

HYPOGLYCEMIC AND ANTI-LIPIDEMIC EFFECTS OF HYDRO-ETHANOLIC EXTRACT OF CEIBA PENTANDRA Linn.

P. Parameshwar, D. Aruna Devi, B. Mahipal Reddy, K. Sathyanarayana Reddy,
E. Ramesh, G. Narender Naik and N. Sravankumar

Jyothismathi institute of Pharmaceutical Sciences,
Timmapur, Karimnagar, AP 505481, INDIA
Corresponding email: drarunadevi.tcps@gmail.com

[Received- 05/08/2011, Accepted-15/06/2012]

ABSTRACT

The hypoglycemic and anti-lipidemic effect of hydro-ethanolic extract of *Ceiba pentandra* Linn (Family-Sterculiaceae) root extracts (CPE) were investigated in normal and alloxan-induced diabetic rats. In the present study, the animals were divided into normal control, diabetic control, diabetic treated and control treated group (n = 6). Effect of oral administration of CPE (300 mg/kg) for 30 days on the level of blood glucose, glycosylated haemoglobin (HbA1C), total cholesterol (TC), triglycerides (TG), phospholipids, low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL), hexokinase, lactate dehydrogenase (LDH) and glucose-6-phosphatase in normal and alloxan-induced diabetic rats were evaluated. When comparing the values of the CPE treated group with those of the control diabetic group, we found that the CPE significantly decreased the elevated blood glucose level, glycosylated haemoglobin, cholesterol, triglycerides, phospholipids, LDL, VLDL. It showed a significant increase in liver glycogen, insulin and HDL level. Treatment with CPE in diabetic rats increased the hexokinase, LDH activity and decreased the glucose-6-phosphatase activity. These results clearly indicated that CPE possess promising anti-diabetic effect in diabetic rats.

Keywords: Antidiabetic, hypolipidemic, alloxan- induced diabetes, blood glucose, *Ceiba pentandra* Linn

INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism. It is a common endocrine disorder and characterized by hyperglycemia resulting from defects in

insulin secretion, insulin action or both¹. *Ceiba pentandra* Linn is a shrub of small tree generally found throughout the warmer parts of India. In animals, hyperglycemia can be induced by partial pancreatectomy or by the

administration diabetogenic drugs such as alloxan, streptozotocin, ditizona and anti-insulin serum. Alloxan causes massive destruction of the β -cells of islets of langerhans, not only destroys pancreatic β -cells but also damages the kidney. The disease is progressive and is associated with high risk of atherosclerosis, kidney and nerve damage as well as blindness. Abnormalities in the regulation of peroxide and transition metal metabolism are postulated to result in the development of the disease as well as its long-term complications.

The present study for the first time reports the antidiabetic activity of CPE of the roots of *C. pentandra* Linn. The results indicate that the plant extract was found to reduce the blood glucose level (BGL) in alloxan-induced diabetic rats. The antidiabetic effect of *C. pentandra* Linn. root extract could be linked to more than one mechanism. The possible mechanism includes the stimulation of β -cells and subsequent release of insulin and activation of the insulin receptors². The plants anti-hyperglycemic action may be by potentiation of pancreatic secretion of insulin, which was clearly evidenced by the increased level of insulin in diabetic rats treated with CPE also acts as a hepatoprotective agent, so this evidently improves the function of liver and maintains glucose uptake, enhanced transport of blood glucose to peripheral tissue and utilization, which may be another mechanism of action.

MATERIALS AND METHODS

Plant material

Fresh roots of *Ceiba pentandra* Linn. Abundant during rainy season was collected from Karimnagar district, Andhra Pradesh, India. They were carefully identified and authenticated by Dr. P. Odelu, Professor of Botany, Shatavahana University, Karimnagar, Andhra Pradesh, India.

