The effect of ocimum basilicum ethanolic extract on some physiological aspects and histopathological changes in alloxanized male rats

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ABSTRACT

The present study was conducted to examine the effect of orally administered of ethanolic extract of *Ocimum basilicum* on blood serum glucose, insulin, hemoglobin A1c, hematology parameters (Hb, PCV, and RBC count) as well as its histological effect on the pancreas, liver and kidney when inducing diabetic type I in rats by alloxan. Twenty –four male rats were divided into three groups randomly, group I : (Negative control) received distilled water. Diabetes was induced in the second and third groups by alloxan injection intraperitoneal, group II : (Positive control) received distilled water. While, group III: (treated) were orally administered 200 mg/kg B.W/day of *Ocimum basilicum* extract for 6 weeks orally. The results showed that *Ocimum basilicum* extract administration significantly ($P<0.05$) decrease in the serum glucose and HbA1 concentrations in concordance with a significant ($P<0.05$) increase insulin level. While, there were significant changes ($P<0.05$)was observed on the Hb, PCV and RBC count. The histopathological changes were observed after administration of *Ocimum basilicum* extract showed a significant islet (beta cells) restoration and improvement histopathologocal changes in liver and kidney. From the results, it can be concluded that extract of *Ocimum basilicum* possess hypoglycemia effect as evidence by amelioration of pancreatic function as well as improving histopathologocal changes of pancreas, liver and kidney in diabetic animals.

Keywords: *Ocimum basilicum*, Physiological parameters, Histopathological, Male rats

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الملخص باللغة العربية

أجريت هذه الدراسة لتقييم تأثير الخلاصة الكحولية للريحان على بعض الجوانب الفسلجية والنسيجية في ذكور الجرذان المصابة بداء السكري من النوع الأول المستحث بالألوكزان الذي تم حقنه بالبريتون بجرعة 100ملغم/ كلغم. استخدمت في هذه الدراسة 24 من ذكور الجرذان وقسمت عشوائيا إلى ثلاث مجموعات، في كل مجموعة ثمانية من الجرذان، فأما المجموعة الأولى فهي مجموعة السيطرة السالبة التي أعطيت 1 مل ماء مقطر عن طريق الفم ، والمجموعة الثانية مجموعة السيطرة الموجبة مصابة بداء السكري المستحث بالألوكزان أعطيت 1 مل ماء مقطر عن طريق الفم، أما المجموعة الثالثة فكانت مجموعة معاملة مصابة بداء السكري المستحث بالألوكزان أعطيت الخلاصة الكحولية للريحان بجرعة 200 ملغم/ كلغم مذابة في 1 مل من الماء المقطر عن طريق الفم ولمدة ستة أسابيع.

أظهرت النتائج انخفاضا معنويا في كل من مستوى السكر في الدم والسكر التراكمي، بينما كان هناك ارتفاع معنوي في مستوى الأنسولين، عدد كريات الدم الحمراء، وتركيز الهيموجوبين وحجم خلايا الدم المرصوصة في مجموعة المعاملة التي أعطيت الخلاصة الكحولية للريحان، نسيجيا، فقد أظهر الفحص النسيجي في مجموعة المعاملة إصلاح تأثير الألوكزان في البنكرياس، الكبد والكلية مقارنة مع مجموعة السيطرة الموجبة (المصابة بداء السكري).

نستنتج من ذلك أن للخلاصة الكحولية للريحان تأثيرا خافضا لسكر الدم والسكر التراكمي وفي تحسين وظيفة البنكرياس وإصلاح التغيرات النسيجية في البنكرياس والكبد والكلية.

INTRODUCTION

Diabetes mellitus is a syndrome characterized by chronic hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion and/or action (1). Chronic elevation of blood glucose eventually leads to long-term complications of diabetes, that leads to various tissue and organs damage that considered major causes of morbidity and mortality in human populations (2). Diabetes is commonly accompanied by other cardiovascular risk factors such as dyslipidemia, hypertension, prothrombic factors and microvascular problems involving eyes, kidney and peripheral nerves (3). However, increased free radical generation and oxidative stress are hypothesized to play an important role in the pathogenesis of diabetes and its late complications (4). The oral hypogly-caemic agents currently used in clinical practice have characteristic profiles of serious side effects such as cholestatic jaundice, aplastic and haemolytic anemias, generalized hypersensitivity reactions, liver failure and diarrhea (5) The side effects of insulin therapy which include insulin allergy, resistance and other late complications like morphological changes in kidneys and severe vascular complications (6). Today, it is estimated that about 80% of the world population relies on botanical preparations as medicine to meet their health needs (7). Several hypoglycaemic plants are potential in ameliorating lipid metabolism abnormalities of diabetes mellitus (8-10). Traditional herbal medicines are generally considered to be safer than synthetic drugs, its widely prescribed today despite the fact that their biologically active compounds are unknown, due to its minimal adverse effects, low costs, economical, effective, and their easy availability as well as to facilitate natural product drug discovery (11,12). *Ocimum_basilicum* (OB) is a plant belonging to Lamiacea family , which is widely cultivated in Asia as a nourishing food and herbal medicine. The *Ocimum basilicum*is considered as one of the most important source of medicine and drugs due to the presence of various phytochemical active compounds like alkaloids, saponins, tannins, phenols, flavonoids, isoflavonoids, proteins, steroids, terpenoids, cardiac glycosides, amino acids, sesquiterpenes, minerals, gums, mucilage, glucas and anthraquinone (13,14). Furthermore, *Ocimum basilicum* had been shown to possess diverse pharmacological properties which may be attributed to its usefulness in folk medicine to treat a wide range of diseases such as diabetes, cardiovascular diseases an antispasmodic, aromatic, digestive, carminative, stomachic and tonic agent. Many studies have established that basil leaves extracts have potent antioxidant, anti-aging, anticancer, antiviral and antimicrobial properties (15- 17). *Ocimum basilicum* has also been used externally for the topical treatment of acne, insect stings, snake bites, and skin infections (18, 19).

