

EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF ETHANOLIC EXTRACT OF *GLYCOSMIS PENTAPHYLLA* IN HYPERLIPIDEMIC WISTAR RATS

Syed Safiullah Ghori*¹, M.A Rizwan Khan¹, Kaab e Alam¹, Abid Hussain Abrar¹,

¹ Pharmacology Research Lab, Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad 500016, Telangana, India

*Anwarul uloom College of Pharmacy, New Mallepally, Hyderabad, Telangana India.

Email: safiullahghori@gmail.com

Phone#: +91 9848256006

ABSTRACT

The present study was designed to perform preliminary phytochemical screening, acute oral toxicity and to evaluate antihyperglycemic activity of whole plant of *Glycosmis pentaphylla* ethanolic extract. *Glycosmis pentaphylla*, whole plant was extracted using ethanol as solvent by soxhlet apparatus. The extract was subjected to preliminary phytochemical screening. Acute oral toxicity studies were performed to determine test dose. The evaluation of antihyperlipidemic activity was done using Triton X 100 and High Fat Diet induced hyperlipidemia models in Wistar albino rats. Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, flavonoids, proteins, and amino acids. Doses up to 2000mg/kg were found to be safe after acute toxicity tests. Cholesterol, triglycerides, HDL, LDL, VLDL, SGOT, SGPT, Total protein and glucose were measured. The results suggested that EGP (ethanolic extract, *Glycosmis pentaphylla*) possess antihyperlipidemic activity against hyperlipidemia induced by Triton X 100 and also High Fat Diet induced experimental models.

Keywords: *Glycosmis pentaphylla*, ethanolic extract, Triton-X, High Fat Diet, hyperlipidemia.

INTRODUCTION

Hyperlipidemia is a condition in which there is abnormal high levels of lipids, elevated serum levels of one or more of total cholesterol, low-density lipoprotein cholesterol, triglycerides, or both total cholesterol and total triglycerides (combined Hyperlipidemia), very low density lipoprotein.¹ Hyperlipidemia is a lifestyle disorder which seriously affects the human health.² It leads to various cardiovascular disorders like angina pectoris, myocardial infarction, hypertension, atherosclerosis, congestive Heart failure.³

Hypercholesterolemia is a metabolic condition that determines the onset of chronic degenerative diseases such as atherosclerosis. The formation of initial lesions appears to originate, more often, from the focal increase in lipoprotein content within regions of the intima, not only due to changes in the permeability of the overlying endothelium, but also mainly because of binding to constituents of the cellular matrix, increasing the residence time of lipid-rich particles within the arterial wall. In the extracellular space of the intima, lipoproteins may undergo changes and evidence points to a pathogenic role for such modifications.⁴

The major side effects of anti-hyperlipidemic agents include muscle toxicity, rhabdomyolysis, psychiatric adverse reactions which include depression, memory loss, confusion and aggressive reactions.^{4,5} These effect the lifestyle again. Hence it is the need of the hour to investigate herbal drugs for treatment of hyperlipidemia which are devoid of the above side effects.

Glycosmis pentaphylla is extensively used in Ayurveda for variety of conditions. However its antihyperlipidemic activity has not been investigated scientifically so far. Keeping in view of pathophysiological complications of hyperlipidemia and therapeutic efficacy of herbal medicines, the plant *Glycosmis pentaphylla* was evaluated for antihyperlipidemic activity by enhancement of plasma cholesterol using laboratory animals which also provided help in understanding the relationship between changes in lipid metabolism and atherogenesis and possible treatments for their reduction.⁶

MATERIAL AND METHODS

Plant material: The plant of *Glycosmis pentaphylla* was collected from a certified ayurvedic wholesaler. The plant was identified and authenticated by Asst Prof. K. Dr. K. Madhava chetty, MSc, Head, Department of Botany, S.V. University, Tirupati

Chemicals:

Triton x 100 (Hi media, Mumbai), Cholesterol (SRL Mumbai), Atorvastatin (Micro labs Pvt Ltd, B, lore) Cholic acid (SRL, Mumbai) Anaesthetic ether-SD Fine chem Ltd., Mumbai, Chloroform-SD Fine chem Ltd .Mumbai. Formaline-SD Fine chem Ltd., Mumbai. All chemicals and reagents were of analytical grade. Diagnostic kits used for estimation of cholesterol, triglycerides, HDL, LDL, VLDL, SGOT, SGPT, Total protein and glucose were procured from Robonik Diagnostic Ltd India. Autoanalyzer (Robonik), Refrigerator centrifuge (MPW-350R), UV-Spectro-photometer (UV-1601, Shimadzu Corporation, Kyoto, Japan), Mini Lyotrap (LTE Scientific Ltd.), Research centrifuge (Remi industries, Mumbai) and homogenizer (Remi Motors, Mumbai). Dhona balance (M/S Dhona instruments Pvt. Ltd., Kolkata, India).

Experimental Animals:

Wistar albino rats of either sex (150–220 g) were obtained from the central animal house of Sigma Institute of Clinical Research and administration Pvt Ltd Hyderabad. The animals were housed at room temperature (22-28 °C) , 12 hr dark and light cycle and given standard laboratory feed and water ad-libitum. The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee (769/2010/CPCSEA).

METHODS

Preparation of extract:

The collected fresh plant material was dried in shade (2 days) and was made to coarse powder with the use of grinder. The powder was weighed separately and transferred to a round bottomed flask and then subjected to continuous heat extraction with soxhlet apparatus using 95% ethanol for 24 hours. Then the extract of ethanol was concentrated. Extract obtained was dried by placing it on a big petriplate on electric water bath (70°C) and then kept in an oven at 30°C for 2 hour. The extract obtained was kept for drying and stored in vacuum desiccators. The percentage yield of the extract was 7.5%.⁷

Qualitative chemical tests:⁸

Ethanol extract of the plant was subjected to chemical tests for the identification of their active constituents.

