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Therapeutic Effect of Asphodelus Tenuifolius Against Streptozotocin Induced Diabetic Rats

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Keywords:

Asphodelus tenuifolius (AT); Streptozotocin STZ; Therapeutic effect

1. Abstract

1.1. Background: Diabetes mellitus is a long-lasting metabolic disorder that hinders the capability of the body to utilize food properly and is affected day by day through our diet, exercise, infection and stress. Presently, the trend is more towards natural plant-based products containing potential bio-active components than towards conventional medicines. For the purpose, out of numerous herbs used in common medicines, the subfamily of Asphodelus tenuifolius, used for several ailments, anti-inflmmation, anti-hypertension and antidiuretics etc.

1.2. Objective: The objective of the present study was to evaluate the therapeutic effect of Asphodelus tenuifolius against streptozotocin induced diabetic rats.

1.3. Method: The current study was based on the in vitro and in vivo properties of Asphodelus tenuifolius. The in vivo study was conducted that includes the TPC, TFC & DPPH. The in vitro study was also administered that includes the body weight and glucose levels of the rats. The thirty rats were divided into six groups; normal control, disease control, drug control, extracts treatment at 250, 500 and 750 mg/kg respectively.

1.4. Results: The results depicted that the maximum decline in blood glucose levels of the streptozotocin induced diabetic rats were observed in T5. However, the body weights of the diabetic rats were maintained.

1.5. Conclusion: Conclusively, Asphodelus tenuifolius plants contain a bio-active component that help to reduce hyperglycemia, maintains body weights in streptozotocin induced diabetic rats.

2. Introduction

Diabetes mellitus, which is commonly known as diabetes is a long-lasting metabolic disorder that hinder the capability of the body to utilize food properly [3] and is affected day by day through our diet, exercise, infection and stress. The insulin hormone is manufactured in pancreas, and it assists the human body to transform food into energy. Diabetes is an absolute cause of morbidity and mortality in the whole world [20].

In the current scenario, due to the increasing burden of diseases, complexity in the treatment regimen, and environmental factors; the healthcare system is facing a challenge in drug therapy management. The high cost of drugs and their associated adverse effects are leading healthcare professionals as well as people to adopt alternative therapy.

About 800 plants are used for the treatment of the diabetes mellitus but only 450 of them have anti diabetic properties and 109 plants are used till date by completing their working principle. In early times traditional plants are used to treat different health issues such as heart diseases, diabetes and so on. The herbal products have less adverse effects on the health of individual as compared with the drugs. Herbal products are helpful in curing the disease for the lifetime whereas the drugs that are prepared chemically are not used for the longer duration due to their adverse effects on the health [24].

Anti-diabetic drugs used have a lot of adverse effects on health such as it changes bone physiology, skeleton health is hindered and have more chances of getting bones fracture [1, 11, 13]. It also increases risk of cardiovascular diseases in individual consuming antidiabetic drugs [5, 22].

Now a day the trend is more towards natural plant-based products containing potential bio-active components than towards conventional medicines. For the purpose, out of numerous herbs used in common medicines, the subfamily of Asphodelus tenuifolius, that is native to the Mediterranean in North Africa and South East Asia, is recognized as Piazi in Urdu and Onion weed in English used newly for several ailments, Burns, Alopecia Areata, Psoriasis, Acne, Nephrolithiasis , Inflammation, Acne & Toothache. Further-

more, there are eighteen more types of Asphodelus species, with a wide variety of classes, and almost all are widely represent the family of Asphodelus Tenuifolius, Asphodelus Ramosus, Asphodelus Microcarpus, Asphodelus Fistulosus and Asphodelus Aestivus [4].

One of the plant Asphodelus tenuifolius has been reported to be used in diabetes treatment but the pharmacological evaluation of anti-diabetic activity of Asphodelus tenuifolius has not been evaluated yet. However, the pharmacological study to validate its traditional practices is limited. Though some pharmacological experimentation has proved its different medicinal values, the anti-diabetic activity of such a plant has not been evaluated until now. If a plant drug is used without careful evaluation, the chance of sub-therapeutic concentration on the body and adverse consequences as well may ensue. So, the careful evaluation is essential for rational drug therapy.

The purpose of the current study is to validate the traditional use of the Asphodelus tenuifolius. It is directly needed to explore new antidiabetic compounds which are more effective and safer. Keeping in mind, one of the plants Asphodelus tenuifolius has been reported to be used in diabetes treatment but the pharmacological evaluation of anti-diabetic activity of Asphodelus tenuifolius has not been evaluated yet. This study aims to evaluate the medicinal (anti-diabetic) value of methanolic extract of the Asphodelus tenuifolius as well as the mechanism behind such activity for rational drug therapy.

