

AMELIORATIVE POTENTIALS OF SAPONINS FROM *HELIANTHUS ANNUUS* ROOTS ON HEPATOPROTECTIVE AND SOME KIDNEY FUNCTION INDICES OF ALLOXAN-INDUCED DIABETIC RATS

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Abstract

The present study evaluated the ameliorative potentials of saponins from *Helianthus annuus* roots on hepatoprotective and some kidney function indices on alloxan-induced diabetes model of rats. Diabetes was induced in rats by a single intraperitoneal injection of alloxan (65 mg/kg body weight) and the animals were orally treated with 100, 200, 300 and 150 mg/kg body weight (bw) of saponins from *Helianthus annuus* roots once daily for three weeks. At the end of the intervention, diabetic untreated animals showed significantly higher alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), serum urea, direct bilirubin and creatinine and lower serum total protein, albumin compared to the normal animals. Histopathological examination of their pancreas revealed corresponding pathological changes in the islets and β -cells. These alterations were reverted to near-normal after the treatment of saponins from *Helianthus annuus* roots at 100, 200, 300 and 500 mg/kg bw when, the effects were more pronounced at 500 mg/kg bw compared to the 100 mg/kg bw treated group. It can be concluded that administration of saponins from *Helianthus annuus* root to diabetic rats did not have any adverse effect on the liver and kidney functions indices in rats, instead it ameliorates the adverse effects of diabetes complications.

Key words: *Helianthus annuus* roots, saponins, hepatoprotective, diabetic rats, kidney function

Introduction

Diabetes mellitus (DM) is a major global health problem and is recognized as one of the leading causes of death worldwide, where the high prevalence of the disease could be attributed to improved nutritional status (1, 2). DM is defined as a metabolic disorder characterized by an elevation of the blood glucose concentration due to absolute or relative lack of insulin leading to hyperglycemia. It is associated with abnormal metabolism of carbohydrates, fat and protein (3, 4, 5). In diabetes, the causes and sites of intervention in biochemical process are diverse and high serum total triglyceride level, high level of transaminase; creatinine and urea have been implicated (6).

Helianthus annuus L. is a folk remedy for bronchiectasis, bronchitis, carbuncles, catarrh, cold, colic, cough, diarrhoea, dysentery, dysuria, epistaxis, eyes, fever, flu, fractures, inflammations, laryngitis, lungs, malaria, menorrhagia, pleuritis, rheumatism, scorpion stings, snakebite, splenitis, urogenital ailments, whitlow, and wounds (7). Anti-hyperglycaemic effects of these plants are due to their capability to improve the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or the facilitation of metabolites in insulin dependent processes (8). *Helianthus annuus* leaves are extensively used for whooping cough, asthma, anti-malaria, insect bites, snake bites, fevers and lung problems. Ojo et al (9) reported the inhibitory ability of *H. annuus* leaf on enzymes linked to diabetes mellitus (α -amylase and α -glucosidase). Despite the wide usage of *Helianthus annuus* in the management of diabetes mellitus, there is no scientific report on the antidiabetic activity of saponins from *Helianthus annuus* roots on hepatoprotective and kidney function in alloxan-induced diabetic rats.

Methods

Chemicals

Alloxan was purchased from Sigma (Sigma-Aldrich, Germany), while thiobarbituric acid was purchased from Fluka (Buchs, Switzerland). Randox kits were purchased from Randox Laboratories Limited, UK. The other reagents used for the execution of the experiment were of analytical grade.

Plant Material

Roots of *Helianthus annuus* were collected at a farm in the suburbs of Ado Ekiti, Nigeria. The plant was identified and authenticated by a plant scientist in the Department of Plant Science, Ekiti State

University, Ado-Ekiti, Nigeria and a voucher specimen number was deposited accordingly at the herbarium of the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria.

Extraction of saponins from *H. annuus* root

A hundred gram (100 g) ground sample was extracted with 500 ml of petroleum ether (40–60 °C) in a soxhlet extractor for 12 h. The air-dried, defatted sample was extracted with methanol (500 ml) for 12 h. The methanol extract was partitioned with n-butanol and water (1:1, v/v). After a thorough shaking, the mixture was allowed to stand overnight and the n-butanol layer was separated in the next day. The aqueous layer was washed five times with aliquots of n-butanol until it became colorless. The pooled butanol layer was evaporated under reduce pressure to give a residue which was dissolved in 100 ml methanol and precipitated by adding a large amount of diethyl ether to obtain a solid crystalline dark brown compound (10).

