International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

HYPOGLYCEMIC AND HYPOLIPIDEMIC ACTIVITIES OF METHANOLIC EXTRACT OF *GLINUS OPPOSITIFOLIUS*

Ghanshyam Panigrahi*[1], Uma Shankar Mishra[1], Sujata Mahapatra[2], Chhayakanta Panda[1], Gourishyam Pasa[1] and Deepak Ku Hati[1]

[1]Department of pharmacology, Royal College of Pharmacy and Health Sciences, Berhampur, Odisha, India
[2]Department of Botany and Biotechnology, Khallikote (Auto) College, Berhampur, Odisha, India

***Corresponding author e-mail:** gspmph@yahoo.co.in

ABSTRACT

The aim of this study has been to investigate the possible hypoglycemic and hypolipidemic effect of methanolic extract of the plant *Glinus oppositifolius*. The methanolic extract of *Glinus oppositifolius* was tested for antihyperglycemic activity in glucose overloaded hyperglycemic rats and hypoglycemic activity in overnight fasted normal rats. The extract was also evaluated for antihyperlipidemic activity in triton-induced hyperlipidemic rats. All the methods were carried out at three dose levels, 100, 200 and 400 mg/kg respectively. The results of the study were expressed as mean ± SEM, n=6 and data was analyzed by using one way analysis of variance test (ANOVA) followed by Bonferroni's Multiple Comparison Test with 5% level of significance ($P<0.05$). The methanolic extract of *Glinus oppositifolius* exhibited significant antihyperglycemic activity ($P<0.05$) at 200 and 400 mg/ kg but did not produce hypoglycemia in fasted normal rats. It also exhibited a significant reduction ($P<0.05$) in serum lipid profile like total cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipopreotein (VLDL) and increase in high density lipoprotein (HDL) in hyperlipidemic rats as compared to hyperlipidemic control statistically. All the activities were found dose dependent. This study supports the traditional claim and the methanolic extract of this plant possessed significant hypoglycemic and hypolipidemic effect could be added in traditional preparations for the ailment of complications associated with hyperglycemic and hyperlipidemic conditions or as an adjuvant with existing therapy.

Keywords: Hypoglycemic, hypolipidemic, lipid profile, *Glinus oppositifolius*, triton

INTRODUCTION

The pandemic of obesity, diabetes and heart disease, based in the lifestyle, poses the greatest threat to our survival for the foreseeable future. The increasing availability of energy dense food and the sedentary lifestyle that is becoming prevalent in both first world and developing nations have led to a worldwide epidemic in type 2 diabetes. Diabetes mellitus is categorized as a metabolic disease characterized by common feature of chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The cause of type 2 diabetes is a combination of resistance to insulin action and an inadequate compensatory insulin-secretory response [1]. The increasing availability of energy dense food and the sedentary lifestyle that is becoming prevalent in both first world and developing nations have led to a worldwide epidemic in type 2 diabetes mellitus.

The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. It was estimated that 2.8% of world population was diabetic in 2000 and this figure would climb to be as high as 4.4% of the world's population by 2030 (most of which will be type 2 diabetes

mellitus). It is predicted that by 2030, India, China and the United States will have the largest number of people with diabetes [2]. Hyperlipidemia is a secondary metabolic dysregulation associated with diabetes. Besides the cause effect relationship with diabetes, elevated serum level of triglycerides, cholesterol and LDL are major risk factors for the premature development of cardiovascular disease like arthrosclerosis, hypertension, coronary heart disease etc. [3]. Increased plasma lipid levels mainly total cholesterol, triglycerides, LDL and VLDL along with decrease in HDL are known to cause hyperlipidemia which is the reason for initiation and progression of atherosclerosis impasse [4].

Elevated lipid levels result from increased absorption through the gut or enhanced endogenous synthesis and therefore two ways are feasible to reduce hyperlipidemia; to block endogenous synthesis or to decrease absorption. Though different types of drugs are available for the treatment of diabetes and hyperlipidemic, there is an increased demand by patients to use natural products for regulate these problems [5].

Since time immemorial, patients with non-insulin dependent diabetes have been treated orally in folk medicine, with a variety of plant extracts. In India, a number of plants are mentioned in ancient literature for the treatment of diabetic conditions. One such drug is *Glinus oppositifolius* of the family Molluginaceae, being used by some local triable people, were selected for the present study.

