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Antidiabetic Effects of *Foeniculum vulgare* in Alloxan-induced Diabetic Male Rats

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ABSTRACT

Background & objective: Diabetes mellitus (DM) has become one of the most challenging health problems of the 21st century. *Foeniculum vulgare* (Mouri in Bengali), an ancient culinary herb, is known to reduce blood glucose levels thus reducing the risk of diabetes. This study was done to observe the antidiabetic role of Mouri in alloxan-induced diabetic male rats.

Methods: This experimental study was carried out in the Department of Physiology, Sir Salimullah Medical College (SSMC), Dhaka from July 2018 to June 2019. A total of 30 healthy *Wistar albino* male rats, 90-120 days old, weighing between 150-180g were included in the study. After acclimatization for 14 days, they were divided into two groups: the control group (Group A) and the experimental group (Group B – diabetic rats treated with Mouri). The control group was again subdivided into Group A1 (normal control group) and Group A2 (alloxan-induced diabetic control group). Each of these groups consisted of 10 rats. All the rats received a basal diet for 21 days. In addition to the basal diet, Group A2 and Group B received a single intraperitoneal injection of alloxan 140 mg/kg on day 1 to induce diabetes. Moreover, Group B received Mouri extract 150 mg/kg/day orally for 21 consecutive days starting from day 1 of the study period. After 12 hours of fasting, blood samples were collected from the tail veins of every rat on day 1 for estimation of Fasting Blood Glucose (FBG) and serum ALT levels. On day 4 FBG levels of all the rats were measured once again. Then all the rats were sacrificed on day 22 and their blood samples were collected from the heart. The outcome variables, such as FBG level, serum levels of insulin, were measured and compared among the study groups. The pancreatic tissue was also collected and histopathology was done by standard laboratory procedure to study their histologic architecture.

Result: In this study, fasting blood glucose level in diabetic rats treated with Mouri at the endpoint of the study (on day 22) was significantly lower (5.51 ± 0.47 mmol/L) than that in alloxan-induced diabetic control (13.52 ± 0.76 mmol/L) ($p < 0.001$) and was almost equal to that in normal control (5.38 ± 0.55 mmol/L) ($p = 0.891$). The serum insulin level in diabetic rats treated with Mouri (11.52 ± 0.84 mmol/L) was almost similar to that of normal control (12.46 ± 1.07 mmol/L) on day 22, whereas it was much higher than that in alloxan-induced diabetic control (8.51 ± 0.68 mmol/L) ($p < 0.001$). Histological study of pancreases of the rats, revealed extravasation of blood in the pancreatic acini, reduced area of islets of Langerhans with atrophy, vacuolation, degeneration, and pyknosis of β-cell nuclei and centrilobular necrosis in the alloxan-induced diabetic control rats.

Conclusion: Mouri (*Foeniculum vulgare*) improves the glycemic status of the alloxan-induced diabetic rats.

Key words: Diabetes mellitus, alloxan, mouri, *Wistar albino* rats, FBG level, serum insulin etc.

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INTRODUCTION:

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, action, or both so that glucose in the blood cannot be absorbed into the cells.[1] The most common acute complications associated with diabetes include diabetic cardiomyopathy, neuropathy, nephropathy, retinopathy, and vasculopathy leading to coronary artery and peripheral vascular diseases.[2] Worldwide the prevalence of diabetes mellitus is increasing day by day and is expected to increase to 592 million by the year 2035.[3] Many drugs such as insulin, sulfonylureas, metformin, and glitazones are used to achieve control over the disease. However, the failure of these drugs to control the disease with side effects & increased management costs has resorted many people to traditional herbal medicines.[4,5] The advantages of herbal medicines are their efficacy, low incidence of side effects, and low cost.[5] For this reason, neem (margosa), tulsi, etc. have been very popular in the treatment of diabetes.[6,7]