Preparation of the plant extract

About 5 kg of roots of *Ceiba pentandra* Linn. were shade dried at room temperature and pulverized using a mixer grinder. About 1 kg of coarse powder was chopped in (1:1 v/v) ethanol and cold macerated for 3 days. During the maceration period occasional stirring was done. After 3 days the suspension was filtered through a fine muslin cloth. The residue was removed and the extract was concentrated on rotaevaporator under reduced pressure and then lyophilized. Finally a dark brown coloured crystal was obtained (yield: 7.8 % w/w, dry weight basis).

Animals

Male Wistar strains of rats, weighing about 150–200 g obtained from the National Institute of Nutrition, Tarnaka, Hyderabad and the animals were kept in the animal house of Jyothismathi institute of Pharmaceutical Sciences, Timmapur, Karimnagar, A.P. at room temperature of 25 - 30°C and at 45 - 55% relative humidity for 12 hr, each of dark and light cycle. The animals were feed with rat pellets (Hindustan Lever Limited, Bangalore, India) and filtered water. Animal studies in the work have been strictly performed as per the Institutional Animal Ethical Committee (IAEC) constituted under the guidelines of Committee for the Purpose of Control and Supervision on experimental Animal (CPCSEA), Ministry of Environment, and Govt. of India.

Induction of diabetes

Alloxan monohydrate (Sigma-Aldrich Co., USA) was used to induce diabetes mellitus in normoglycemic rats³. Animals were allowed to fast for 16 hrs and were injected intraperitoneally (i.p.) with freshly prepared alloxan monohydrate in sterile normal saline in a dose of 120 mg/kg body weight. Blood glucose level was measured after 72 hrs of alloxanisation by one-touch glucometer (Accu-chek sensor) of Roche Diagnostics,

Germany, and it was confirmed by testing for glucosuria using glucose indicator sticks. Rats showing fasting blood glucose levels (>250 mg/dl) were selected for the study.

Experimental design

The normoglycemic animals were divided into four groups of six animals in each group. The animals were fasted overnight before the experimental schedule began but allowed free access to water.

Group I – Normal control, normal healthy rats received water saline only.

Group II – Diabetic control the rats were made diabetic by an i.p. injection of single dose of alloxan monohydrate (120 mg/kg) in normal saline.

Group III – Diabetic rats treated with hydro-ethanolic extract of *C. pentandra* root (CPE) (300 mg/kg body weight) orally for 30 days.

Group IV – Control treated normoglycemic rats received only CPE (300 mg/ kg body weight, oral route) for 30 days. The dose of 300 mg/kg was selected for the study based on our preliminary screening tests.

Preparation of tissue homogenate⁴

The tissues of 100 mg were homogenized in 0.1 M cold tris-HCl buffer (pH 7.4) in a Potter-Elvehjam homogenizer fitted with a Teflon plunger at 600 rpm for 30 min. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C and the supernatant was used for enzyme assays. Lipids were extracted from liver and kidney tissues and used for assaying biochemical parameters.

Biochemical parameters⁵⁻⁸

Blood glucose was estimated by GOD-POD method using a commercial kit (Span diagnostics, India). Plasma insulin was assayed by AxSYM autoanalyser (Abbott Laboratory, Abbott Park, IL, USA), TC, TG and HDL were analyzed by kits (Roche diagnostics, GmbH, D-68298 Mannheim, Germany) on Hitachi auto analyzer. LDL, VLDL, liver glycogen, phospholipids,

HbA1C, hexokinase, LDH and glucose-6-phosphatase were evaluated.

Statistical analysis

All values are expressed as the Mean \pm SEM of 6 animals, statistical significance was estimated by analysis of variance (ANOVA). Dunnet's test was used for multiple comparisons, $p < 0.05$ implies significant

