Ameliorating Effect of Saponin from *Phoenix pusilla* root on Streptozotocin Induced Diabetic Animal Model.

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Abstract

Plant bioactive compounds are being studied for its applications in medicinal field, food industries, cosmetics preparations and other fields. Many plants are exploited for their medicinal property but some plants remain unnoticed. *Phoenix pusilla* is well-known for its traditional medicinal value however very less scientific proofs are available about this plant. The oral acute toxicity and *in vivo* antidiabetic and anti-inflammatory activity of the ethanol extract of *Phoenix pusilla* root and partially purified saponin of *Phoenix pusilla* were analysed in the present study. Acute toxicity testing of both the test drugs revealed no toxicity at a single concentration of 2000mg/kg body weight. So the safest dose level for checking the pharmaceutical activity of the test drugs were fixed within this concentration. *In vivo* study was carried out by injecting streptozotocin and after 3days, experimental rats with glucose levels greater than 250mg/dl were chosen for the study. Experimental animals were treated with the test drugs, positive control drug and a non-diabetic control group were maintained and checked for lipid profile, blood glucose level, antioxidant enzymes, renal markers, liver makers and inflammatory markers levels. According to the results obtained, both the crude extract, partially purified saponin were found to have hypoglycemic, antioxidant,

hypolipidemic and anti-inflammatory effect. Comparatively, partially purified saponin was highly effective than the crude extract of root.

Keywords: Saponin, hypoglycemic, antioxidant, toxicity, antidiabetic, anti-inflammatory activity.

Introduction

Herbal medicine consumption has become a practice again because of the side effects and high cost of prevailing chemical drugs (Dar et al., 2017). Most of the developing countries, prefer herbal medicine to modern medicine, due to their historical and cultural background (Ashwin Prakash Karurkar et al., 2022). The Vedas and the Bible described the use of herbal remedies and healthcare preparations. In countries like China, India, Japan, Pakistan, Sri Lanka and Thailand traditional medicine utilization for treatment is common (Singh, 2015).

Phoenix is the genus with nearly 14species that are native to tropical and subtropical regions (Elsafy et al., 2015). *Phoenix pusilla* (PP) is a shrub that can grow up to 3meters. In India, PP is commonly seen in coromandel coast of Tamilnadu (Chengalpattu, Thanjavur, Salem, South Arcot, Trichy, Tirunelveli) and South Kerala (http://envis.frlht.org/). All the parts of PP have traditional uses like leaves are used to make mats, brooms, baskets, fruits are used to treat fever, pith is used in treating gonorrhoea and eaten as food by starving people. Roots are used as disposable toothbrush (http://www.asia-medicinalplants.info/phoenix-pusilla-gaertn/).

Toxicity testing is the important step of drug development. Toxicity can be assessed using cell lines or by administering the drugs to the experimental animals. This testing aids determining “No observed adverse effect levels” (NOAEL) (Parasuraman 2011). In acute toxicity testing, a single dose of the test compound is administered orally or dermally or by inhalation (Ashwin Prakash Karurkar et al., 2019). Acute toxicity studies provide information that will be useful in designing future work and also the dose selection can be made for long term studies (Arome and Chinedu 2013).

A recent survey reports that the occurrence of diabetes in 2019 was 9.3%, and this will increase and reach the projected percentage of 10.2 in 2030 and 10.9 in 2045. Globally, men’s diabetic percentage was more than women’s in 2018 (Asrar Ahamed et al., 2018).

Also, it was identified that the prevalence of diabetes was high in higher income countries compared to low income countries. In the top 10 countries lists of diabetic survivors, India is in the second place, and this position is expected to be same in 2045 (Saeedi et al., 2019). Many research reports that this disease is becoming more prevalent because of life style, food pattern changes (Mitra 2019; Nanditha et al., 2019). About PP only less scientific proofs are available but the literature survey reveals that all the parts of PP have traditional medicinal applications, and so in this present study toxicity of PP root ethanol extract (PPE) and partially purified saponin (PPS) were checked for its further use to treat human sickness.

Materials & methods

PP collection and extraction

PP roots were collected from Viramangudi village, Thanjavur district, authenticated, dried and stored. PPE was obtained by extracting 100g of PP root powder with ethanol in soxhlet apparatus for 24 hours, solvent was allowed to evaporate, concentrated and the yield was noted (5g) (Sankar and Shoba, 2017).

Saponin isolation (PPS)

PPS was isolated by Akaniro-Ejim et al., 2016 procedure.