Acute toxicity study

Acute toxicity studies were performed according to OECD-423 guidelines category IV substance (acute toxic class method). (Organization for economic Co-operation and development, 2001).⁹ Animals were individually observed for changes in skin, mortality, general behavioral pattern, tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma a for time period of 14 days.

Experimental Models For Evaluation Of Anti Hyperlipidemic Activity

The antihyperlipidemic activity was performed by two experimental models, triton x 100 induced hyperlipidemia and high fat diet induced hyperlipidemia.

Triton X 100 Induced Hyperlipidemia model¹⁰

Thirty Wistar rats were randomly divided into 5 groups of 6 each. The group II to V, hyperlipidemia was induced in wistar albino rats by single intraperitoneal injection of Triton x 100 (100mg/kg) in physiological saline solution after overnight fasting for 18 hrs. The first group was given standard pellet diet, water and orally administered with 5% Tween 80. The II to V group animals were injected i.p. with solution of Triton -x-100mg /kg body weight. After 72 hours of triton injection, the second group received a daily dose of 5% Tween 80 (p.o) for 7 days. The third was administered with the standard Atorvastatin 10mg/kg, p.o. for 7 days. IV and V group was administered a daily dose of EGP 200 and 400 mg/kg suspended in 5% Tween 80, p.o., for 7 days, after inducing hyperlipidemia. Food was withdrawn 10h prior to the blood sampling.

High fat Induced Hyperlipidemia model¹⁰

The chronic hyperlipidemia induced in wistar albino by feeding HFD once a day for 28 days. To the group I Normal diet was given once a day for 28 days. Group II was given HFD once a day for 28 days. Group III was given HFD with standard dose of Atorvastatin 10mg/kg once a day for 28 days. Group IV was given HFD with EGP 200mg/kg. Group V was given HFD with EGP 400mg/kg. The feeding and treatment schedule is represented in fig. given below

Biochemical estimations

On the 8th day, blood was collected by retro-orbital sinus puncture, under mild ether anaesthesia in both the experimental models. The collected samples were centrifuged for 15 minutes 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:¹¹

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): 0.02ml standard and 2.00 ml reaction solution, while for the blank sample (B): 0.02 ml 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):¹²

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 10 min. The absorbance of standard and test against reagent blank was read at 505 (500-540 nm).

Estimation of HDL-cholesterol:¹³

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 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 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 reaction solution was added to it. In an 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 546 nm

Estimation of glucose:¹⁴

CHOD-PAP method was used to estimate the serum glucose level. For this 2ml 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 10min at +15 to 25c and then centrifuged for 15min at 4000rpm. Within 2hr after centrifugation, the clear supernatant was used for the determination glucose. 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 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 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

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

Histopathological studies:

At the end of the study period, animals from both the experimental groups were sacrificed liver and aorta were dissected out, washed, 5µm thick section slides were prepared and stained with heamatoxyline-eosin and examined by light microscopy.

Statistical analysis

All the data expressed as mean± SEM and evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparisons test using Prism Graphpad version 5.0 . Values of P<0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Results Of Triton-X-100 Induced Hyperlipidemia

1. Effect on serum Total cholesterol level

Rats given with triton x 100 intraperitoneal injection had increased serum TC level (132.2±2.76) when measured on 8 day. This was significantly higher (p<0.001) when compared to serum TC levels in normal control rats. Triton x 100 animals treated with Atorvastatin (10mg/kg, p.o. once daily) had serum TC level of (88.49±0.33) when measured on day 7. This was significantly lower (p<0.001) when compared to serum TC levels in control (Triton x 100) rats (132.2±2.76). Triton x 100 animals treated with extract EGP200 and

400mg/kg p.o. once daily had serum level of (98.15±8.32 and 86.50±6.83) when measured on day 8 as shown in (fig no 2A). These values were significantly lower ($p<0.001$ and $p<0.001$) when compared to serum TC levels in control (Triton x 100) rats (132.2±2.76).

2. Effect on serum Triglycerides level

Rats given with triton x 100 intraperitoneal injection had increased serum TC level (109.3±1.76) when measured on 8 day. This was significantly higher ($p<0.001$) when compared to serum TG levels in normal control rats (85.89±0.28). Triton x 100 animals treated with Atorvastatin (10mg/kg, p.o. once daily) had serum TG level of (74.33±0.59) when measured on day 8. This was significantly lower ($p<0.001$) when compared to serum TG levels in control (Triton x 100) rats (109.3±1.76). Triton x 100 animals treated with extract EGP 200 and 400mg/kg p.o. once daily had serum TG level of (81.00±0.48 and 77.23±0.53) when measured on day 8 (fig no 2B). These values were significantly lower ($p<0.001$ and $p<0.001$) when compared to serum TC levels in control (Triton x 100) rats (109.3±1.76).

3. Effect on serum HDL-C level

Rats given with triton x 100 intraperitoneal injection had increased serum HDL level (52.19±4.96) when measured on 8 day. This was significantly lower ($p<0.01$) when compared to serum HDL levels in normal control rats (71.15±0.77). Triton x 100 animals treated with Atorvastatin (10mg/kg, p.o. once daily) had serum HDL level of (67.95±3.57) when measured on day 8. This was significantly higher ($p<0.01$) when compared to serum HDL levels in control (Triton x 100) rats (52.19±4.96). Triton x 100 animals treated with extract EGP 200 and 400mg/kg p.o. once daily had serum HDL level of (57.57±4.19 and 66.09±2.46) when measured on day 8 (fig no 2C). The extract 200mg/kg was showed no significant changed and 400mg/kg was significantly higher $p<0.05$ when compared to serum HDL levels in control (Triton x 100) rats (52.19±4.96).