RESULTS

Effect on glucose, liver glycogen, plasma insulin and HbA1C^{9,10}

Treatment with CPE on blood glucose level (BGL), insulin, glycogen and HbA1C are depicted in Table 1. The rats exposed to alloxan developed diabetes as evident from the significant elevation in BGL as compared to normal control rats. A significant decrease ($p < 0.001$) in BGL was observed in diabetic rats treated with CPE. The extract failed to produce hypoglycemic activity in normal treated animals as it shows non significance. Liver glycogen level was significantly decreased in diabetic rats as compared to normal control rats. Administration of CPE significantly ($p < 0.001$) increased the liver glycogen level. The insulin levels in diabetic control rats were decreased significantly compared to those in normal rats. In diabetic treatment group insulin levels are significantly increased ($p < 0.001$). The normal rat treated with the CPE alone has no significant change. Alloxan-induced diabetic rats shows a significant ($p < 0.001$) increase in the levels of HbA1C compared to normal control rats indicating poor glycemic control. Treatment with 300 mg/kg body weight CPE decreased HbA1C significantly. However the normal control rats and control treated rats with CPE have no significant difference between them.

Effect on lipid profile^{11,12}

Comparison of results obtained from control rats and diabetic rats revealed that there was a significant increase of TC and TG levels in

serum, liver and kidney. Administration of CPE shows a significant decrease in TC and TG level in diabetic rats (Table 2 and 3). However the control rats treated with CPE shows a significant decrease ($p < 0.05$) in liver TC levels. A significant increase ($p < 0.001$) in LDL, VLDL levels were observed in the serum of alloxan-induced diabetic rats compared to control rats, whereas the HDL level was decreased significantly ($p < 0.01$). The treatment with CPE reduced LDL, VLDL and improved HDL significantly in diabetic treated rats (Table 4). In diabetes induced rats the serum phospholipid level was significantly increased ($p < 0.001$). Whereas, the phospholipids in liver and kidney were decreased significantly when compared to the normal control rats (Table 5). The control treated rats have no significant difference.

Hepatic hexokinase, LDH and glucose 6-phosphatase

The activities of carbohydrate enzymes are represented in Table 6. Activity of hexokinase and LDH in liver is markedly decreased while glucose-6-phosphatase activity increased significantly in diabetic control rats. Treatment with CPE in diabetic rats increased the hexokinase, LDH activity and decreased the glucose-6-phosphatase activity.

DISCUSSION

Liver is an insulin dependent tissue, which plays a vital role in glucose and lipid homeostasis and is severely affected during diabetes. Decreased glycolysis impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver. Increase in liver glycogen can be brought about by an increase in glycogenesis and/or decrease in glycogenolysis. So the CPE might have stimulated glycogenesis and/or inhibited glycogenolysis in the diabetic rat liver. HbA1C was found to increase in patients with

diabetes mellitus to about 16% and the amount of increase is directly proportional to the fasting BGL. The CPE reduces the elevated HbA1C in diabetic rats. Under normal circumstances, insulin activates enzyme lipoprotein lipase and hydrolysis triglycerides. In uncontrolled type-II diabetes mellitus, observed an increase in TC, TG, LDL and VLDL cholesterol with decrease in HDL cholesterol, which contribute to coronary artery disease. The abnormal high concentration of serum lipids in the diabetic subject is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. The marked hyperlipidaemia that characterize the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots.

Administration of CPE reduced TC, TG, LDL, VLDL and improved HDL level. Excess of fatty acids in plasma produced by the alloxan-induced diabetes promotes the liver conversion of some fatty acids into phospholipids and cholesterol. These two substances along with excess of TG formed in the liver may be discharged into the blood in the lipoproteins. As a result serum phospholipid is elevated, whereas the phospholipids in the liver and kidney were decreased. Treatment with CPE normalized the condition. The reduction in hepatic hexokinase and LDH are mainly due to leakage of these enzymes into the blood as a result of alloxan toxicity.

Higher activity of glucose-6-phosphatase provides H^+ which binds with $NADP^+$ to form NADPH which is helpful in the synthesis of fats from carbohydrates. When glycolysis slows down because of cellular activity, pentose phosphate pathway that is still active in liver provides NADPH, which converts acetyl radicals into long chain fatty acids

during diabetes mellitus. However treatment of alloxan-induced diabetic rats with CPE for 30 consecutive days could restore the normal metabolism by shifting the balance from lipid metabolism to carbohydrate metabolism. Thus, the results of the present investigation clearly indicate that the roots of *C. pentandra* Linn. possess possible usefulness in the treatment of diabetes mellitus.