Aims of the study:

The present study was aimed at investigate the effects of *Ocimum basilicum* extract on blood glucose level, serum insulin hormone, hemoglobin A1c (HbA1c), Hb, PCV, RBC count and histological profile in pancreas, liver and kidney in animals induced diabetic type I.

MATERIALS AND METHODS

Plant preparation:
The fresh leaves of *Ocimum basilicum* were bought from the local market in Basra city/Iraq. The fresh leaves were collected, washed with distilled water and then dried under the shade at room temperature for six days. The dried leaves were cut into small pieces and ground into fine powder by using electric mill for 3 minutes. 50 gms of the powder were put in the round bottle flask, 200 ml of ethanol (70%) were added to flask and extracted for 12 hrs. at 70 °C. The extract was cooled and filtered with Whatman No. 1 filter paper. The filtrate was dried at room temperature and dryness powders were kept in tight closed container and stored at 4°C until use in the experimental procedure.

Experimental animals (rats):
The experiment was performed on twenty- four healthy male rats (*Rattus norvegicus*) weighing between (250 ± 25) gm and aged (12) weeks. Rats were kept for adaptation period of two weeks at the animal house of College of Veterinary Medicine / University of Basra. The animals were housed as four rats to each cage under optimum conditions (12 hrs. light/ dark cycle) and temperature of 25 ±2°C. These conditions were maintained throughout the duration of the experiment. The animals were fed with standard diet (pellet) and provided with water *ad libitum*.

Experimental design

Induction of diabetes: Diabetes was induced in overnight fasting rats by a single intraperitoneal injection of alloxan monohydrate (Sigma Ltd, USA) at dose 100 mg / kg body weight (20). Each 100 mg of alloxan was dissolved in 1ml of normal saline. Immediately after alloxan injection water replaced by 5% glucose solution for 24 hrs. in order to overcome sudden hypoglycemia (21). Diabetes was conformed 72 hrs. after induction, the rats were fasted for 12 hrs. and blood was taken from tail artery of the rats (22). The animals showing blood glucose level estimated by GOD-POD enzymetic colorimetric method (23).The animals were stabilized for a week and rats with blood glucose level more than 200 mg/dl were considered diabetic and selected for the study. Normal and diabetic rats

was randomly assigned to three groups (n = 8 in each group) as follows: Group I: (Negative control) the rats were received distilled water (1 ml). Group II: (Positive control) diabetic rats were received distilled water (1ml). Group III: (Diabetic treated) the rats were received *Ocimum basilicum* ethanolic extract (200 mg/ kg B.W) dissolved with distilled water (1ml). All treatment were continued for 6 weeks were administered by gastric intubation orally as single dose daily. After 6 weeks overnight fasting, rats of all groups were anaesthetized using ether solution inhalation. Blood samples were immediately collected from the heart and placed in plain tubes to clot at room temperature. The serum separated by centrifuge at 3000 rpm for 10 minutes. The serum used for glucose and insulin determined was done by using special enzymatic kits. Other blood samples were collected into tubes with anticoagulant (EDTA) which were used for hematological parameters. Immediately after blood collection, animals were sacrificed, Pancreas, liver and kidney carefully excised, washed with normal saline remove any red blood cells (erythrocytes) and clots.

Biochemical assay: The blood glucose concentration was measured by the glucose oxidase Method (23), the insulin level was determined using Elisa kit (24). While, the glycosylated hemoglobin (HbA1c) was determined using a hemoglobinA1c assay kit (Randox Lab., Ltd., UK) according to (25).

Haematological assay: Haematological values were measured by following standard methods at end of the experimental period. The RBC count was obtained by hematocytometer (Neubaure improved chamber) and using Hayme's solution as a diluting fluid and a special pipette for dilution (26,27). The microhematocrit method is used to calculate the percentage of PCV by the use of heparinized capillary tubes which contain heparin, the hematocrit value was obtained by service device (Hematocrit reader) (28). Hb concentrations estimated by Sahli apparatus (29).

Histological preparation: Small specimens of pancreas, Liver and Kidney from all groups were fixed in 10% neutral buffered formalin, dehydrated in ascending concentrations of ethyl alcohol (70-100%). Fixed specimens were embedded in paraffin wax and sections of 5µm thickness were cut. Slides were stained with Heamatoxylin and Eosin (H and E) for histological examination (30).