***In vivo* study**

Animal maintenance

Healthy young adult male Wistar albino rats of 6-8 weeks, 150-200g (±20g) obtained from TANUVAS, Madhavaram, Chennai was used for the study. Animals were fed with water and pellet diet (Sai Meera Foods, Banglore). Experimental animals were kept in the cage for 7 days before toxicity test for making animals to get adapted to the laboratory conditions. Room temperature was maintained at 22ºC + 3ºC. 50-60% humidity and 12 hours light and dark cycle were maintained (Subha and Geetha, 2017). All the procedures were carried out by following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, New Delhi. (IAEC no: 15/321/PO/Re/S/01/CPCSEA)

Test drugs preparation

Crude ethanol extract: PPE suspended in 0.5% carboxy methyl cellulose.

Partially purified saponin: Saponin was suspended in water.

Acute toxicity study

Toxicity study is an important screening test of new drugs to be done before they are approved for human use. The importance of toxicity study is that, it helps to understand the mode of action of the drugs, safety dose level identification, dose response information etc. Toxicity studies are differentiated in to acute toxicity and sub-acute toxicity study. An acute toxicity study provides information about dose selection of drugs for further study, future study design, LD50 level etc (Arome and Enegide 2013). Single or multiple doses is administered to check the adverse effect in short duration. In sub-acute toxicity, repeated doses are administered for longer duration to check the adverse effects, additionally this study helps in designing and determining the doses for the chronic study (Eaton and Gallagher 2010).

Dose administration and observation

Organization for Economic and commercial development guidelines (OECD-423) were followed to carry out the acute toxicity test (Emmanuel *et al*., 2018). Toxicity study was conducted by selecting the animals randomly and the selected animals were fasted for 12 hours. Animals were weighed before the dose was administered. PPE and PPS were the two test samples studied for their toxicity effect. For each test sample, two groups (control and test) of three male albino Wistar rats were maintained and administrated with single dose (2000 mg/kg body weight) orally by gavage using a feeding needle.

After dosage administration, the animals were not provided food for further 4hours. The animals were observed for every 30minutes for the initial period of 4hours and thereafter daily until the 14 th day of dosing for any toxicity (Emmanuel et al., 2018; Saleem et al., 2017). All the observations are systematically recorded with individual records being maintained for each animal.

***In vivo* antidiabetic activity of PPE and PPS**

Diabetes induction and animal selection

50mg/Kg body weight of streptozotocin was injected intraperitoneally. The animals were allowed to drink 5% glucose solution overnight to avoid hypoglycemic shock. After 3days of streptozotocin injection, the animals having fasting blood glucose level more than 250mg/dl were considered as diabetic rats and selected for *in vivo* study.

Experimental Design

Diabetic rats were divided into 7 groups with six animals in each group.

Group I: Normal control received 2 ml Luke warm water for 21 days orally

Group II: Diabetic control received 2 ml Luke warm water for 21 days orally.

Group III: Diabetic rats treated with standard glibenclamide 5mg/kg orally once a day for 21 days.

Group IV: Diabetic rats treated with test drug (PPE) 100 mg/kg body weight suspended in 2 ml of luke warm water once a day orally for 21 days.

Group V: Diabetic rats treated with test drug (PPE) 200 mg/kg body weight suspended in 2 ml of luke warm water once a day orally for 21 days.

Group VI: Diabetic rats treated with drug (PPS) 50 mg/kg body weight suspended in 2 ml of luke warm water once a day orally for 21 days.

Group VII: Diabetic rats treated with drug (PPS) 100 mg/kg body weight suspended in 2 ml of luke warm water once a day orally for 21 days.

The dose was established from the result of toxicity studies.

The weight of the animals was recorded individually prior to the commencement of the experiment and at intervals of 7 days for 21 days experimental period to obtain the change in body weight of the rats.

Blood collection

All the experimental rats were fasted overnight and then the blood was withdrawn through puncturing the retro orbital sinus on the 0^{th} day, 7^{th} day, 14^{th} day and 21^{st} day of the post induction period to determine blood glucose levels by glucose oxidase method with glucose analyzer. On the 21^{st}day the blood was collected by cardiac puncture, under mild ether anesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and they were used for various biochemical experiments.

Preparation of tissue homogenate

After the experimental duration, the animals were sacrificed under mild chloroform anesthesia. The pancreas was excised, immediately washed with cold saline and 10% homogenate pancreatic tissues was prepared with 0.1M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 1000g for 10 min at 4℃ in a refrigerated centrifuge to remove the nuclear debris. The supernatant was used for further assay (Sankar and shoba, 2017).

***In vivo* antioxidant activity assay**

The concentration of oxidative stress markers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and lipid peroxidation inhibition (LPO) were measured in pancreas.

Superoxide dismutase assay

To the tube containing 750µl of ethanol and 150 µl of chloroform, 100 µl of homogenate sample was added and centrifuged. From the supernatant collected, 500 µl was added to 0.6 mM of EDTA (0.5ml) and 0.1M buffer (1ml, carbonate-bicarbonate buffer). 500 µl of epinephrine (1.8mM) was added to initiate the reaction and the absorbance was measured at 480nm. The enzyme activity is expressed as 50% inhibition of epinephrine autooxidation. The enzyme activity was expressed as Units/mg protein (Mishra and Frideovich, 1972).