Ethical considerations

All the experimental protocols will be conducted according to the guidelines for the care and use of experimental animals. A total of 30 Wistar rats of 150 ± 39 g will be procured from the Biochemistry Animal House at Ekiti State University, Ekiti State, Nigeria. All animals will receive humane care according to the principles of Laboratory Animal Care of the National Society of Medical Research and in accordance with the principles of laboratory animal care (NIH Publication; Guide for the care and use of laboratory animals, No. 85–23, revised 1985). The animals will be maintained according to the rules and regulations of the Ekiti State University, Animal Ethics Committee.

Animals

Thirty albino rats with an average weight of 150 ± 39 g were obtained from Animal Unit Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria. They were divided into six groups of five animals each and allowed to acclimatize to experimental condition for two weeks. They were housed in clean cages and maintained under standard laboratory conditions (temperature 25 ± 2 °C with dark/light cycle 12/12 h). They were fed ad libitum on rat pellets (Top Feeds, Nigeria) and water. The principles of Laboratory Animal Care (Public Health Services, 1986) were followed throughout the duration of the experiment.

Experimental protocol

Diabetes was induced through a single intraperitoneal injection of a freshly prepared alloxan (Sigma-Aldrich, Germany) solution in normal saline at a dose of 150 mg/kg body weight. Seventy-two hours later, the rats with moderate diabetes having glycosuria and hyperglycemia (i.e. with blood glucose levels of 200–300 mg/dl) were chosen for the experiments. The rats (n = 30) were divided equally into 6 groups. Group I served as normal control, and were given 2 ml saline by gavage, group II served as diabetic control, groups A–D were diabetic rats treated with saponin at doses of 100, 200, 300 and 500 mg/kg body weight respectively for 21 days. After 21 days of treatment, the rats were weighed and sacrificed by decapitation and the blood collected into clean dry beakers for serum preparation and the liver, kidney and pancreas was used for the determination of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP). The tissues (liver and kidney) were removed into 0.25 M ice cold sucrose solution in a ratio of 1:5 w/v.

Biochemical parameters

Alkaline phosphatase (ALP) (EC. 3.1.3.1) activity (in the serum, liver and kidney) was determined using Para- Nitrophenyl phosphate (PNPP) (11). Alanine aminotransferase (ALT) (EC. 2.6.1.2) and aspartate aminotransferase (AST) (EC. 2.6.1.1) (in the serum, liver and kidney) were assayed as described by (12). Serum albumin, bilirubin, globulin, protein, creatinine and urea were measured using an Automated Chemistry Analyzer (Labmax Pleno, Labtest Co. Ltd., Lagoa Santa, Brazil).

Statistical analysis

The data are expressed as mean \pm S.E.M. (standard error of mean). The differences among groups were analyzed by the one-way analysis of variance (ANOVA). Inter-group comparisons were done using Duncan's Multiple Range Test (DMRT) with 95% confidence intervals. The SPSS 20.0 (SPSS Inc., Chicago, USA), was used for this analysis. For fasting blood glucose, repeated measures ANOVA followed by Duncan Multiple Range Test was used. Statistical different is expressed at $p < 0.05$.

Results

Table 1 showed that the effect of the saponin extract from *Helianthus annuus* roots on the concentration of total protein, albumin and bilirubin in the serum of the experimental animals. The results showed that serum bilirubin of diabetic rats

increased when compared with normal rats and extract treated rats. However, treatment with saponin extract from *Helianthus annuus* roots lowers significantly the serum bilirubin concentration compared to the diabetic control ($p < 0.05$). Compared with the normal control group, the levels of serum albumin and total protein were significantly ($p < 0.05$) reduced in the untreated diabetic rats. However, the reductions were attenuated towards to control values in the saponin extract treated animals. The data for serum urea and creatinine are presented in Table 2. At the end of the experimental period, the concentrations of the above-mentioned serum parameters were increased in the diabetic control group compared to the normal control group. On the other hand, oral administration of saponin extract from *H. annuus* roots to diabetic animals significantly ($p < 0.05$) ameliorated these alterations in 100 mg/kg, 200 mg/kg, 300 mg/kg and 500 mg/kg groups respectively.

