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In-vivo evaluation of Antihyperglycemic, Antidyslipidemic and Antioxidant activities of hydro-ethanolic *Pithecellobium dulce* (Roxb.) Benth. *pod extract* in alloxan induced diabetic swiss albino male mice

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ABSTRACT

As medicinal plants are suitable alternatives for synthetic and chemical drugs hence this study was performed to evaluate the role of hydro-ethanolic pod extract of Pithecellobium dulce (Roxb.) Benth,in alloxan induced diabetic male mice by measuring glucose levels, lipid profile and antioxidants activity in a period of 45 days treatment. Diabetes was induced in mice by using single intraperitonial injection of alloxan monohydrate (150mg/kg body weight). All mice of one group were orally administered by a single dose of 300mg/kg BW/day of PDPE extract whereas mice of another diabetic group were treated with glibenclamide (10mg/kg of bw.) for 45 days. After this period of treatment serum was used to analyze fasting blood glucose levels, and lipid profile and various homogenates viz. liver, kidney, pancreas were used for the estimation of hepatic glycogen content and enzymatic and non-enzymatic antioxidants such as Superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and Thiobarbituric acid reactive substances (TBARS). The effects of PDPE treatment were compared with that of reference antidiabetic drug Glibenclamide along with diabetic and normal control (DC & NC) group. 45 days treatment of PDPE significantly ($P<0.05$) lowered FBG levels in diabetic mice whereas, hepatic glycogen content was elevated with normalized lipid profile. Moreover, the activity of enzymatic and non- enzymatic antioxidants was normalized to such an extent that helped in reduction of oxidative damage in the tissues of diabetic animals. The findings of the present study indicated significant anti-hyperglycemic, antidyslipidemic and anti-oxidant activity in P.dulce pods.

Key words: Antidiabetic, Antioxidantive, Antidyslipidemic, Oxidative stress, lipid profile, Diabetes Mellitus, *Pithecellobium dulce (Roxb.) Benth.*

INTRODUCTION

Diabetes Mellitus (DM) is a heterogenous chronic disorder considered inadequate insulin secretion, action or both which causes hyperglycemia by altering carbohydrate, lipid and proteins from the body's metabolism (1). Beside this, Beta cells destruction in the diabetes is highly sensitive to Reactive Oxygen Species (ROS), which are continuously produced as a result of oxygen

metabolism during mitochondrial oxidative phosphorylation cause oxidative stress (2). Further, Elevated level of this oxidative stress plays a major action in the development of micro-vascular and cardiovascular complications of diabetes (3). Antioxidants are known to have important role in diabetes as they protect beta cells from oxidation by inhibiting the peroxidation chain reaction
Lipoprotein abnormalities on the other hand are common in DM and contribute significantly to its complications such as atherosclerosis. Moreover, these abnormalities are also related with age, obesity, ethanol, antihypertensive drugs, diet etc (4).
Several synthetic drugs/ allopathic medicines are used for the treatment of DM. These drugs have limitations due to undesirable adverse effects such as hypoglycemia, weight gain, inability to arrest pancreas degeneration etc (5). Several herbal medicines were previously reported to possess beneficial implications for the management of diabetes and its complications which can also be used to manage diabetes induced oxidative stress (6).
The present study is focused to evaluate the antihyperglycemic, antidyslipidemic and antioxidative properties of *Pithecellobium dulce* (Roxb.) Benth, (jungle jalebi) which belongs to the family Fabaceae. It is an evergreen, small to medium sized plant found in the regions of tropical America and cultivated throughout the plains of india and also in andamans (7). Few parts of this plant have been reported to possess astringent, emollient, antidiabetic and abortificient properties. According to ethno-botanical data, *P. dulce* possesses an antidiabetic effect, which has never been experimentally demonstrated (8).

MATERIALS AND METHODS

Chemicals:

Alloxan monohydrate was purchased from SDFine chemical (Mumbai, India). Other chemicals used for this study were of analytical grade and obtained from HIMEDIA (India), SRL (India), CDH (India), Qualigens (India/ Germany) kits used for the estimation of lipid parameters were purchased from Erba Mannheim (Transasia Biomedicals Ltd, Daman, India).

