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ORIGINAL ARTICLE

Efficacy and Safety of Fennel in Alloxan-induced Male Diabetic Rats

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ABSTRACT

Background & objective: Diabetes mellitus is a growing global health concern characterized by chronic hyperglycemia and associated complications, including hepatotoxicity. Traditional herbal remedies like *Foeniculum vulgare* (fennel) have recently garnered attention for their potential therapeutic benefits. This study investigates the hepatoprotective roles of fennel in alloxan-induced male diabetic rats.

Methods: An experimental study was conducted at Sir Salimullah Medical College, utilizing 30 Wistar albino male rats (90-120 days old). Following a 14-day acclimatization, the rats were divided into three groups: normal control, alloxan-induced diabetic control, and an experimental group treated with fennel extract (150 mg/kg/day) for 21 days post-alloxan induction. Blood samples were collected for glucose and insulin measurement, and liver function tests (Total Bilirubin, ALT, AST) were performed. Histopathological examinations of liver tissues were also conducted.

Results: Initial fasting blood glucose levels were comparable across groups; however, by Day 22, the alloxan-induced diabetic control group showed significantly higher levels compared to the fennel-treated group ($p < 0.001$). At the end of the study on Day 22, significant differences were observed in serum levels of total bilirubin, ALT, and AST among the three experimental groups ($p < 0.001$). The alloxan-induced diabetic group (Group A2) showed the highest levels of these liver function parameters, while the normal control group (Group A1) had the lowest. Post-hoc analysis indicated that Group A2 had significantly elevated levels compared to both the fennel-treated group (Group B) and Group A1 ($p < 0.001$ for all comparisons). Histopathological examination revealed that all rats in Group A1 exhibited normal liver histology, whereas all rats in Group A2 displayed abnormal findings. In Group B, 80% of the rats showed normal liver histology, while 20% had mild changes. These results highlight significant differences in liver histopathological outcomes across the three groups ($p < 0.001$).

Conclusion: The findings suggest that *Foeniculum vulgare* effectively protects against alloxan-induced hepatotoxicity in male diabetic rats, evidenced by improved biochemical parameters and histological integrity of liver tissues. This study underscores the potential of fennel as a therapeutic option for managing diabetes and its associated hepatic complications.

Key words: Diabetes mellitus, alloxan, fennel, hepatoprotective roles, Wistar albino rats etc.

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INTRODUCTION:

Diabetes is a cluster of metabolic disorders characterized by chronic hyperglycemia due to impaired insulin secretion and action. This condition is associated with various complications, including diabetic cardiomyopathy, neuropathy, nephropathy, retinopathy, and vasculopathy, which contribute to significant health burdens.[1,2] With the increasing global prevalence of diabetes, expected to reach 300 million cases by 2025, there is a growing demand for effective treatments.[3]

While conventional therapies, such as insulin and metformin, can help manage diabetes, they often come with side effects and rising costs, leading many patients toward traditional herbal medicines.[4,5] Among these, *Foeniculum vulgare* (fennel), a member of the Apiaceae family, has gained attention for its potential health benefits. Rich in vitamins (such as niacin & vitamin C) and minerals (including calcium and iron), it contains bioactive compounds like trans-anethole and flavonoids that exhibit anti-inflammatory, antioxidant, and antispasmodic properties.[6-11] Recent research indicates that fennel extract mitigates metabolic disturbances and oxidative stress associated with diabetes.[12] Another study demonstrated that it improved the histological structure of the islets of Langerhans in the pancreas and induced hypoglycemia in diabetic rats.[13] Alloxan, known as a cytotoxic glucose analog and a potent diabetogenic agent, induces diabetes by damaging pancreatic beta cells, which decreases the size of the islets of Langerhans.[14] The free radicals generated by alloxan inhibit glucokinase, induce lipid peroxidation leading to reduced glucose-induced insulin secretion and subsequent hyperglycemia.[15] Lipid peroxidation induced by free radicals is believed to contribute to the development of various pathological conditions including hepatotoxicity. Notably, alloxan-induced diabetic rats treated with *Foeniculum vulgare* demonstrated a significant reduction in blood glucose levels alongside hepatoprotective effects.[16] Furthermore, the hepatoprotective properties of *Foeniculum vulgare* have been validated in hepatotoxic rat models.[17] Fennel seed extract and its active compound trans-Anethole (TA) can protect the liver against diabetes-induced hepatic injury in rats, probably via hypoglycemic and antioxidant effects.[18]

Although evidence of its antidiabetic properties exists, including improvements in metabolic disturbances associated with diabetes,[12] the primary focus of this study is to explore the hepatoprotective and nephroprotective roles of fennel in the context of alloxan-induced diabetic male rats. Given the limited research on its protective effects in kidney and liver health specifically within diabetic models, this study aims to investigate the potential hepatoprotective roles of Foeniculum vulgare in alloxan-induced male rats.

