthoma- atherosclerosis
- Chemical induced cardiovascular diseases atherosclerosis
- Carbon disulfide induced cardio vascular diseases atherosclerosis
- Hutuhinson Gilford syndrome-atherosclerosis
- Progeria-atherosclerosis
- Smoking
- High amounts of sugar in the blood due to insulin resistance of diabetes
- Obesity
- Physical inactivity [6,7,8]

SYMPTOMS OF ATHEROSCLEROSIS:

Unfortunately, atherosclerosis produces no symptoms until the damage to arteries is severe enough to restrict blood flow.

Restriction of blood flow to the heart muscle due to atherosclerosis can cause angina pectoris or a myocardial infarction (heart attack).

Narrowing of the arteries supplying blood to the brain may cause transient ischemia attacks (symptoms and signs of stroke listing less than 24 hrs) and episodes of dizziness, or ultimately, to a stroke itself.[7,8,9,10,11,12,13,14]

MATERIALS AND METHODS

Plant material:

The plant material used for the study is: The ethanolic extract of *Polygonum glabrum* plant.

Collection of plant material:

The aerial part of *Polygonum glabrum* was collected from Tirumala hills, Tirupati, Andhra Pradesh. India. It was identified and authenticated by Prof. Madhava Chetty, K., Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for future reference.

Table 1:Atherogenic diet composition

Composition	Normal diet (%)	Atherogenic diet (%)
Protein (Milk	12	10
Carbohydrates (Wheat flour)	71	61
Sugar	05	05
Fat (Butter)	05	16
Salts	04	04
Vitamins	01	02
Fibers	02	01
Cholesterol	--	01
Total Weight	100g	100 g

Dosing of animals:

The animals were dosed with the test and the standard drugs orally based on the body weights of the animals. The animals were dosed with the extracts for about 14 days. During dosing of animals, the body weights of the animals and the food consumed by the animals were taken on successive days.

Grouping of animals: Ant atherosclerosis

The animals were divided into four groups. Each group contains five animals. Grouping is as follows:

Group 1: Normal Group (Tween 80)

Group 2: Control Group (HFD)

Group 2: Extract I- *Polygonum glabrum* + HFD(200 mg/kg) Group 3:

Extract II- *Polygonum glabrum* + HFD (400 mg/kg) Group 4:

Standard-Atorvastatin + HFD (10 mg/kg)