Foeniculum vulgare is a member of the family Apiaceae, derived from the Latin word Foenum which means hay. It is a hardy, umbelliferous herb, with yellow flowers and feathery leaves, growing wild in most parts of Europe, but is generally considered indigenous to the shores of the Mediterranean. *Foeniculum vulgare* contains vitamins like niacin, pyridoxine, riboflavin, vitamin A, vitamin C etc., and minerals like calcium, copper, iron, zinc, magnesium, etc.[8] Among the main components of *Foeniculum vulgare*, are trans-anethole, flavonoids, limonene and α-pinene.[9,10] *Foeniculum vulgare* is chiefly known as a culinary herb, commonly used as a household remedy for various medicinal purposes.[11] Mature fruits and oil of *Foeniculum vulgare* are used as a constituent of pharmaceutical & cosmetic products. *Foeniculum vulgare* has shown anti-inflammatory, antioxidant, and antispasmotic activities.[12,13] Flavonoid increases insulin secretion[14] and have anti-oxidants[10,15] and hepatoprotective effects.[16] Trans-anethole ameliorates hyperglycemia by regulating key enzymes of carbohydrate metabolism.[17]

Recently, it has been observed that *Foeniculum vulgare* extract has improved metabolic disturbances and oxidative stress that are associated with diabetes.[18] Another study showed *Foeniculum vulgare* improved the histological structure of islets of Langerhans of pancreatic beta cells and caused hypoglycemia in diabetic rats.[19] Alloxan is known as a cytotoxic glucose analog and a prominent diabetogenic agent, which could induce diabetes by causing necrosis of pancreatic beta cells in experimental animals, leading to the decrease in size of the islets of Langerhans.[20] Free radicals generated by alloxan inhibit glucokinase thereby decreasing glucose-induced insulin secretion with consequent hyperglycemia.[21] Alloxan-induced diabetic rats treated with *Foeniculum vulgare* showed a significant decrease in blood glucose levels with a hepatoprotective effect.[22] However, limited studies done on its anti-diabetic efficacy and safety in human beings limit generalization of its findings to reference populations. Besides, no such study on its anti-diabetic activities has been conducted in our country. Hence, this study was designed to observe the antidiabetic effects of *Foeniculum vulgare* in alloxan-induced diabetic male rats.

METHODS:

This Experimental study was conducted in the Department of Physiology, Sir Salimullah Medical College, Dhaka over a period of 1 year from 1st July 2018 to 30th June 2019. A total of 30 healthy *Wistar albino* male rats, aged 90–120 days weighing from 150–180 g were included in the study. Rats were purchased from the animal house of the Department of Pharmacy, Jahangir Nagar University, Savar, Dhaka. The study commenced by obtaining ethical clearance from the Institutional Ethics Committee, Sir Salimullah Medical College, Dhaka. All the rats were kept in the animal house of the Institute of Nutrition and Food Science, Dhaka University where the experiment was carried out. After acclimatization for 14 days, rats were randomly divided into two groups – Group A and Group B. Group A (the Control group), consisted of 20 rats. This group was again subdivided into Group A1 (Normal control group) consisting of 10 rats and Group A2 (Alloxan-induced diabetic control group) consisting of 10 rats.

Animal preparation and inclusion:

Before intervention, the rats were kept at 23 ± 2°C room temperature for 14 days under 12-hourly dark-light cycle. During this period, the animals had free access to food and water *ad Libitum*. The total period of study was 21 consecutive days counted from the day of intervention. At the beginning of the study period (day 1) initial body weight was measured and at the end of the study period (day 22), their final body weight was measured. After 12 hours of overnight fasting, on day 1 (before alloxan injection) blood samples (approximately 1 ml) were collected from the tail veins of all the rats by aseptically severing the tip of the tails with a sharp sterile blade to estimate fasting blood glucose levels. Rats with normal levels of fasting blood glucose were included in the experiment.