Preparation of plant extract:

Taxonomically identified plant *P. dulce* Pods were collected from the Khejari nursery, Jaipur, Rajasthan, India. Shade dried pods were subjected to size reduction to a course powder which was then soxhlet extracted with 50% hydroethanol. This extract was then concentrated to dryness under reduced pressure at 60±1ºC in a rotator vaccum evaporator. The extract was then dried at 40-45ºC in hot air oven till solid to semisolid mass was obtained. During experimental period, the suspension of pods extract was prepared in 20% tween 20 in normal saline.

Animal Care and monitoring:

The healthy Swiss albino male mice (*Mus Musculus*) of 4-6 months old and of 25-35gms in weight were procured from the C.C.S. Haryana Agricultural University, Hissar, India. All the animals were housed under standard laboratory environment 12:12 h L: D cycle light, 23±2ºC temperature and 55±5% relative humidity. For feeding standard rat pellet diet and tap water *ad libitum* were provide to the experimental animals. All these animals were maintained and treated as per the directions of Institutional Animal Ethical Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Introduction of diabetes and treatment with drugs:

In this study, all the animals were divided in four groups Normal Control (NC), Diabetic Control (DC), Glibenclamide Treated (GT) and *P.dulce* pod extract treated (PDPE). After 18 hours fasting period, the mice from all the groups were made diabetic by single intra-peritoneal injection of

alloxan monohydrate. For single mice the alloxan is used 150mg/kg body weight which is freshly dissolved in normal saline (9). Subsequent of alloxan administration, the food and water were freely accessible to the treated animals and to control the drug induced hypoglecemic shock they were provided with 5% glucose solution to drink overnight. After one week of alloxan injection, the Fasting Blood Glucose (FBG) levels were determined by means of one touch ultra glucometer (Dr. Morpen's Glucometer) and with compatible blood glucose strips (10). Animals with 140mg/dl or above FBG levels were considered as diabetic (11, 12) and were selected for treatment with drug (10mg/kg body weight) or pod extract (300mg/kg body weight). The drug and the pod extract were administered orally, once in a day for 45 days.

Estimation of FBG levels:

FBG levels of all the four experimental groups were noted by glucometer (Dr. Morpen One Touch Glucometer) at th time interval (Before inducing, zero day and 45^{th} day) of the experiment by collecting a drop of blood from the tail vein of each animal. FBG levels were expressed in mg/dl.

Estimation of lipid parameters:

Serum from all the four groups were isolated from the blood collected from overnight fasting experimental mice through the retrorbital plexus on day 45^{th}. Isolated serum was employed for the estimation of lipid profile, total cholesterol (TC), triglyceride (TG), and HDL-cholesterol using diagnostic kits (Erba Mannheim cholesterol kit, Transasia Bio- Medicals Ltd., Daman). VLDL and LDL- cholesterol were calculated as per Friedevald's equation. VLDL-cholesterol = serum triglyceride/5; LDL-cholesterol = serum total cholesterol – VLDL-cholesterol – HDL cholesterol. Results were expressed in mg/dl.

Preparation of tissue homogenate and estimation of antioxidants:

After 45 days of experimentation, the animals were sacrificed by cervical dislocation and were dissected under aseptic conditions. Then, Liver, Pancreas and Kidney were removed from each animal, freed from adhering tissues, washed with ice-cold normal saline solution (0.9%) and blotted dry and weighted. Thereafter, each tissue was seperately homogenized in ten times of its volume of 0.2M tris HCl with the help of homogenizer. The homogenate was filtered through cheese cloth to remove any lumps that may be present. The filtrate was centrifuged at 10,000 r.p.m. for 20 minutes at 4°C. The supernatant so obtained was used for estimation of Hepatic glycogen content which was measured by anthrone-H_2SO_4 method, with glucose as standard (13) and enzymatic and non-enzymatic antioxidants viz., Superoxide dismutase (SOD) (14), Catalase (CAT) (15), glutathione peroxidise (GPx) (16), Reduced glutathione (GSH) (17) and thiobarbituric acid reactive substances (TBARS) (18).