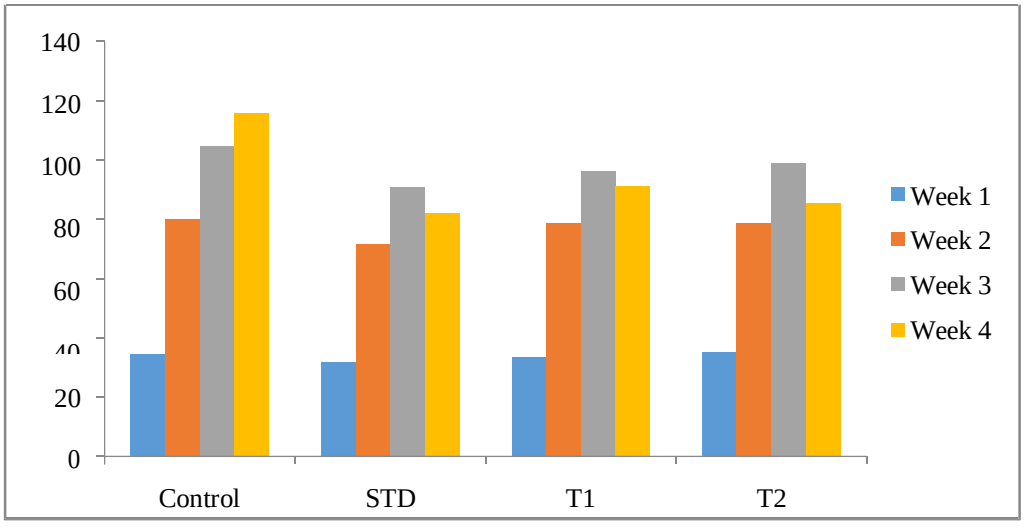
Table 2: Grouping of animals

	Differences in body weights (gm) (Mean ± SEM)			
Group (n=5)	Week 1	Week 2	Week 3	Week 4
Group I Normal control group	30.5 ± 3.52	37.5 ± 1.5	40.6 ± 3.6	44.12 ± 3.1
Group II Negative control group HFD	34.6 ± 6.48	79.9 ± 0.1	104.8 ± 2.1	115.8 ± 1.0
Group III Positive control group Orlistat 50mg/kg b.w. p.o	31.9 ± 2.24	71.6 ± 3.8	90.8 ± 6.1	82.1 ± 8.1
Group IV T_1 PG 200mg/kg b.w. p.o	33.6 ± 2.5	78.5 ± 2.9	96.2 ± 1.5	91.2 ± 6.8
Group V T_2–PG 400mg/kg b.w. p.o	35.2 ± 6.1	78.8 ± 2.0	99.0 ± 8.2	85.51 ± 2.6

Dosing of animals:

The animals were dosed with the test and the standard drugs orally based on the body weights of the animals. The animals were dosed with the extracts for about 14 days. During dosing of animals, the body weights of the animals and the food consumed by the animals were taken on successive days.

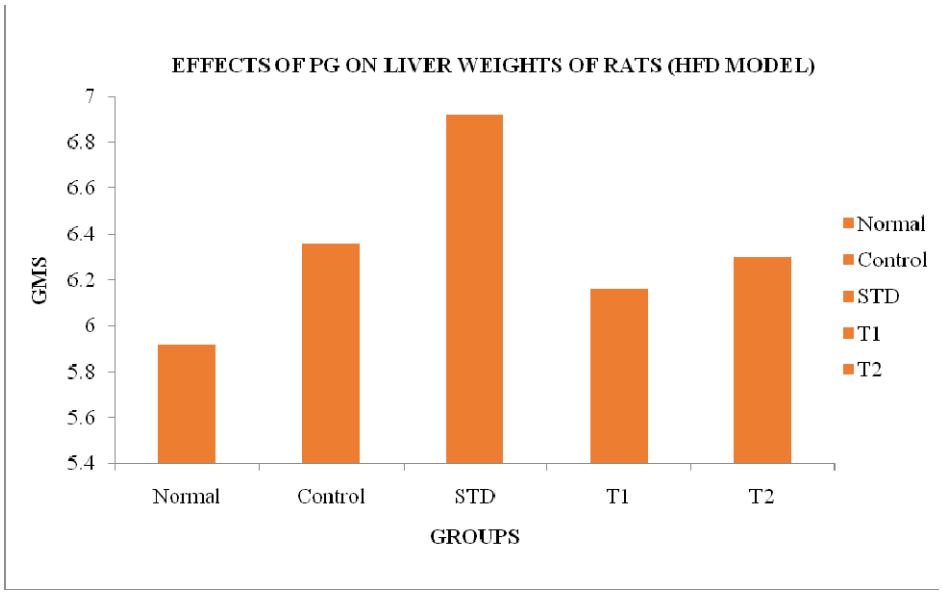
- → The rats were treated with test and standard drugs by oral gavage for 14 days.
- → After this time i.e., 20 hrs after the last application of the test compounds the animals are anaesthetized with anaesthetic ether and 1.2ml of blood is withdrawn by retro orbital puncture.
- → The blood samples will be collected on the 14th day for estimating biochemical parameters
- → The blood samples were taken from the rats after overnight fasting.
- → Biochemical parameters were determined after treatment.
- → The serum was labeled with the animal number and the estimations were made. The serum enzymes SGOT, SGPT and ALP level and the lipid profile (total 7cholesterol HDL, LDL, VLDL and triglyceride level) and total protein was determined enzymatically on prietest bio chemistry analyser. SOD,GSH,MDA were determined by using UV Spectrophotometer



Graph 1 : Effect of PG on body weights of rats

Table 3: Effects of PG on liver weights of rats (HFD MODEL)