REFERENCES:

1. Bhandarkar MR, Khan A. Anti hepatotoxic effect of *Nymphaea stellata* against carbon tetra chloride-induced hepatic damage in albino rats. *J. Ethnopharmacol.* 2004; 91: 61-64.
2. Babu V, Gangadevi T, Subramoniam A. Antidiabetic activity of ethanolic extract of *Cassia Kleinii* leaf in streptozotocin-induced diabetic rats and isolation of an active fraction and toxicity evaluation of the extract. *Indian J. Pharmacol.* 2003; 35: 290-296.
3. Bartosikova L, Necas J, Suchy V, Kubinova R, Vesela D, Benes L, et al. Monitoring of antioxidant effect of Morine in alloxan-induced diabetes mellitus in the laboratory rat. *Acta Veter. Brno.* 2003; 72: 191- 200.
4. Grover JK, Vats V, Rathi SS. Anti-hyperglycemic effect of *Eugenia jambolana* and *Tinospora cordifolia* in experimental diabetes and their effect on key metabolic enzymes involved in carbohydrate metabolism. *J. Ethnopharmacol.* 2000; 73: 461-470.
5. Baquer NZ. Glucose over utilization and under utilization in diabetes and effects of antidiabetic compounds. *Anal. Real. Acad. Farma.* 1998; 64: 147-180.
6. Prince PSM, Menon VP, Pari L. Hypoglycemic activity of *Syzigium cumini* seeds: effect on lipid peroxidation in alloxan diabetic rats. *J. Ethnopharmacol.* 1998; 61:1-7
7. Bopanna KN, Kannan J, Sushma G, Balaraman R, Rathod SP. Antidiabetic and anti hyperlipaemic effects of neem seed kernel powder on alloxan diabetic rabbits. *Indian J. Pharmacol.* 1997; 29: 162- 167.
8. Koenig R, Peterson CM, Jones RL, Sandek C, Lehrman M, Cerami A. Correlation of glucose regulation and hemoglobin A1c in diabetes mellitus. *N. Engl. J. Med.* 1976; 295: 417-420.
9. Koida H, Oda T. Pathological occurrence of glucose-6- phosphatase in liver disease. *Clin. Chem. Acta* 1959; 4: 554-561.
10. Brandstrup N, Kirk JE, Bruni C. Determination of hexokinase in tissues. *J. Gerontol.* 1957; 12: 166-171.
11. Olch J, Less M, Solane SGH. A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 1957; 26: 497- 509.
12. Arroll VV, Longly RW, Joseph HR. Determination of glycogen in liver and muscle by use of anthrone reagent. *J. Biol. Chem.* 1956; 220: 583- 593.

Figures and Tables:

Table 1. Effect of CPE on concentration of BGL, liver glycogen (LG), HbA1C and insulin in control and experimental rats.

Groups	Glucose (mg/dl)	Cholesterol		
		Serum (mg/dl)	Liver (mg/g), wet tissue	Kidney (mg/g), wet tissue
I	80.08 ± 0.35	167.85 ± 1.06	22.50 ± 0.28	18.40 ± 0.46
II	275.68 ± 0.35***	355.40 ± 1.62***	27.73 ± 0.61 ***	28.14 ± 0.67 ***
III	89.88 ± 0.44***	183.31 ± 1.72***	22.72 ± 0.46***	23.37 ± 1.65**
IV	79.65 ± 0.31	150.36 ± 9.39	20.45 ± 0.56 *	17.69 ± 0.39

Values are Mean ± SEM (n = 6).