Statistical analysis: Data are presented as means ± SE. Statistical analysis was performed using one way analysis of variance (ANOVA). The values were considered to be significantly different when the P value was less than 0.05 compared to the respective control group.

RESULTS

The results showed a significant ($P< 0.05$) decreased in serum glucose concentration and hemoglobin A1c. This reduction was proportional with a significant ($P<0.05$) increased in insulin level in diabetic rats treated with *Ocimum basilicum* extract when compared to the diabetic group and control group (table 1).

Table(1): Blood glucose, hemoglobin A1c (HbA1c) and serum insulin level from control group, diabetic group and diabetic group treated with *Ocimum basilicum* extract

Groups	Blood glucose mg/dl	HbA1c %	Insulin µ Iu/ml
Negative control	79.37±2.33 C	4.43 ± 0.09 B	7.23±0.12 A
Positive diabetic	234.87 ±4.99 A	9.35 ± 0.13 A	3.58±0.05 C
Treated	96.72±2.79 B	5.15 ± 0.29 C	6.48 ±0.14 B

The different letters mean significant differences at ($p < 0.05$) level as compared with control group. Values are expressed as mean ± SE

The results indicated that a significantly ($P< 0.05$) increase in hemoglobin concentration, PCV and RBC count in diabetic group treated with *Ocimum basilicum* extract when compared to the diabetic rats and control group (table 2).

Table (2): Hemoglobin concentration (Hb), PCV and RBC count from control group, diabetic group and diabetic group treated with *Ocimum basilicum* extract

Groups	Hb g/100ml	PCV %	RBC count $x10^6$cell/ mm^3
Negative control	12.54±0.08 A	44 ± 0.49 A	4.84±0.11 A
Positive diabetic	8.25±0.17 C	29.55±0.44 C	2.92±0.07 C
Treated	11.77 ±0.26 B	42.47 ±0.50 B	3.95 ±0.14 B

The different letters mean significant differences at ($p < 0.05$) level as compared with control group. Values are expressed as mean ± SE

Histological findings:
The Pathological changes after administration of alloxan (100mg/kg B.W) which revealed in the Pancreas show vacuolation of islet (figure 1). The liver show diffuse vacuolation in hepatocytes and congestion in central vein (figure 2). While, in the kidney was seen atrophy of glumerulus and cortical areas of vacuolated and dilated tubules (figure 3). These changes were compared with negative control (figures 4-6). While, after administration of *Ocimum*

basilicum extract (200mg/kg B.W) it was observed that pancreas included restoration of the islet within normal limites (figure 7)). The liver showed hepatocytes and central vein within normal limites (figure 8). Whereas, kidney showed cortical areas tubules and glomerulus within normal limites (figure 9). These changes were compared with diabetic group (Positive control).

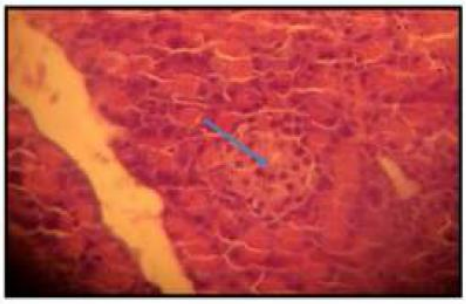

Figure (1):Pancreas section from control rats showing normal islet → H&EX100

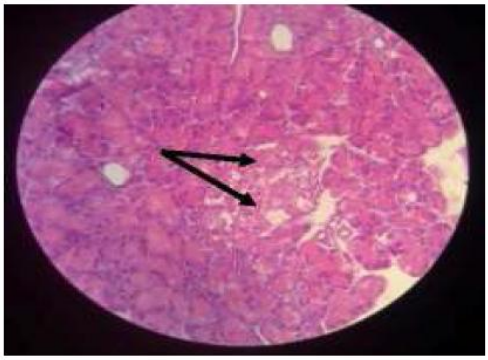

Figure (2): Pancreas section from diabetic rats, administered alloxan 100mg/kg of single dose IP showing vacuolation of islet (→) H&EX100

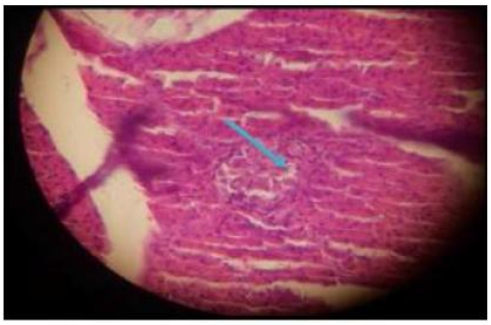

Figure (3):Pancreas section from diabetic rats treated with *Ocimum basilicum* extract 200 mg/kg for 6 weeks, demonstrates islet within normal limites (→) H&EX100

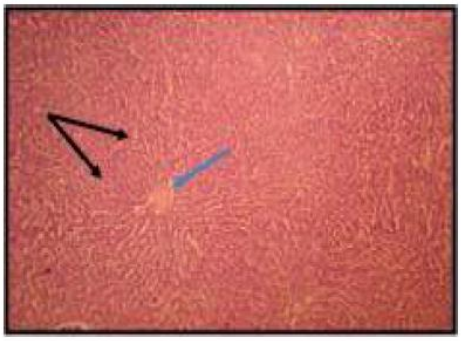

Figure (4): Liver section showing normal heypatocytes (→) central vein (→) in control rats H&EX100