Groups (n = 5)	**Liver weights (g) (Mean ± SEM)**
Group I Normal control	5.92 ± .44
Group II Negative control HFD.	6.36 ± 0.65
Group III-Positive control Orlistat 50mg/kg b.w. p.o.	6.92 ± 0.91**
Group IV T1 – MEDB-200mg/kg b.w. p.o	6.16 ± 0.52*
Group V T2 – MEDB-400mg/kg b.w. p.o.	6.30 ± 0.18**



Graph 2 : Effect of PG on Liver weights of rats (HFD MODEL)

BIOCHEMICAL PARAMETERS:

a) SERUM LIPID PROFILE:

Rats fed with high fat diet (HFD) showed impairment in normal lipid profile, leading to increased total cholesterol, triglyceride, LDL-C, VLDL-C while HDL-C was decreased. PG at 200mg/kg bw showed significant reduction ($p<0.05$), while, PG at 400mg/kg bw significantly decreased ($p<0.01$) the total cholesterol levels were highly significant reduction of $p<0.001$ was observed with orlistat at 50mg/kg bw.

Significant reduction of triglycerides, $p<0.05$ was seen with PG 200 mg/kg bw and the values were found to be <0.01 with PG 400 mg/kg bw whereas highly significant reduction $p<0.001$ was seen with orlistat at 50mg/kg bw.

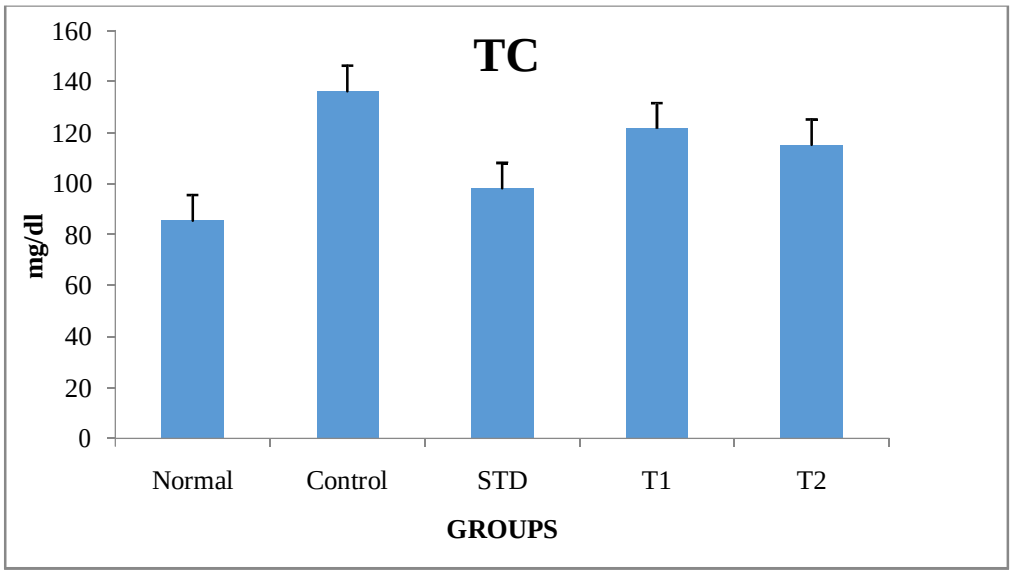
LDL and VLDL were significantly reduced $p<0.05$ with PG at 200mg/kg bw but with PG 400 mg/kg bw and orlistat at 50mg/kg bw the value of LDL was found to be $p<0.01$.Whereas HDL-C levels were significantly increased with PG 400 mg/kg bw and orlistat at 50mg/kg bw $p<0.01$ when compared to normal and untreated groups.

b) LIVER FUNCTION TEST:

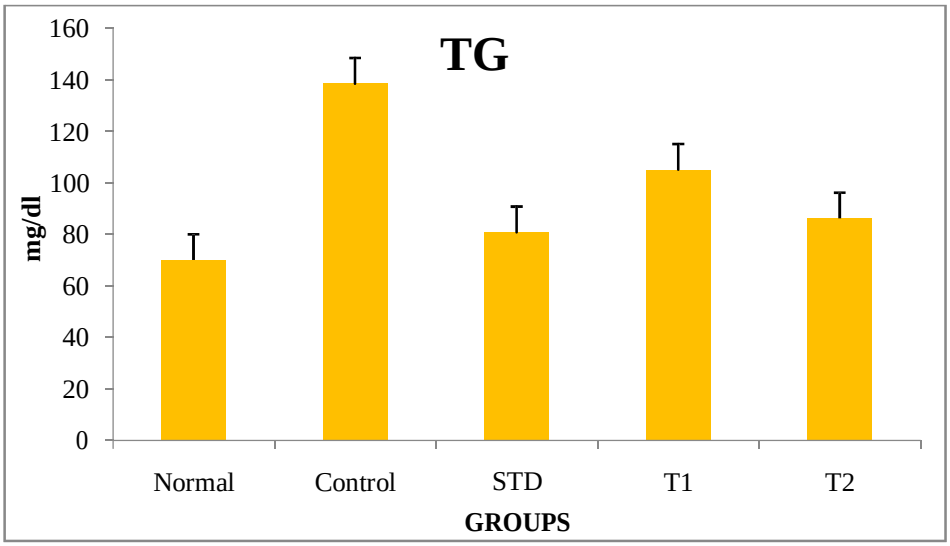
Animals treated with high fat diet (HFD) showed increased levels of marker enzymes SGOT, SGPT and ALP but upon administration of EEPG significantly reduced the levels. P<0.05 was seen with PG 200 mg/kg bw and p<0.01 was seen with PG 400 mg/kg bw and orlistat at 50mg/kg bw p<0.01.P<0.01 value was seen in the levels of ALP with administration of PG at 200mg/kg bw and PG 400 mg/kg bw but p<0.001 was seen with orlistat at 50mg/kg bw.

Table 4: Effect of PG on Total Cholesterol and Triglyceride levels in HFD rats

Groups (n = 5)	**Total Cholesterol (mg/dl) Mean ± SEM**	**Triglycerides (mg/dl) Mean ± SEM**
Group I Normal control	85.53 ± 1.79	70.12 ± 2.59
Group II Negative control (HFD)	136.25 ± 1.82	138.54 ± 1.85
Group III Positive control Orlistat 50mg/kg b.w. p.o	98.15 ± 1.06***	80.83 ± 4.86***
Group IV T_1 – PG 200mg /kg b.w. p.o	121.56 ± 1.78*	105.15 ± 2.20*
Group V T_2 – PG 400mg/kg b.w. p.o.	115.2 ± 1.87**	86.24 ± 1.95**



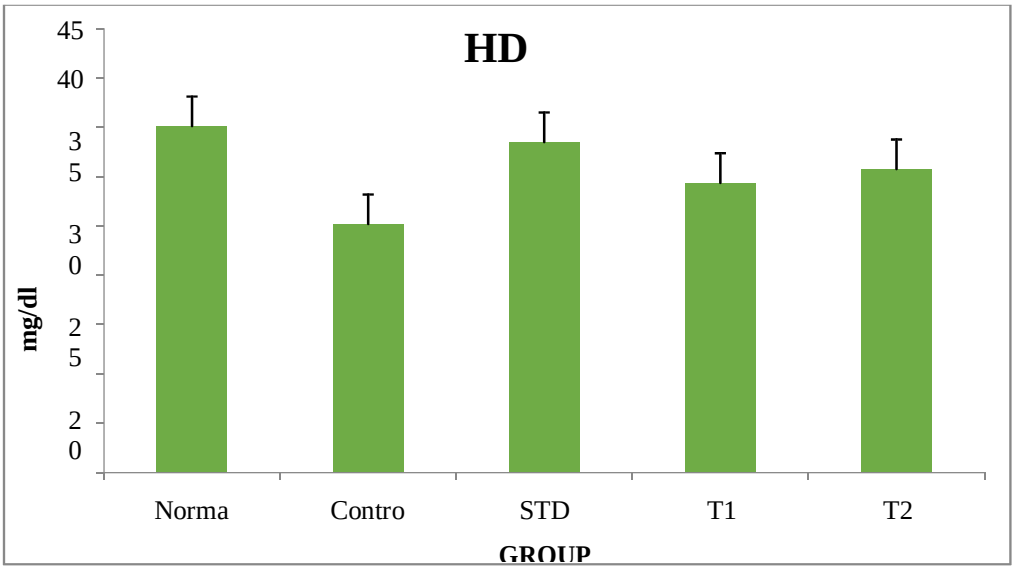
Graph 3: Effect of PG on Total Cholesterol of rats (HFD MODEL)