3. Material & Methods

3.1. Study Design

The experimental design study will be applied to study the therapeutic effect of Asphedolus Tenuifolius against streptozotocin induced diabetic rats.

3.2. Study Population

Albino rats (n = 30) with body weight (130-200g appx).

3.3. Sampling Unit

Healthy and streptozotocin induced diabetic rats.

3.4. Procurement of Raw Material

The raw material includes Asphodelus tenuifolius plant was procured from the fields of Mianwali.

3.5. Identification of the Plant

The plant was identified by plant taxonomist from the Botany Department of the GC University Lahore. They confirmed the name of the plant “Asphodelus tenuifolius” and issued the voucher.

3.6. Washing, Drying & Powdering of the Plant

The plant was washed thoroughly with water. It was shade dried by the fan in room temperature for 36 hours. Then the plant was grinded and the powder was prepared by commercial electrical steel blender. The extract was ready to use.

3.7. Preparation of Extract

3.7.1. Maceration: Methanol is taken as a solvent because more photochemical were soluble in methanol. It has higher absorbency. 2 kg plant is taken with 4 liters of solvent (methanol) for 5 days in the can. Soaked the plant in the methanol in the air tight container and stored. The lid of can was tightly closed with aluminum foil and left at room temperature in the laboratory to avoid evaporation of solvent. Every day container was occasionally shacked to mix the powder with the solvent.

3.7.2. Filtration: The soaked material was filtered through the many layers of muslin cloth after 5 days, for 3 times for coarse filtration and again through filter paper to obtain particle free filtrate solution. The extract was left at the bottom and the powder is at the top, then the filtrate was gathered.

3.7.3. Extraction: The plant was extracted by rotary evaporator. Firstly, distilled water was filled in the warm water bath. The filtrate solution was put in the round boiling flask meanwhile attach the boiling flask with sample. Vacuum was closed carefully. The rottery evaporator was turned on. Then the temperature was increased at 65-degree C. So that the solvent was heated, methanol was evaporated and converted into liquid form; it was ensured that solvent had been reduced to one third of its actual volume at low pressure. After this the heating bath was turned off, increased the boiling flask released the vacuum pressure. Only the extract was left behind. The extract was put into the petty dishes and the round boiling flask was removed from the clamp. The petri dish was put into the hot air oven for the 3 days for drying. The extract produced was quite sticky like paste and had blackish green color. It was stored in the 0 degree C temperature. The yield measured in weighing balance was grams.

3.7.4. Storage of the Extract: The extract was stored in the bottle and kept in the refrigerator for 4 degree celcius.

3.8. Phytochemical Analysis

The phytochemical compounds present in the plant extract were tested by using the analyzing photochemical constituent method.

3.8.1. Determination of Total Phenolic Compounds (TPC): Following the procedure of Singleton et al, [23], Folin Ciocalteu method was used for evaluating the total phenolic content. 0.5 ml of the sample was used with the 250 µL of Folin- Ciocalteu reagent with 750 µL of 20% sodium carbonate solution and it made a solution of 5ml with the distilled water. With the help of streptophotometer the absorbance was recorded after 2 hours at 765 nm.

3.8.2. Determination of Total Flavonoid Content (TFC): Following the procedure of Zou et al. [25], caloric method was used for evaluating the total flavonoid content. 50 µL of extract was used with the 2ml of distilled water with 0.15ml of 5% $NaNO_2$ solution and it made a solution of 5ml with the distilled water. The absorbance was recorded at 510 nm.

3.8.3. DPPH Radical Scavenging Assay: The DPPH radical scavenging assay was evaluated by using the procedure of Muller et al., [14]. Keeping the sample at the room temperature for 30 mins, in the 4ml of sample 1ml DPPH was added. With the help of spectrophotometer the absorbance was recorded at 520 nm.

3.9. Experimental Animal

Albino rats weighing 130-200g were selected for the study and purchased from the Biochemistry Department of University of Veterinary & Animal Sciences Lahore. The rats were carried out in the Animal House of Punjab University College of Pharmacy. The rats were kept in neat and clean environment and in pathogenic free cages. The bedding of the cages was changed on daily basis. The temperature of the animal house was 22 Degree Celsius and the humidity was 55%. Throughout the experiment, the rats were kept at natural photoperiod of 12 hours light and 12 hours darkness. All rats received water and food for the 2 weeks for the onset of the experiment to estimate the normal growth and behavior. Institutional guidelines were kept in mind while performing the experiment.