METHODS:

Study Design and Setting:

This experimental study was conducted at the Department of Physiology, Sir Salimullah Medical College (SSMC), Dhaka, over a one-year period from July 1, 2018, to June 30, 2019. A total of 30 healthy Wistar albino male rats, aged 90 to 120 days and weighing between 150 and 180 grams, were included in the study. The rats were procured from the animal house at the Department of Pharmacy, Jahangir Nagar University, Savar, Dhaka. Prior to the commencement of the study, ethical clearance was obtained from the Institutional Ethics Committee at Sir Salimullah Medical College. All animals were housed in the animal facility of the Institute of Nutrition and Food Science, Dhaka University. Following a 14-day acclimatization period, the rats were randomly divided into two groups: Group A (Control) and Group B (Experimental Group, n= 10). Group A was further subdivided into Group A1 (Normal Control, n=10) & Group A2 (Alloxan-induced Diabetic Control, n = 10).

Animal Preparation:

During the acclimatization period, the rats were maintained at a room temperature of 23 ± 2°C under a 12-hour light/dark cycle with ad libitum access to food and water. The total study duration was 21 days, starting from the day of intervention. Initial body weights were recorded on Day 1, and final body weights were measured on Day 22. On Day 1, after an overnight fasting period of 12 hours,

approximately 1 ml blood samples were collected from the tail veins of all rats to assess fasting blood glucose levels. Only rats exhibiting normal fasting blood glucose levels were included in the experiment.

Intervention:

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

Follow-up and Laboratory Procedures:

On Day 4, fasting blood glucose levels were reassessed, and rats with levels between 11-20 mmol/L were selected for further experimentation. On Day 22, all rats were anesthetized using a chloroform (30%) vapor, followed by sacrifice. Blood samples (approximately 5 ml) were collected from the heart using a sterile syringe and placed in clean, dry test tubes with proper identification. After allowing blood to clot, samples were centrifuged at 4000 rpm for 10 minutes, and the supernatant serum was stored in labeled Eppendorf tubes at -80°C for biochemical analysis. Fasting blood glucose & serum insulin levels were measured as indicators of glycemic control. Biochemical investigations were performed using a semi-autoanalyzer in the Department of Physiology, SSMC, while serum insulin levels were evaluated in the Department of Biochemistry, Bangabandhu Sheikh Mujib Medical University (BSMMU). Histopathological examination of pancreatic tissues was conducted by preparing histological slides that were subsequently analyzed microscopically, with photomicrographs taken in the Department of Pathology, SSMC.

Sample Collection:

The pancreases of the sacrificed rats were carefully collected and rinsed in ice-cold saline, then dried on tissue paper. The weight of each pancreas was recorded using an electronic balance. Tissue samples were fixed in 10% formalin for histological processing. Histopathological findings were classified as normal or abnormal based on criteria established by Adeyemi et al.[19]

Laboratory Investigations:

Fasting blood glucose levels were measured using the glucose oxidase (GOD-POD) method with a semi-autoanalyzer,[20] while serum insulin levels were determined by the chemiluminescent microparticle immunoassay (CMIA) method using the ARCHITECT Plus ci4100 system.[21]

The serum total bilirubin level was quantified using the Dimethyl sulfoxide (DMSO) colorimetric method with a semi-autoanalyzer. Serum alanine aminotransferase (ALT) levels were measured using the recommended kinetic UV method, as described by Schumann et al.[22] also via a semi-autoanalyzer. Similarly, serum aspartate aminotransferase (AST) levels were determined using the same kinetic UV method.[22] For histological examination, the preparation of pancreatic and liver tissues was carried out. The outcome variables assessed in this study included serum levels of total bilirubin, ALT, & AST, creatinine, urea uric acid etc. and histological architecture of liver