Glinus oppositifolius is a branched herb growing all over India. This species has been found in other tropical parts of Asia, Africa and North Australia. It is an annual prostrate weed commonly found in paddy fields after harvesting, riversides, and open sands of seashores. The leaves are used as vegetable for cooking purposes, as well as an expectorant and antipyretic agent. Dried and powdered stems with leaves of the herb are used for treating jaundice and abdominal pain.

A decoction of fine powder of the aerial parts of the plant is used in the treatment of malaria [6]. A maceration of pounded plant material with oil or water is used in the treatment of wound. *Glinus oppositifolius* are used by the traditional healers for treating joint pain, inflammation, diarrhoea, intestinal parasites, fever boils and skin disorders [6]. Ethanolic extract of the plant has been reported to depress central nervous activity [7]. The leaves contain spergulagenic, spergulagenin A and a trihydroxy ketone. A bioactive pectic polysaccharide isolated from *Glinus oppositifolius* is found to possess immunomodulating property [8].

MATERIALS AND METHODS

Plant material: *Glinus oppositifolius* plants were collected from local areas of Berhampur, Odisha. The taxonomical identification of the plant specimen was done by Dr. P. Lakshminarasimhan, scientist, Central National Herbarium, Botanical Survey of India, Howrah (Authenticated no. CNH/23/2011/ Tech.II/483). Voucher specimen was preserved in the Department of Pharmacognosy of the Royal College of Pharmacy and Health Sciences, Berhampur for further verification. The plant materials were air dried under shade, coarsely powdered and kept in airtight container until further use.

Animals: As per the OECD draft guidelines 423 received from CPCSEA, young female albino mice were used for acute toxicity study. Whereas other in vivo methods were carried out by using Sprague-Dawley (SD) rats of both sexes. All the animals for the in vivo studies, with no prior drug treatment, were procured from the animal house of R.C.P.H.S., Berhampur and housed in polypropylene cages with clean sterilized husk bedding (six mice or three rats/ cage). Bedding was changed every alternate day to maintain proper hygienic condition. Animals were maintained under controlled room temperature (22 ± 2°C) and humidity (55 ± 5°C) with a 12:12 hour light: dark cycle.

The animals were fed with standard laboratory food diet made in-house recommended by National institute of nutrition (NIN), Hyderabad and pure drinking water *ad libitum*. The animals were acclimatized to laboratory hygienic conditions in the departmental laboratory for 7 days before commencing the experiment. The ethical clearance was granted for the study by Institutional animal ethics committee (IAEC) of Royal College of Pharmacy and Health Sciences, Berhampur, (bearing registration number 1018/C/06/CPSEA and date of registration 19th Dec 2006) in resolution number 02/ 09/IAEC held on 26/10/2009.

Chemicals: Glibenclamide and Simvastatin were obtained from Dr. Reddy's Laboratories, Hyderabad. Experimental hyperlipidemic agent, Triton -WR 1339, was purchased from Sigma, USA. Blood glucose test-strips of Ascensia Entrust of Bayer Health Care and Diagnostic kits of Crest Biosystems, a division of Coral clinical systems, India were purchased. All other chemicals used for study were of analytical grade.

Preparation of extracts: Dried and powdered plant material (400 gm) was extracted by successive extraction process using soxhlet apparatus. Solvents were chosen depending upon their increase in polarity like Petroleum Ether (60-80° C), Chloroform, Methanol and Water. The extraction was carried out for 72 hours for each solvent. All the extracts were dried using rotary vacuum evaporator and freeze dryer. Their percentage yields were determined and stored in dessicator until further use.

Phytochemical screening: Different extracts obtained from the above extraction process were analyzed for presence of various phytoconstituents such as alkaloids, glycosides, flavones, tannins, terpenes, sterols, saponins, fats and sugars by the method of preliminary phytochemical study (color reactions) [9, 10, 11].

Acute toxicity studies: The acute oral toxicity studies of extracts were carried out as per the OECD guidelines, draft guidelines 423 adopted on 17th December 2001 received from CPCSEA, Ministry of social justice and empowerment, Govt. of India. Administration of the stepwise doses of methanolic extracts of *Glinus oppositifolius* from 100 mg/kg up to the dose 2000 mg/kg to young female albino mice and observed the signs of toxicity up to 72 hr in the tested animals [12]. The female albino mice 25-30 gm were divided into different groups of six animals each.