3.10. Acute Oral Toxicity Determination

This toxicity test was executed by OECD guidelines given, on the rats 12 hours fasting and healthy to check the toxicity level of extract before conducting full experiment [17]. The groups (test) of the rats were observed with 50mg/kg to 4000mg/kg dose for the extract, on the other hand control group only got vehicle. The both groups test and control were examined for acute symptoms of toxicity or the behavior changes from 30 minutes to 4 hours, for mortality upto 72 hours. The rats were examined for 14 days to consider any changes or death [10].

3.11. Extract and Drug Dose: Preparation and Administration

The extract and drug dose study was performed as per guidelines of the OECD.

3.12. Baseline Parameters Measurement

1. Blood glucose level (BGL)

2. Body weight

3.13. Hypoglycemic Activity Measurement (n = 30)

• Group 1 (Normal Control): 5 Rats

• Group 2 (Diabetic Control): 5 Rats

• Group 3 (Standard Drug Treatment): 5 Rats – 5mg/kg body weight of Glucophage (once morning)

• Group 4 (Extract Treatment): 15 Rats – On 3 sub-groups

a. Sub-group A: (5 Rats – 250mg/kg)

b. Sub-group B: (5 Rats – 500mg/kg)

c. Sub-group C: (5 Rats – 750mg/kg)

Note

The blood was gathered from the heart of the rats by cardiac puncture on the 21st day and it was supposed to place at the room temperature for the clotting and then for the purpose of serum separation stored at 4 °C at 3000 rpm. The animals were

given mild anesthesia and their liver, kidney and pancreas was anatomize and stored at −20 °C after rinsing with cold saline for further studies.

3.14. Route of Administration

The Streptozotocin solution was given by intraperitoneal injection whereas extract and tab glucophage were given orally with the help of dossier.

3.15. Preparation of Different Doses

• Streptozotacin was given at a dose of 55mg/kg

• Extract of Asphodelus tenuifolius was administered at three different doses 250mg/kg, 500mg/kg and 750mg/kg.

• 500mg/kg dose of Glucophage was given.

• Glucose solution was orally administered at dose 2g/kg.

3.16. Induction of Diabetes

The diabetes was induced by the intraperitoneal injection 55mg/kg body weight of strepzotocin, fresh 0.1 M Citrate Buffer in the rats fasted overnight. After the 12 to 14 days of the induction of streptozotocin rats were showed the blood glucose level more than 250 mg/dl and were analyzed as diabetic [7, 16, 21].

By following the method mentioned above, streptozotocin was induced in the 12 hours fasted rats. The dose was injected intraperitoneally. After that, the weight of the rats was checked for the calculation of the dose of streptozotocin. The blood glucose of the rats was measured through tail vein. Then the streptozotocin was dissolved in the saline and induced immediately. The rats were gripped in the hands and the site of injection was swabbed with the povidine iodine solution to inject streptozotocin. The sterile insulin syringe was used to calculate the streptozotocin amount that was injected in the abdominal cavity of the rats. After the streptozotocin induction, the food and water were provided to rats. After 48 hours of induction of streptozotocin the blood glucose levels of the rats were measured by glucometer. The rats with blood sugar of more than 200mg/dl were considered diabetic.

3.17. Dosing

The extract of plant was given to rats daily with the help of dossier and blood glucose was checked on alternate days by glucose strips of glucometer for 21 days.

3.18. Oral Glucose Tolerance Test

Oral Glucose Tolerance Test was performed on the rats that are fasting for 12 hours. The rats were separated into 6 groups & after half hour examination of extracts, glucose was observed at dose of 2g/kg. The analysis of the glucose for blood was done at half, 1, 2 & 3 hours before by using glucose oxidase peroxidase strips and glucometer [6].

3.19. Euthanasia of Rats

The rats were starved for the whole night and enthanized by using chloroform anesthesia at the end of the experiment. The rats were enthanized by the use of the possible acceptable techniques, agents and equipements by the competent individual keeping in mind IACUC protocols. Rats were not merged during enthanasia. Enthanasia was not taken place before other rats. To avoid stress in the rats, the rats that were waiting for sacrifice were kept in the separate place until they were enthanized. Chloroform was put on the cotton pad and left in the glass jar. Rat was placed in the glass jar and covered in a proper way. After this, rat became unconscious within 2 minutes. The fading eye color and breathing of the rat were examined.

3.20. Sample Collection

Blood sample was taken from the rats through cardiac puncture into pretreated vial of ethylene diamine tetra acetic (EDTA) acid. Organs of the rats such as pancreas and kidney were preserved in 10% formalin solution and sent for the histopathological studies.