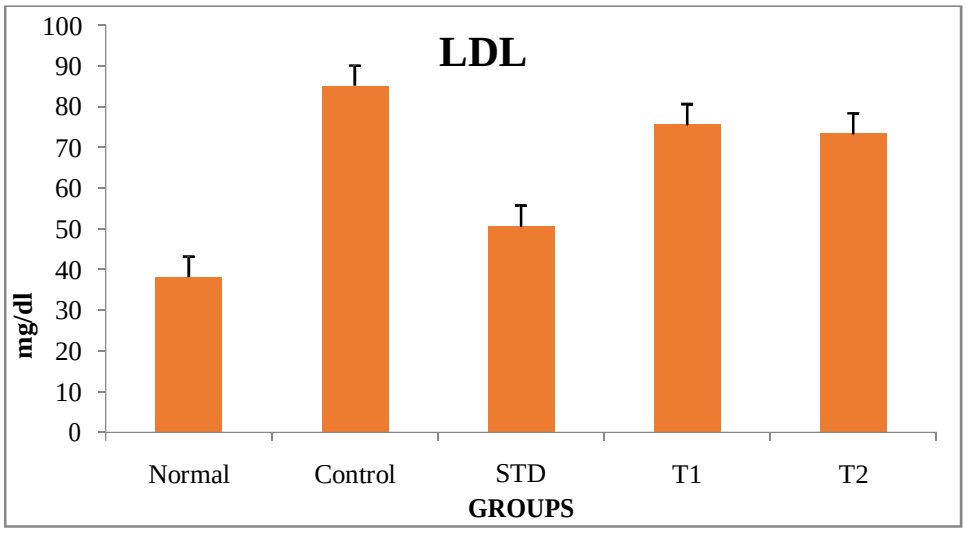
Graph 4: Effect of PG on Triglycerides of rats (HFD MODEL)

Table 5: Effect of PG on HDL, LDL AND VLDL levels in rats

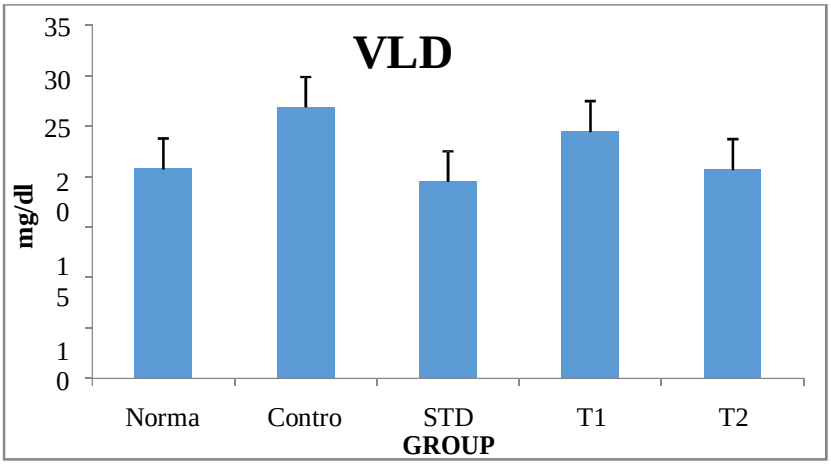
Groups (n = 5)	**HDL (mg/dl) Mean ± SEM**	**LDL (mg/dl) Mean ± SEM**	**VLDL (mg/dl) Mean ± SEM**
Group I Normal control	35.12 ± 3.84	38.19 ± 1.51	20.81 ± 2.17
Group II Negative control HFD	25.21 ± 2.81	85.16 ± 1.82	26.89 ± 2.89
Group III Positive control Orlistat 50mg/kg b.w. p.o	33.51 ± 4.98**	50.76 ± 2.89**	19.52 ± 2.76**
Group IV T1 –PG 200mg/kg b.w. p.o	29.38 ± 2.42*	75.67 ± 3.62*	24.51 ± 3.76*
Group V T_2 – PG 400mg/kg b.w. p.o	30.78 ± 1.89**	73.45 ± 3.56**	20.75 ± 3.96**