Table 3 showed that the effect of the saponin extract from *Helianthus annuus* roots on the alanine transaminase (ALT) in the liver, kidney and serum of the experimental animals. There was significant ($p < 0.05$) increase in the liver and serum of diabetic control group compared to the normal control group, while significantly ($p < 0.05$) reduced values in the kidney of diabetic control group compared to normal control group. Oral administration of saponin extract from *H. annuus* roots to diabetic animals significantly ($p < 0.05$) ameliorated these alterations in 100 mg/kg, 200 mg/kg, 300 mg/kg and 500 mg/kg groups respectively.

Table 4 showed that the effect of the saponin extract from *Helianthus annuus* roots on the aspartate transaminase (AST) in the liver, kidney and serum of the experimental animals. There was significant ($p < 0.05$) increase in the liver, kidney and serum of diabetic control group compared to the normal control group. Oral administration of saponin extract from *H. annuus* roots to diabetic animals significantly ($p < 0.05$) reduced these alterations in doses administered.

Table 5 showed that the effect of the saponin extract from *Helianthus annuus* roots on the alkaline transaminase (AST) in the liver, kidney, pancreas and serum of the experimental animals. There was significant ($p < 0.05$) increase in the liver, kidney, pancreas and serum of diabetic control group compared to the normal control group. Oral administration of saponin extract from *H. annuus* roots to diabetic animals significantly ($p < 0.05$) reduced these alterations in doses administered.

Discussion

Plants have always been an exemplary source of drugs and mainly currently available drugs have been directly or indirectly obtained from botanicals (13). In the present study, saponin extract from *H. annuus* roots considerably lowers the total and direct bilirubin levels in alloxan-induced diabetic rats in addition to attenuated the reduced values of total protein and serum albumin in diabetic rats. However, these attenuations further indicate that saponin extract from *H. annuus* root might aid in the recovery of animals from some metabolic disorders associated with diabetes mellitus.

Alloxan a toxic glucose analog widely used to induce experimental diabetes in animals. It selectively destroys insulin-producing cells in the pancreas in animals, and has been shown to establish a redox cycle with the formation of superoxide radicals and undergoes dismutation to hydrogen peroxide with the formation of hydroxyl radicals (14, 15, 16). Hyperuremia and hypercreatininemia have been reported to occur in alloxanized diabetic rats (17), and such elevated levels of biomolecules (Table 2) in the present study did not only suggest disturbance in the metabolism of these substances, but also agrees with previously published reports. The levels of the molecules in the serum of the animals were normalized by the saponin extract from *H. annuus* roots to the control group.

Enzyme activities in the tissues are usually used as a 'marker' to ascertain early toxic effects of administered foreign compounds to experimental animals (18, 19, 20, 21, 22). ALP is a membrane bound enzyme while ALT and AST are cytosolic enzymes. These enzymes are highly concentrated in the liver and kidney and are found only in the serum in significant quantities when the cell membrane becomes leaky and completely ruptured (20). (21) has documented that rise in serum level with corresponding decrease in tissue level of these intracellular enzymes is an index of damage to liver and kidney cells. In this present study, increase in serum enzymes activities without concomitant alteration in the tissue levels of the enzymes may implied that the serum elevation in the diabetic groups might be from single intraperitoneal administration of alloxan at a dose of 65 mg/kg body weight has been reported to cause oxidative damage to pancreatic beta cells in rats (23), which was gradually reversed in saponin extract from *H. annuus* roots administration (Table 3 and Table 4). Furthermore, in comparison with ALT, AST is less specific for the detection of hepatic injury (24). This is due to the fact that; the half-life of ALT is much

longer compared to AST in experimental animals. Therefore, in a condition of acute liver injury, there will be an increase in both serum ALT and AST levels. However, the serum AST level may likely return to normal more rapidly compared to the level of serum ALT, which make ALT more sensitive in assessing hepatic damage in disease conditions (25, 26, 27). Hence, the hepatoprotective ability of saponin extract from *H. annuus* roots is supported by the reduced serum ALT, ALP and urea levels in the diabetes treated groups of 100 mg/kg, 200 mg/kg, 300 mg/kg and 500 mg/kg respectively compared to the diabetic control group (Table 4 and Table 5). The excellent performance of saponin extract from *H. annuus* roots in reversing the negative effects of alloxan on diabetic rats may due to the present of the antioxidant effect of saponin. Furthermore, saponin extract from *H. annuus* roots may have exert its action through insulinomimetic.