3.21. Plan for Data Management and Analysis

Statistics 8.1 was used for data entry and analysis after consultation with a statistician. The p value was calculated and a value <0.050 was considered significant. Appropriate statistical test was applied for different variables analysis and data was presented with the help of tables, charts and graphs where necessary.

3.22. Ethical Consideration

The study was organized by following the animal care and handling criteria given by the Animal Ethical Committee which were planned by using the guidelines of National Institute of Health, Islamabad. Written consent was taken from the Institutional Review Board (IRB) of the University of the Punjab, Lahore, Pakistan (Table 1).

Table 1: Treatments with different interventions

Normal Control (5 rats)	Diabetic Control (5 rats)	Standard Drug Treatment (5 rats)	Extract Treatment (15 rats)		
T_0	T_1	T_2	T_3	T_4	T_5
Normal Saline	No Extract Given	Standard Drug Glucophage	250mg/kg	500mg/kg	750mg/kg

$T_{0=}$ Normal Control
$T_{1=}$ Disease Control
$T_{2=}$ Standard Drug Control
$T_{3=}$ Extract Treatment 250mg
$T_{4=}$ Extract Treatment 500mg
$T_{5=}$ Extract Treatment 750mg

4. Results

4.1. Antioxidant Assay

4.1.1. Total Phenolic Content: The total phenolic content was observed as 508.18 mg GAE/g extract in the Asphodelus tenuifolius.

In the study Khalfaoui et al., [7] reported that Asphodelus Tenuifolius plant, chloroform extract have rich phenolic content 40.99 mg GAE/g , Ethyl Acetate extract phenolic content is 24.04 mg GAE/g, the butanol extract have the lowest phenolic concentration in this extract 10.54 mg GAE/g.

Mahboub, Slimani, Hechifa, Merad, & Khelil [12] stated that total phenolic content that is 101.82 µg GAE/g observed from lyophilized samples of Asphodelus tenuifolius harvested from septentrional Algerian Sahara.

On the other hand, Munir, Sarfraz, Hussain, Shahid & Sultana [15] observed Asphodelus tenuifolius from Pakistan with total phenolic contents ranging from 53.40 to 76.23 mg GAE/g for different solvent extracts.

Al-Laith, Alkhuzai & Freije [2] reported the data on Asphodelus tenuifolius from Bahrain with total phenolic contents ranging from 139.66 to 442.44 mg GAE/g for harvested sites.

Kalim, Bhattacharyya, Banerjee & Chattopadhyay [8] executed a study to evaluate the antioxidant capacity of the plants that are used in the Unani system of medicine. In the results he concluded that Asphodelus Tenuifolius total phenolic content is 15.74 ± 0.98 mg GAE/g.

4.1.2. Total Flavonoids Content: The total flavonoids content was observed as 94.29 mg RutinE/g extract in the Asphodelus tenuifolius.

In the study [7] reported that Asphodelus Tenuifolius plant, chloroform extract have the highest flavonoid content 213.07 mg CE/g, Ethyl Acetate extract flavonoid content is 202.89 mg CE/g, the butanol extract have the lowest flavonoid content 62.85 mg CE/g.

Mahboub, Slimani, Hechifa, Merad, & Khelil [12] found a good level of flavonoid contents with a value of 16.10 µg QE/g observed from lyophilized samples of Asphodelus tenuifolius harvested from septentrional Algerian Sahara.

On the other hand, Munir, Sarfraz, Hussain, Shahid & Sultana, [15] stated other data on Asphodelus tenuifolius from Pakistan found flavonoid contents in the range of 165.82 to 312.12 mg QE/g for different solvent extracts.

Kalim, Bhattacharyya, Banerjee & Chattopadhyay [8] executed a study to evaluate the antioxidant capacity of the plants that are

used in the Unani system of medicine. In the results he concluded that Asphodelus Tenuifolius total flavonoid content is 11.98 ± 0.74 mg QEE/g.

4.1.3. DPPH Assay: The DPPH assay was observed from 100 to 750 ppm as 132.65, 132.03, 135.39, 136.31, 135.70, 138.45, 147.61, 141.81, 143.94 and 175.70 mg AAE/g extract in the Asphodelus tenuifolius.

Kalim, Bhattacharyya, Banerjee & Chattopadhyay [8] executed a study to evaluate the antioxidant capacity of the plants that are used in the Unani system of medicine. In the results he concluded that Asphodelus Tenuifolius DPPH value is 2.006 ± 0.002 µg/ml.

In the study [7] reported that Asphodelus Tenuifolius plant, chloroform extract have the DPPH value IC50 25 µg/mL, Ethyl Acetate extract DPPH value is IC50 45 µg/mL, the butanol extract have the DPPH value IC50 92 µg/mL (Table 2).