Statistical Analysis :

Results are expressed as mean ± Standard Error of Mean (SEM). Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test using SPSS (version 16.0) and student's't'-test using Sigma Plot (version 8.0). The values of $P<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Effect on Fasting Blood Glucose Levels:

Alloxan reduces the production of insulin by β cells of islets of langerhans which thus, leading to the insulin deficiency and finally hyperglycemia, a major class of diabetes complications (19). Glycogenolysis and/or gluconeogenesis are the states of increased blood glucose level in diabetic

mice as compared to normal control (20). Alloxan injection to all the three groups (DC, SD and PDPE) resulted in significant (P<0.05) increment in the blood glucose levels as compared to normal control group (NC). 45 days treatment of PDPE significantly reduced the level of glucose by 38.06% (i.e. from 208.28 ± 15.3 mg/dl to 129 ± 4.3mg/dl) to normalization. Reduced levels of FBG by treatment of PDPE can be compared by lowering effect of glibenclamide (GT). After 45 days of dosage GT produced 53.54% reduction (from 206 ± 35.37mg/dl to 95.7 ± 18.1mg/dl) in FBG levels (Table 1). The present study revealed that the treatment of PDPE significantly decreased the level of FBG in diabetic mice. By reducing the increased level of blood glucose this extract can correspond to the presence of some compounds indicating antidiabetic potential of it. In alloxan induced diabetic mice, insulin was secreted but not satisfactorily to manage blood glucose level, thus leading to the significant increment of FBG levels in alloxan induced diabetic mice. However, 45 days treatment of diabetic mice with PDPE (300mg/kg body wt) caused a significant decline in FBG levels, which might be due to increased consumption of peripheral glucose or potentiating of the insulin effects.

Table 1: FBG levels after 45 days treatment:

Days	NC	DC	ST	PDPE
Before Induction	106.14±13.18	102±3.36	99±17.54	102.7±3.8
After Induction	106.14±13.18 b	185.57±13.91 a	206±35.37 a	208.28±2.7 a
45	103.71±9.8 a	163.71±16.96 a	95.7± 18.1 b c	129±9.54 a c

Values are mean±SEM of 7 observations, Units for FBG mg/dl

*Before induction (Basal values); Student's't' –test is significant at P<0.05. a: significant (P<0.05) difference, b: insignificant difference (P>0.05) compared to basal values; c: significant (P<0.05) difference compared to values obtained after alloxan injection

Effect on Hepatic glycogen content:

Hepatic glycogen content decreased significantly (P>0.05) in diabetic control group (DC) by 57.94% as compared to normal control group (NC). 45 days treatment of diabetic mice with PDPE significantly (P<0.05) increased the depletion of glycogen content by 79.18% as compared to diabetic control, which can also be compared to glibenclamide treated group (GT, 80.23% increase) (Table 2). It has been reported that normalization of reduced hepatic glycogen content was subsequent to insulin treatment in diabetic mice (21). Glycogen is primarily an intracellular storable form of glucose in various tissues and directly reflects insulin action, as insulin promotes its deposition by exciting glycogen synthetase and inhibiting glycogen phosphorylase. In diabetic mice the observed depletion of liver glycogen level was reliable with earlier results and representing that it was probably be due to the loss of glycogen synthetase activating system and improved activity of glycogen phosphorylase (22, 23). Similarly, administration of PDPE significantly increases the hepatic glycogen content in 45 days treated diabetic mice and thus confirming its insulin potentiating effects.

Table 2: Hepatic glycogen content of 45 days treated animals:

Parameters	NC	DC	ST	PDPE
Hepatic Glycogen Content (mg/gm tissue)	45.89±12.14	19.30±9.8 a	97.62±13.4b c	92.7±6.8 b

Values are mean±SEM of 7 observations
Student's't' –test is significant at P<0.05. a: significant (P<0.05)difference, a*: insignificant difference (P>0.05) compared to NC, b: significant difference (P<0.05) to DC, b*: insignificant difference (P>0.05) to DC, c: significant difference (P<0.05) to ST, c*: insignificant difference (P>0.05) to ST.

Effects on Serum Lipid Profile:
Moreover, altered carbohydrate metabolism, diabetes also escort metabolic disorders of fat and lipid (24). Dyslipidemia in diabetes is may be due to the extreme mobilization of fat from adipose tissues because of underutilization of glucose (25). An obvious increase was observed in the level of serum TC, TG, LDL, and VLDL-Cholesterol whereas the level of HDL is significantly (P<0.05) depleted in Diabetic Control group (DC) as compared to the Normal Control group (NC). 45 days treatment of PDPE caused a significant (P<0.05) reduction in TC, TG, LDL and VLDL with simultaneous increase in HDL-Cholesterol, as compared to diabetic control mice (Table 3). Results revealed from the study were consistent with several earlier studies (26). PDPE antidyslipidemic action was might be due to the lipid peroxidation inhibition, since DM is associated with an increase in lipid peroxides with reduce antioxidant enzymes (27).