4. Effect on serum LDL-C level

Rats given with triton x 100 intraperitoneal injection had increased serum LDL level (18.40±4.79) when measured on 8 day. This was significantly higher ($p<0.001$) when compared to serum LDL levels in normal control rats (4.167±0.84).

Triton x 100 animals treated with Atorvastatin (10mg/kg, p.o. once daily) had serum LDL level of (5.683±3.69) when measured on day 8. This was significantly lower ($p<0.01$) when compared to serum LDL levels in control (Triton x 100) rats (18.40±4.79). Triton x 100 animals treated with extract EGP 200 and 400mg/kg p.o. once daily had serum LDL level of (24.38±8.58 and 15.97±2.93) when measured on day 8 (fig no 2D). These values were showed no significant changed in LDL level when compared to serum LDL levels in control (Triton x 100) rats (18.40±4.79).

5. Effect on serum VLDL-C level

Rats given with triton x 100 intraperitoneal injection had increased serum VLDL level (21.88±0.36) when measured on 8 day. This was significantly lower ($p<0.001$) when compared to serum VLDL levels in normal control rats (17.15±0.04). Triton x 100 animals treated with Atorvastatin (10mg/kg, p.o. once daily) had serum VLDL level of (14.87±0.12) when measured on day 8 (fig no 5). This was significantly lower ($p<0.01$) when compared to serum VLDL levels in control (Triton x 100) rats (21.88±0.36). Triton x 100 animals treated with extract EGP 200 and 400mg/kg p.o. once daily had serum VLDL level of (16.20±0.10 and 15.47±0.09) when measured on day 8. These values were significantly lower ($p<0.01$ and $p<0.001$) when compared to serum VLDL levels in control (Triton x 100) rats (21.88±0.36).