4.2. Bio-evaluation Trial

The study was conducted on the albino rats; the body weights and glucose levels were measured before and after the induction of the diabetes via streptozotocin. The rats were divided into six groups; normal control, disease control, standard drug control, and other three were of extract treatment at 250mg, 500mg and 750mg respectively. After the induction of diabetes in rats, the Asphodelus tenuifolius was given to the rats as extract treatment at different levels to check which one is effective in lowering the glucose level in rats (Table 3).

Table 2: Total Phenolic & Flavonoids content mean absorbance and mg GAE/g extract values of Asphodelus tenuifolius

Sample	Total Phenolic Content (1000 ppm)	Total Flavonoids Content (1000 ppp)
Mean absorbance	2.59	0.118
Extract	508.18±18.26 mg GAE/g	94.29±3.5 mg RutinE/g

Table 3: DPPH Assay with mean absorption and mg AAE/g extract of Asphodelus tenuifolius

Sr. No	Conc. Ppm	Mean Absorption	% Inhibition	Mg AAE/g Extract
1	100	0.319	32.42	132.65
2	150	0.32	32.28	132.03
3	200	0.316	33.05	135.39
4	250	0.315	33.26	136.31
5	300	0.316	33.12	135.7
6	350	0.313	33.76	138.45
7	400	0.303	35.87	147.61
8	450	0.309	34.53	141.81
9	500	0.307	35.02	143.94
10	750	0.272	42.35	175.7

4.2.1. Body Weights: The initial body weight was measured for the groups in grams. The body weight of T0, T1, T2, T3, T4 & T5 was 255±11, 199±8, 179±7, 241±10, 192±6 and 260±11 grams respectively.

The weights on week 2 were recorded for T0, T1, T2, T3, T4 & T5 as 241±10, 178±7, 189±8, 135±5, 134±4 and 231±10 grams respectively.

Rath, Kar, Panigrahi & Maharana [19] conducted a study to evaluate the antidiabetic effect in the rats and he concluded that in control group the weight was gradually decreasing till the end of the research. Then the diabetic rats were given 200 and 400 mg/kg of dose which showed the gain in the body weight as compared with the metmorfin treated group.

Pandit, Phadke & Jagtap [18] executed a study on the antidiabetic effect on the rats and he concluded that the weight was decreased in the streptozotocin induced rats as compared with others. Given plant at all the doses such as 25, 50 and 100mg/kg showed the improvement in the weight as compared to diabetic control group which loses the weight of the rats till the end of the study.

4.2.2. Glucose Levels: The initial glucose level was measured for the groups in mg/dl. The glucose levels of T0, T1, T2, T3, T4 & T5 was 87±3, 62±2, 62±1, 56±2, 63±2 and 69±1 mg/dl respectively.

The glucose levels increased after the induction of the streptozotocin in the rats. The glucose levels on week 2 were recorded for T0, T1, T2, T3, T4 & T5 as 92±3, 307±14, 349±11, 169±7, 254±11 and 159±6 mg/dl respectively.

Rath, Kar, Panigrahi & Maharana [19] conducted a study on the antidiabetic effect on the rats and at the end of the study he concluded that, hyperglycemia was controlled in the treated groups. The metformin was also useful in showing the significant results for decrease in blood glucose level (Figure 1-13).

Pandit, Phadke & Jagtap [18] concluded a study on streptozotocin induced diabetic rats. It was concluded that high blood serum level was decreased on 21st day of the study in rats. The glibenclamide

was also useful in showing the significant results compared with diabetic control for lowering the blood glucose level.