The control group received 10 ml/kg body weight of distilled water orally. The other groups received the extracts of *Glinus oppositifolius* at dose levels of 100, 500, 1000, 1500, 2000 mg/kg body weight through oral route. After administration of dose the animals were observed continuously for the first 4 hr and occasionally up to 24 hr and at the end of 72 hr [13] for recording mortality, if any. Additional observations like behavioral changes, somato motor activity, tremors, convulsions, tonic extension, strub tail, muscle spasm, loss of righting reflex, ataxia, sedation, hypnosis, lacrimation, diarrhea, salivation, writhing, changes in skin, fur, eyes, mucous membranes etc were recorded. One tenth of upper limit dose and its half dose and double dose were selected as the levels for examination of therapeutic activity.

Oral Glucose Tolerance Test: After acclimation for 7 days, the oral glucose tolerance test was performed in overnight fasted normal rats[14]. All the rats were randomly divided into five groups (n=6). Group 1 was received only vehicle 1 ml/100 g and served as control group animals; group 2, treated with 5 mg/kg of glibenclamide; the remaining three groups were treated with 100, 200 and 400 mg/kg of methanolic extracts of Glinus *oppositifolius*. The rats were fasted for 12h (free access to water) and administered the above drugs to respective groups. Zero minute blood sugar level was determined from overnight fasted animals. After 30 minutes of the drug treatment (p.o.) the rats of all groups were orally fed with glucose 4 gm/kg. Blood glucose concentration was determined after 30, 60, 90 and 120 minutes of glucose loading. The blood samples were collected from the tail tip and measured by using glucometer and blood glucose test-strips.

Hypoglycemic activity: The hypoglycemic activity was performed in overnight fasted normal rats as per the method described by Jarald *et al*., 2008. All the rats were randomly divided into five groups of six rats each. Group 1 was kept as control, and was given a single dose of 1 ml/100 g of the vehicle; group 2 was treated with glibenclamide (5 mg/kg) as the hypoglycemic reference drug. Groups 3, 4 and 5 were treated with methanolic extract at three dose levels i.e. 100, 200 and 400 mg/kg (p.o.).

The rats were fasted for 12h (free access to water) and administered the above drugs to respective groups. Zero minute blood sugar level was determined from overnight fasted animals i.e. before oral administration of drug. The blood glucose concentration was also measured after 30, 60 and 120 minutes of oral administration of drug. The blood samples were collected from the tail tip of the rats and measured the glucose concentration by using glucometer and blood glucose test-strips [14].

Triton-induced hyperlipidemic model: Several studies showed that systemic administration of triton WR 1339 (ionic surfactant) in fasted rats causes elevation in plasma lipid level. Triton Wr-1339 has been widely used to block clearance of triglyceride-rich lipoproteins to induce acute hyperlipidemia in several animals [15]. This model is widely used for a number of different aims [16] particularly, in rats it has been used for screening natural or chemical hypolipidemic drugs [17]. After 7 days acclimation, hypolipidemic activity was studied in triton WR 1339 induced hyperlipidemic rats.

All the rats were randomly divided into six groups of six rats each. Group 1 and 2 were received only vehicle 10 ml/kg and served as normal control and hyperlipdemic control groups respectively; group 3, treated with Simvastatin 10mg/kg and served as standard animals; the remaining three groups were

treated with 100, 200 and 400 mg/kg of methanolic extracts of *Glinus oppositifolius*. The different drugs were administered to the respective group of animals for 5 consecutive days. Hyperlipidemia was induced on 6th day of the experiment by single injection of 400 mg/kg of triton WR 1339) in 0.15 M NaCl, intraperitoneally, to 12 hour fasted animals. Normal control animals were injected with normal saline.

Drugs and extracts were administered orally to the respective groups, immediately as well as 20 h after triton injection. Rats were not fed but had free access to water during the experiment period. After 4 h of second dose, the blood of all animals were collected and used for the study the lipid profiles i.e. serum cholesterol, triglycerides, HDL cholesterol by using auto analyzer and commercially available kits. LDL cholesterol and VLDL were calculated by using Friedewald's formula [18].