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
-Normal size of the area of islets of Langerhans	-Intracellular microvacuolation	-Decreased size of area of islets of Langerhans
-Normal β-cell membrane	Pale cytoplasm	-Pyknosis of β-cell nucleus
-Normal β-cell nucleus		

Histopathological findings of liver:

Normal:	Mild change:	Moderate change
Normal architecture of -hepatic lobule -central vein	Less/absence of lymphocytic and Kupffer cells infiltration	Presence of centrilobular necrosis
Normal structure of -hepatocyte -portal tract	Less/absence of centrilobular necrosis	Disorganization of hepatic sinusoids, dilated blood vessels
The normal orientation of hepatic sinusoids		Infiltration of lymphocytes and Kupffer cells
		Vacuolar degenerative changes in hepatocytes and pyknotic nucleus

Statistical Analysis:

Data analysis was performed using the Statistical Package for Social Science (SPSS) for Windows, version 22. Continuous data were expressed as mean ± SD, and comparison between groups was conducted using an unpaired t-test. Categorical data were presented as frequencies and percentages, with comparisons made using the Chi-square (χ^2) test. An ANOVA test was utilized for continuous data comparisons among the three groups, followed by a post-hoc Hochberg test for intergroup comparisons. A significance level of 5% was established, with a p-value of <0.05 considered statistically significant.

RESULTS:

Fasting Blood Glucose and Serum Insulin Levels in Different Groups of Rats

The fasting blood glucose levels are summarized in Tables I and II. On Day 1, the mean (±SD) fasting blood glucose levels were comparable across all groups ($p = 0.698$). By Day 4, however, fasting blood glucose levels were significantly elevated in both Group A2 and Group B compared to Group A1 ($p<0.001$ for both comparisons), while no significant difference was observed between Group A2 and Group B ($p=0.568$). On Day 22, the fasting blood glucose levels remained significantly higher in Group A2 when compared to both Group A1 ($p<0.001$) and Group B ($p<0.001$). Furthermore, mean serum insulin levels were significantly lower in Group A2 compared to Group A1 ($p<0.001$) and Group B ($p<0.001$).

Liver Function Parameters: Serum Total Bilirubin, ALT, and AST Levels

At the endpoint of the study on Day 22, the serum levels of total bilirubin, ALT, and AST demonstrated significant heterogeneity among the three experimental groups ($p < 0.001$ in each case). Group A2 exhibited the highest levels of all liver function parameters, while Group A1 displayed the lowest. Post-hoc analysis using the Hochberg test revealed that the serum total bilirubin, ALT, and AST levels in Group A2 were significantly elevated compared to those in Group B ($p < 0.001$ for all parameters), the levels of which were again significantly higher than those in Group A1 ($p < 0.001$ for all parameters) (Tables III and IV).

Distribution of rats by histological changes in the liver

Histopathological examination of the liver revealed normal histology in 100% of the rats in Group A1, while 100% of the rats in Group A2 exhibited abnormal histopathological findings. In Group B, the majority (80%) of rats displayed normal liver histology, whereas 20% demonstrated mild histological changes. These findings indicate significant differences in the histopathological outcomes across the three groups ($p < 0.001$) (Table V & VI and Photomicrograph 1 to 4).

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

Groups	Fasting blood glucose (mmol/L)			Serum insulin (µU/ml)
	Day 1	Day 2	Day 3	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 by Post-hoc Hochberg test

Groups	Fasting blood glucose			Serum insulin
	Day 1 p-value	Day 2 p-value	Day 3 p-value	Day 22 p-value
A1 vs A2 vs B	0.698	< 0.001S	< 0.001S	< 0.001S
A1 vs A2	1.000	< 0.001 S	< 0.001	< 0.001S
A1 vs B	1.000	< 0.001S	1.000	0.071
A2 vs B	1.000	0.568	< 0.001S	< 0.001S

s = significant.