Conclusion

In conclusion, various doses of saponin extract from *H. annuus* roots possess strong antidiabetic activity via improving a number of organ specific diabetic-complications related parameters in diabetic rats which might be due to the antioxidant effect of the saponin in *H. annuus* roots. Hence, saponin extract from *H. annuus* roots may be a potential anti-diabetic natural product with no considerable side effects.

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References

1. Uebanso T, Arai H, Taketani Y, Fukaya M, Yamamoto H, Mizuno A, Uryu K, Hada T, Takeda E. Extracts of *Momordica charantia* suppress postprandial hyperglycemia in rats. *Journal of Nutritional Science and Vitaminology* 2007; 53: 482–488.
2. Ogbonnia S, Adekunle AA, Bosa MK, Enwuru VN. Evaluation of acute and subacute toxicity of *Alstonia congensis* Engler (Apocynaceae) bark and *Xylopiya aethiopicum* (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. *African Journal of Biotechnology* 2008; 7: 701–705.
3. Shah JG, Patel MS, Patel KV, Gandhi TR. Evaluation of anti-diabetic and antioxidant activity of *Centrathrum anthelmintica* in STZ-induced diabetes in rats. *The International Internet Journal of Pharmacology* 2008; 6: 1–10.
4. Sharma VK, Kumar S, Patel HJ, Hugar S. Hypoglycemic