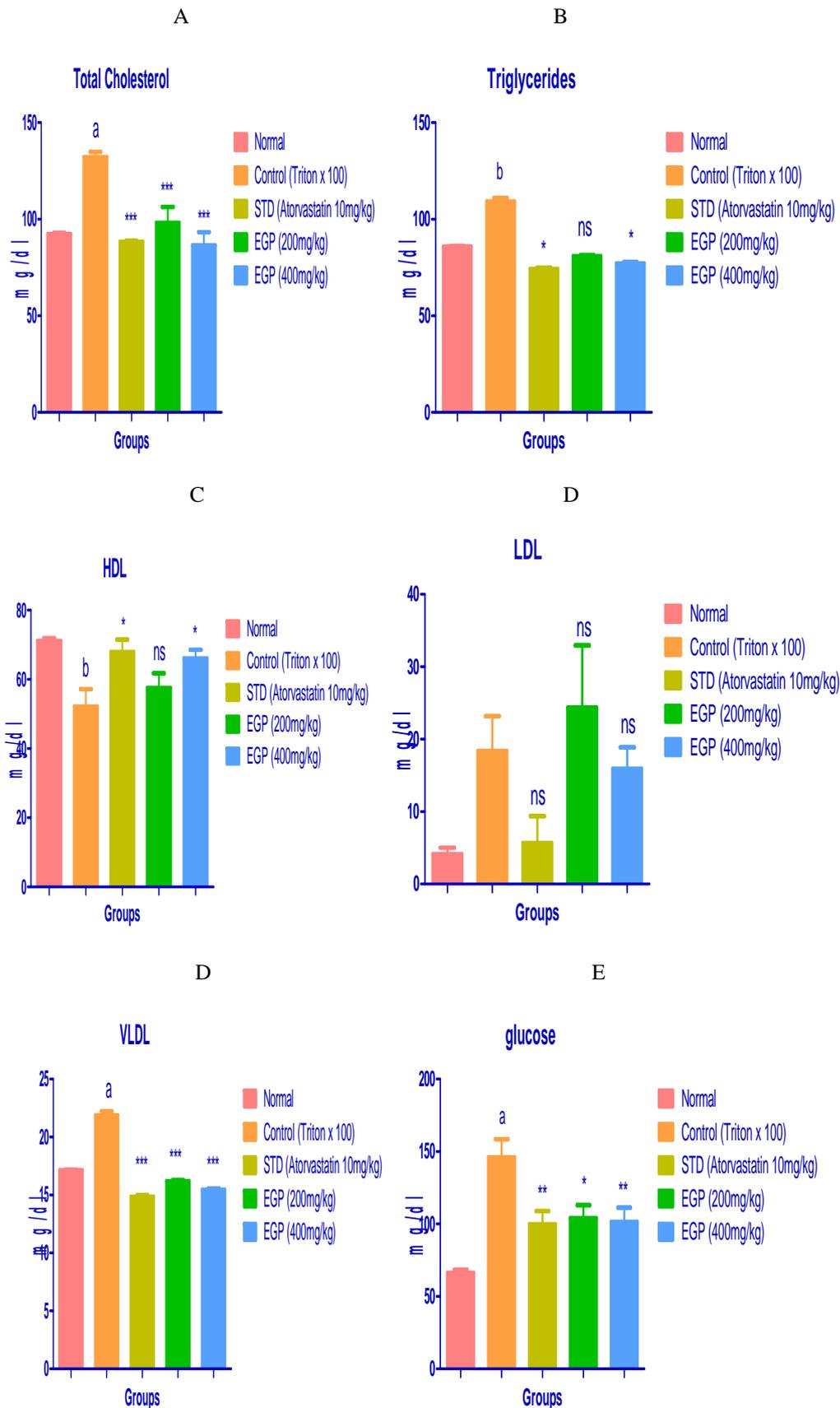
6. Effect on serum glucose level

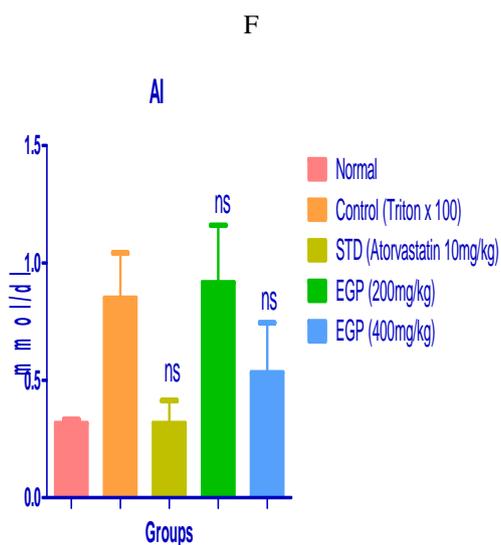
Rats given with triton x 100 intraperitoneal injection had increased serum glucose level (146.0±12.59) when measured on 8 day. This was significantly higher ($p<0.001$) when compared to serum glucose levels in normal control rats (68.21±2.10). Triton x 100 animals treated with Atorvastatin (10mg/kg, p.o. once daily) had serum glucose level of (99.99±8.87) when measured on day 8. This was significantly lower ($p<0.01$) when compared to serum glucose levels in control (Triton x 100) rats (146.0±12.59). Triton x 100 animals treated with extract EGP 200 and 400mg/kg p.o. once daily had serum glucose level of (104.0±9.03 and 101.6±9.61) when measured on day 8 (fig no 2E). This was significantly lower ($p<0.05$ and $p<0.01$) when compared to serum glucose levels in control (Triton x 100) rats (146.0±12.59).

8. Effect on AI

Rats given with triton x 100 intraperitoneal injection had increased atherogenic index (0.85±0.19) when measured on 8th day. There was no significant increased AI in control when compared to AI in normal control rats (0.31±0.01). Triton x 100 animals treated with Atorvastatin (10mg/kg, p.o. once daily) had AI of (0.31±0.09) when measured on day 8. This was significantly lower ($p<0.01$) when compared to AI in control (Triton x 100) rats (0.85±0.19). Triton x 100 animals treated with extract EGP 200 and 400mg/kg p.o. once daily had AI of (0.91±0.24 and 0.53±0.21) when measured on day 8 as shown in (fig no 2F). These values were exhibited no significantly lower AI when compared to AI in control (Triton x 100) rats (0.85±0.19).

Fig.2: Effect of ethanolic extract of *Glycosmis pentaphylla* on various biochemical parameters in triton x induced hyperlipidemic rats.

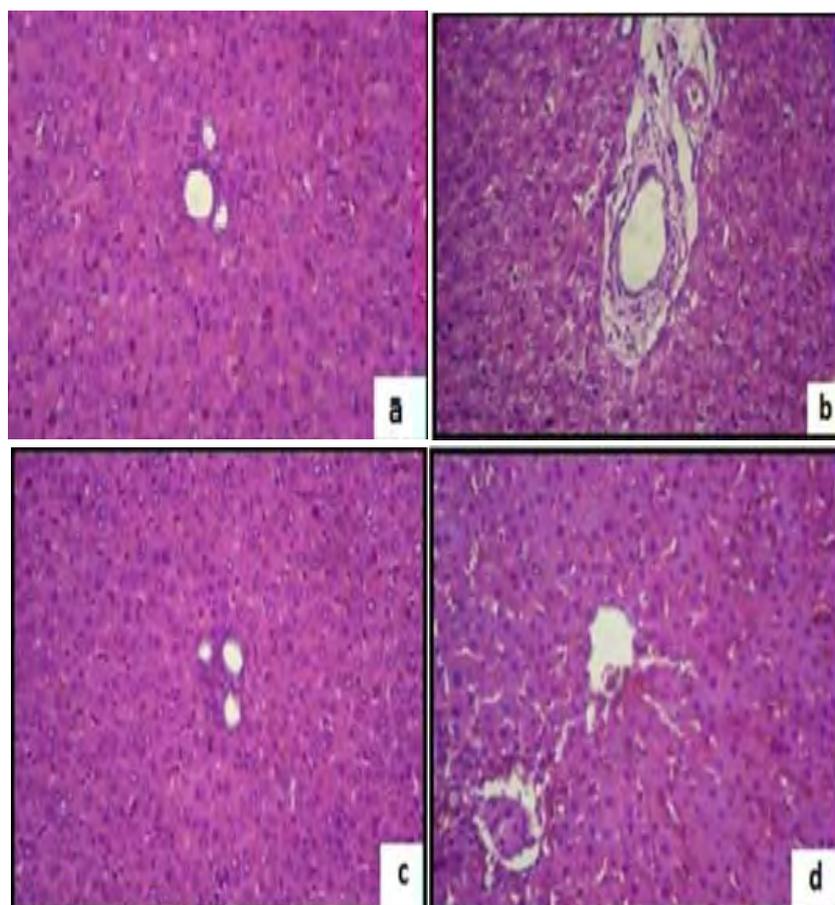




A-F: Total Cholesterol, Triglycerides, HDL, LDL, VLDL, Glucose & Atherogenic Index

All the values are Mean ± SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, ***p<0.001 as compared to control and ^ap<0.001 as when compared to normal.

Histopathological changes in liver of animals in triton x-100 induced hyperlipidemia



(a) normal group showing normal architecture;

(b) Control (Triton x-100) showing fatty infiltration and granular degeneration

(c) Standard (Atorvastatin 10mg/kg) treated group showing negligible cytoplasmic fatty infiltration and granular degeneration

(d) EGP 200mg/kg treated group showing mild to moderate cytoplasmic fatty infiltration and granular degeneration.