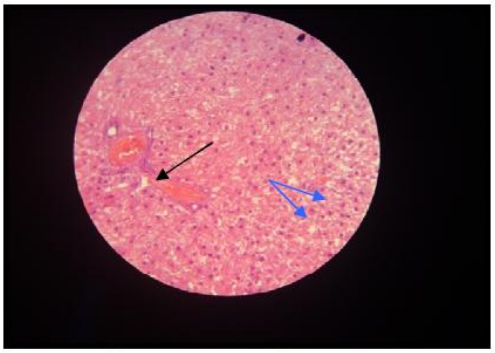

Figure (5): Liver section from diabetic rats , administered alloxan 100mg/kg single dose IP, demonstrates diffuse vacuolation of the hepatocytes (→) and congestion of the central vein (→) H&EX100

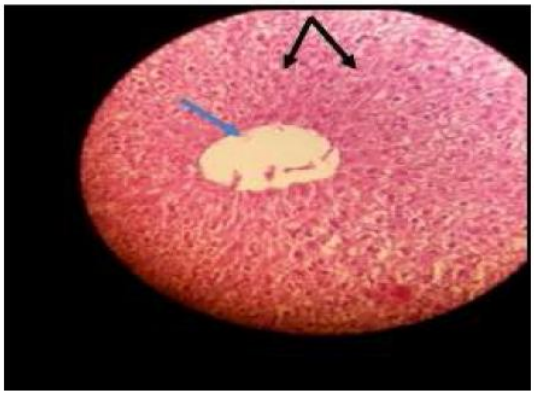

Figure (6): Liver section from diabetic rats treated with Ocimum basilicum extract 200mg/kg for 6 weeks, demonstrates hepatocytes (⟶) and central vein (⟶) within normal limits H&EX100

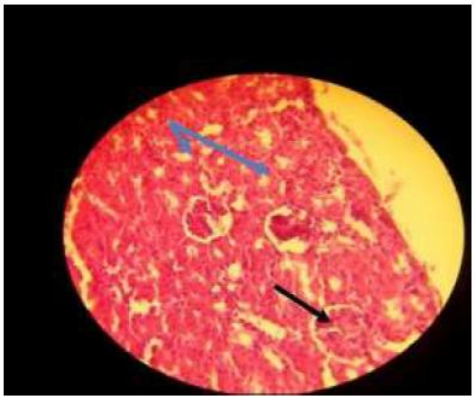

Figure (9): Kidney section from diabetic rats treated with Ocimum basilicum extract 200mg/kg for 6 weeks, demonstrates glomerulus (⟶) and cortical areas tubules within normal limits (⟶) H&EX100

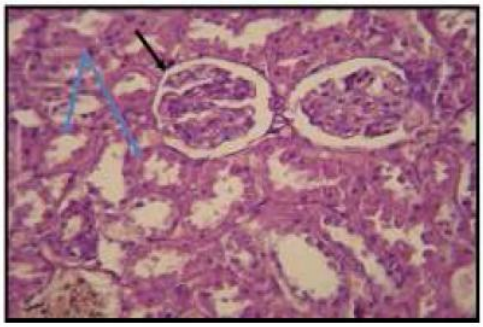

Figure (7): Kidney section showing normal glomerulus (⟶) and cortical areas tubules (⟶) in control rats H&EX400

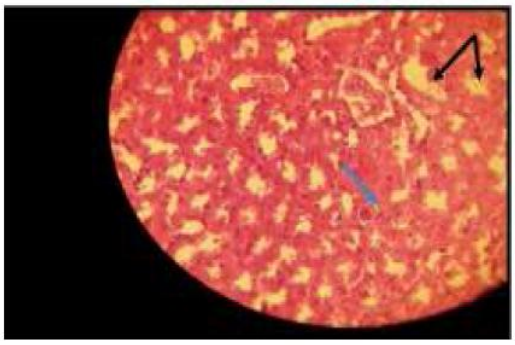

Figure (8): Kidney section from diabetic rats, administered alloxan 100mg/kg single dose IP, demonstrates atrophy of glomerulus (⟶) and cortical areas of vacuolated and dilated tubules (⟶) H&EX100

DISCUSSION

Diabetes mellitus is poised to become one of the largest global health problems in the 21st century because of its influences on multiple organ systems leading to serious complications therefore efforts remain necessary to discover new hypoglycaemic agents from plants (31). It is widely accepted that medicines of herbal origin play an essential role in treating diverse diseases since they are enriched of bioactive photochemical ingredients that might offer effective safe and potency as a therapeutic herb (32).