Statistical Comparison: I vs. II; II vs. III; I vs. IV. ***P<0.001

Table 2. Concentration of Cholesterol level in serum, liver and kidney

Groups	Triglycerides		
	Serum (mg/dl)	Liver (mg/g), wet tissue	Kidney (mg/g), wet tissue
I	106.47 ± 0.88	30.84 ± 0.46	17.81 ± 0.37
II	180.68 ± 2.75***	43.36 ± 2.58***	39.31 ± 0.52***
III	164.14 ± 4.58**	37.39 ± 0.82*	34.52 ± 1.40**
IV	108.57 ± 0.86	32.69 ± 0.63	17.64 ± 0.55

Values are Mean ± SEM (n = 6).

Statistical Comparison: I vs. II; II vs. III; I vs. IV. ***P<0.001, ** P <0.01, * P <0.05.

Table 3: Effect of CPE on concentration of serum triglycerides

Groups	Triglycerides		
	Serum (mg/dl)	Liver (mg/g), wet tissue	Kidney (mg/g), wet tissue
I	41.49 ± 1.78	82.58 ± 0.91	13.93 ± 0.79
II	34.92 ± 0.79**	133.59 ± 1.53 ***	35.53 ± 1.15***
III	39.40 ± 0.20*	117.71 ± 1.51***	21.35 ± 0.62***
IV	41.50 ± 0.89	80.49 ± 0.77	14.33 ± 0.38

Values are Mean ± SEM (n = 6).

Statistical Comparison: I vs. II; II vs. III; I vs. IV; ***P<0.001, ** P <0.01, * P <0.05.

Table 4. Effect of CPE on concentration of serum HDL, LDL and VLDL.

Groups	Phospholipids		
	Serum (mg/dl)	Liver (mg/g), wet tissue	Kidney (mg/g), wet tissue
I	140.90 ± 2.43	46.09 ± 2.43	29.30 ± 0.96
II	249.16 ± 4.61***	31.52 ± 1.20***	20.44 ± 0.88***
III	168.15 ± 2.35***	39.90 ± 1.39**	25.34 ± 0.53**
IV	154.30 ± 2.85	41.57 ± 1.08	27.20 ± 0.91

Values are mean ± S.E.M (n = 6).

Statistical Comparison: I vs. II; II vs. III; I vs. IV; *** P<0.001, ** P <0.01, * P <0.05.

Table 5. Effect of oral administration of CPE on phospholipids.

Groups	Phospholipids		
	Serum (mg/dl)	Liver (mg/g), wet tissue	Kidney (mg/g), wet tissue
I	140.90 ± 2.43	46.09 ± 2.43	29.30 ± 0.96
II	249.16 ± 4.61***	31.52 ± 1.20***	20.44 ± 0.88***
III	168.15 ± 2.35***	39.90 ± 1.39**	25.34 ± 0.53**
IV	154.30 ± 2.85	41.57 ± 1.08	27.20 ± 0.91

Values are Mean ± SEM (n = 6).

HYPOGLYCEMIC AND ANTI-LIPIDEMIC EFFECTS OF HYDRO-ETHANOLIC EXTRACT

Statistical Comparison: I vs. II; II vs. III; I vs. IV; ****P*<0.001, ***P*<0.01.

Table 6: Activities of hexokinase, LDH and glucose-6-phosphatase in liver of normal and experimental animals

Groups	Hexokinase Unit ^a / g protein	LDH Unit ^b /g tissue	Glucose-6-phosphatase Unit ^c /mg protein
I	136.67 ± 4.28	2192 ± 152.45	0.172 ± 0.06
II	98.92 ± 1.89***	1532 ± 79.63***	0.236±0.038***
III	123.48 ± 2.78***	1840 ± 85.78***	0.193 ± 0.028***
IV	142.78 ± 3.21	2124 ± 146.78	0.168 ± 0.02

a, μ moles of glucose phosphorylated / min

b, IU/g: international unit, the amount of enzyme that catalyzes one mole of substrate/min/g tissue.

c, μ moles of Pi liberated / min

Values are Mean ± SEM (*n* = 6).