High Fat Diet Induced Hyperlipidemia

1. Effect on serum Total cholesterol level

Rats fed with HFD for 28 days had increased serum TC level (132.1 ± 0.56) when measured on 28 day. This was significantly higher ($p < 0.001$) when compared to serum TC levels in normal control rats (81.69 ± 1.58). HFD rats treated with Atorvastatin (10mg/kg, p.o. once daily) had serum TC level of (74.39 ± 1.26) when measured on day 28th. This was significantly lower ($p < 0.001$) when compared to serum TC levels in control (HFD) rats (132.1 ± 0.56). HFD rats treated with extract EGP200 and 400mg/kg p.o. once daily had serum level of (103.7 ± 2.58 and 87.37 ± 2.82) when measured on day 28. These values were significantly lower ($p < 0.001$ and $p < 0.001$) when compared to serum TC levels in control (HFD) rats (132.1 ± 0.56).

2. Effect on serum Triglycerides level

Rats fed with HFD for 28 days had increased serum TG (125.5 ± 2.44) when measured on 28 day. This was significantly higher ($p < 0.001$) when compared to serum TG levels in normal control rats (79.93 ± 2.16). HFD rats treated with Atorvastatin (10mg/kg, p.o. once daily) had serum TG level of (70.78 ± 1.46) when measured on day 28. This was significantly lower ($p < 0.001$) when compared to serum TG levels in control (HFD) rats (125.5 ± 2.44). HFD rats treated with extract EGP 200 and 400mg/kg p.o. once daily had serum TG level of (28.62 ± 1.54 and 27.63 ± 2.29) when measured on day 28. These values were significantly lower ($p < 0.01$ and $p < 0.01$) when compared to serum TC levels in control (HFD) rats (125.5 ± 2.44).

3. Effect on serum HDL-C level

Rats fed with HFD had increased serum HDL level (20.61 ± 1.00) when measured on 28 day. This was significantly lower ($p < 0.01$) when compared to serum HDL levels in normal control rats (28.49 ± 1.23). HFD rats treated with Atorvastatin (10mg/kg, p.o. once daily) had serum HDL level of (31.97 ± 0.90) when measured on day 28. This was significantly higher ($p < 0.001$) when compared to serum HDL levels in control (HFD) rats (20.61 ± 1.00). HFD rats treated with extract EGP 200 and 400mg/kg p.o. once daily had serum HDL level of (28.62 ± 1.54 and 27.63 ± 2.29) when measured on day 28. These values were significantly higher ($p < 0.01$ and $p < 0.01$) when compared to serum HDL levels in control (HFD) rats (20.61 ± 1.00).

4. Effect on serum LDL-C level

Rats fed with HFD had increased serum LDL level (64.71 ± 1.60) when measured on 28 day. This was significantly lower ($p < 0.001$) when compared to serum LDL levels in normal control rats (40.35 ± 1.00). HFD rats treated with Atorvastatin (10mg/kg, p.o. once daily) had serum LDL level of (34.41 ± 1.53) when measured on day 28. This was significantly lower ($p < 0.001$) when compared to serum LDL levels in control (HFD) rats (64.71 ± 1.60). HFD rats treated with extract 200 EGP and 400mg/kg p.o. once daily had serum LDL level of (54.67 ± 1.33 and 41.38 ± 0.27) when measured on day 28 (**fig no3B**). These values were significantly lower ($p < 0.001$ and $p < 0.001$) when compared to serum LDL levels in control (HFD) rats (64.71 ± 1.60).

5. Effect on serum VLDL-C level

Rats fed with HFD had increased serum VLDL level (24.99 ± 1.13) when measured on 28 day. This was significantly lower ($p < 0.001$) when compared to serum VLDL levels in normal control rats (14.93 ± 1.23). HFD rats treated with Atorvastatin (10mg/kg, p.o. once daily) had serum VLDL level of (12.00 ± 0.17) when measured on day 28. This was significantly lower ($p < 0.001$) when compared to serum VLDL levels in control (HFD) rats (24.99 ± 1.13). HFD rats treated with extract EGP 200 and 400mg/kg p.o. once daily had serum VLDL level of (19.80 ± 0.57 and 18.81 ± 0.79) when measured on day 28 (**fig no 3A**). This was significantly lower ($p < 0.05$ and $p < 0.01$) when compared to serum VLDL levels in control (HFD) rats (24.99 ± 1.13).