In the present study, alloxan was used as a diabetogen. It induces diabetes by destroying β-cells of the pancreas partially, through production of reactive oxygen species (33). In contrast in untreated diabetic rats, blood glucose levels increased due to the insulinopenia and the consequent insulin resistance (34). The oral administration of *Ocimum basilicum* extract resulted in a significant reduction in serum glucose level in diabetic rats treated. This indicated an enhanced glucose utilization triggered by insulin production from the beta cells. The profound medical effects of this herb may be attributed to its pharmaceutical potentiality due to presence of the active phyto-compounds like flavonoids, triterpenoids, alkaloids, saponins, tannins and polyphenols contents (13) These compounds are known bioactive antidiabetic principle (35). These findings are in agreement to pervious researches carried out on different ocimum species extracts (36- 38) reported that *Ocimum* species extracts has the ability to attenuate of hyperglycemia and ameliorate diabetic complications via suppressing blood sugar levels and increasing liver glycogen storage. On the other hand, Aqueous *Ocimum basilicum* extract may act via inhibition of hepatic glucose production and/or

renal glucose reabsorption, improving insulin action or stimulation of glucose utilization by the peripheral tissues (39).However, insulin level was found decreased in alloxan-induced diabetic rats. In general, several studies have demonstrated that alloxan has a β-cell cytotoxic , which significantly induced diabetes by damaging the a β-cell that causes reduction in insulin release (40, 41).There is a significant increase in serum insulin level was observed when alloxan diabetic animals were treated with *Ocimum basilicum* extract. These results have proved that the extract of *Ocimum basilicum* has a potent significant hypoglycemic effect comparable to that of effect by stimulating insulin secretion from β cells of pancreatic islets, the effects of this herb may be attributed to its flavonoids, *Ocimum basilicum*are rich source of flavonoids which have been shown to possess various biological properties related to antioxidant mechanisms (42). So it can be concluded that the extract has the potential to enhance the glucose-dependent insulin release from the pancreatic beta cells and thereby decrease the blood glucose level in alloxan-induced diabetic rats also improving insulin action (43). Moreover, further studies revealed protective effect of *Ocimum basilicum* extract on pancreatic beta cells in diminishing hyperglycemia-related oxidative stress. Indeed, it was reported that oxidative stress may have significant effect in the Glucose Transport Protein (GLUT) or at insulin receptor increasing serum glucose levels and scavengers of oxidative stress may have an effect in reducing serum glucose level in diabetes due to its strong antioxidant (44, 45). The rate of formation of HbAIC has been observed to be proportional to blood glucose level (46). The HbAIC is considered a reliable index in glycaemic control (47). In the diabetic group, HbAIC level increased significantly suggesting glycosylation of Hb in the presence of hyperglycaemia, glycosylated Hb shows reduced affinity to oxygen a process that aid free radical release (48). In extract treated, marked decrease in HbAIC concentration was observed when compared to that of diabetic animals indicating decrease in blood glucose level and recovery to Hb. A number of medicinal plants have been reported to reduce HbAIC formation due to its strong antioxidant (49). From our results, a significant decrease in hemoglobin concentration, PCV and RBC count in diabetic rats as compared with normal control indicates that the anemia occurring in DM is due to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia. On the other hand, oxidation of these glycosylated membrane proteins and hyperglyceamia in DM cause an increase in the production of lipid peroxides, which in turn cause the hemolysis of RBCs (50). However, the administration *Ocimum basilicum* extract caused increase in the hemoglobin concentration, PCV and RBC count in diabetic treated rats this may be due to the decreased level of blood glucose and/or due to lowered lipid peroxide level in RBC membrane leading to a decreased susceptibility of RBC to hemolysis. (51, 52). This in agreement with the (53, 54) demonstrated that administration of *Ocimum basilicum* in low and high dose by SRBC titre method where a good increasing values were observed in RBC, haemoglobin count and antibody in Wister albino rat this may be attributed to the presence high amount of phenolic compounds which have redical scavenging activity. Similar results were obtained by (55), who found that administration of aqueous extracts of *Ocimum basilicum* caused an increasing RBC count in *Clarias batracus*. Damage of pancreas, renal and liver tissues observed in the present study may be resulted from the increase in lipid peroxidation and decrease of antioxidant enzymes in the pancreas, kidney and liver following exposure to alloxan induced diabetes, administration of *Ocimum basilicum* extract improved the histological changes in the pancreas could be attributed to its major flavonoides components which are known to regenerate the residual beta cells after damaging effect by the diabetogenic agent (56) . This results revealed protective effect of *Ocimum basilicum* extract on pancreatic beta cell due to antidiabetic action and antioxidant properties (57, 58). Recovery of renal and hepatic tissues with treatment of the extract could be explained by the regenerative capability of the extract renal tubules and hepatocytes. The results seem to be in accordance with findings of other authors(59-62) showed that *Ocimum basilium* leaf extract suppressed histopathological alterations in liver and kidney of rats and restored creatinine, urea as well as liver function enzymes to its normal values. Similar findings were shown by (63-65) concluded that improvement liver and kidney morphology and function associated with administration of *Ocimum* species extract this explain their hepato-renal protective effect on its damage seen in diabetic rats. Similar finding were shown by (66, 67) demonstrated that dietary treatment of *Ocimum sanctum* normalized a high level of serum creatinine in diabetic rats, indicating its protective effect on renal glomerular filtration ability.

CONCLUSION

It was concluded that *Ocimum basilicum* extract possess hypoglycemia effect as evidence by amelioration of pancreatic function as well as improving of pancreas, liver and kidney structures in diabetic animals.