Table III. Serum total bilirubin, ALT and AST levels in different groups of rats

groups	S. total bilirubin (mg/dl)	Serum ALT (U/L)	Serum AST (U/L)
A1 (n = 10)	0.70 ± 0.26	41.00 ± 3.53	40.00 ± 5.14
A2 (n = 10)	2.52 ± 0.49	87.10 ± 11.26	61.10 ± 5.32
B (n = 10)	1.15 ± 0.18	63.30 ± 5.31	50.00 ± 7.30
p-value	< 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 IV. Multiple comparison of by Post-hoc Hochberg test

Comparing groups	Serum total bilirubin	Serum ALT	Serum AST
	p-value	p-value	p-value
A1 vs A2 vs B	< 0.001	< 0.001	< 0.001
A1 vs A2	< 0.001	< 0.001	< 0.001
A1 vs B	0.017	< 0.000	0.003
A2 vs B	< 0.000	< 0.000	< 0.001

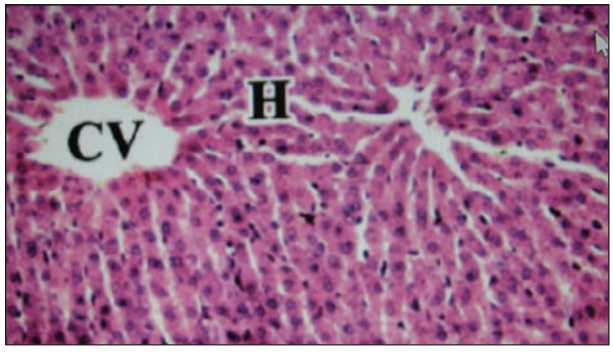
Table V. Histopathological observation of livers in different groups of rats

Group	Observation	Result/Findings
Group A1 (n=10) (Normal control group)	• Architecture of -hepatic lobule -central vein • Structure of -hepatocyte -portal tract • Orientation of hepatic sinusoids	Normal hepatic structure
Group A2 (n=10) (Alloxan - induced diabetic control group)	• Presence of centrilobular necrosis • Disorganization of hepatic sinusoids • Infiltration of lymphocytes and Kupffer cells • Vacuolar degenerative changes in hepatocytes and pyknotic nucleus • Dilated blood vessels	Moderate histological changes
Group B (n=10) (Diabetic rats treated with Mouri)	• Restoration of the normal architecture of hepatic sinusoids • Less/absence of lymphocytic and Kupffer cell infiltration • Less/absence of centrilobular necrosis	Normal histological findings in 8 rats and mild histological changes in 2 rats

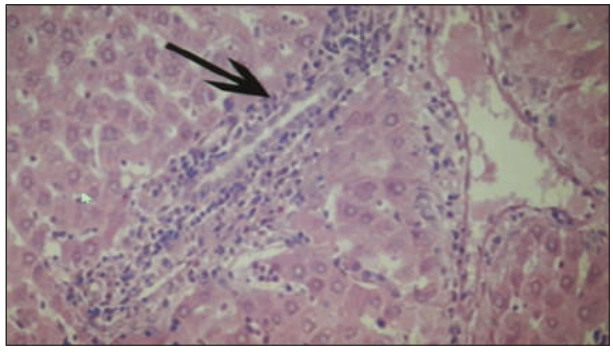
Table VI. Comparison histological architectures among different groups of rats

Comparing groups	P findings	Serum ALT	p-value
	Normal	Normal	
A1 (n=10)	10(100.0)	0(0.0)	
A2 (n=10)	0(0.0)	10(100.0)	< 0.001
B (n = 10)	8(80.0)	2(20.0)	

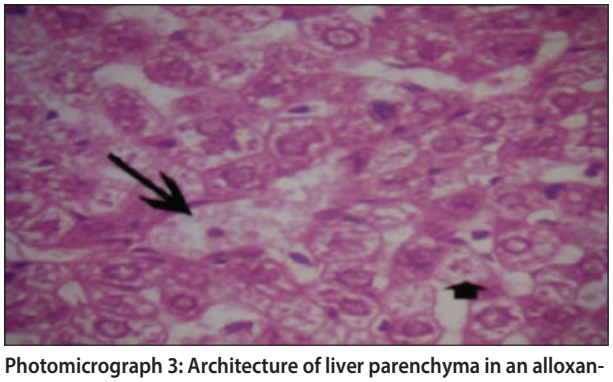
Data were analyzed using **Chi-square (χ^2)** and were expressed as **n(%).**



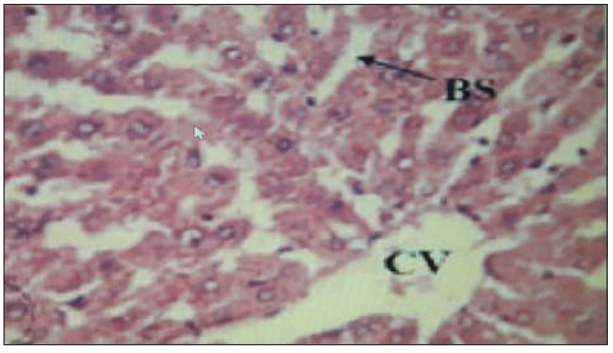
Photomicrograph 1: Architecture of liver parenchyma in a normal control group of rat (here CV represents central vein and H represents hepatic cord in X 400)