Collection of blood and estimation of biochemical parameters: The blood was collected from the rat tail vein for the estimation of blood sugar by using glucometer and blood glucose test-strips of Ascensia Entrust of Bayer Health Care. For estimation of other biochemical parameters, blood was drawn from the retro-orbital plexus of the rats (fasted for 12 h), by sterilized capillary tubes, under light ether anesthesia, into eppendorf tubes.

The blood samples were allowed to coagulate for 30 minutes at room temperature and then they were centrifuged at 3000 rpm for 10 minutes. The serum used as specimen, should be free from hemolysis and must be separated from the clot promptly. The resulting upper serum layer was collected in properly cleaned, dried, and labeled eppendorf tubes and were stored at 2-8^{o}C for further analysis of the lipid profiles.

Statistical analysis: The values are expressed as mean ± SEM. The results were analyzed for statistical significance using one-way ANOVA (and nonparametric), followed by Bonferroni's Multiple Comparison Test (Graph pad prism 5.04 version). *P* <0.05 was considered statistically significant.

RESULTS

Preliminary phytochemical screening: The percentage yields of petroleum ether, chloroform, methanol and aqueous were found to be 2.7, 4.4, 6.8 and 9.3 % w/w respectively. The petroleum ether contained fats, alkaloids and terpenoids. The chloroform extract contained steroids and alkaloids. Methanol extract contained carbohydrates, saponins, alkaloids, tannins, flavons and flavonoids. Aqueous extract contained carbohydrates, alkaloids, saponins, flavons and flavonoids. Several papers have recently reported the hypoglycemic and hypolipidemic effects of phenolic compounds such as flavonoids, flavons, tannins etc.

The phytochemical studies of different extracts of *Glinus oppositifolius* revealed that the methanolic extract of the plant was the most active extract as it contains phenolic compounds such as flavons, flavonoids, tannins etc. Hence, the methanolic extract of *Glinus oppositifolius* (MEGO) was taken up for further studies.

The effect of methanolic extracts in glucose loaded hyperglycemic animals: The antihyperglycemic effect in glucose loaded hyperglycemic rats (shown in table-1) were studied after administration of methanolic extract of *Glinus oppositifolius* at the dose of 100, 200 and 400 mg/kg and glibenclamide 5 mg/kg to respective groups.

After 30 minutes of the glucose load, there was a significant rise in the blood glucose level of the control animals and at the end of two hours, the glucose level declined. The extract exhibited significant antihyperglycemic effect at 200 and 400 mg/kg dose levels after glucose load, compared to control group animals. There was no significant difference found between the dose of 400 mg/kg of extract and glibenclamide treated animals.

The effect of methanolic extract in fasted normal rats: The hypoglycemic effect in fasted normal rats were evaluated, after administration of the methanolic extract at the dose of 100, 200 and 400 mg/kg and glibenclamide 5 mg/kg to respective group and the results are given in table-2. After 30 min. of drug administration up to the end of 2 hours the blood glucose levels of the standard animals were declined. The extract did not show any hypoglycemic activity at any dose level.

The effect of methanolic extract in Triton-induced hyperlipidemic model: As expected, administration of triton WR1339 led to elevation of serum lipid levels, which were maintained over a period of study in hyperlipidemic control group.

The results were comparable with reference standard simvastatin. There was a significant elevation in serum lipids in triton-induced hyperlipidemic control rats when compared with normal control. The methanolic extract of *Glinus oppositifolius* at 200 and 400 mg/kg dose levels showed significant serum lipid

lowering effects in hyperlipidemic rats and reduced serum lipids significantly ($P<0.05$) as compared to hyperlipidemic control statistically (shown in table-3 & figure-1).

DISCUSSION

The present study was undertaken to examine the hypoglycemic and hypolipidemic activity of methanolic extract of *Glinus oppositifolius*. Antihyperglycemic effect was studied on glucose loaded rats and hypoglycemic effect was studied on the normal rats. Hypolipidemic effect of the methanolic extract was evaluated by using triton induced hyperlipidemic model. Effective blood glucose control is the key for preventing or reversing diabetic complications and improving the quality of life in patients with diabetes.