Graph 5: Effect of PG on HDL of rats (HFD MODEL)



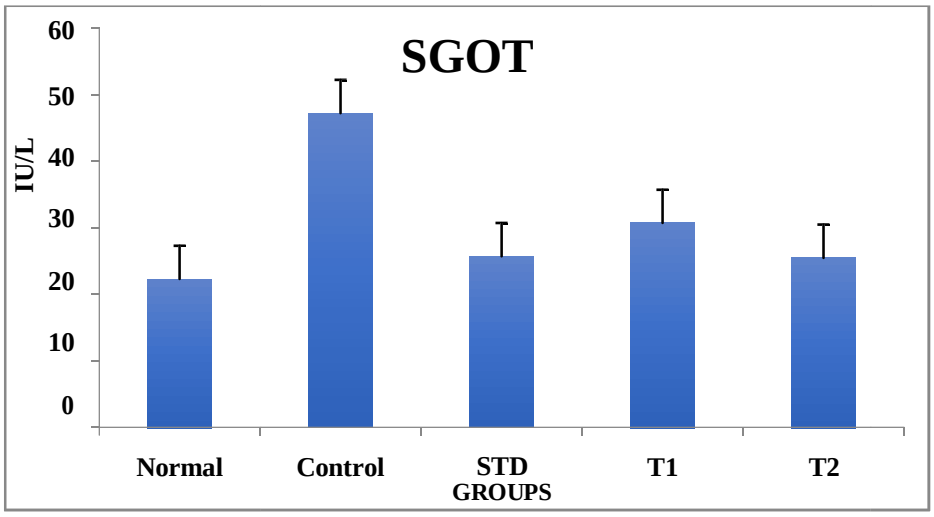
Graph 6: Effect of PG on LDL of rats (HFD MODEL)



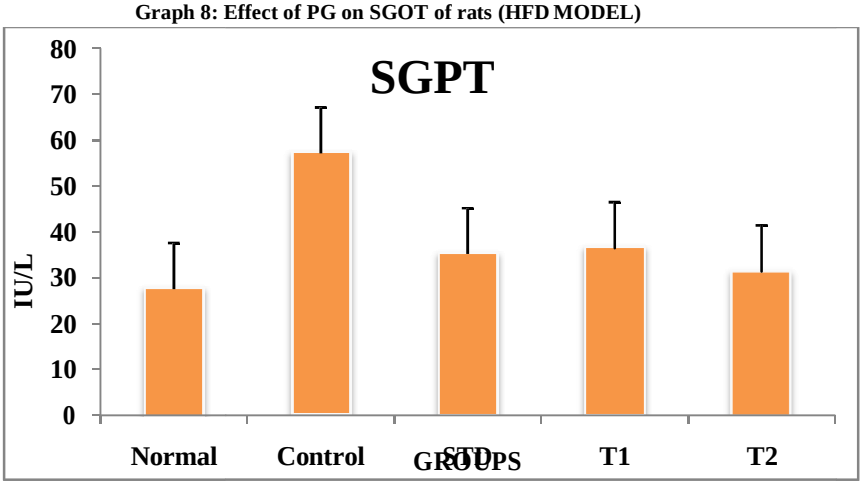
Graph 7: Effect of PG on VLDL of rats (HFD MODEL) Table 6:

Effect of PG on SGOT, SGPT AND ALP levels in rats

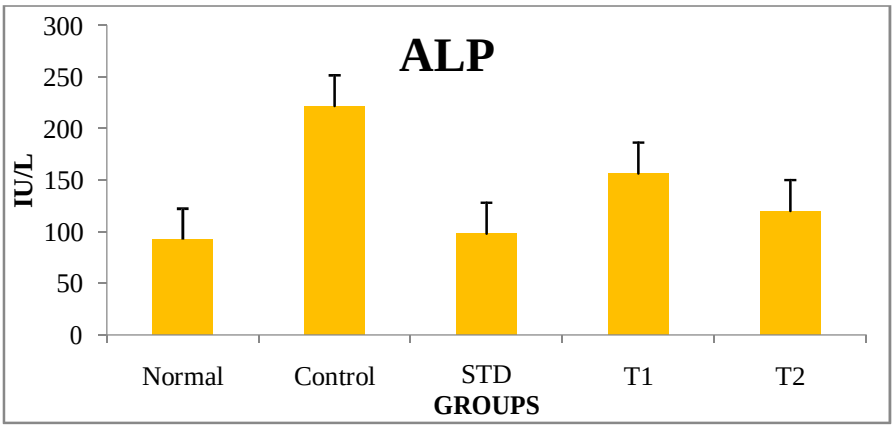
Groups (n = 5)	**SGOT (IU/L) Mean ± SEM**	**SGPT (IU/L) Mean ± SEM**	**ALP (IU/L) Mean ± SEM**
Group I Normal control	22.36 ± 3.89	27.52± 3.89	92.46 ± 4.89
Group II Negative control HFD	47.29 ± 4.15	57.100 ± 5.78	221.78 ± 2.79
Group III Positive control Orlistat 50mg/kg b.w. p.o	25.76 ± 4.89**	35.16 ± 4.89**	98.15 ± 3.88***
Group IV T_1 –PG 200mg/kg b.w. p.o	30.78 ± 4.89*	36.45 ± 3.53*	156.53 ± 4.88**
Group V T_2 – PG 400mg/kg b.w. p.o	25.52 ± 4.89**	31.36 ± 4.12**	120.16 ± 5.19**



Graph 8: Effect of PG on SGOT of rats (HFD MODEL)



Graph 9: Effect of PG on SGPT of rats (HFD MODEL)



Graph10: Effect of PG on ALP of rats (HFD MODEL)

HEPATIC MORPHOLOGY AND HISTOPATHOLOGY:

The livers of untreated groups were found to be yellow in color and appeared bulky, whereas livers of the treated groups were found to be normal and less bulky. Histological analysis is shown in the figure , where liver of untreated rats exhibited a typical signs of fatty mass i.e. showing accumulation of fat droplets through the liver acini. When treated with EEPG and Orlistat smaller degree of lipid accumulation and fewer pathological signs were observed in a dose dependent manner.

CARDIAC RISK INDICATOR:

Atherogenic index of plasma and % protection of HFD treated animals were calculated from their Triglycerides and HDL-C values. There was a dose dependent reduction in atherogenic index of plasma with EEPG administered at all two doses (200 and 400 mg/kg bw p.o.) and Orlistat exhibited maximum reduction in AIP which is as follows:

IN HFD MODEL:

- T_1 –2.93
- T_2 – 2.64
- Orlistat – 2.35

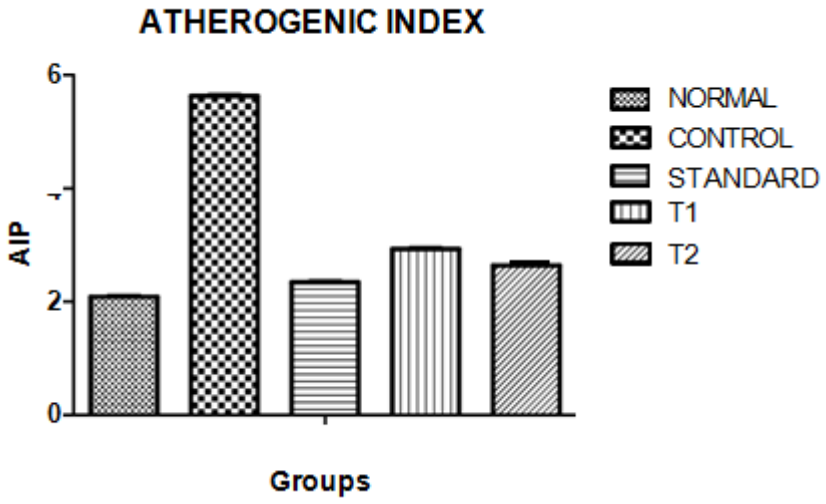
Table 7 : Atherogenic index and percentage protection with PG: (HFD MODEL)