Intervention:

The Group A1 received a basal diet and normal saline 20 ml/kg/day orally for 21 days starting from day 1 of the study period. In addition to the basal diet, Group A2 and Group B received a single intraperitoneal injection of alloxan 140 mg/kg on day 1 to induce diabetes. Besides, Group B received Mouri extract 150 mg/kg/day by oral gavage for 21 consecutive days in the morning between 9-10 AM (starting from day 1 of the study period).

Follow-up, lab procedures and measurement of outcome variables:

On day 4, the fasting blood glucose levels of all the rats were again measured. Then rats with fasting blood glucose levels 11-20 mmol/L were selected for this experiment. On day 22, all the rats were anesthetized with the help of chloroform (30%) and were sacrificed. Then blood samples (approximately 5 ml) were collected from the heart using a sterile disposable syringe and were taken in separate clean and dry test tubes with proper identification numbers. The test tubes were kept in a standing position till clotting of blood occurred. Then blood was centrifuged at a rate of 4000 rpm for 10 minutes. After that, supernatant serum was collected in a labeled eppendorf tube and preserved in a deep freeze for biochemical analysis. For the assessment of glycemic control, fasting blood glucose and serum insulin levels were measured. All the above-mentioned biochemical investigations were performed in a semi-autoanalyzer in the Department of Physiology, SSMC except serum insulin level, which was estimated in the Department of Biochemistry, BSMMU. To find the histopathological changes of pancreatic tissue, histological slides were prepared, and observed under the microscope and photomicrographs were taken in the Department of Pathology, SSMC.

Collection of pancreas and liver samples:

The pancreases of the sacrificed rats were collected and washed in ice-cold saline and wiped on tissue paper. The weight of the pancreas was measured by an electric balance analyzer. Then pancreas samples were fixed in 10% formalin for subsequent histological processing. Preparation of the pancreas for histological examination was done according to Ambrogi's (1960) method. Histological findings were categorized by normal and abnormal changes according to Adeyemi et al.[23] as follows:

Histopathological findings of the pancreas

Normal	Mild change	Moderate change
Normal pancreatic acini	Loss of integrity of β-cell membrane	Extravasation of blood in pancreatic acini
The normal size of the area of Islets of Langerhans	Intracellular micro vacuolation	Decreased size of the area of islets of Langerhans
Normal β-cell membrane	Pale cytoplasm	Pyknosis of β-cell nucleus
Normal β-cell nucleus		

Laboratory investigations:

Fasting blood glucose level was measured by glucose oxidase (GOD-POD) method in a semi-autoanalyzer. Serum insulin level was measured by the chemiluminescent micro-particle immunoassay (CMIA) method in the ARCHITECT Plus ci4100 system.[24] The variables measured to evaluate the outcome were fasting blood glucose & serum insulin.

Data analysis:

Statistical analysis was done using (Statistical Package for Social Science) SPSS for Windows, version 22. While data presented on a continuous scale were expressed as mean ± SD and were compared between two groups using an Unpaired t-Test, the categorical data were expressed as frequencies and corresponding percentages & were compared between groups using Chi-square (χ^2) Test. The ANOVA test was performed for the comparison of continuous data among three groups with a post-hoc Hochberg test being done to make an intergroup comparison. The level of significance was fixed at 5% and a p-value<0.05 was considered significant.

RESULTS:

The mean fasting blood glucose level on day 1 was almost similar in all groups (p=0.698). However, on day 4 this level was significantly higher in Group A_2 and Group B compared to that in Group A_1 (p<0.001 in each case), whereas this level was almost similar between Groups A_2 and B (p=0.568). However, the mean FBS level on day 22 was significantly dropped in group B (5.51 ± 0.47 mmol/L) compared to that in group A2 (p < 0.001) and was almost similar to that in group A1 (p = 0.891). The mean serum insulin was significantly lower in group A2 in comparison to group A1 (p < 0.001) and group B (p < 0.001) (Table I & II).