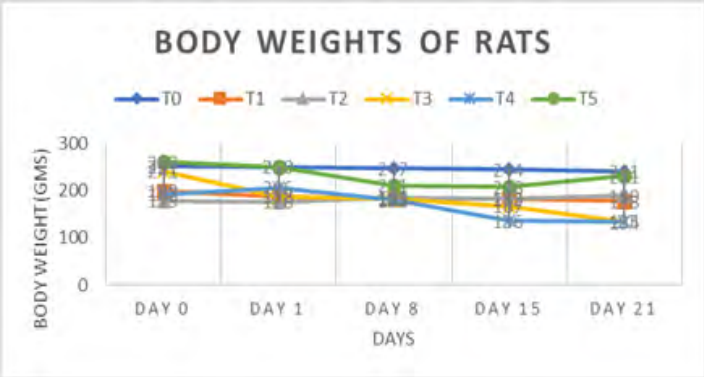

Figure 1: Difference between the body weights after the induction of diabetes

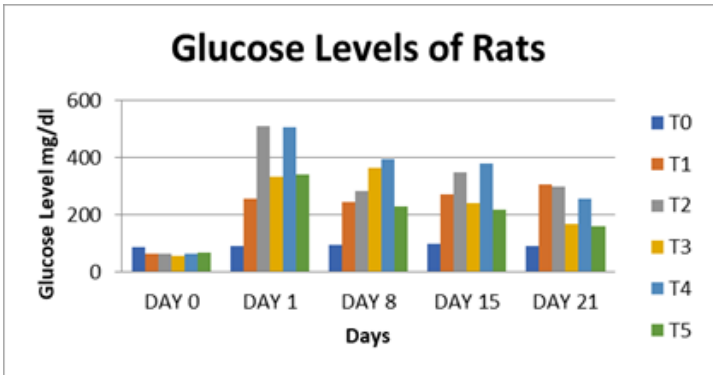

Figure 2: Difference between the glucose levels after the induction of diabetes

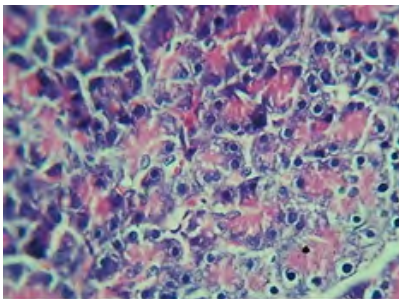

Figure 3: Pancreatic Histopathology of T_0 (Normal Control)

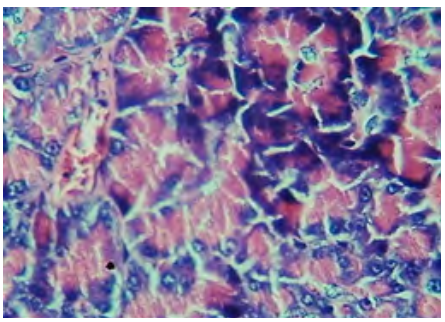

Figure 4: Pancreatic Histopathology of T_1 (Disease Control)

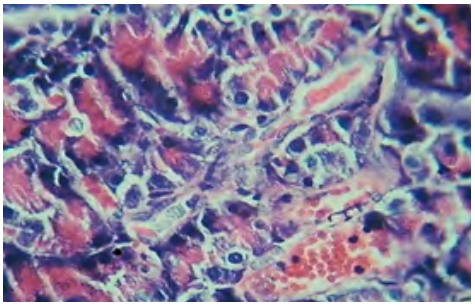

Figure 5: Pancreatic Histopathology of T_2 (Drug Control)
(For treatment, extract of ASPHODELUS TENUIFOLIUS (AT) is used)

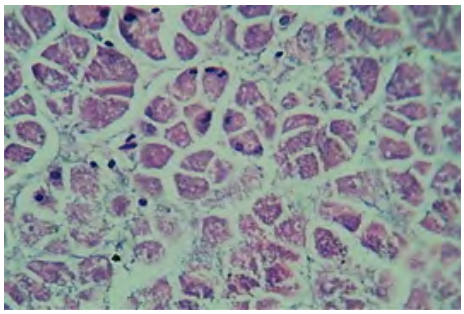

Figure 6: Pancreatic Histopathology of T_3 (AT methanolic extract 250mg/kg)

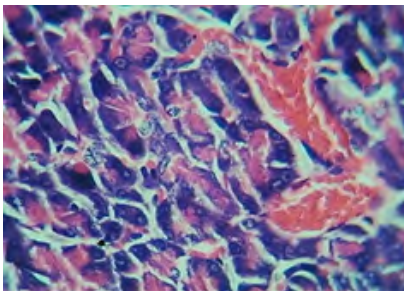

Figure 7: Pancreatic Histopathology of T_4 (AT methanolic extract 500mg/kg)

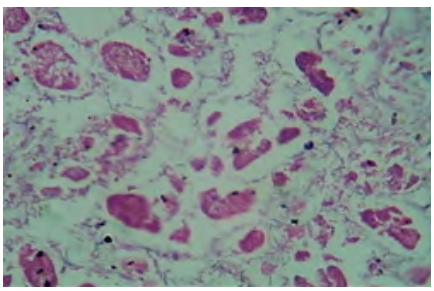

Figure 8: Pancreatic Histopathology of T_5 (AT methanolic extract 750mg/kg)

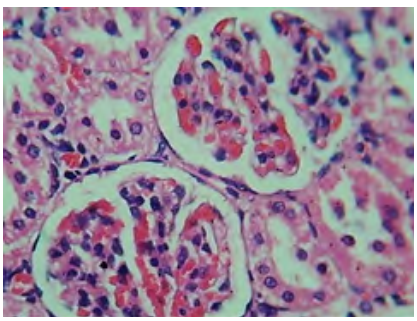

Figure 9: Kidney Histopathology of T_0 (Normal Control)