Thus, sustained reduction in hyperglycemia will decrease the risk of developing microvascular complications and most likely reduce the risk of macrovascular complications [19]. On the basis of this statement, we have selected the glucose induced hyperglycemic model to screen the antihyperglycemic activity of the plant extracts. In the glucose loaded hyperglycemic model, the plant tested for antihyperglycemic activity exhibited significant antihyperglycemic activity at the dose level of 200 and 400 mg/kg. Excessive amount of glucose in the blood induces the insulin secretion. This secreted insulin will stimulate peripheral glucose consumption and control the production of glucose through different mechanisms [20].

However, from the study (glucose control), it was clear that the secreted insulin requires more than 2 h to bring back the glucose level to normal. In case of the methanolic extract and drug treated groups, the glucose levels did not exceed the control group, giving an indication regarding the supportive action of the extracts and drug in the glucose utilization. The methanolic extract of the plant, when tested for hypoglycemic activity, did not exhibit hypoglycemic activity, suggesting its mechanism might be similar to biguanides. Biguanides do not increase insulin secretion; they promote tissue glucose uptake and reduce hepatic glucose output, thereby producing antihyperglycemic effect and not hypoglycemic effect [20].

There was marked increase in the level of serum total cholesterol, triglycerides, LDL, VLDL and decrease in the level of good cholesterol carrier HDL in the animals treated with triton. Elevated level of blood cholesterol especially LDL was the major risk factor for the coronary heart disease and HDL as cardio protective protein. Treatment with 200 and 400 mg/kg methanolic extract of *Glinus oppositifolius* significantly decreased the level of cholesterol, triglycerides, VLDL and LDL as compared to hyperlipidemic control. There was significant increase in HDL as compared to control. This effect may be due to the increased activity of lecithin: cholesterol acetyl transferase which incorporates free cholesterol, free LDL into HDL and transferred back to VLDL and intermediate density lipoprotein. Decrease in the triglyceride level may be due to the increase in activity of the endothelium bound lipoprotein lipase which hydrolyzes the triglyceride into fatty acid or due to inhibition of lipolysis so that fatty acids do not get converted to triglyceride. Lipoproteinlipase releases fatty acids from chylomicrons and very low-density lipoproteins (VLDL) in the circulation.

Half of those are taken up for storage. Catecholamines can suppress lipolysis via their action on 2-adrenoceptors, but they mostly have the opposite effect of insulin and, by acting on β-adrenoreceptors, increase cyclic AMP and the phosphorylation of hormone-sensitive lipase, stimulating lipolysis and the release of fatty acids in the circulation. Hepatic cholesterol synthesis is accelerated by triton WR 1339. Moreover, triton physically alters very low density lipoproteins rendering them refractive to the action of lipolytic enzymes of blood and tissues, preventing or delaying their removal from blood [21]. Hence the hypolipidemic effect of extracts could be due to an increased catabolism of cholesterol into bile acids.

CONCLUSION

The results obtained from the pharmacological screening have led to the conclusions that, methanolic extract of *Glinus oppositifolius* has shown potential activity in decreasing the serum glucose level and have significant antihyperlipidemic activity. This research supports the inclusion of this plant in traditional antidiabetic preparations. Hence it can be exploited as a hypoglycemic and hypolipidemic therapeutic agent or adjuvant in existing therapy for the treatment of diabetes associated with hyperlipidemia.

ACKNOWLEDGEMENTS

The authors thank to Royal College of Pharmacy and Health Sciences, Berhampur, Odisha, for providing required facilities to carry out this research work.

Table 1: The effect of methanolic extract in glucose loaded hyperglycemic animals

Groups	Blood glucose concentration (mg/dl) at different time				
	0 min	30 min	60 min	90 min	120 min
Vehicle	89.33 ± 3.63	146.16 ± 3.41	159.83 ± 3.38	142.16 ± 3.66	124.17 ± 3.42
Glibenclamide	86.5 ± 3.76	113.83 ± 4.42***	94.16 ± 2.98***	76.83 ± 3.57***	69.83 ± 2.80***
MEGO-100	[a] 88.66 ± 3.11	141.33 ± 3.48	160.83 ± 3.74	136.5 ± 2.17	117.67 ± 3.12
MEGO-200	[a] 85.16 ± 3.39	[a] 128.66 ± 3.07*	133.66 ± 4.13***	109.66 ± 3.07***	98.17 ± 3.54***
MEGO-400	[a] 87.66 ± 1.78	[a] 119.5 ± 3.21***	[a] 108.83 ± 3.28***	[a] 88.66 ± 3.20***	[a] 80.50 ± 3.12***