Histopathological examination of the pancreas revealed normal findings in all the rats of group A_1 and abnormal findings in all the rats of group A_2. In Group B, 70% of rats exhibited normal histology of the pancreas, and 30% showed mild histological changes. There were significant differences in histopathological findings of the pancreas among the groups of rats (p < 0.001). The detailed histological findings are given in Tables III - IV and pics 1-3.

Table I. Fasting blood glucose and serum insulin levels among different groups of rats on different days of evaluation

Groups	Fasting blood glucose (mmol/L)			Serum insulin (µU/ml)
	Day 1	Day 4	Day 22	Day 22
A1 (n=10)	4.88 ± 0.58	5.14 ± 0.47	5.38 ± 0.55	12.46 ± 1.07
A2 (n=10)	5.08 ± 0.63	11.90 ± 0.55	13.52 ± 0.76	8.51 ± 0.68
B (n=10)	5.06 ± 0.52	11.60 ± 0.47	5.51 ± 0.47	11.52 ± 0.84
p-value	0.698	< 0.001	< 0.001	< 0.001

Data were analyzed using **ANOVA statistics (F)** & were expressed as **mean ± SD.** Group A1: Normal control group; Group A2: Alloxan-induced diabetic control group; Group B: Alloxan-induced diabetic rats treated with Mouri

Table II. Multiple comparisons of FBS and serum insulin by Post-hoc Hochberg test

Groups	Fasting blood glucose			
	Day 1	Day 4	Day 22	Serum insulin
A1 vs A2 vs B	0.698	< 0.001[S]	< 0.001[S]	< 0.001[S]
A1 vs A2	1.000	< 0.001 [S]	< 0.001	< 0.001[S]
A1 vs B	1.000	< 0.001[S]	0.891	0.071
A2 vs B	1.000	0.568	< 0.001[S]	< 0.001[S]

s = significant.

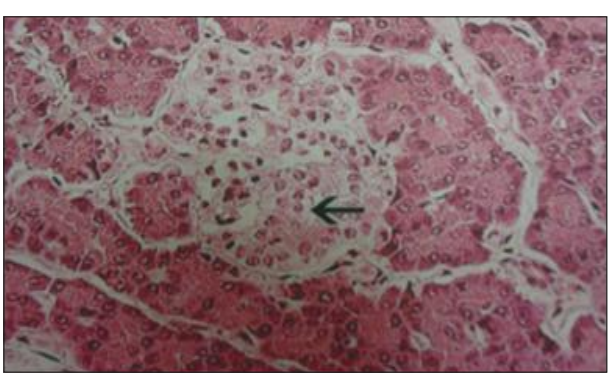
Table III. Histopathological changes in the pancreas of different groups of rats

Group	Observation	Result/Findings
Group A1 (n=10) (Normal control group)	Architecture of -Pancreatic acini -Area of islets of Langerhans - β-cell membrane - β-cell nucleus	Normal histological findings
Group A2 (n=10) (Alloxan-induced diabetic control group)	-Extravasation of blood in pancreatic acini -Pyknosis of β-cell nucleus -The area of islets of Langerhans decreased with atrophy and vacuolation	Moderate histological changes
Group B (n=10) (Alloxan-induced diabetic rats treated with Mouri)	-Less/absence of integrity of β-cell membrane -Normal β-cell nucleus -Normal area of islets of Langerhans	Normal histological findings in 7 rats and mild histological changes in 3 rats

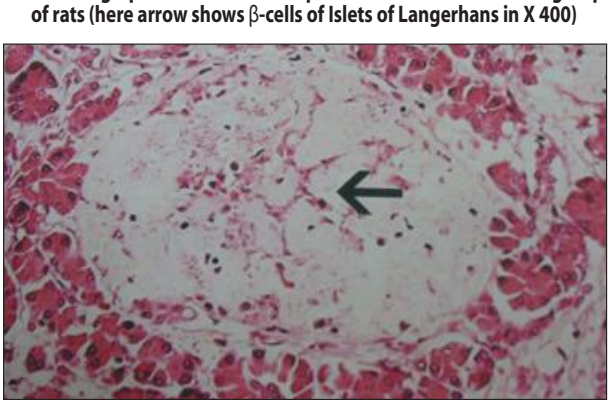
Table IV. Comparison of histopathology of pancreas in different groups of rats