Catalase (CAT) assay

Catalase (CAT) activity was estimated by the method of Sinha *et al*., 1973. 100 µl of tissue homogenate, 1000 µl of phosphate buffer (0.01M, pH 7) and 400 µl of H_2O_2 were pipette in to the tube and incubated. The reaction was stopped by the addition of 3.0 ml dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). Tubes were kept in the boiling water bath for 10 minutes. The absorbance at 530 nm was then measured and CAT activity was expressed as µM of H_2O_2 consumed/min/mg of protein.

Glutathione peroxidase (GPx) assay

Glutathione peroxidase (GPx) was measured by the method described by Rotruck et al., 1973. The reaction mixture was prepared by pipetting 0.2 ml of tissue homogenate, 200 µl of phosphate buffer (0.4M, pH7), 100 µl of sodium azide (10mM), 200 µl of reduced glutathione and 100 µl of hydrogen peroxide (0.2M). The tubes were then incubated at room temperature for 10 minutes and after incubation the reaction was stopped by adding 400 µl of 10% trichloroacetic acid. Then the tubes were centrifuged for 20 minutes at 3200g. The supernatant was then assayed for glutathione content using Ellman's reagent (19.8 mg 5,5'-dithiobisnitrobenzoic acid [DTNB] in 100 ml 0.1% sodium citrate). The activities were expressed as µg of GSH consumed/ min/mg protein.

Lipid peroxidation inhibition assay

To the tube containing 1.5ml of glacial acetic acid (20%) of pH 3.5, 0.1ml of homogenate/ standard (malonidialdehyde, MDA) was added. Then 0.2ml of sodium dodecyl sulphate (SDS, 8.1%), 0.8 % of thiobarbituric acid (TBA, 1.5ml), 0.7ml of distilled water were added and the tubes were incubated for 1 hour at 95℃ with marble on the top of each tube. After incubation, the tubes were cooled, n-butanol/pyridine mixture (5ml) and water (1ml) were added, mixed well and centrifuged for 10 minutes at 4000rpm. The organic layer was separated and the absorbance was measured.

The concentration of malondialdehyde (MDA) formed was measured spectrophotometrically at 532 nm. The standard used in this assay was 1,1,3,3-Tetraethoxypropane (TEP), a form of MDA. TBARS concentration was expressed as nmol of malondialdehyde (MDA) per mg protein (Ohkawa et al., 1979).

Serum lipid profile analysis in experimental animals

Commercially available kits were used for serum lipid profile determination and it was purchased from Accurex Biomedical Pvt. Ltd., Mumbai, India.

Very low density lipoprotein (VLDL)

VLDL was calculated by using Friedewald's formula

VLDL (mg/dl) = TG/5 (Friedewald et al., 1972).

Renal markers estimation in drug treated and control groups

Urea and creatinine in serum was estimated by following the procedure mentioned by Gopinathan and Rameela, 2015; Jaffe, 1886 respectively.

Liver marker enzymes assay

Aspartate transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured in the serum of test drug treated and control group (King, 1965).

Assay of Alkaline phosphatase

100 µl of serum sample was mixed with 1.5ml of carbonate buffer, 100 µl of magnesium chloride, 1ml of disodium phenyl phosphate and incubated for 15 minutes at room temperature. Then 1ml of Folin-phenol reagent, same volume of sodium carbonate were added and incubated for 10 minutes. The colour developed was read at 640nm. The activity of ALP was expressed as moles of phenol liberated/min/mg of protein (Kind and King method, 1972) 26.

Inflammatory markers analysis in experimental animals

TNF-alpha and IL-6 were assayed based on the protocol provided by the kit *manufacturer (Accurex Biomedical Pvt. Ltd., Mumbai, India).*

***Estimation of C-Reactive protein** (CRP)*

Competitive ELISA method reported by Burger *et al*., 1998 was followed to measure the CRP level in the sample. In this method, the wells were coated with phosphoryl choline bovine serum albumin conjugate (20mM carbonate buffer) and kept for overnight incubation at 4 ^{o}C.

After incubation, the wells were washed with PBS containing 0.5 % tween-20 (washing buffer). Then 0.1ml of sample, equal volume of human CRP conjugated to horse raddish peroxidase were added to the wells and incubated for 15 minutes at room temperature.

Again the wells were washed with washing buffer provided in the kit, 0.1 ml of tetramethylbenzidine (substrate) was added to all the wells and incubated for 15 minutes. Finally, the reaction was stopped by adding 0.1 ml of 2M sulphuric acid. The plate was read at 450nm (Burger et al.,1998).