REFERENCES

1. Akinnuga AM.; Bamidele O.; Ebunlomo OA.; Adeniyi OS. and Adeleyea GS. (2010). Hypoglycaemic effects of dietary intake of ripe and unripe *lycopersicones culentum* (tomatoes) on

streptozotocin-induced diabetes mellitus in rats. J. Biol. Sci. 10: 50-53.
2. Lyra R.; Oliveira M.; Lins D. and Cavalcanti N. (2006). Prevention of type 2 diabetes mellitus. Arq. Bras. Endocrinol. Metab. 50: 239-249.
3. Bhandari U.; Kanojia R. and Pillai K K.(2005). Effect of ethanolic extract of *Zingiber officinale*on dyslipidemia in diabetic rats. J. Ethnopharmacol. 97:227-230.
4. Zangiabadi N.; Sheibani V. ; Asadi-Shekaari M.; Shabani M. and Jafari M. (2011). Effects of melatonin in prevention of neuropathy in STZ-induced diabetic rats. Am. J. Pharmacol. Toxicol. 6:59-67.
5. Stephen ND. (2006). Insulin Oral hypoglycemic agent and the Pharmacology of endocrine Pancreas. In: Goodman & Gillman's The Phamacological Basis of Therapeutics. Burnton LL, Lazo JS, Parker KL Eds; 11th ed.; McGraw-Hill Medical Publishing Division, New York. pp. 1613-1641.
6. Al-Attar AM. (2010). Physiological study on the effect of *acalyphawil kesiana* leaves extract on streptozotocin-induced experimental diabetes in male mice. Am. Med. J. 1: 51-58.
7. Ogbera AO.; Dada O.; Adeyeye F. and Jewo PI. (2010). Complementary and alternative medicine use in diabetes mellitus. West. Afr. J. Med. 29(3): 158-162.
8. Thompson Coon J S. and Ernst E.(2003). Herbs for serum cholesterol reduction: A systematic view. J. Fam. Pract. 52: 468-478.
9. Hicham H.; Mohammed A. and Souliman A. (2009). Sweet basil (*Ocimum basilicum* L.) improves lipid metabolism in hypercholesterolemic rats J. Clinic. Nutr. Metab. 4:181-186.
10. Patil RN.; Patil RY.; Ahirwar B. and Ahirwar D. (2011). Evaluation of antidiabetic and related actions of some indian medicinal plants in diabetic rats. Asian Pacific, J. Trop. Med. 4:20-23.
11. Bhandari M.; Jong-Anurakkun N.; Hong G. and Kawabata J.(2008). A-Glucosidase and a-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (Bergeniaciliata, Haw). Food. Chem. Age. Res. Rev. 106:247-252.
12. Dallak M.; Al-Khateeb M.; Abbas M.; Elessa R. and Al-Hashem F. (2009). Invivo, acute, normohypoglycemic, antihyperglycemic, insulinotropic actions of orally administered ethanol extract of *Citrullus colocynthis* (L.) schrab pulp. Am. J. Biochem. Biotechnol. 5: 118-125.
13. Daniel VN.; Daniang IE. and Nimyel ND.(2011). Phytochemical analysis and mineral elements composition of *Ocimum basilicum* obtained in Jos Metropolis, Plateau State, Nigeria. Int. J. Eng. Technol. 11(6):161-165.
14. Bilal A.; Ahmed A.; Bilal SN.; Jahan N. and Hajra S. (2012). Phytochemical and pharmacological studies on *Ocimum basilicum* L. Int. J. Curr. Res. Rev. 4(23):73-83.
15. Eddouks M.; Maghrani M.; Lemhadri A.; Ouahidi ML. and Jouad H. (2002). Ethnopharmacological survey of medicinal plants used for the treatment of diabetes mellitus, hypertension and cardiac diseases in the south-east region of Morocco (Tafilalet). J. Ethnopharmacol. 82: 97-103.
16. Almeida I.; Alviano DS. and Vieira DP. (2007). Antigiardial activity of *Ocimum basilicum*_essential oil. Parasitol. Res. 101 (2): 443-452.
17. Aymjad K. (2013). Antimicrobial activity of ethanolic extract of *Ocimum basilicum* leaf from Saudi Arabia. J. Bio.Tech.:1-4.
18. Supawan B.; Chanida P. and Nijsiri R. (2007). Chemical compositions and antioxidative activities of essential oils from four *Ocimum species* endemic to Thailand. J. Health Res. 21(3): 201-206.
19. Anuradha G.; Sundar BS.; kumar JS. and Ramana MV. (2014). Synthesis and characterization of silver nanoparticles from *Ocimum basilicum L. var.thyrsiflorum* Europ. J. Acad. Ess. 1(5): 5-9.
20. Al-Aumar L W K. (1994). The effect of garlic on blood glucose and cholesterol level in normal and diabetic rabbits. Master thesis. College of Veterinary Medicine, University of Baghdad (Arabic).
21. ALShamony LA.; ALKazaraji SM. and Twaji H A.(1994). Hypoglycemic effect of *Artemisia herbaand* effect of valuable extract on some blood parameters in diabetic animals. J. Ethnopharmacol. 43: 167-171.
22. Burcelin R.; Eddouks M.; Maury J.; Kande J.; Assan R. and Girard J. (1995). Excessive glucose production, rather than Insulin resistance, account for hyperglycemia in recent onset streptozocin-diabetic rats. Diabet. 35: 283-290.
23. Trinder P. (1969). Determination of blood glucose using an oxidase-peroxidase system with a non carcinogenic chromogen. J. Clin. Pathol. 22: 158-161.
24. Clark PMS. and Hales CN. (1994). How to measure plasma insulin. Diabet. Metab. Rev. 10: 79-90.
25. Parker KM.; England JD.; Da Costa J.; Hess RL. and Goldstein D E. (1981). Improved colorimetric assay for glycosylated hemoglobin. Clin. Chem. 27:669–672.
26. Schalm OW.; Jain N C. and Carrol E J. (1975). Veterinary hematology. Leo and Febiger, Philadelphia. Pp: 140-152.
27. Sood R. (1996). Hematology for student and paractitioners. 4th ed., Jaypee brothers medical publishers, (P) LTD. India. Pp: 318- 325.
28. Dasic JV.and Lewis SM. (1984). Practical hematology 6th ed. Edinburgh, Churchill. Pp: 40-55.
29. Coles E H. (1986). Veterinary clinical physiology. 4th ed. W.B. Saunders company, Philadelphia, London. P: 16.
30. Luna L G. (1993). Manual of histological staining methods of the Armed Forces Institute of Pathology. 3rd ed. New York, Mc graw-Hill.
31. Vats VJK.; Grover S. and Rathi S.(2002). Evaluation of antihyperglycemia and hypoglycemia effect of *Trigonella foenum-graecum* Linn, *Ocimum sanctum* Linn in normal and alloxanized diabetic rats. J. Ethnopharmacol. 79:95-100.