Groups	HP findings		p-value
	Normal	Abnormal	
A1 (n=10)	10(100.0)	0(0.0)	
A2 (n=10)	0(0.0)	10(100.0)	< 0.001
B (n = 10)	7(70.0)	3(30.0)	

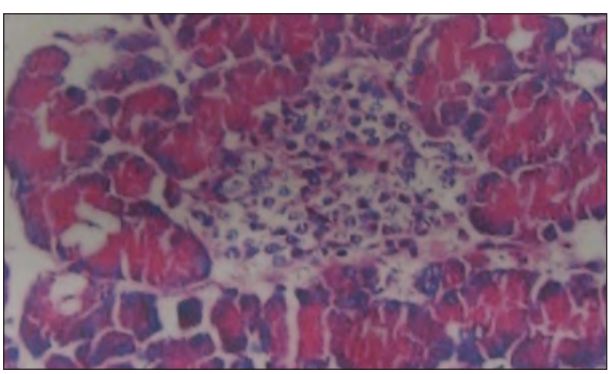
Data were analysed using **Chi-square** (χ^2) and were expressed as **n(%).**



Photomicrograph 1: Architecture of pancreas in a normal control group of rats (here arrow shows β-cells of Islets of Langerhans in X 400)



Photomicrograph 2: Vacuolar changes with pyknosis of some nuclei (arrow mark) of β cells of islets of Langerhans in an alloxan-induced diabetic control rat (X 400)



Photomicrograph 3: Restoration of the normal architecture of islets of Langerhans of the pancreas in a diabetic rat treated with Mouri (X 400)

DISCUSSION

The present study was carried out to evaluate the antidiabetic effects of Mouri (*Foeniculum vulgare*) in alloxan-induced diabetic male rats. For this study, fasting blood glucose and serum insulin levels were estimated to assess the efficacy of Mouri. Moreover, histological examination of pancreatic tissues was also done to observe the microscopical abnormalities in the pancreas and liver.

In this study, the fasting blood glucose level in diabetic rats treated with Mouri at the endpoint of the study (on day 22) was significantly lower than that in alloxan-induced diabetic control and was almost equal to that in normal control. A similar finding was also observed by different researchers.[13,25] In contrast, no significant blood glucose-lowering effect of Mouri was found by some researchers.[26] The serum insulin level study shows

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that diabetic rats treated with Mouri had almost the same level of insulin as that of normal control on day 22, whereas it was much lower in alloxan-induced diabetic controls. Several researchers observed a similar finding.[13,18,27]

In the present study, abnormal histological changes, such as extravasation of blood in pancreatic acini, decreased area of islets of Langerhans with atrophy and vacuolation, degeneration, and pyknosis of β cell nuclei were observed invariably in the alloxan-induced diabetic control group. These findings are also in agreement with those of some other investigators. Whereas 70% of diabetic rats treated with Mouri showed almost normal histological architecture of the pancreas and 30% showed only minimal histological changes indicating that Mouri is safe.[28-31] On the contrary, Ozbek et al.[25] observed no improvement in the pancreatic architecture of diabetic rats, which might be due to the low dose of the supplementation.

CONCLUSION:

From the findings of the study, it appears that *Foeniculum vulgare* can effectively treat alloxan-induced diabetic rats with a reduction of FBS levels to almost that of the normal level after 21 days of treatment. The lower level of fasting blood glucose and the normal level of serum insulin in the diabetic rats treated with Mouri suggest that Mouri extract could improve glycemic status in alloxan-induced diabetic rats. Furthermore, minimal histopathological changes observed in the pancreas of diabetic rats treated with Mouri suggest it is safe for the pancreas.