6. Effect on serum glucose level

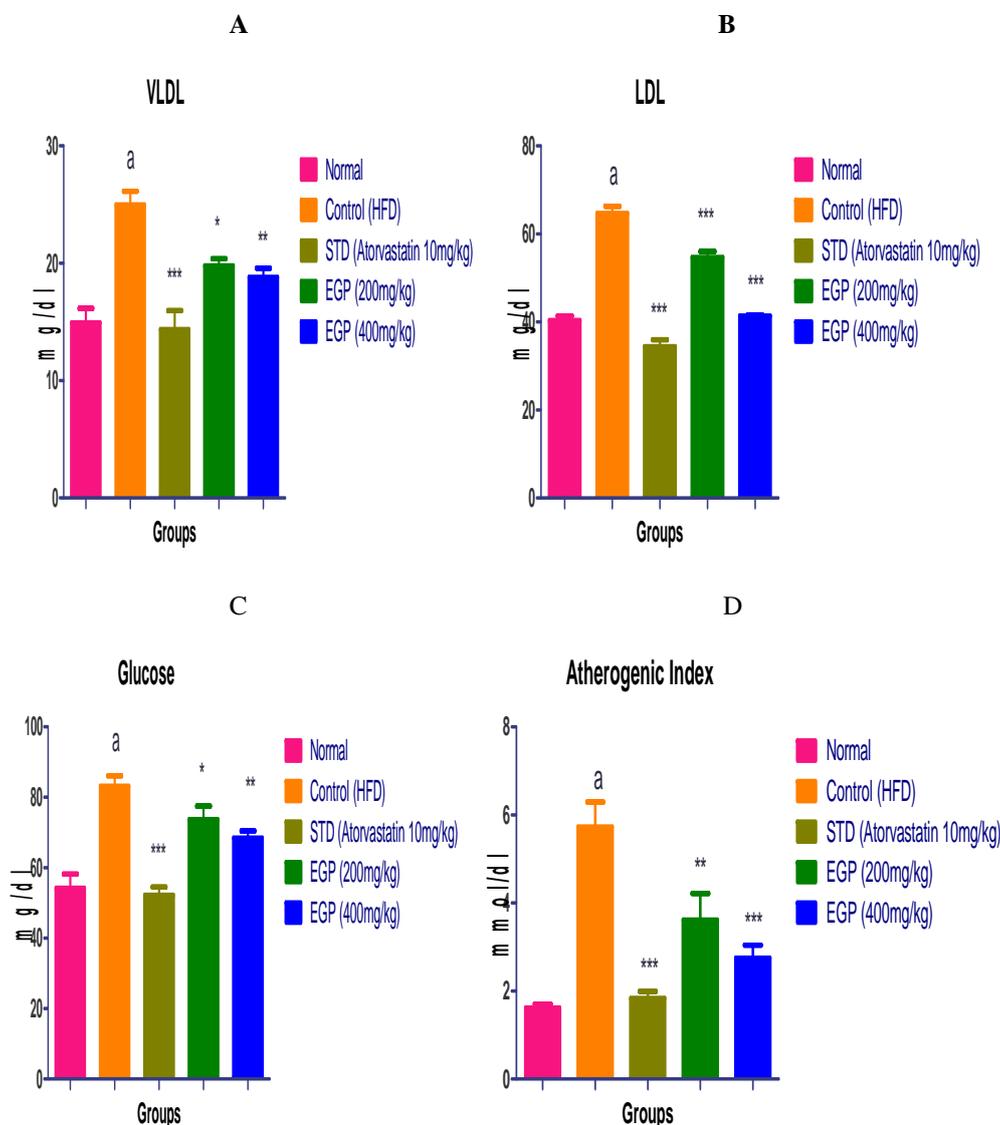
Rats fed with HFD had increased serum glucose level (83.19 ± 2.90) when measured on 28 day. This was significantly higher ($p < 0.001$) when compared to serum glucose levels in normal control rats (54.24 ± 3.95). HFD rats treated with Atorvastatin (10mg/kg, p.o. once daily) had serum glucose level of (52.18 ± 2.34) when measured on day 28. This was significantly lower ($p < 0.001$) when compared to serum glucose levels in control (HFD) rats (83.19 ± 2.90).

HFD rats treated with extract EGP 200 and 400mg/kg p.o. once daily had serum glucose level of (73.71 ± 3.84 and 68.47 ± 1.96) when measured on day 28 (**fig no 3C**). These values were significantly lower ($p < 0.05$ and $p < 0.01$) when compared to serum glucose levels in control (HFD) rats (83.19 ± 2.90).

8. Effect on AI Rats fed with HFD had increased atherogenic index (5.73 ± 0.56) when measured on 28th day. This was significantly higher ($p < 0.001$) when compared to AI in normal control rats (1.61 ± 0.08). HFD rats treated with Atorvastatin (10mg/kg, p.o. once daily) had AI of (1.83 ± 0.16) when measured on day 28. This was significantly lower ($p < 0.001$) when compared to AI in control (HFD) rats (5.73 ± 0.56). HFD rats treated with

extract EGP 200 and 400mg/kg p.o. once daily had AI of (3.61±0.59 and 2.75±0.29) when measured on day 8 (fig no 3D). These values were significantly lower ($p < 0.01$ and $p < 0.001$) when compared to AI in control (HFD) rats (5.73±0.56).

Fig No.3 Effect of ethanolic extract of *Glycosmis pentaphylla* on biochemical parameters in High Fat Diet induced hyperlipidemic rats.



All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, *** $p < 0.001$ as compared to control and ^a $p < 0.001$ as when compared to normal.

Results of histopathological studies in High fat diet induced Hyperlipidemia

1. Normal Group: Light microscopy of the aortic sections of normal group showed normal histology of the tunica intima, media and adventia. The intima was composed of a continuous layer of endothelial cells. (fig no 4A)