Histopathology of Pancreas

Pancreatic tissue sections were prepared by fixing the tissue in formalin, then dehydration was done with grades of alcohol. Dehydrated tissue was then embedded in wax to make blocks. 5µm section was made using microtome and the thin section was fixed on to the slide. Then the sections were stained with hematoxylin-eosin and observed under a microscope.

Statistical Analysis

All the values were expressed as Mean ± SD. The differences between control and treatment groups were tested for significance using ANOVA followed by Dunnet's t test. $p<0.05$, $P<0.01$ were considered significant using Graph pad version 8.3 (Vemuri et al., 2018).

Results & Discussion

Acute toxicity test of both PPE and PPS was performed using an oral dose of 2000 mg/kg and revealed no toxicity sign. From the results obtained, it was understood that the lethal dose was greater than the concentration checked for the toxicity. Various parameters that were checked during the study include touch response, alertness, grooming, writhing etc (Table 1).

Both the test drugs showed positive sign of alertness, pile erection and touch response, rest of the parameters were found to be negative. It was also found that the experimental animals administrated with test drugs responded as like the control group. Furthermore, it was understood that the administered dose of test drugs was not causing any deleterious effects and so antidiabetic as well as anti-inflammatory study were carried out by fixing the dose within the checked dose level in the acute toxicity study. Along with the above mentioned parameters, the weight change among the test animals was noted (Table 2). It was observed that with PPE administration, the weight change in experimental animals during the study period was 202.3±6.64, 202.7±7.42 and 203.2±2.70. PPS administration showed the values of 210.5±27.75, 211.7±31.67, 213.4±32.67 on 1^{st}, 7^{th} and 14^{th} day respectively. Water and food intake of test animals was similar to the control group (Table 3, 4). Body weight, water and food intake observations in the test drug administered groups revealed no statistically differences from control group. So it was clear that the test drugs were not affecting the normal activity of experimental animals.

Glucose level analysis in experimental animals was done at intervals of 7 days for 21 days period, revealing that the sugar level variation was dose dependent. Analysis of glucose on the 7th day in all the groups showed an average value of more than 250mg/dl except in normal control. Fourteenth day observation showed a reduction in glucose level in the drug treated groups and the final experimental day results revealed that the test drugs were found to be effective as positive control in reducing the glucose level (Figure 1). An uncontrolled blood glucose increase in group-II animals was found, which seems to be due to a defect in secretion or effect of insulin. A lowering blood glucose effect was observed in all the drug treated groups. This lowering effect may be due to the active components of the plant extract which are involved in the inducing insulin secretion or may possess insulin mimec effect.

Body weight records, showed that in the diabetic control group (group II) animals weight loss was seen. On the other hand, the test drug treated group showed an improvement in weight gain. It was found that with the dose of 200mg/kg body weight of PPE, the positive variation seen in weight was from 182.85±4.42 on the 0th day to 196.20±3.43 on 21st day. Similarly, PPS (100mg/dl) showed statistically significant weight gain compared with diabetic control. Weight record of PPE and PPS treated group when compared with normal control, positive control group showed the effectiveness of the test drugs in weight improvement (Figure 2). Body weight reduction seen in diabetic control may be due to

increased fat, protein (Bansode et al., 2017) catabolism, which is the important change usually observed in type 2 diabetes.

In all the enzymatic antioxidant assays, the activity was dose dependent (as the concentration of drug increased, the activity observed was high)**.** Superoxide dismutase activity was found to be 6.92±0.59 U/mg of protein in PPS treated group at the concentration of 100mg/kg body weight, it was the highest value observed in the test drug treated groups IV, V, VI, VII and it was evident that the test drugs have effective antioxidant activity as group-III. Catalase activity assay revealed the same kind of result as SOD result (Figure 3). Among the test drugs PPS was found to be more effective than PPE. PPS showed 9.41±1.21 U/mg of protein (100mg/kg body weight) glutathione peroxidase activity, while PPE at the highest concentration showed 8.21±1.03 U/mg of protein. Lipid peroxidation inhibition value obtained for groups IV, V, VI & VII showed that the test drugs presence lowers the peroxidation of lipid when compared with the untreated group. Glibenclamide treated group showed statistically significant value compared with group II. Pancreatic antioxidant enzyme level in the diabetic control indicates that the stress was encountered by the experimental animals due to streptozotocin injection. Oxidative stress associated with diabetes if untreated found to cause vascular disease (Asmat et al., 2016). Defects in antioxidant role and oxidation of glucose, glycation of protein lead to free radical generation, which inturn plays a vital role in the pathogenesis of diabetes. Hyperglycemic condition of diabetes is considered as an important oxidative stress that affects liver and leads to metabolic disturbance in carbohydrate, protein and lipid (Yazdi et al., 2019).