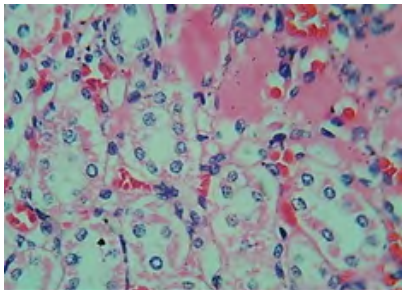

Figure 10: Kidney Histopathology of T_1 (Disease Control)

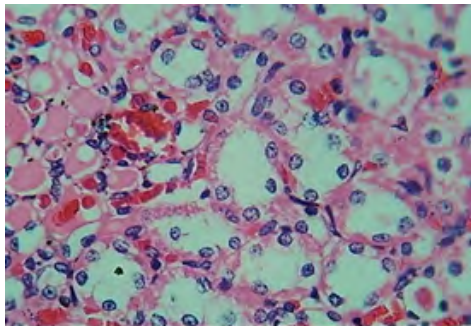

Figure 11: Kidney Histopathology of T_2 (Drug Control)
(For treatment, extract of ASPHODELUS TENUIFOLIUS (AT) is used)

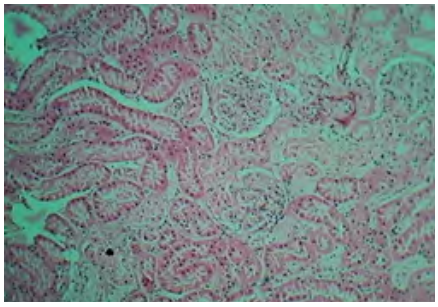

Figure 12: Kidney Histopathology of T_3 (AT methanolic extract 250mg/kg)

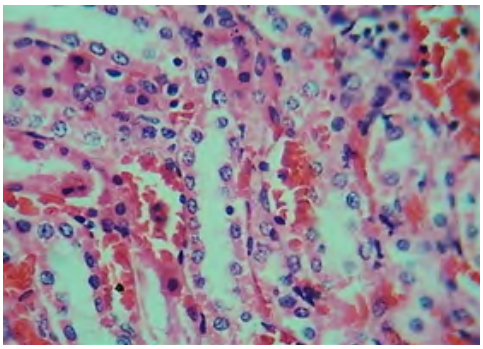

Figure 13: Kidney Histopathology of T_4 (AT methanolic extract 500mg/kg)

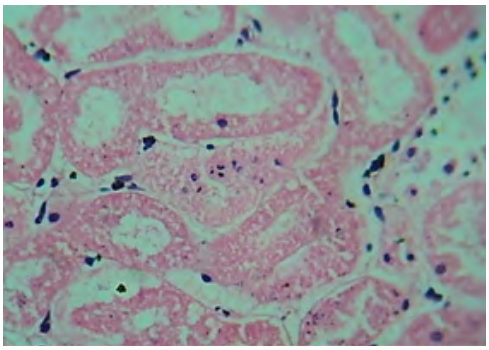

Figure 14: Kidney Histopathology of T_5 (AT methanolic extract 750mg/kg)

4.3. Pancreas

4.3.1. Pancreas histopathology:

(a) Normal control: Normal rats showing intact architecture. There are normal islets of langerhans. Exocrine and endocrine portions are present. There is no inflammation, no fibrosis. Hemorrhage and necrosis are not seen.

(b) Disease control: Diabetic untreated rats showing altered architecture with shrinkage of islets of langerhans. Exocrine and endocrine portions are present. There is mild chronic inflammation present. There is no fibrosis. Hemorrhage and necrosis are not seen.

(c) Drug control: Metformin treated rats showing intact architecture with normal islet of langerhans. Endocrine and exocrine portion are present. There is mild chronic inflammation. There is no fibrosis, hemorrhage and necrosis seen (Table 4).

(For treatment, extract of ASPHODELUS TENUIFOLIUS (AT) is used)

(d) AT (methanolic extract) 250mg/kg showing altered architecture, Islets of langerhans are damaged and leads to shrinkage. Exocrine portion is normal while endocrine portion is degenerated. There is no inflammation in this group of rats. No fibrosis, hemorrhage and necrosis seen.

(e) AT (methanolic extract) 500mg/kg showing intact architecture with shrinkage of islet of langerhans. Exocrine portion is normal while endocrine portion is shrinked. There is no chronic inflammation present. Hemorrhage, fibrosis and necrosis are not seen.