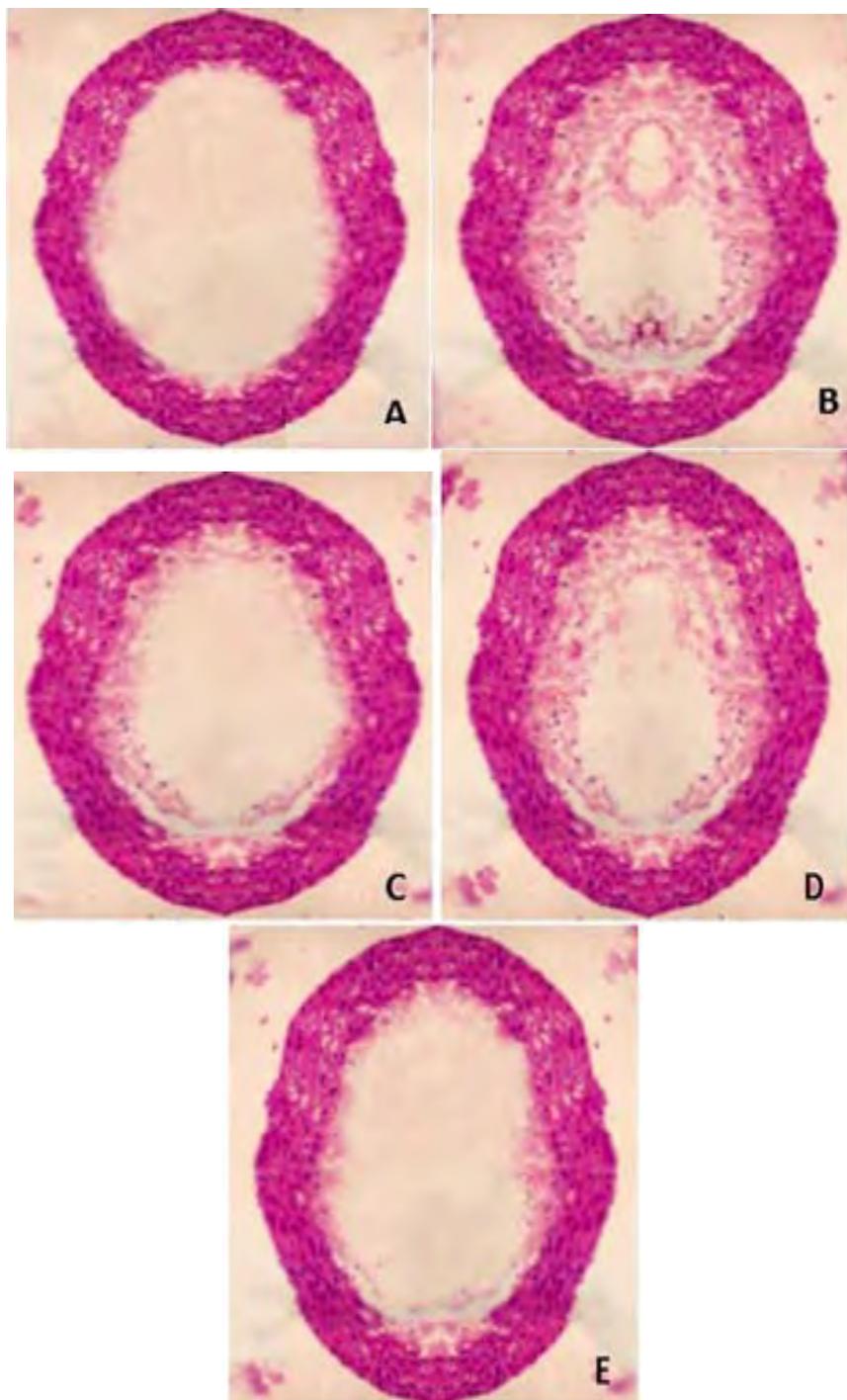
2. Control Group: Light microscopy of the aortic sections of HFD control group showed thickening of vascular wall of aortic musculature with fatty tissue. Also there is a formation of neointima containing vascular smooth muscles cells of tunica media. (fig no 4B)

3. Standard Group: Light microscopy of the aortic sections of HFD and Atorvastatin 10mg/kg showed thickening of vascular wall of aortic musculature with fatty tissue. Moderately decreased the formation of neointima and vascular smooth muscle cells of tunica media are less in the neointima. (fig no 4C)

4. EGP 200mg/kg group: Light microscopy of the aortic sections of HFD and ethanolic extract of 200mg/kg treated group showed thickening of vascular wall of aortic musculature with fatty tissue. Mild decrease of formation of neointima containing vascular smooth muscle cells of tunica media. (fig no 4D)

5. EGP 400mg/kg: Light microscopy of the aortic sections of HFD and 400mg/kg treated group showed thickening of vascular wall of aortic musculature with fatty tissue. There is mild formation of neointima containing vascular smooth muscle cells of tunica media and almost there is a appearance of normal architecture.(fig no 4E)

Fig.4: Effect of ethanolic extract of *Glycosmis pentaphylla* on histopathological changes in aorta of rats fed with HFD for 28 days



A: Normal group ,B: Control (HFD) group,C: Standard (Atorvastatin 10mg/kg, D:EGP (200mg/kg) ,
E: EGP (400mg/kg

DISCUSSION

The dried and powdered whole plant material of *Glycosmis pentaphylla* was subjected to soxhlet extraction with 90 % ethanol .The yield of the extract was 10 % w/w. Phytochemical analysis of the extract showed different phytoconstituents viz. glycosides, phytosterols, triterpinoids, alkaloids and flavonoids. Phytoconstituents like glycosides, triterpinoids, Saponins, alkaloids and flavonoids are known to have antihyperlipidemic property. It

has been well established that nutrition plays an important role in the etiology of hyperlipidemia and atherosclerosis¹⁵.

Cholesterol feeding has been often used to elevate serum cholesterol levels to assess the hypercholesterolemia-related metabolic disturbances in animals^{16,17}. Cholesterol feeding alone however does not affect the serum TG level. It is assumed that a high level of saturated fat in addition to cholesterol is required to significantly elevate serum TG level in rat model.¹⁸ Drugs interfering cholesterol biosynthesis are active in I, while drugs interfering with cholesterol excretion and metabolism are active in phase II, EGP treatment significantly decreased total lipids in phase I and phase II. The hypolipidemic activity of EGP was evident in both synthesis and excretory phases of triton induced hyperlipidemia in rats.

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 stimulates the cholesterologenesis rate¹⁹. 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.