In diabetes, the abnormality in lipid metabolism is based on the extent of insulin lack, insulin resistance, obesity and other secondary causes (Andallu et al., 2009). Lipid profile analysis on 21st day of treatment showed that the cholesterol level in the diabetic control group was 172.81±7.43mg/dl, glibenclamide treated group showed 69.13±7.3 mg/dl, group IV, V showed 80.11±4.23, 78.13±6.16 mg/dl and 81.10±6.40, 72.90±8.4 mg/dl observed in groupVI, group VII respectively. LDL level in diabetic control group was 127.80±0.90mg/dl, at the highest concentration of test drugs (PPS, PPE) it was 51.3±3.14, 52.10±3.20mg/dl respectively. The results observed for cholesterol, LDL prove the hypocholesteremic potential of the test drugs.

A similar kind of effect was seen with TG and VLDL values in the experimental group, PPS was significant as a positive control in VLDL level maintenance and triglyceride

value comparison indicating that the PPS was efficacious. HDL quantity improvement was seen in test drug treated groups as observed in the glibenclamide treated group. Group II HDL value shows the impact of diabetes in lowering the good cholesterol. Creatinine and urea serum levels were controlled by PPE and PPS (Figure 4). Increase in good cholesterol level, decrease in VLDL and triacylglycerol concentration in the test drug (PPE, PPS) treated groups is the positive indicator of the lipid profile controlling effect of the drugs, whereas in the group II animals, the lipid profile controlling effect was seen in reverse which shows the impact of diabetes on lipid metabolism.

Another important complication of diabetes is nephropathy. It was found that all the complications of diabetes were found to associated with oxidative stress. Advanced glycation end product formation in the kidney is the major cause for diabetic nephropathy. Kidney damage in diabetic condition is mainly due to the glycation product formation which attacks the proteins and lipids of kidney. The leaf extract of *Punica granatum* also showed an effective potential in decreasing the formation of glycation end product which shows the significance of the extract to be used in the management of diabetic complications (Mestry et al., 2017). Concentration dependent reduction was seen in the level of liver marker enzymes (ALT, AST & ALP) in the drug treated groups i.e., with a high concentration of drug the marker enzyme level was less. Test drugs were found to be comparable with glibenclamide. PPS was more effective than PPE in preventing liver injury (Figure 5). Liver marker assay showed the hepatoprotective effect of the PPE and PPS. Similarly, the renal markers assay results showed that the drugs were effective in protecting the kidney against damage. PPE and PPS showed a significant and controllable effect on inflammatory markers. Mechanism that underlies liver problem in diabetes includes: triglycerides increase causes oxidative stress, promotes inflammatory signal that inturn stimulate collagen synthesis, growth factors of connective tissue and activates liver stellate cells, which then leads to fibrosis and cirrhosis (Li et al., 2019).

Inflammatory markers (IL-6, TNF-α and CRP) assayed on 21st day of streptozotocin treatment in group II experimental animals showed 5.52±0.51pg/ml, 16.05±1.10pg/ml and 3.7±0.23mg/l, whereas in highest concentration of test drugs treated group (V and VII), IL-6 was 3.96±0.42, 3.22±0.60pg/ml, TNF-α level was 13.66±0.32, 10.21±0.28 and CRP value was 3.86±0.11, 2.96±0.53. So the results of groups V and VII compared with group II show

that the treatment lowered the marker concentration (Figure 6). As in other parameters analyzed, PPS showed effective action against the inflammatory markers.

Histopathological study of the pancreas showed that in group I, islets were normal (Figure 7a). Diabetic control group (Figure 7b), showed that the insulin secreting cells were affected, degeneration of islet cells was seen whereas in the glibenclamide treated group (Figure 7c) effective recovery of islets was observed. Similarly, in drug treated groups, the improvement in islets normalisation was found to be based on concentration (Figure 7d, e, f). PPS highest concentration (Figure 7g) drug treated group showed effective improvement in pancreas normalization as positive control. Patel et al., 2015, carried out an evaluation of the hypoglycemic effect of Shivlingi seeds ethanol extract and saponin fraction by inducing diabetes using streptozotocin in experimental animals. They found that both ethanol extract and saponin fraction showed effective antidiabetic, antioxidant, anti-hyperlipidemic activity and also showed effective regeneration of pancreas as seen with PPE and PPS.

Saponins exhibit antidiabetic property by insulintropic effect, protecting beta cells of pancreas, activates the enzymes for glucose utilization. Saponin isolated from Sea cucumber, steroidal saponin from the fruit of bitter melon, triterpenoid saponin from drumstick primula (Singh et al., 2014) were among the few examples which were scientifically proven for its antidiabetic activity (Barky et al., 2017). Likewise, diosgenin, hexadecanoic acid, stigmasterol are some active compounds that have been proven for their antidiabetic activity (Bharti et al., 2018).