(f) AT (methanolic extract) 750mg/kg show intact architecture,with shrinkage of Islet of langerhan. Exocrine portion is normal while endocrine portion is also shrinked in this group. There is no inflammation and hemorrhage. Fibrosis and necrosis are not seen (Table 5-7).

Table 4: Mean squares for Body Weights

Source	DF	DAY 0	DAY 1	DAY 8	DAY 15	DAY 21
Groups	5	4443.88^{NS}	3830.74^{NS}	2297.06*	2448.05*	4733.38*
Error	16	1831.9	1023.94	690.69	1167.19	1494.03
Total	21					

Table 5: Effect of Asphodelus tenuifolius based dietary treatments on body weights

Treatments	DAY 0	DAY 1	DAY 8	DAY 15	DAY 21
T0	251±10 ab	250±9 a	247±7 a	244±11 a	241±9 a
T1	199±8 abc	188±7 b	182±7 b	182±6 b	178±5 ab
T2	179±8 c	176±7 b	185±6 b	183±5 b	189±3 ab
T3	241±10 abc	188±8 b	183±6 b	167±5 b	135±2 b
T4	192±7 bc	206±9 ab	180±5 b	136±4 b	134±6 b
T5	260±12 a	250±8 a	211±5 ab	208±7 ab	231±2 a

Table 6: Mean squares for Glucose Levels

Source	DF	DAY 0	DAY 1	DAY 8	DAY 15	DAY 21
Groups	5	478.714^{NS}	112940^{NS}	46791.4*	42522.8*	46409.9*
Error	16	86.598	19487	12582	22296.7	21089.4
Total	21					

Table 7: Effect of Asphodelus tenuifolius based dietary treatments on glucose levels

Treatments	DAY 0	DAY 1	DAY 8	DAY 15	DAY 21
T0	87±3 a	92±4 c	96±1 c	98±3 b	92±2 c
T1	62±2 b	255±9 bc	243±10 abc	271±12 ab	307±11 a
T2	62±2 b	512±13 a	282±13 ab	347±8 a	299±4 ab
T3	56±2 b	333±11 ab	365±12 ab	240±11 ab	169±7 b
T4	63±1 b	505±14 a	394±12 a	381±15 a	254±8 ab
T5	69±2 b	339±9 ab	229±10 bc	216±8 ab	159±7 b

4.4. Kidney

4.4.1. Kidney histopathology

(a) Normal control: Normal rats showing the normal shape and ratio of cortex and medulla. The shape and number of glomeruli is normal. The shape and size of renal tubules is normal. There is no inflammation in normal rats. Deposition and necrosis are not seen. There is no edema and vascular congestion.

(b) Disease control: Diabetic untreated rats showing normal shape and number of glomeruli. The shape and ratio of cortex and medulla is normal. The shape and size of renal tubules is normal. There is no inflammation, edema and vascular congestion. Deposition and necrosis are not seen.

(c) Drug control: Metformin treated rats showing normal shape and ratio of cortex and medulla. The shape and number of glomeruli is normal. Renal tubules shape and size is normal.

Deposition and necrosis are not seen. There is no acute or chronic inflammation, edema and vascular congestion.

(For treatment, extract of ASPHODELUS TENUIFOLIUS (AT) is used)

(d) AT (methanolic extract) 250mg/kg showing normal shape and structure of glomeruli, normal shape and ratio of medulla (inner part) cortex (outer part). Altered shape and size of renal tubules. There is moderate chronic inflammation present. Edema is seen. Deposition and vascular congestion are not seen. Focal tubular necrosis is seen.

(e) AT (methanolic extract) 500mg/kg showing normal shape and structure of glomeruli, normal shape and ratio of medulla (inner part) cortex (outer part). Normal shape and size of renal tubules. There is no chronic inflammation present. Edema is seen. Deposition, necrosis and vascular congestion are not seen in this group of rats.

(f) AT (methanolic extract) 750mg/kg, Rats in this group show the good response at 750mg/kg dose among the other two groups as there is normal shape and ratio of cortex and medulla. Normal shape and number of glomeruli. Normal shape and size of renal tubules. There is no chronic inflammation present. Deposition is not seen. Edema is not seen. Vascular congestion, Necrosis and Hemorrhage are not seen.

5. Conclusion

From this study we conclude that Asphodelus tenuifolius helps to lower the blood glucose levels. It also helps to maintain the

body weights. The high TPC & TFC content was observed such as 508.18 mg GAE/g extract & 94.29 mg RutinE/g extract resprectively. Anti-inflammatory activity of the plant was observed. It also has an anti-oxidant potential.

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