32. Seth SD. and Sharma B. (2004). Medicinal plants ofindia. Indian. J.Med. Res. 120:9-11.
33. Hamden K.; Carreau S.; Lajmi S.; Aloulou D. and Kchaou D.(2008). Hyperglycaemia, stress oxidant, liver dysfunction and histological changes in diabetic male rat pancreas and liver: Protective effect of 17 beta-estradiol. Steroids.73: 495-501.
34. Harrigan RA.; Nathan MS. and Beattie P. (2001). Oral agent for the treatment of type 2 diabetes mellitus:pharamcology toxicology and treatment. Ann. Emerg. Med. 38: 68-78.
35. Jung M.; Park M.; Lee HC.; Kang YH.; Kang ES. and Kim S.K. (2006). Antidiabetic agents from medicinal plants. Curr. Med. Chem. 13(10): 1203-1218.
36. Ifesan BOT.; Ijarotimi O S. and Osundahuns OF. (2006). Evaluation of the antioxidant activity of *Ocimum* species. J. Food Technol. 4(4): 318.
37. Mohammed A.; TankoY.; Okasha MA.; Magaji RA. and Yaro AH. (2007). Effects of aqueous leaves extract of *Ocimum gratissium* on blood glucose levels of streptozocin-induced diabetic wistar rats. Afri. J. Biotech. 6 (18): 2087-2090.
38. Umar IA.; Mohammed A.; Dawod FA; Kyrai H. and Abdullahi M.(2012). Antidiabetic action of the aqueous extract of *Ocimum suave* in alloxan-induced diabetic rats. Afri. J. Biotech. 11(38):9243-9247.
39. Malaisse WJ. (1982). Alloxan toxicity to the pancreatic Beta cell, a new hypothesis. Biochem. Pharmacol. 31:3527-3534.
40. Reema D. and Pradeep B.(2010). Astudy of the antidiabetic activity of *Barleria prionitis* Linn. Indian. J. Pharmacol. 42(2):70-73.
41. Mbaka GO.; Ogbonnia SO.; Oyeniran KJ. and Awopetu PI.(2012). Effect of *Raphia hookeri* seed extract on blood glucose, glucosylated hemoglobin and lipid profile of alloxan –induced diabetic rats. Brit. J. Med. Med. Res. 2(4):621-635.
42. Zakaria Z; Azia R. ; Lachimanan YL.; Sreenivasan S. and Rathinam X. (2008). Antioxidant activity of *Coleus blumei*, *Orthosiphon stamineus, Ocimum basilicum* and *Menthaar vensis* from Lamiaceace family. Int. J. Nat. Eng. Sci. 2(1): 93-95.
43. Devasagayam A.; Manisha M.; Priyanjali D.; Jayant L. and Saroj G. (2007). Indian herbs and herbal drugs used for the treatment of diabetes. J. Clin. Biochem. Nutr. 40: 163-173.
44. Dinanath DP.; Dnyandeo KM. and Gurumeet CW.(2011). Antibacterial and antioxidant study of *Ocimum basilicum* labistea (sweet basil). J. Adv. Pharma. Edu. Res. 2:104-112.
45. Ugwu MN.; Umar IA.; Utu-Baku AB.; Yakubu OB. and Okafor AI. (2013). Antioxidant status and organ function in streptozotocin- induced diabetic rats treated with aqueous, methanolic and petroleum ether extracts of *Ocimum basilicum* leaf. J. App. Pharma. 3(4):75-79.
46. Anitha L. and Chandralekha K. (2010). Effect of supplementation of spirulinaon blood glucose, glycosylated hemoglobin and lipid profile of male noninsulin dependent diabetics. Asian. J. Exp. Biol. Sci. 1(1): 36-46.
47. Koenig RJ.; Peterson CM.; Jones RL.; Saudek C.; Lehrman M. and Cerami A. (1978). Correlation of glucose regulation and hemoglobin AIc in diabetes mellitus. New Eng. J. Med. 295: 417- 420.
48. Bunn HF.; Shapiro R.; McManns M.; GarrickL.; MacDonald HH.; Gallop PM. and Gabby KH. (1979). Structural heterogenicity of human hemoglobin A due to non-enzymic glycosylation. J. Biol. Chem. 254: 3892-3898.
49. Bera TK.; De D.; Chatterjee K.; Ali K M. and Ghosh D. (2010). Effect of Diashis, a polyherbal formulation, in streptozotocin-induced diabetic male albino rats. Int. J. Ayurveda Res. 1(1):18-24.
50. Wolff S P. (1993). Diabetes mellitus and free radicals. Brit. Med. Bull. 49: 642-652.
51. Dorman HJ. and Hiltunen R. (2010). *Ocimum basilicum* L.: phenolic profile and antioxidant-related activity. Nat. Prod. Commun. 5(1): 65-72.
52. Nahak G.; Mishra RC. and Sahu RK. (2011). Phytochemical investigation invitro antioxidant evalualion of some *ocimum* species. J. Pharma. Res. 4(7):233-235.
53. Chinnasamy S.; Regini G.; Kingston C.; Kavitha A.; Sukumaran S. and Raj A. (2007). Potential anti-inflammatory properties of crude alcoholic extract of *Ocimum basilicum* L. in human peripheral blood mononuclear Cells. J. Heal. Sci. 53(4): 500-505.
54. Joshi SC. and Jain PK.(2014). Areview on hypolipidaemic and antioxidant potential of some medicinal plants. J. Pharm. Sci. 3(11):357-380.
55. Nahak G. and Sahu R K.(2014). Immunomodulatary activity of aqueous leaf extract of *Ocimum basilicum* linn in *Clarias batrachus*. Int. J. Pharm. Sci. 6(6):433-440.
56. Kakkvarthy BK.; Gupta S.; Gambir SS. and Gode KD.(1980). Pancreatic Beta cell regeneration a noval antidiabetic mechanism of *Pterocarpus marsupium* Roxb. Indian. J. Pharmacol. 12:1237.
57. Jayasinghe C.; Gotoh N.; Aoki T. and Wada S. (2003). Phenolics composition and antioxidant activity of sweet basil (*Ocimum basilicum* L.). J. Agric. Food Chem. 51(15): 4442-4449.
58. Gülçin I.; Elmastas M. and Aboul-Enein HY. (2007). Determination of antioxidant and radical scavenging activity of Basil (*Ocimum basilicum* L. Family Lamiaceae) assayed by different methodologies. Phytother. Res. 21(4): 354-361.
59. Gbadegesin MA. and Odunola OA. (2010). Aqueous and ethanolic leaf extracts of *Ocimum basilicum* (sweet basil) protect against sodium arsenite-induced hepatotoxicity in Wistar rats. Nig. J. Physiol. Sci. 25:29 – 36.
60. Zaveri M.; Desai N. and MovaliyaV. (2011).Effect of *Ocimum basilicum* on cisplatin models of acute renal failuer. Adv. Res. Pharma. Bio. 1(2): 91-99.
61. Orass SK. (2012). Pharmacological effect of aqueous leaves extract of *Ocimum basilicum* against liver toxicity induced by acetaminophen in male rats. J. Agric. Res. 1 (1):13-28.