In the present study Triton x-100 and high fat diet model was chosen for the induction of hyperlipidemia. Triton-x-100 model is used as a acute model for induction of hyperlipidemia in rats. Rats given triton x intraperitoneally showed significantly higher levels of TC, TG, LDL and glucose (P<0.001) whereas decreased levels of AI, VLDL-C, HDL-C were observed when compared to serum levels in control rats. The above parameters were significantly reversed with the treatment of standard drug atorvastatin (i.e) decreased levels in TC, TG, LDL, VLDL, GLUCOSE, HDL-C and AI respectively. Rats given HFD intraperitoneally showed significantly higher levels of TC, TG, LDL and glucose whereas decreased levels of AI, VLDL, HDL were observed when compared to serum levels in control rats. The above parameters were significantly reversed with the treatment of standard drug atorvastatin (i.e) decreased levels in TC, TG, LDL, VLDL, glucose, HDL-C and AI respectively. The treatment with test drug 200mg/kg and 400mg/kg also significantly lowered the elevated levels of TC, TG, VLDL, glucose respectively in both the experimental models. Triton induced hyperlipidemia in rats is an acute model for the primary screening of antihyperlipidemic agents which induces hyperlipidemia in two phases. Triton acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extra hepatic tissues, thereby increasing blood lipid levels.²⁰

There was also increase in the levels of HDL-C after the treatment of 200mg/kg and 400 mg/kg in both the models. The possible mechanism of protective action of the test drug may be due to the activity of LCAT 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.

CONCLUSION:

In conclusion, the findings of the study suggest that EGP (Ethanol extract of *Glycosmis pentaplylla*) is a potent antihyperlipidemic, antihypertriglycerolemic drug lowering LDL, VLDL and increasing HDL levels in both the models, Triton x 100 and HFD. The mechanism has an indication towards inhibiting cholesterol and triglyceride synthesis.

ACKNOWLEDGEMENT

The authors are thankful to Dr. A Venkateshwar Reddy, Principal, Anwarul uloom college of pharmacy and Sicra Labs, Hyderabad for providing support in this research.

REFERENCES

- [1] Grundy S.M. and Vega G.L. Hypertriglyceridemia: causes and relation to coronary heart disease - *Semin. Thromb. Hemost.* 1988; 14: 249-164
- [2] Ross R., Glomset J.A, Engl N. J. *Med.* 1976; 295: 369-420
- [3] Austin M.A. Plasma triglyceride as a risk factor for coronary heart disease. The epidemiologic evidence and beyond - *Am. J. Epidemiol.* 1989; 129: 249- 259.
- [4] Kim HY, Jeong da M, Jung HJ, Jung YJ, Yokozawa T, Choi JS. Hypolipidemic effects of *Sophora flavescens* and its constituents in poloxamer 407-induced hyperlipidemic and cholesterol-fed rats. *Biol Pharm Bull* 2008 Jan; 31(1):73-8.
- [5] Kourounakis, Victoratos P, Peroulis N, Stefanou N, Yiangou M, Hadjipetrou L, et al. Experimental hyperlipidemia and effect of NSAIDs. *Exp Mol Pathol* 2002; 73(2):135-8.
- [6] Jan Fedacko, Singh RB, Chaithiraphan S, Viola Vargova, Brian Tomlinson, Fabien De Meester, Anna Gvozdjakova. Clinical Manifestations of Adverse Effects of Statins, Oxidative Stress and Possible Role of Antioxidants in Prevention. *The Open Nutraceuticals Journal.* 2010;3: 154-165.
- [7] C.K. .Kokate, A.P.Poroth and S.B. Gokhale (2007). *Text Book of Pharmacognosy.* 42 nd Edition, Pune :NiraliPrakashan, 2007, 108-109.

- [8] Sudhass, Karthic R, Naveen, Rengaramanujam. Antihyperlipidemic activity of spirulina platensis in triton x 100 induced hyperlipidemic rats. *HJD Med* 2011 Oct; 3 (2);32-7
- [9] OECD guidelines for the testing of chemicals (Acute oral toxicity – up and down procedure). Adopted 23rd march 2006. [cited 2008 Jun 20]; Available from: URL: www.oecd.org.
- [10] Mukesh SS and MB. Antihyperlipidemic activity of Salacia chinensis root extracts in triton induced and atherogenic diet-induced hyperlipidemic rats. *Indian J Pharmacol.* 2012;44(1):88–92
- [11] Cholesterol CHOD-PAP method, end point. Robonik diagnostics Navi Mumbai. 2010.
- [12] Triglycerides GPO-Trinder's method, end point. Robonik diagnostics Navi Mumbai. 2010.
- [13] HDL-cholesterol Phosphotungstic acid method, end point. Robonik diagnostics Navi Mumbai. 2010.
- [14] Glucose Trinder's method, end point. Robonik diagnostics Navi Mumbai. 2010.
- [15] Holmgren PR and Brown AC. serum cholesterol levels of nondiabetic and streptozotocin diabetic rats fed a high cholesterol diet. *Artery* 20;P:337-45.
- [16] Hozumi T, Yoshida M, Ishida Y, Mimoto H, Sawa J, Doi K and Kazumi T. Long term effects of dietary fiber supplementation on serum glucose and lipoprotein levels in diabetic rats fed a high cholesterol diet. *Endocrinol J* 1995;42:187-92.
- [17] Watts GF, Jackson P, Mandalia S, Burnt JN, Lewis ES, Coltart DJ and Lewis B. Nutrient intake and progression of coronary artery disease. *Am J Cardiol* 1994;73:328-32.
- [18] Oda A, Bannai C, Yamoka T, Katori T, Matsushima T and Yamashita K. Inactivation of Cu-Zn-SOD by in vitro glycosylation in erythrocytes of diabetic patients. *HormMetab res* 1994;26:1-4.
- [19] Davidson MH. Safety profiles for the HMG-CoA reductase inhibitors: treatment and trust. *Drugs.* 2001;61(2):197-206.
- [20] Umesh K, Patil, S. Saraf and VK Dixit. Hypolipidemic activity of seeds of *Cassia tora* Linn. *Journal of Ethnopharmacology* 2004;90:249-52.