Table 3: Effect of 45 days treatment of P. dulce podextract on serum lipid profile in alloxan induced diabetic mice:

Parameters (mg/dl)	NC	DC	ST	PDPE
TC	98.42±13.9	230±13.34 a	149.7±9.3 b	229.4±17.1 b c
TG	113.86±14.6	171.70±15.29 a	71.8±5.2 b	118.4±14.8 b c
HDL	40.86±13.3	24.4±5.47 a	55.7±3.9 b	65±5.0 b c
LDL	34.71±21.02	170.7±14.1 a	79.57±8.8 b	140.8±14.9 b c
VLDL	22.85±2.96	34.28±2.9 a	14.28±1.11 b	23.8±3.8 b c

Values are mean±SEM of 7 observations
Student's't' –test is significant at P<0.05. a: significant (P<0.05) difference compared to NC; b: significant (P<0.05) difference compared to DC; c: significant (P<0.05) difference compared to GT and c*: insignificant difference compared to GT

Effect on Enzymatic and Non-enzymatic antioxidants:
Several reports indicate alterations in enzymatic and non-enzymatic antioxidants during oxidative stress caused by diabetes (28) that formulates β-cells susceptible to damage by free radicals. Moreover, the diabetogenic role of alloxan also involves in the production of ROS and therefore distorted levels of antioxidant defence mechanism (29). GSH that containing thiol group is most important defence that works against the oxidative stress by reacting with peroxides and hydroperoxides (30). Moreover, lipid peroxidation is also a major feature of chronic diabetes (31). Results from this study showed significant increase in the level of TBARS in all the three tissues and decrease in the GSH level and antioxidant enzymes such as SOD, CAT, GSH-Px in liver, kidney and pancreas in alloxan induced diabetic control group (Table 4). These observations were in support to the earlier findings (29, 32), demonstrating reduction in GSH content of tissue's on alloxan induced diabetic mice, as GSH is converted to its oxidised form due to the over production of reactive oxygen species. But treatment of diabetic mice with PDPE for 45 days decreases TBARS content. At the same time GSH content also increased significantly (P<0.05), demonstrating that PDPE could either increase in the biosynthesis GSH or reduced level of oxidative stress and finally supportive in the degradation of GSH. Similar findings were also

reported previously by various authors (21). Besides TBARS and GSH, antioxidant enzymes such as SOD, CAT, GSH-Px levels were elevated. The 45 days treatment of PDPE restored the level of SOD in hepatic and renal tissue to 139.80 ± 4.5 and 121.65 ± 5.16 as compared to diabetic control group and reduced in pancreatic tissue to 89.2 ±12.46. Level of CAT and GSH-Px in 45 days treated PDPE group were significantly normalised in hepatic, pancreatic and renal tissues. Moreover, 45 days treated glibenclamide group could not restore enzymatic antioxidants content, as significantly (P<0.05) lower values of enzyme activites can be seen under GT. The antioxidant activity of PDPE might be due to the inhibition of glycation antioxidant enzyme (33).

Table 4: Activity of antioxidants in various organs of alloxan induced diabetic mice after 45 days treatment of *P. dulce* pod extract:

Parameters		NC	DC	ST	PDPE
Hepatic	SOD[1]	190.20±15.41	140.7±5.144 a	132.67±2.51 b*	139.80±4.5 b*c*
	CAT[2]	208.20±9.43	136.30±6.61 a	150.50±7.85 b	215.30±35.56 b c
	GPx[3]	217.5±12.7	122.7±10.3 a	148.4±6.5 b	187.3±12.8 b c
	GSH[4]	39.2±2.9	12.3±2.1 a	19.2±1.6 b*	27.4±2.2 b c*
	TBARS[5]	34.2±4.4	432.14±16.9 a	134.51±1.4 b	49.14±3.9 b c
Pancreatic	SOD[1]	220.3±14.5	197.40±27.9 a	208.4±15.95 b*	89.2±12.46 b c
	CAT[2]	214.30±8.93	153.30±2.85 a	194.20±9.51 b	208.90±18.29 b c
	GPx[3]	172.1±22.3	89.01±9.75 a	105.16±20.2 b	140.2±9.4 b c
	GSH[4]	11.9±1.8	7.9±1.4 a*	15.2±2.3 b	9.2±1.6 b* c
	TBARS[5]	63.75±9.07	462.4±13.98 a	107.8±3.91 b	68.5±4.9 b c
Renal	SOD[1]	198.5±26.07	124.20±12.17 a	113.8±9.12 b*	121.65±5.16 b* c*
	CAT[2]	268±2.64	146.40±5.76 a	196.5±3.96 b	275.8±46.95 b c
	GPx[3]	167.8±8.6	84.23±14.6 a	96.2±5.7 b*	149.6±10.1 b c
	GSH[4]	27.36±2.6	8.74±1.6 a	7.5±2.7 b*	17.4±1.8 b c
	TBARS[5]	13.25±1.28	102.24±14.8 a	21.6±2.53 b	6.3±3.4 b c