Group (n=5)	Atherogenic index of plasma (AIP)	Percentage protection
Group I Normal control	2.09	
Group II Negative control HFD	5.63	
Group III Positive control Orlistat 50mg/kg b.w. p.o	2.35	59.7 %
Group IV T_1 – MEDB 200mg/kg b.w. p.o	2.93	49.2 %
Group V T_2 – EEPG 400mg/kg b.w. p.o	2.64	55.32 %

Table- Values are expressed as Mean ± SEM (n=5)

*p<0.05, **p<0.01, ***p<0.001 was considered significant compare normal and untreated groups

EFFECT OF EEPG ON ATHEROGENIC INDEX IN HFD MODEL



Graph 11: Effect of PG on Atherogenic Index of rats (HFD MODEL)

SUMMARY AND CONCLUSION

Phytochemical screening of the extract shows the presence of chemical constituents like Alkaloids, steroids, fixed oils, cardio tonic aglycones, flavonoids ,saponins ,carbohydrates, proteins, resins. Acute toxicity tests were performed according to the OECD guide line no.423, LD50 value was found to be 200mg/kg and 400mg/kg.Anti atherosclerotic activity was performed by using the high fat diet induced method. In the present study an increase in plasma HDL-cholesterol with a concomitant percentage decrease from other lipid was observed. It can be concluded from the present data that the levels of total serum cholesterol, triglyceride and MDA which are actually raised in atherogenic diet, can be lowered significantly with Polygonum glabrum. And total proteins and antioxidant parameters SOD, GSH which which are actually lowered in atherogenic diet can be raised significantly with **Polygonum glabrum .**From this we can conclude that the extract (***Polygonum glabrum***.) Showed the anti atherosclerotic activity.

BIBLIOGRAPHY

[1] Henriette's Herbal, http://metalab.unc.edu/hermed/
[2] Herbal Information Center,http://www.kcweb.com/herb/herbmain.htm
[3] Herbal Garden, http://www.herbalgardens.com/
[4] American Heart Association.2001 Heart and stroke statistical update. Dallas (TX): American Heart Association; 2000.
[5] Humphrey LL, FuR, RogersK, FreemanM, HelfandM. Homocysteine level and coronary heart disease incidence: a systematic review and meta-analysis. Mayo ClinProc 2008;83(11):1203–12.
[6] Hardman, Limbard, Gilman: Goodman & Gilman's pharmacological basis of therapeutics. McGraw Hill medical publishers, 10th edition, 977-986.
[7] Rang, Dale, Ritter, Flower, and Henderson: A Text Book of Rang & Dale's Pharmacology. Elsevier churchillivingstone publishers, 7th edition, 285-293, 604.
[8] Raja B Singh MD, Sushma A Mengi PhD, Yan-Jun Xu MD PhD, Amarjit S Arneja MD, Naranjan S Dhalla PhD MD (Hon) DSc (Hon) Pathogenesis of atherosclerosis: A multifactorial process Exp Clin Cardiol Vol 7 No 1 Spring 2002
[9] Ramu B, Sathish Kumar M, Ramakrishna M (2015) Current Regulatory Scenario for Conducting Clinical Trials in India. Pharmaceut Reg Affairs. 4:137. doi: 10.4172/2167-7689.1000140.
[10] Lederle FA. Management of small abdominal aortic aneurysms. Ann Intern Med 1990;113(10):731–2.
[11] Kent KC, Zwolak RM, Jaff MR, et al. screening for abdominal aortic aneurysm: a consensus statement. J VascSurg 2004;39(1):267–9.
[12] Bandameedi R, Mohammed S, Soma H. A case study on National List of Essential Medicines (NLEM) in India and WHO EML 2015-overview. *Pharm Regul Aff.* 2016;5:159.
[13] Rang, Dale, Ritter, Flower, and Henderson: A Text Book of Rang & Dale's Pharmacology. Elsevier churchillivingstone publishers, 7th edition, 285-293, 604.
[14] Stephen P Adams, Michael Tsang, James M Wright Lipid lowering efficacy of atorvastatin (Review)