*The results were expressed as mean ± SEM, n=6. The difference between groups was analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's Multiple Comparison Test with 5% level of significance (P<0.05). ***P< 0.001, **P< 0.01 and *P< 0.05; compared Standard and Test groups vs Glucose loaded control and 'a'- indicates there is no significant difference between standard and test drug at P< 0.05 significant level.*

Table 2: The effect of methanolic extract in fasted normal rats

Groups	Blood glucose (mg/dl) at different time				
	0 min	30 min	60 min	90 min	120 min
Vehicle	85.50 ± 2.88	84.33 ± 3.24	87.67 ± 2.72	83.67 ± 2.14	84.83 ± 3.04
Glibenclamide	84.17 ± 3.27	59.83 ± 3.75***	47.67 ± 2.74***	39.83 ± 1.82***	35.17 ± 2.21***
MEGO-100	86.83 ± 3.74	89.83 ± 3.87	85.17 ± 2.51	87.67 ± 4.37	82.67 ± 2.73
MEGO-200	85.67 ± 3.22	84.33 ± 2.67	84.83 ± 3.86	87.17 ± 3.09	84.17 ± 3.22
MEGO-400	84.67 ± 3.17	87.33 ± 1.54	86.83 ± 2.69	83.67 ± 3.86	85.33 ± 3.82

*The results were expressed as mean ± SEM, n=6. The difference between groups was analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's Multiple Comparison Test with 5% level of significance (P<0.05). ***P< 0.001, **P< 0.01 and *P< 0.05; compared Standard and Test groups vs Vehicle control and 'a'- indicates there is no significant difference between standard and test drug at P< 0.05 significant level.*

Table 3: The effect of methanolic extract in triton-induced hyperlipidemic model

Groups	Lipid profiles (mg/dl)				
	Total Cholesterol	Triglycerides	HDL	LDL	VLDL
Normal control	61.83 ± 1.54	55.17 ± 1.64	32.67 ± 1.28	18.13 ± 2.16	11.03 ± 0.33
Hyperlipidemic control	193.17 ± 3.58[#]	114.33 ± 1.93[#]	17.83 ± 1.11[#]	152.47 ± 4.42[#]	22.87 ± 0.39[#]
Standard	118.83 ± 2.97***	79.67 ± 2.33***	29.83 ± 1.14***	73.07 ± 2.67***	15.93 ± 0.47***
MEGO-100	192.33 ± 2.33	116.33 ± 1.52	18.17 ± 1.35	150.90 ± 3.49	23.27 ± 0.30
MEGO-200	169.83 ± 2.57**	104.17 ± 2.14*	20.33 ± 1.23*	128.67 ± 2.14**	20.83 ± 0.43*
MEGO-400	[a] 131.83 ± 2.23***	[a] 92.50 ± 3.06***	[a]25.83 ± 1.25**	[a] 87.50 ± 2.85***	18.50 ± 0.61***

The results were expressed as mean ± SEM, n=6. The difference between groups was analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's Multiple Comparison Test with 5% level of significance (P<0.05).
[#]P< 0.001, [##]P< 0.01 and [###]P< 0.05; compared Normal control vs Hyperlipidemic control.
****P< 0.001, **P< 0.01 and *P< 0.05; compared Standard and Test groups vs Hyperlipidemic control.*
And 'a'- Indicates there is no significant difference between standard and test drug at P< 0.05 significant level.