Conclusion

In all the developing countries, diabetes prevalence is high due to the lack of awareness, physical activity, unhealthy food and alcohol intake. Also, the drugs utilized in diabetes treatment are costly and have side effects. So the plant-based drugs could be a good substitute for diabetes treatment and management.

Conflicts of interest

The authors do not have any conflict of interest to declare.

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Table 1: Effect of of PPE and PPS any changes in behavioural pattern observed during Acute toxicity study

S. No	Experimental Animal 2000mg mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	Control group	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	PPE Test group	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	PPS Test group	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1.Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15. Lacrimation 16. Exophthalmos 17. Diarrhea 18. Writhing 19. Respiration 20. Mortality. (+ Normal, - Not found)

Table 2: Changes in Body Weight observed in PPE administrated test group

Experimental Animals 2000mg mg/kg	**DAYS**		
	1	**7**	**14**
Control group	200.1±65.7	201.3 ± 41.1	201.6 ±2.1
PPE Test group	202.3± 6.6	202.7 ±7.4	203.2 ± 2.7
PPS Test group	210.5± 27.8	211.7 ± 31.7	213.4 ± 32.7
P value (p)*	NS	NS	NS

N.S- Not Significant, n = 3, values are expressed as mean ± SD (One-way ANOVA followed by Dunnett's test)

Table 3: Effects on Water intake (ml/day) of Wistar albino rats group when exposed to *Phoenix pusilla* ethanol extract

Experimental Animals 2000mg mg/kg	**DAYS**		
	1	**7**	**14**
Control	54 ± 3.2	54±6.1	54.3±5.4
PPE Test group	53.5±1.3	53.8±6.7	54.2±5.6
PPS Test group	60.4±2.3	60.6±1.1	60.9±6.2
P value (p)*	NS	NS	NS

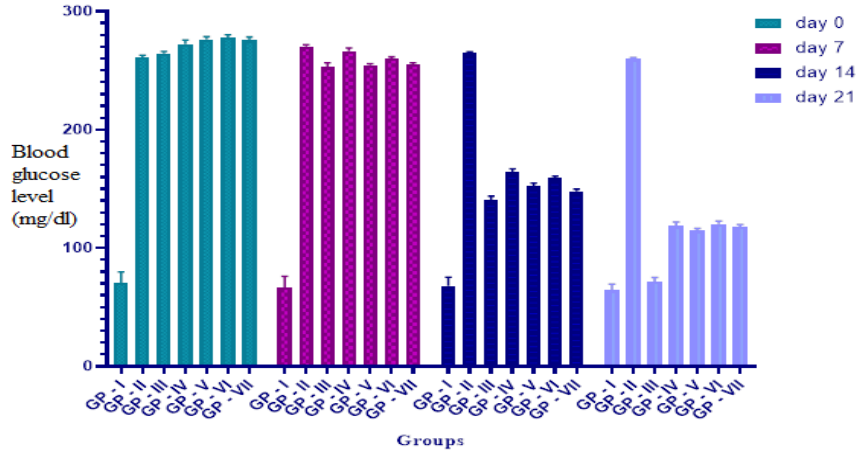
N.S- Not Significant, n = 3 values are expressed as mean ± SD (One-way ANOVA followed by Dunnett's test)

Table 4: Effects on Food intake (gm/day) of Wistar albino rats group exposed to *Phoenix pusilla* crude extract

Experimental Animals 2000mg mg/kg	DAYS		
	1	7	14
Control group	56±2.8	56.2±2.9	57.7±8.9
PPE Test group	58.6±5.4	58.4±5.2	59.8±6.7
PPS Test group	39.4±1.6	39.3±1.2	39.2±6.2
P value (p)*	NS	NS	NS

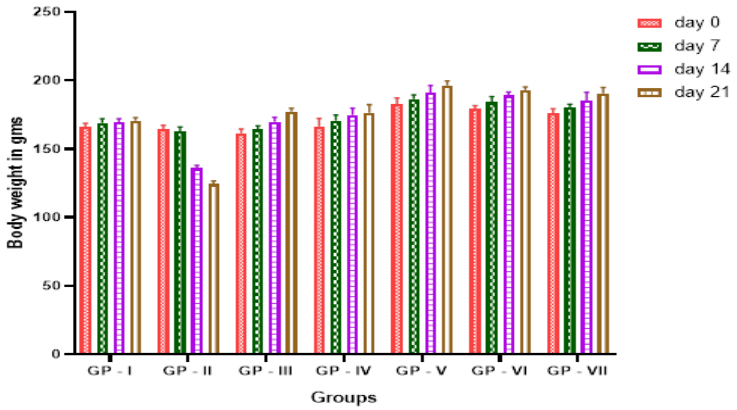
N.S- Not Significant, values are expressed as mean ± SD (n = 3). (One-way ANOVA followed by Dunnett's test)