Statistical Comparison: I vs. II; II vs. III; I vs. IV; ****P*<0.001, ***P*<0.01

Fig. 1: concentration of BGL, LG, HbA1C and insulin

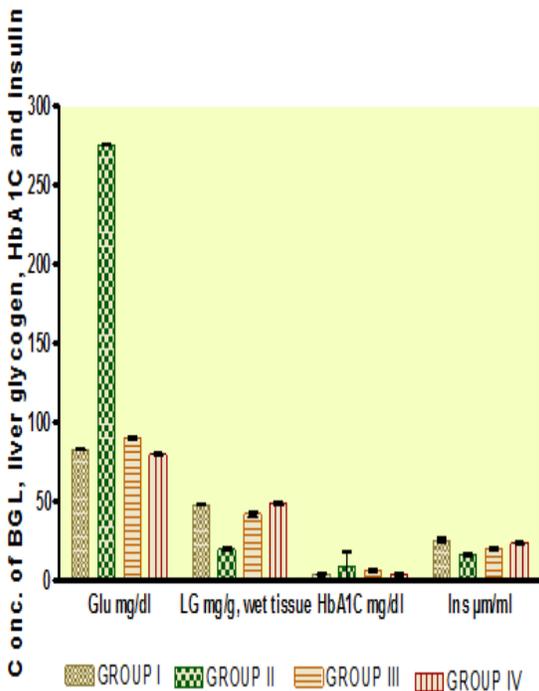


Fig. 2: Concentration of Cholesterol level in serum, liver and kidney

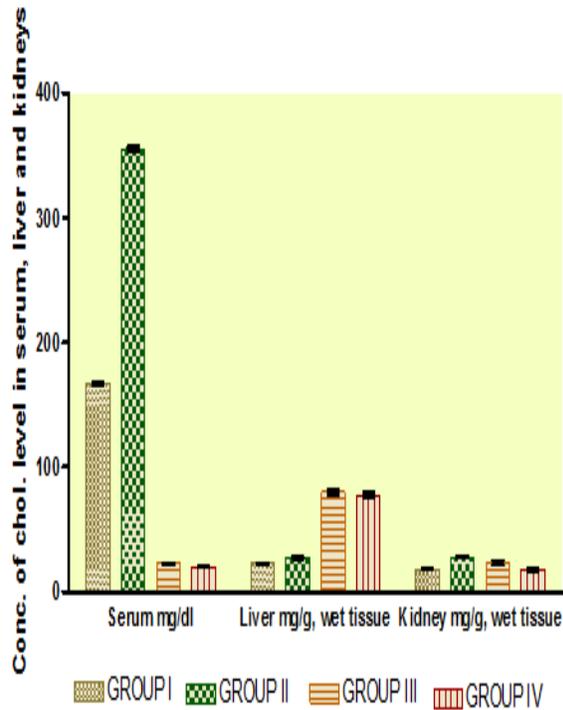


Fig. 3: Concentration of serum triglycerides

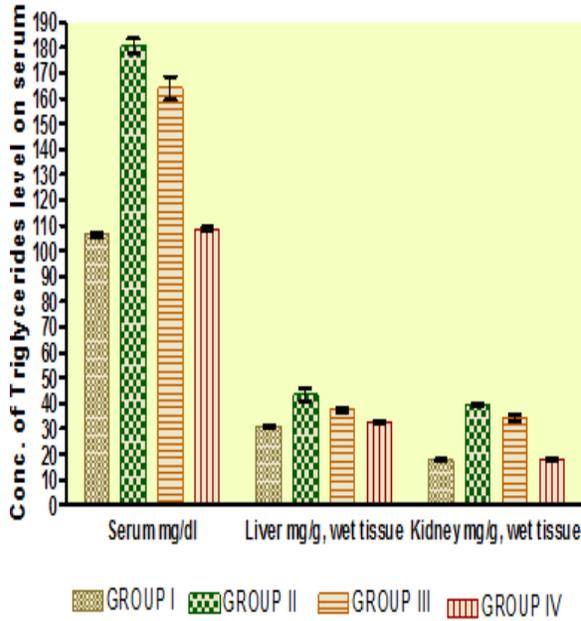


Fig. 4: concentration of serum HDL, LDL and VLDL.