62. Sakr S A. and Al-Amoudi W M.(2012). Effect of leave extract of *Ocimum basilicum* on deltamethrin induced nephrotoxicity and oxidative stress in albino rats. J. Appl. Pharma. Sci. 2 (05): 22-27.
63. Sharma MK.; Kumar M. and Kumar A. (2002). *Ocimum sanctum* leaves extract provides protection against mercury induced toxicity inSwiss albino mice. Indian J. Exp. Biol. 40: 1072–1082.
64. Arfa MM. and Rashed AM. (2008). The modulative biochemical effect of extract of *Ocimum gratissimum* as anti-oxidant on diabetic albino rats Egypt. J. Comp. Pathol. Clinic. Pathol. 21(3): 69 – 87.
65. Suanarunsawat T.; Ayutthaya WD.; Thirawarapan S. and Poungshompoo S. (2014). Anti-Oxidative, Anti-Hyperglycemic and Lipid-Lowering Effects of Aqueous Extracts of *Ocimum sanctum* L. leaves in diabetic rats. Food. Nutr. Sci. 5:801-811.
66. Suanarunsawat T. and Songsak T. (2005). Anti-hyperglycemic and anti-hyperlipidemic effect of dietary supplement of white *Ocimum sanctum* Linn. before and after STZ-induced diabetes mellitus. Int. J. Diabet. Metab. 13:18–23.
67. Gupta S.; Mediratta PK.; Singh S.; Sharama KK and Shukia R. (2006). Antidiabetic, antioxidant and antihypercholesterolamic effects of *Ocimum sanctum*(linn) seed oil. Indian. J. Exp. Biol. 44(4):300-304.