REFERENCES

1. American Diabetic Association (ADA). Diagnosis and classification of diabetes. *Diabetes Care* 2012;35(1): S64-9.
2. Gao Q, He B, Zhu C, Xiao Y, Wei L, Jia W. Factors associated with lower extremity atherosclerotic disease in Chinese patients with type 2 diabetes mellitus: A case-control study. *Medicine* 2016;95(51).
3. International Diabetes Federation . 6th edn. IDF; Brussels: 2013. IDF diabetes atlas.www.idf.org/diabetesatlas.
4. King H, Aubert RE, Herman WH. Global Burden of Diabetes, 1995-2025, Prevalence, numerical estimates, and projections. *Diabetes Care* 1998;21(9):1414-1431.
5. Gupta S, Mediratta PK, Singh S, Sarma KK, Shukla R. Antidiabetic, anti hypercholesterol aemic and anti-oxidant effect of Ocimum sanctum seed oil. 2006;44:300-304.
6. Das AR, Mostofa M, Hoque ME, Das S, Sarkar AK. Comparative Efficacy of Neem (Azadirachta Indica) and Metformin Hydrochloride (Comet®) In Streptozotocin Induced Diabetes Melitus in Rats. *Bangladesh Journal of Veterinary Medicine* 2010;8(1):75 – 80.
7. United States Department of Agriculture (USDA) Food Composition *Databases*,2018.[online].[viewed19th June 2019].Availablefrom:https://www.ans/usda.gov/nutrition /nutrient/usda-nutrition-database.
8. Kazemi M, Mousavi E, Kharestani H. Chemical compositions and antimicrobial activities of essential oils of Varthemia Persica, Foeniculum Vulgare and Ferula Lycia. *Bacteriology* 2012;5:42-52.
9. Salama ZA, El Baz FK, Gaafar AA, Zaki MF. Antioxidant activities of phenolics, flavonoids and vitamin C in two cultivars of fennel (Foeniculum vulgare Mill.) in responses to organic and bio-organic fertilizers. *Journal of the Saudi Society of Agricultural Science*s 2015. Available in: http://dx.doi.org/10.1016/j.jssas.2013.10.004.
10. Khorshidi J, Mirahmadi FS, Tabatabaei FM. Influence of oil content and yield of foeniculum vulgare mill. CV. Soroksary seeds by adapting different plant. *J Med Plant Res* 2014;8(6):282.
11. Anwar F, Ali M, Hussain AI, Shahid M. Antioxidant and antimicrobial activities of essential oil and extracts of fennel (Foeniculum vulgare Mill.) seeds from Pakistan. *Flavour and Fragnance Journal* 2009;24:170-176.
12. Dunia TN, Al-Aridhi. The Effect of Foeniculum Vulgare Alcoholic Extract on Some Metabolic Changes in Liver and Kidneys of Alloxan Diabetic Mice. *Iraqi Journal of Medical Science* 2014;12:1681-6579.
13. Soares JMD, Leal AEBP, Silva JC, Almeida JRGS, Oliveira HP. Influence of flavonoids on mechanism of modulation of insulin secretion. *Pharmacognosy* 2017;13(52):639-646.
14. Dua A, Garg G, Mahajan R. Polyphenols, flavonoids and antimicrobial properties of methanolic extract of fennel (Foeniculum vulgare Miller). *European Journal of Experimental Biology* 2013;3(4):203-208.
15. Jin X, Qian J, Lu Y. The role of hepatoprotective effect of a flavonoid-rich extract of Salvia plebeia R.Br. on carbon tetrachloride induced acute hepatic injury in mice. *Journal of Medicinal Plants Research* 2011:5, [viewed 06 April 2017] Available online at http://www.academicjournals. org/JMPR.
16. Sheikh BA, Pari L, Rathinam A, Chandramohan R. Trans-anethole, a terpenoid ameliorates hyperglycemia by regulating key enzymes of carbohydrate metabolism in streptozotocin induced diabetic rats. *Biochimie* 2015; 112:57- 65.