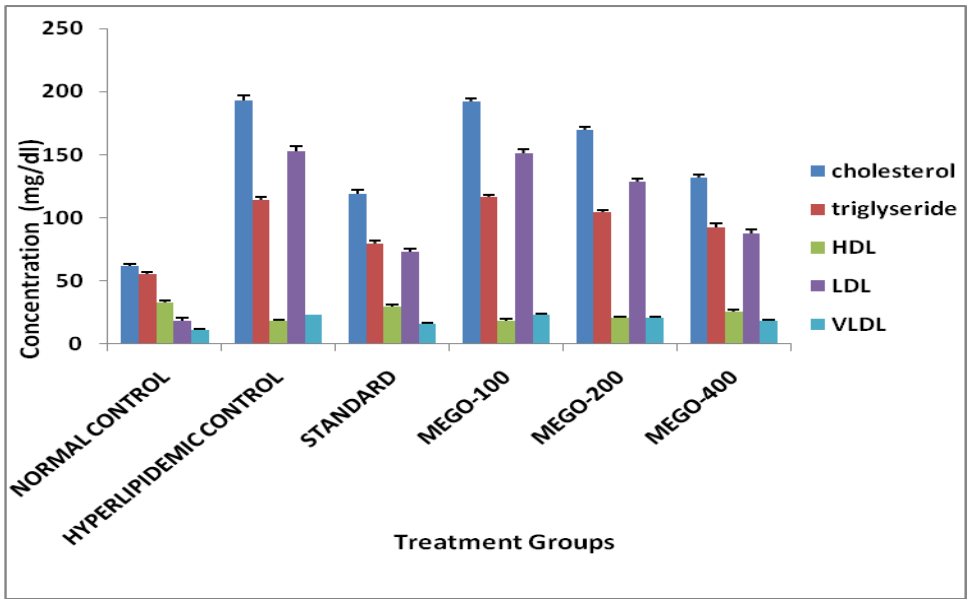

Figure 1: The effect of methanolic extract in triton-induced hyperlipidemic model

REFERENCES

1. American Diabetes Association. Diabetes Care, 2005; 28: 37-42.
2. Wild S, Roglic G, Green A, Sicree R, King H. Diabetes Care, 2004; 27: 1047-53.
3. Ansarullah, Jadeja RN, Thounaojam MC, Patel V, Devkar RV, Ramachandran AV. Int J Green Pharm, 2009; 3: 119-24.
4. Ghule BV, Ghante MH, Saoji AN, Yeole PG. Indian J Exp Biol, 2006; 44: 905-9.
5. Venkatesh S, Reddy GD, Reddy BM, Ramesh M, Apparao AV. Fitoterapia, 2003; 74: 274-9.
6. Diallo D, Hveem B, Mahmoud MA, Berge G, Paulsen BS, Maiga A. Pharm Biol, 1999; 37(1): 80-91.
7. Ghani A. Medicinal plants of Bangladesh. 2nd ed., Dhaka, Bangladesh; The Asiatic Society of Bangladesh: 2003.
8. Inngjerdingen KT, Debes SC, Inngjerdingen M, Hokputsa S, Harding SE, Rolstad B. J Ethnopharmacol, 2005; 101: 204-214.
9. Kokate CK, Purohit AP, Gokhale SB. Text book of pharmacognosy. 33rd ed., Pune; Nirali Prakashan: 2001.
10. Khandelwal, KR. Practical Pharmacognosy. 14th ed., Pune; Nirali Prakashan: 2005. 146-55.
11. Evans WC. Trease and Evans Pharmacognosy. 15th ed., Edinburgh London, New York, Philadelphia, St Louis, Sydney, Toronto; W.B. Saunders: 2002.103-104, 227, 247-250, 336, 471, 519-520, 545-7.
12. Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA). OECD Guidelines for the testing of chemicals, revised draft guidelines 423: Acute Oral toxicity- Acute toxic class method, revised document. India: Ministry of Social Justice and Empowerment; 2000.
13. Ghosh MN. Fundamentals of Experimental Pharmacology. 3rd ed., Kolkata; Hliton & Company: 2005; 190-7.
14. Jarald EE, Joshi SB, Jain DC. Ind J Pharmacol, 2008; 40 (6); 256-60.
15. Schurr PE, Schultz JR, Parkinson TM. Lipids, 1972; 7: 69-74.
16. Li YY, Jiang CJ, Wan XC, Zhang ZZ, Li DX. Acta Biochim Biophys Sin, 2005; 37: 364- 70.
17. Harbowy ME, Balentine DA. Crit Rev Plant Sci, 1997; 16: 415-80.
18. Friedwald WT, Levy RT, Fredrickson DS. Clin Chem, 1972; 18: 499-502.
19. Latha M, Pari L, Sitasawad S, Bhonde R. Life Sci, 2004; 75: 2003-14.
20. Andrew JK. Diabetes. New York; Churchill Living Stone: 2000.
21. Friedman M, Byers SO. Am J Physiol, 1957; 190: 439-45.