Figure 1: Effect of PPE and PPS on Blood glucose level in control and drug treated groups



Values indicate mean ± SEM for six animals

Figure 2: Effect of PPE and PPS on body weight of rats during treatment

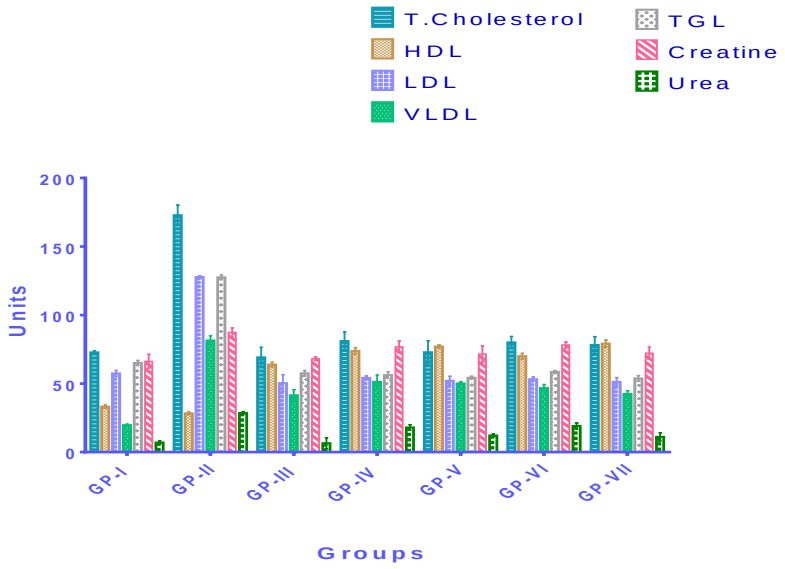


Values indicate mean ± SEM for six animals

Figure 3: Effect of PPE and PPS on Antioxidant status in experimental animal

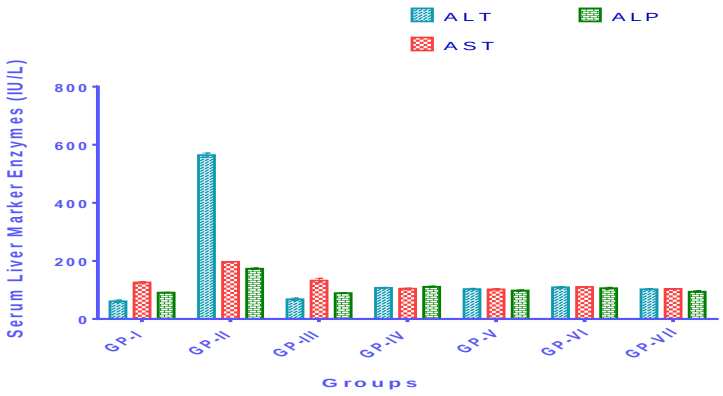
Values indicate mean ± SEM for six animals

Figure 4: Effect of PPE and PPS on Lipid profile and renal makers level observed in experimental animals



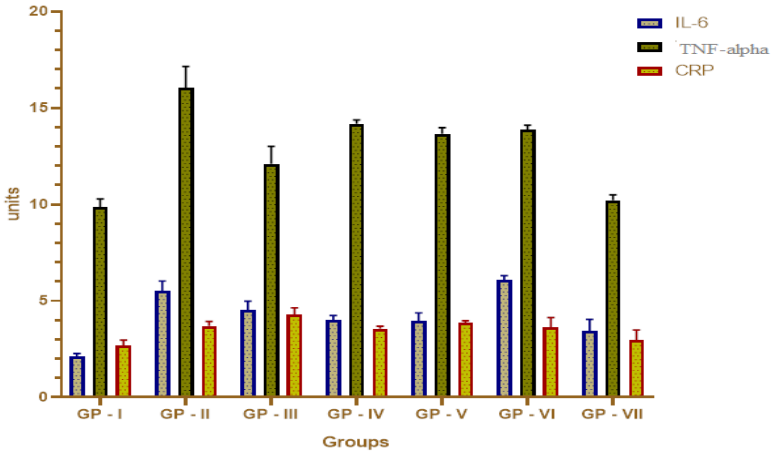
Values indicate mean ± SEM for six animals

Figure 5: Effect of PPE and PPS on Liver marker enzymes in drug treated and control group animals