17. Osman NN, Jambi EJ, Aseri NH. Assessment of antidiabetic and antioxidant activities of Cassia angustifolia and Feoniculum vulgare in diabetic rats. *International Journal of Pharmaceutical Research & Allied Sciences* 2017;6: 149-162.

18. Abou El-Soud N, El-Laithy N, El-Saeed G, Salah Wahby M, Khalil M, Morsy F, Shaffie N. Antidiabetic Activities of Foeniculum Vulgare Mill. Essential Oil in Streptozotocin-Induced Diabetic Rats. *Macedonian Macedonian Journal of Medical Sciences* 2011;4:139-146.

19. Dunn JS, Kirkpatrick DE, Gilmour MK, Kirkpatrick J, McLetchie NGB. Further observations on the effects of alloxan on the pancreatic islets. *J physiol* 1944;103: 233-243.

20. Lenzen S. The mechanism of alloxan & streptozotocin induced diabetes. *Diabetologia* 2008;51:216-226.

21. El Araby B, Farrag D, Ahmed I, Zahkouk SA. Effect of Foeniculum vulgare and Propolis on Liver in Alloxan Diabetic Rats. *Advances in Biological Research* 2017;11: 311-318.

22. El-Baz FK, Salama ZA, Abdel BHH, Gaafar AA. Hepatoprotective Effect of Sweet Fennel (Feoniculum vulgare L.) Methanol Extract against Carbon Tetrachloride Induced Liver Injury in Rats. *Int J Pharm Sci Rev Res* 2014;25(36):194-201.

23. Adeyemi, D.O., Komolafe, O.A., Adewole, O.S., Obuotor, E.M., Abiodun, A.A., Adenowo, T.K., 2010. Histomorphological and morphometric studies of the pancreatic islet cells of diabetic rats treated with extracts of Annona muricata. Folia Morphol (Warsz). 69(2), pp. 92-100.

24. Robbins, D.C., Tager, H.S., Rubenstein, A.H. 1984. Biologic and clinical importance of proinsulin. *N Engl J Med*. 310(18), pp. 1165-75.

25. Ozbek H, Ozturk M, Bayram I, Ugras S, Citoglu GS. Hypoglycemic and Hepatoprotective Effects of Foeniculum vulgare Miller Seed Fixed Oil Extract in Mice and Rats. *Eastern Journal of Medicine* 2003;8:35-40.

26. Udia PM, Takem LP, Ufot UF, Antai AB, Owu DU. Insulin and alpha amylase levels in alloxan-induced diabetic rats and the effect of Rothmannia hispida (K. Schum) Fagerl leaf extract. *The Journal of Phytopharmacology* 2016;5:1-5.

27. Jayant SK, Srivastava N. Effect of Ocimum sanctum against alloxan induced diabetes and biochemical alterations in rats. *Integrative Obesity and Diabetes* 2016;2:1-4.

28. Shaffie NM, Morsy FA, Gamal el Din A, Sharaf HA. Effect of Caraway, Coriander and Fennel on the structure of Kidney and Islets of Langerhan in Alloxan-Induced Diabetic Rats: Histological and Histochemical Study. *Journal of American Science* 2010;6:405-418.

29. Jimoh A, Tanko Y, Mohammed A. Anti-diabetic effect of methanolic leaf extract of Cissus cornifolia on alloxan-induced hyperglycemic in Wistar rats. *Annals of Biological Research* 2013;4:46-54.

30. Al-Qudah MMA, Haddad MA, EL-Qudah JMF. The effects of aqueous ginger extract on pancreas histology and on blood glucose in normal and alloxan monohydrate-induced diabetic rats. *Biomedical Research India* 2016;27: 350-356.

31. Elkotby D, Hassan AK, Emad R, Bahgat I. Histological Changes in Islets of Langerhans of Pancreas in Alloxan-Induced Diabetic Rats Following Egyptian Honey Bee Venom Treatments. *International Journal of Pure and Applied Zoology* 2018;6:1-6.