- activity of *Ficus glomerata* in alloxan induced diabetic rat. *International Journal of Pharmaceutical Sciences Review and Research* 2010; 1:18–22.
5. Dinesh KB, Analava M, Manjunatha M. Azadirachtolide: an anti-diabetic and hypolipidemic effects from *Azadirachta indica* leaves. *Pharmacognosy Communication* 2011; 1: 78–84.
 6. Anaja HP. Relation of plasma fructosamine in lipid profiles in diabetic patients. *International Diabetes Digest* 1995; 6: 64–66.
 7. Ngondi JL, Fossouo Z, Djioetsa EJ, Oben J. Glycaemic variations after administration of *Irvingia gabonensis* seeds fractions in normoglycaemic rats. *African Journal of Traditional, Complementary and Traditional Medicine* 2006; 4: 94–101.
 8. Saini S, Sharma S. Antidiabetic Effect of *Helianthus Annuus* L., Seeds ethanolic extract in Streptozotocin- Nicotinamide Induced Type 2 Diabetes Mellitus. *Int J Pharm Pharma Sci* 2013;5: 2 382-387
 9. Ojo OA, Ojo AB, Ajiboye BO, Olayide I, Fadaka AO. *Helianthus annuus* Leaf Ameliorates Postprandial Hyperglycaemia by Inhibiting Carbohydrate Hydrolyzing Enzymes Associated with Type-2 Diabetes. *Iranian Journal of Toxicology* 2016; 10 (5): 17-22.
 10. Adanlawo IG, Akanji MA. Effect of chronic administration of saponin extract from the fruits of *Solanum anguivi* Lam. on alkaline phosphatase activities of some rat tissues. *Nigerian Journal of Biochemistry and Molecular Biology* 2003; 18: 59–62.
 11. Wright PJ, Leathwood PD, Plummer DT. Enzymes in rat urine. phosphatase. *Enzymologia* 1972; 42: 317-327.
 12. Reitman S, Frankel S. A colourimetric method for determination of serum glutamate-oxaloacetate and pyruvate transaminase. *American Journal Clinpathology* 1957; 28: 56-59.
 13. Ojo OA, Oloyede OI, Olarewaju OI, Ojo AB, Ajiboye BO, Onikanni SA. Toxicity studies of the crude aqueous leaves extracts of *Ocimum gratissimum* in albino Rats. *IOSR Journal of Environmental Science, Toxicology and Food Technology* 2013; 6: 434-439.
 14. Szkudelski T. The mechanism of alloxan and streptozotocin action in β cells of the rat pancreas. *Physiology Research* 2001; 50: 536–546.
 15. Vanhorebeek I, De Vos R, Mesotten D, Wouters PJ, De Wolf-Peeters C, Van den Berghe G. Protection of hepatocyte mitochondrial ultrastructure and function by strict blood glucose control with insulin in critically ill patients. *Lancet* 2005; 365: 53–59.
 15. Vanhorebeek I, Ellger B, De Vos R, Boussemaere M, Debaveye Y, Perre SV, Rabbani N, Thornalley PJ, Van den Berghe G. Tissue-specific glucose toxicity induces mitochondrial damage in a burn injury model of critical illness. *Critical Care Medicine*. 2009; 37: 1355–1364.
 16. Sokeng DS, Rokeya B, Mostafa M, Nahar N, Mosihuzzaman N, Ali L, Kamtchouing P. Antihyperglycemic effect of *Bridelia ndellensis* ethanol extract and fractions in streptozotocin-induced diabetic rats. *Afr J Trad CAM* 2005; 2: 94-102.
 17. Akanji MA, Ngaha EO. Effect of repeated administration of Berenil on urinary enzyme excretion with corresponding tissue pattern in rats. *Pharmacology Toxicology* 1989; 64: 272-279.
 18. Adesokan AA, Akanji MA. Effect of administration of aqueous extract of *Enantia chlorantha* on the activities of some enzymes in the small intestine of rats. *Nig. J. Biochem Mol Biol* 2004; 18: 103-105.
 19. Adesokan AA1, Oyewole OI, Turay BMS. Kidney and Liver Function Parameters in Alloxan-Induced Diabetic Rats Treated with Aloe Barbadensis Juice Extract. *Sierra Leone Journal of Biomedical Research* 2009; 1 (1): 33-37.
 20. Ojo OA, Oloyede OI, Olarewaju OI, Ajiboye BO. Evaluation of transaminase activity of aqueous extract of *Ocimum gratissimum* in the liver and kidney of albino rats. *International Journal of Biological and Medical Research* 2013; 4(4): 3650-3653.
 21. Ojo OA, Oyinloye BE, Ajiboye BO, Ojo AB, Habiba M, Olarewaju OI. Dichlorvos induced oxidative stress in rat brain, Protective effects of the ethanolic extract of *Alstonia boonei* stem bark. *Asian J Pharm* 2014; 8(4): 216-221,
 22. Weaver DC, McDaniel M, Lacy PE. Alloxan uptake by isolated rat islets of Langerhans. *Endocrinology* 1978; 102: 1847-1855.
 23. Center SA. Interpretation of liver enzymes. *Vet. Clin. North Am. Small An. Pract* 2007; 37: 297-333.
 24. Ramaiah SK. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. *Food Chemistry Toxicology* 2007; 45: 1551-1557.
 25. Meyer D, Harvey JW. Hepatobiliary and skeletal muscle enzymes and liver function tests. In: Meyer, D., Harvey, J.W. (Eds.), *Veterinary Laboratory Medicine: Interpretation and Diagnosis*. WB. Saunders Co, St. Louis, MO, 2004; 169-192.
 26. Ojo OA, Ajiboye BO, Oyinloye BE, Ojo AB, Olarewaju OI. Protective effect of *Irvingia gabonensis* stem bark extract on cadmium induced nephrotoxicity in rats. *Interdiscip Toxicol* 2014; 7(4): 208-214.

Table 1. Some Liver function indices of rats administered with Saponin extract from *Helianthus annuus* Roots in alloxanized diabetic rats

Parameter	Control	Diabetes untreated	Group A (100mg/kg)	Group B (200mg/kg)	Group C (300 mg/kg)	Group D (500mg/kg)
Total bilirubin (mg/dl)	0.91±0.30 ^a	7.18±0.84 ^c	1.32±0.13 ^{ab}	1.64±0.35 ^{ab}	1.87±0.15 ^{ab}	2.30±0.17 ^b
Direct bilirubin (mg/dl)	1.62±0.17 ^a	7.04±0.75 ^c	1.93±0.07 ^{ab}	2.92±0.25 ^b	2.52±0.33 ^{ab}	2.70±0.11 ^{ab}
Albumin (g/l)	13.71±0.83 ^e	2.80±0.60 ^a	15.17±0.15 ^d	11.65±0.75 ^c	11.47±0.86 ^c	7.34±0.38 ^b
Total protein (g/l)	16.39±0.63 ^c	3.53±0.10 ^a	16.25±0.15 ^c	12.71±0.21 ^b	11.67±0.34 ^d	10.51±0.01 ^e