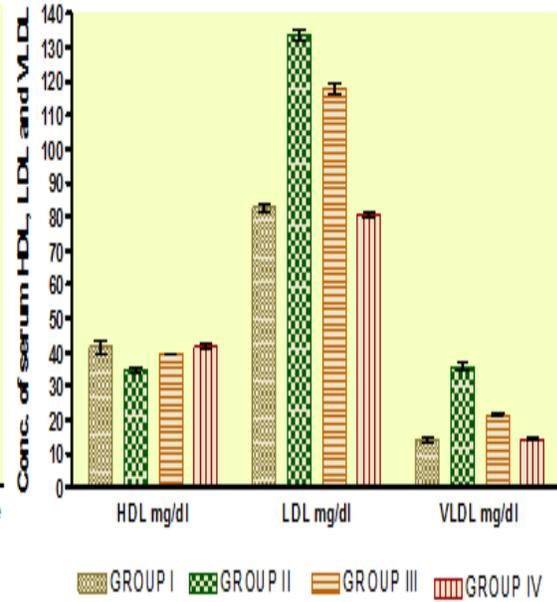


Fig. 5: Concentration of phospholipids in Serum, liver and kidney

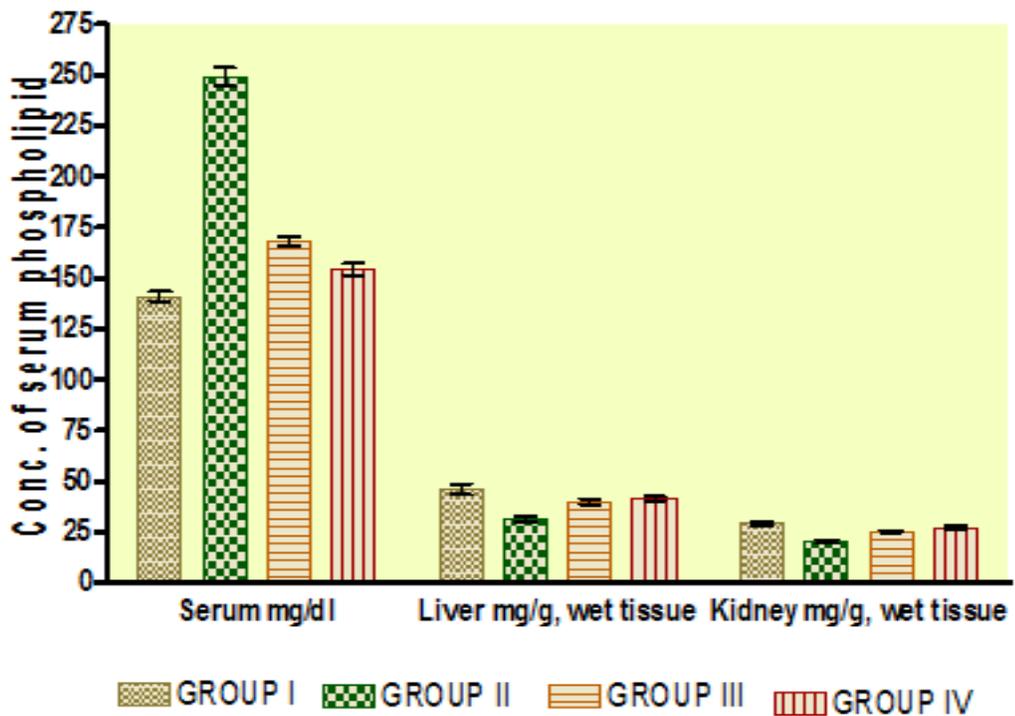


Fig. 6: Activities of hexokinase, LDH and glucose-6-phosphatase in liver