Photomicrograph 2: Architecture of liver parenchyma in an alloxan-induced diabetic control group rat (here black arrow shows mononuclear cell infiltration in X 400)



Photomicrograph 3: Architecture of liver parenchyma in an alloxan-induced diabetic control group of rats (here black arrow shows an area of necrotic cells and the blackhead arrow indicates vacuolar degenerative changes in hepatocytes in X 400)



Photomicrograph 4: Restoration of the normal architecture of liver parenchyma in an alloxan-induced diabetic rat treated with Mouri (X 400)

DISCUSSION

The present study aimed to evaluate the hepatoprotective effects of *Foeniculum vulgare* (fennel) in alloxan-induced diabetic male rats. Accordingly, serum levels of total bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were analyzed to evaluate liver function and the safety profile of fennel. A histological examination of hepatic tissues was conducted to investigate microscopic structural changes in both the pancreas and liver.

At the endpoint (day 22) of the study, the fasting blood glucose levels in diabetic rats treated with Mouri were significantly lower than those in the alloxan-induced diabetic control group, approximating the levels found in the normal control group. This finding aligns with results reported by other researchers.[9,23] However, some studies have reported a lack of significant blood glucose-lowering effects of fennel, highlighting the need for further investigation in this area.[24] The serum insulin levels in diabetic rats treated with fennel were comparable to those in the normal control group on day 22 but significantly higher than those in the alloxan-induced diabetic controls. This observation is consistent with the finding of Udia et al.[25]

At the end of the study, the alloxan-induced diabetic control group exhibited a significant increase in serum total bilirubin levels compared to the normal control group. In contrast, the bilirubin levels in diabetic rats treated with fennel were significtly

lower than those in the alloxan-induced diabetic controls, which is consistent with observations from other investigations.[26-28] Serum levels of ALT and AST were notably elevated in the alloxan-induced diabetic control group relative to the normal control group. However, these enzyme levels were significantly lower in the fennel-treated group than in the diabetic controls, which is supported by several studies.[16,17,29-32] Conversely, some research indicates elevated serum ALT and AST levels in diabetic rats treated with fennel, though the reasons for these findings remain unexplained.[24]

Histological analysis revealed significant abnormalities, including centrilobular necrosis, disorganization of hepatic sinusoids, lymphocytic infiltration, Kupffer cell infiltration, vacuolar degeneration of hepatocytes, pyknotic nuclei, and dilated blood vessels in all rats from the alloxan-induced diabetic control group. These findings align with previous studies.[33,34] Conversely, 80% of the diabetic rats treated with fennel displayed nearly normal histological architecture, while 20% exhibited only minimal changes, suggesting a favorable safety profile for fennel. However, Ozbek and associates[24] reported no improvement in hepatic architecture in diabetic rats treated with fennel, without providing explanations for their findings.

CONCLUSION

The results of this study indicate that fennel extract may effectively ameliorate the effects of alloxan-induced diabetes in rats, demonstrated by a negligible increase in serum total bilirubin levels compared to both normal and diabetic control groups. Furthermore, the serum levels of ALT and AST in fennel-treated diabetic rats were not significantly elevated when compared to the alloxan-induced diabetic control group, suggesting that fennel does not exert hepatotoxic effects. While alloxan-induced diabetic rats commonly exhibited significant hepatic architectural abnormalities, those treated with fennel showed normalization of liver structure, indicating its potential to mitigate the detrimental effects of alloxan on liver tissues.

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 (Baltimore)*, 2016;95(51):e5230. doi: 10.1097/MD.0000000000005230.
3. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. *J Ethnopharmacol*, 2002;81(1): 81-100.
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. Sharma US, Kumar A. Anti-diabetic effect of Rubus ellipticus fruit extracts in alloxan induced diabetic rats. *Journal of Diabetology*, 2011;2:4.
6. Kazemi M, Mousavi E, Kharestani H. Chemical compositions & antimicrobial activities of essential oils of Varthemia Persica, Foeniculum Vulgare & Ferula Lycia. *Bacteriology*, 2012;5:42-52.
7. Salama ZA, El Baz F K, 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 Sciences*, 2015. Available in: http://dx.doi.org/10.1016/j.jssas.2013.10.004.
8. 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.
9. 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.
10. 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.
11. 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;5:1558-1563. [viewed 06 April 2017] Available online at http://www.academicjournals.org/JMPR.
12. 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.