*Values are expressed as mean of five determinations ± SEM

*Row values with different superscripts are significantly (p<0.05) different

Table 2: Some Kidney function parameters of rats administered with Saponin extract from *Helianthus annuus* Roots in alloxanized diabetic rats

Parameter	Control	Diabetes untreated	Group A (100mg/kg)	Group B (200mg/kg)	Group C (300 mg/kg)	Group D (500mg/kg)
Urea (mg/dl)	3.69±0.33 ^a	19.67±2.23 ^c	3.74±0.47 ^a	5.31±0.80 ^b	5.39±0.92 ^b	9.27±0.12 ^d
Creatinine (mg/dl)	0.30±0.07 ^a	2.07±0.21 ^d	0.36±0.06 ^{ab}	0.40±0.06 ^{ab}	0.80±0.18 ^b	1.65±0.19 ^c

*Values are expressed as mean of five determinations ± SEM

*Row values with different superscripts are significantly (p<0.05) different

Table 3: ALT Activity (U/L) of Liver, Kidney and Serum of Rats administered with Saponin extract from *Helianthus annuus* Roots in alloxanized diabetic rat

Tissues	Control	Diabetes untreated	Group A (100mg/kg)	Group B (200mg/kg)	Group C (300mg/kg)	Group D (500mg/kg)
Liver	2.69±0.94 ^a	11.67±3.04 ^b	3.69±0.43 ^a	4.12±1.12 ^c	4.42±1.03 ^c	6.23±0.43 ^d
Kidney	6.36±0.65 ^b	2.73±0.21 ^a	5.18±1.29 ^b	5.89±1.09 ^b	5.59±0.61 ^b	7.51±0.29 ^c
Serum	2.24±0.60 ^a	3.62±0.16 ^b	1.40±0.16 ^a	1.40±0.16 ^a	1.40±0.16 ^a	2.26±0.12 ^a

*Values are expressed as mean of five determinations ± SEM

*Row values with different superscripts are significantly (p<0.05) different

Table 4: AST Activity (U/L) of Liver, Kidney and Serum of Rats administered with Saponin extract from *Helianthus annuus* Roots in alloxanized diabetic rat

Tissues	Control	Diabetes untreated	Group A (100mg/kg)	Group B (200mg/kg)	Group C (300mg/kg)	Group D (500mg/kg)
Liver	5.67±1.78 ^a	21.02±2.48 ^b	5.58±1.43 ^a	5.65±1.15 ^a	5.88±0.73 ^a	8.09±1.58 ^c
Kidney	4.59±1.15 ^a	10.09±0.64 ^b	2.59±0.50 ^a	4.13±0.73 ^a	3.45±0.96 ^a	4.49±1.07 ^a
Serum	3.96±0.45 ^b	6.92±0.64 ^c	2.06±0.25 ^a	2.27±0.31 ^a	2.57±0.22 ^a	3.99±0.21 ^b

*Values are expressed as mean of five determinations ± SEM

*Row values with different superscripts are significantly (p<0.05) different

Table 5: ALP Activity (U/L) of Liver, Kidney Pancreas and Serum of Rats administered with Saponin extract from *Helianthus annuus* Roots in alloxanized diabetic rat

Tissues	Control	Diabetes untreated	Group A (100mg/kg)	Group B (200mg/kg)	Group C (300mg/kg)	Group D (500mg/kg)
Liver	3.81±0.41 ^a	12.44±0.98 ^b	3.52±0.55 ^a	3.94±1.24 ^a	4.02±0.85 ^a	5.31±1.07 ^c
Kidney	4.60±0.95 ^a	16.27±1.88 ^b	3.94±0.66 ^a	4.52±0.91 ^a	4.32±0.62 ^a	3.91±0.55 ^a
Pancreas	2.79±0.45 ^a	16.57±2.08 ^b	3.19±0.45 ^a	4.30±1.20 ^a	5.03±1.02 ^c	6.27±0.92 ^c
Serum	2.19±0.30 ^a	7.39±0.60 ^c	1.93±0.26 ^a	2.43±0.44 ^a	1.93±0.26 ^a	4.29±0.46 ^b

*Values are expressed as mean of five determinations ± SEM

*Row values with different superscripts are significantly (p<0.05) different