Values indicate mean ± SEM for six animals

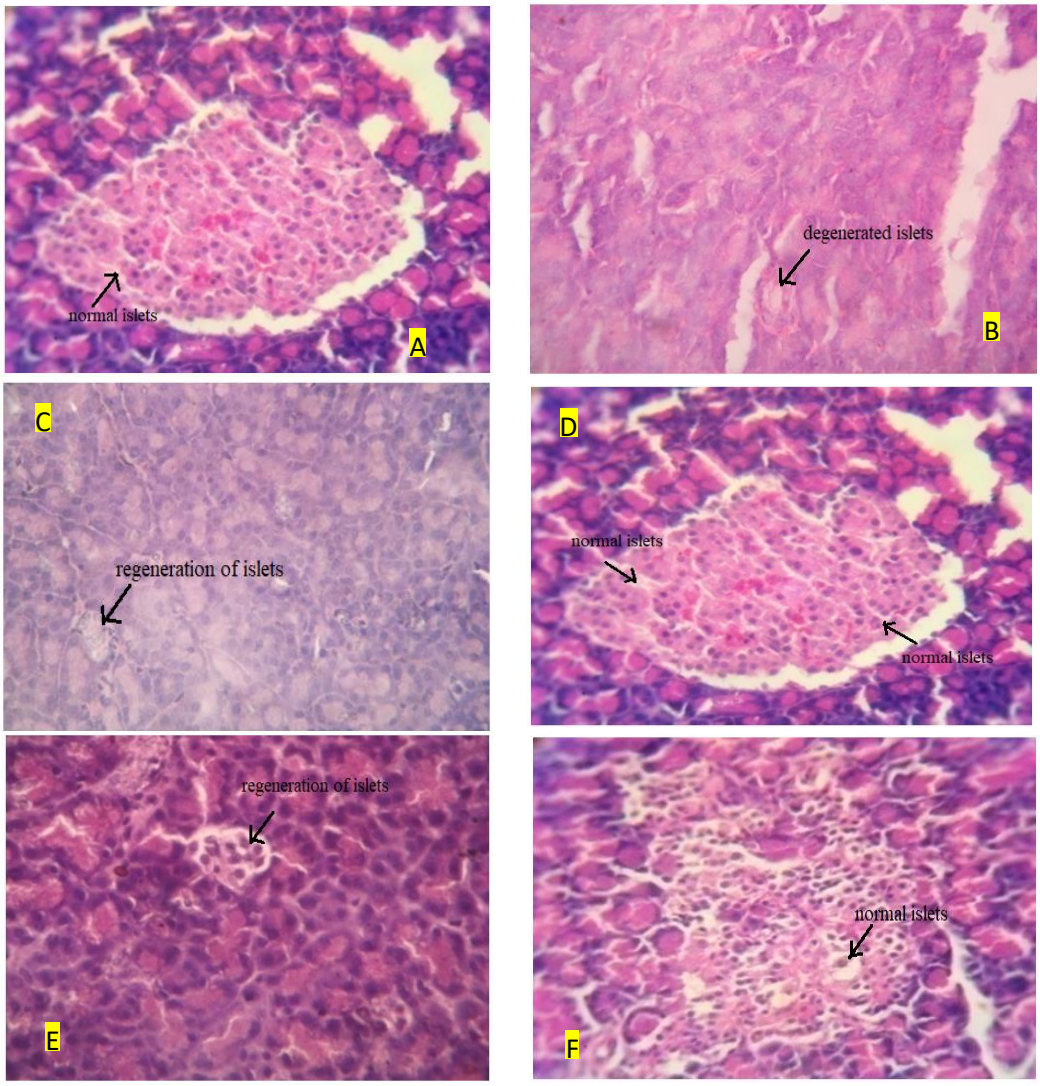
Figure 6: Effect of PPE and PPS on levels of

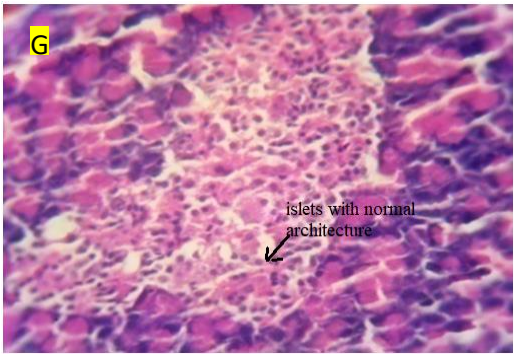


Inflammatory markers in drug treated and untreated groups

Values indicate mean ± SEM for six animals

Figure 7: Effect of PPE and PPS on Histopathology of Pancreas in STZ induced Diabetic animal model





A: Normal control (Normal pancreatic islet cells), B: Streptozotocin Induced Diabetic control (expansion and dilated islet cells), C: Glibenclamide treated group (absence of dilation and prominent hyperplastic of islets), D: PPE treated group (100mg/Kg body weight) (moderate expansion of pancreatic islets, showing prominent hyper plastic islet); E: PPE treated group (200mg/Kg body weight) (absence of dilation and prominent hyperplastic of islets); F: PPS treated group 50mg/Kg body weight (mild expansion and absence of dilations) and G: PPS treated group 100mg/Kg body weight (Isltes with normal architecture).