13. 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.

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

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

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

17. El-Baz F K, Salama ZA, Abdel Baky HH, 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.

18. Samadi-Noshahr Z, Hadjzadeh MA, Moradi-Marjaneh R, Khajavi-Rad A. The hepatoprotective effects of fennel seeds extract & trans-Anethole in streptozotocin-induced liver injury in rats. *Food Sci Nutr*, 2020;9(2):1121-1131. doi: 10.1002/fsn3.2090.

19. Adeyemi DO, Ukwenya VO, Obuotor EM, Adewole SO. Anti-hepatotoxic activities of Hibiscus sabdariffa L. in animal model of streptozotocin diabetes-induced liver damage. *BMC Complement Altern Med*, 2014;14:277.

20. Trinder P. Quantitative determination of glucose. *Ann Clin Biochem*, 1969;6:24-33.

21. Robbins DC, Tager HS, Rubenstein AH. Biologic and clinical importance of proinsulin. *N Engl J Med*, 1984;310(18): 1165-75.

22. Schumann G, Bonora R, Ceriotti F, Férard G, Ferrero CA, Franck PFH. IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37°C. Part 5, Reference Procedure for the Measurement of Catalytic Concentration of Aspartate Aminotransferase. *Clin Chem Lab Med*, 2002;40(7): 725–733.

23. Mhaidat NM, Ahmed S, Abu-zaiton Alzoubi KH, Al-zoubi W, Alazab RS. Antihyperglycemic Properties of Foeniculum vulgare Extract in Streptozocin-Induced Diabetes in Rats. *International Journal of Pharmacology*, 2015;11(1):72-75.

24. 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.

25. 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.

26. 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.

27. Olusanya AR, Ifeoluwa BS, Jumoke AA, Khadijat B. *Diabetes Food Plan*. 2018. Available in: http://dx.doi.org/10.5772 /intechopen.71529.

28. Kumar V, Ranjan S, Akhouri V, Siddiqui Md S, Sharma B. Anti-hyperglycemic and Antioxidant Action of Azadirachta Indica Leaf in Alloxan Induced Type-I Diabetes Mellitus in Experimental Diabetic Swiss Albino Mice: A Histopathological Investigation. *International Journal of Pharmaceutical Sciences & Research*, 2018;9:1673-1682.

29. Gad El-Karim DR, Oda SS, Tohamy HG, Hashem MA. The Effect of Metformin & Ginseng on Alloxan-Induced Diabetic Rats: Hematological, Biochemical and Histopathological Studies. *Alexandria Journal of Veterinary Sciences*, 2017; 55:60-66.

30. Markkas N, Govindharajalu M. Protective effect of Mollugo cerviana extract on liver markers in alloxan induced diabetic rats. *The Journal of Phytopharmacology*, 2018; 7:1-4.

31. Mehta RK, Supriya K, Chaudhary J, Rasal VP, Mehta RK. Effect of Cucumis sativus Linn. in Liver Pathogenicity of Alloxan Induced Diabetic Rat. *Acta Scientific Medical Sciences*, 2018;2:03-07.

32. Hanan MS, Sohair SA, Hanan AA, Areej AY. Hepatoprotective effect of fennel and tiger nut on biochemical parameters and DNA damage in rats. *Egypt. J. Chem. Environ. Health*. 2015. 1, pp.18-37.

33. Oyebadejo S, Bassey E, Oyewunmi A, Archibong V, Usoro E. Histopathological Study of the Liver of Alloxan Induced Diabetic Rats and Macerated Allium Sativum (Garlic) Ameliorative Effect. *Asian Journal of Biomedical and Pharmaceutical Sciences*, 2014;4:72-77.

34. Lucchesi AN, Cassettari LL, Spadella CT. Alloxan-induced diabetes causes morphological and ultrastructural changes in rat liver that resemble the natural history of chronic fatty liver disease in humans. *Journal of Diabetes Research*, 2015;(5):1-11. Z