Values are mean±SEM of 7 observations

[1]Units/min/mg Protein, [2]µ molesH_2O_2 decomposed/min/mg Protein, [3]µg GSH consumed/min/mg Protein, [4]mg/gm tissue and [5]nm TBARS/mg Protein. Student's't' –test is significant at P<0.05. a: significant (P<0.05)difference, a*: insignificant difference (P>0.05) compared to NC, b: significant difference (P<0.05) to DC, b*: insignificant difference (P>0.05) to DC, c: significant difference (P<0.05) to ST, c*: insignificant difference (P>0.05) to ST.

Figure1: Effect of 45 days treatment of PDPE on SOD in alloxan induced diabetic mice:

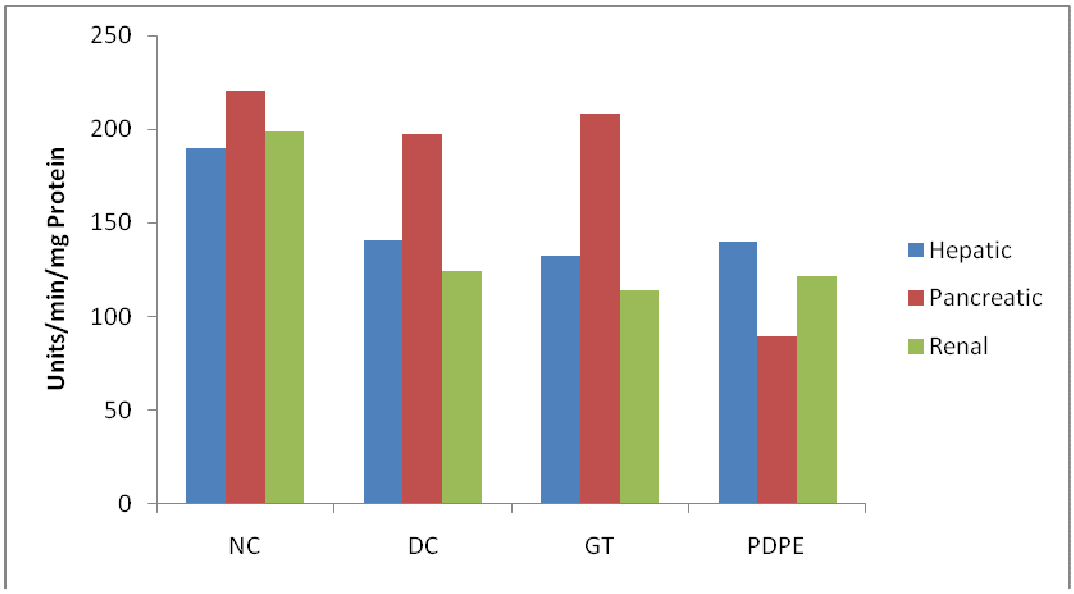


Figure2: Effect of 45 days treatment of PDPE on CAT in alloxan induced diabetic mice:

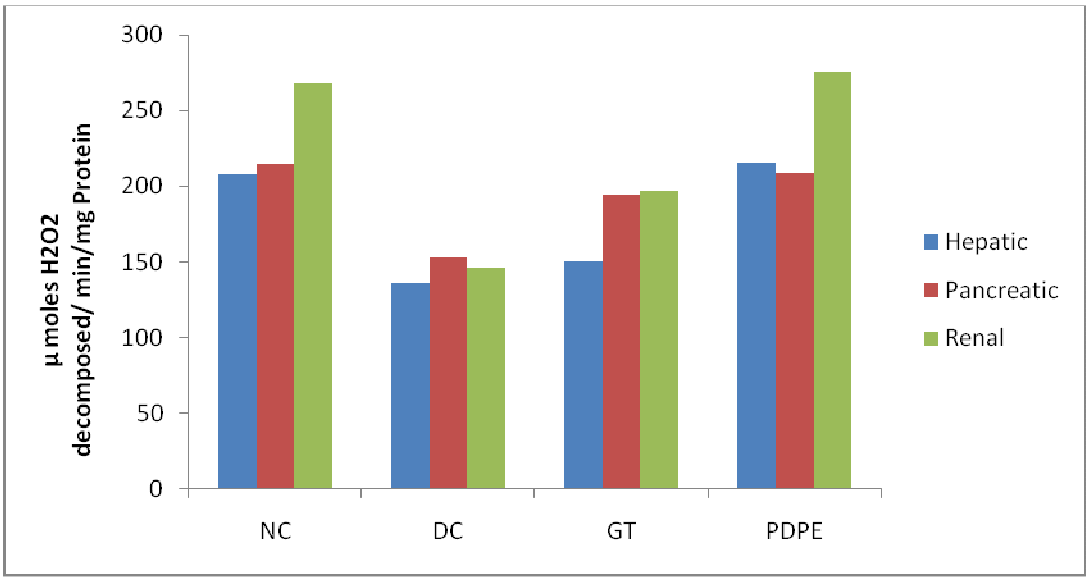


Figure 3: Effect of 45 days treatment of PDPE on GSH-Px in alloxan induced diabetic mice:

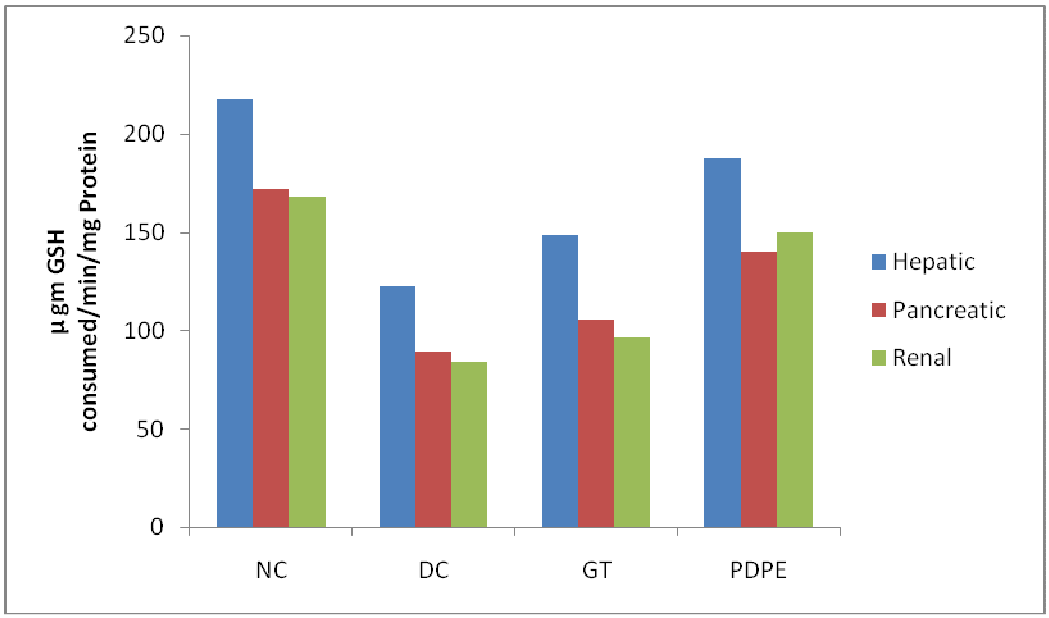


Figure 4: Effect of 45 days treatment of PDPE on GSH in alloxan induced diabetic mice:

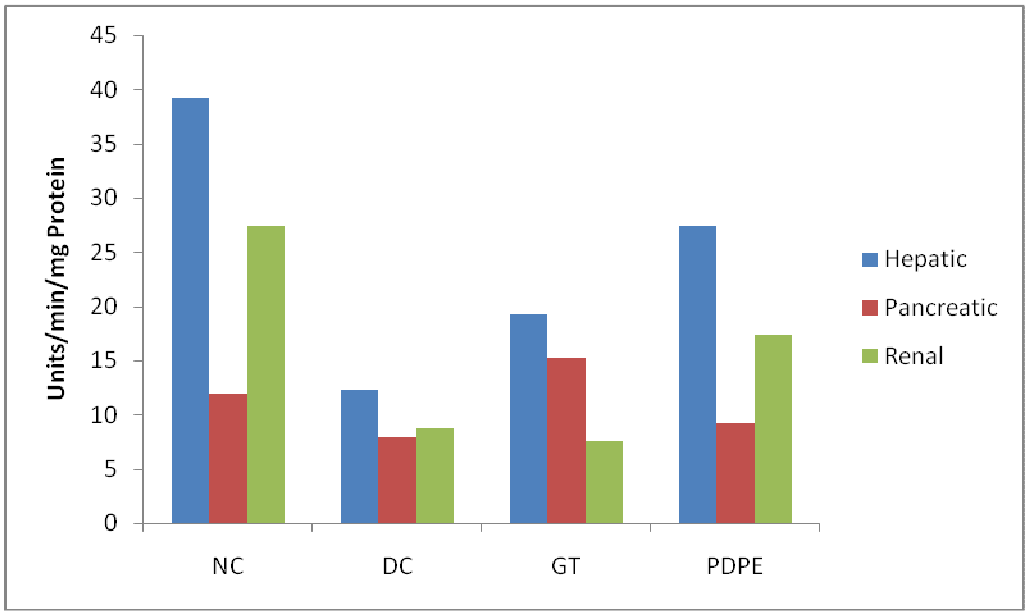
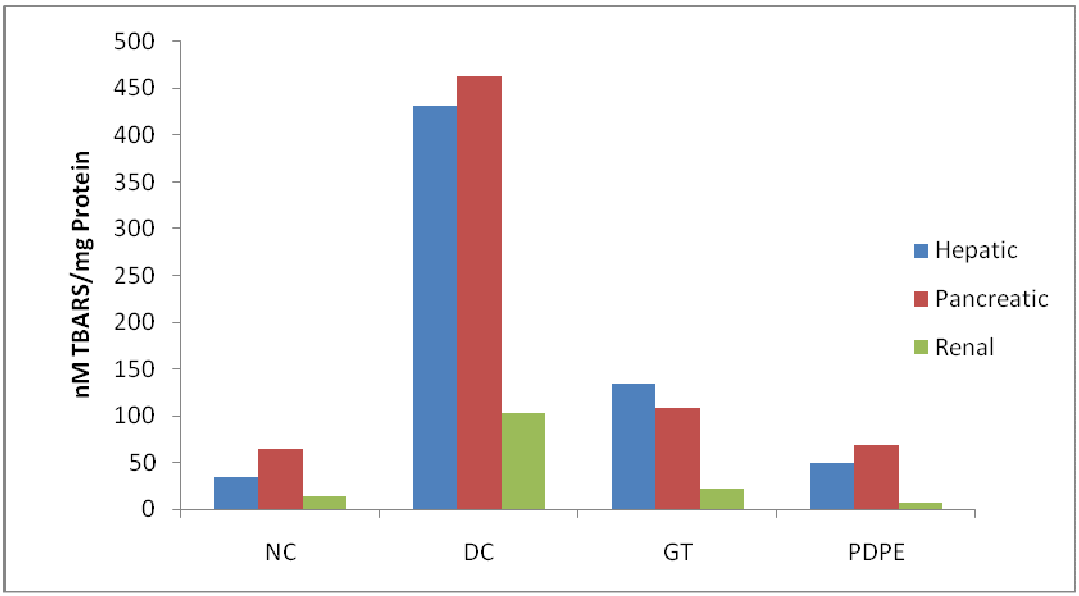


Figure 5: Effect of 45 days treatment of PDPE on TBARS in alloxan induced diabetic mice:



CONCLUSION

Results obtained from the preset study indicated significant anti-hyperglycemic, anti-dyslipidemic and anti-oxidant activities in the pods of *P.dulce* significant decline in Fasting Blood Glucose levels and increase of hepatic glycogen content by Pods of *P.dulce* were might be due to the increased consumption of peripheral glucose or potentiating effects of the insulin. Consequently, the pods were also being helpful in the inhibition of lipid peroxidation. Moreover, the antioxidant activity was normalised by Pods of *P.dulce* to such an extent that it helped in the reduction of oxidative damage caused in the tissues of alloxan induced diabetic animals. Thus, it can be concluded that Pods of *P.dulce* may find use for the management of oxidative stress during diabetes and its late complications.

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