STUDY THE EFFECT OF ETHANOLIC EXTRACT OF *CERATOINA SILIQUA*, GLIMEPHAN AND METFORMIN ON SEMEN FLUID QUALITY IN DIABETIC MALE GUINEA PIG INDUCED BY ALLOXAN

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(Received 15 May 2019 ,Accepted 15 July 2019)

Key words: *Ceratonia siliqua*, Glimephan, metformin.

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ABSTRACT

This study was carried out in an animal house of the Collage of Veterinary Medicine/ University of Basrah, An attempt has been done to induce diabetic by alloxan in male guinea pigs and investigation of the effects of diabetic on semen fluid quality. Moreover, the present study aimed to evaluate the ameliorating effect of ethanolic extract of *Ceratonia siliqua*, metformin and Glimphan in experimentally induced in diabetic male guinea pigs. The study was applied on (30) adult male guinea pigs, their weight ranged between (800-1000g) and aged between 6-6.5 months. The male guinea pigs were divided randomly into five groups, each group consist of six guinea pig as the following:

Group1:- Male guinea pigs at (Negative controls) administrated normal saline3ml orally for 30 days.

Group2:- Male guinea pigs at(Positive control) given alloxan (150mg/kg B.W. I.P) for three days and remain for 30 days.

Group3:- Male guinea pigs given alloxan (150mg/kg B.W. I.P.) for three days, then treated with Glimphan drug (0.1mg/kg orally administration) for 30 days.

Group4:- Male guinea pigs given alloxan (150mg/kg I.P.) for three days, then treated with Metformin drug(15mg/kg orally administration) for 30 days.

Group5:- Male guinea pigs given alloxan 150mg/kg I.P. for three days, then treated with ethanolic extract of *Ceratonia siliqua* fruit (500mg/kg B.W. orally administration) for 30days.

At the end of experimental period, the blood samples were collected from heart by cardiac puncture, the serum was isolated to be used for the analysis of biochemical parameters such as glucose concentration, FSH, LH and testosterone concentrations. The results showed a significant (P≤0.05) increase in glucose concentration of the serum diabetic male guinea pig group as compared with (-ve) control group, whereas the results were revealed a significant(P≤0.05)decrease of glucose concentration guinea pigs treated with ethanolic extract of *Ceratonia siliqua*. The results obtained a significant decrease (P≤0.05) in body weight, FSH, LH and testosterone concentrations in serum and semen, sperm motility, sperm concentration, total sperm cell, live-dead sperm and significant (P≤0.05) increase in sperm abnormalities. While guinea pigs that are treated with ethanolic extract of *Ceratonia siliqua* the showed a significant (P≤0.05) increase in body weight. In addition, this extract improved the reproductive system by significantly increasing FSH, LH and testosterone concentrations in serum and semen, sperm motility, sperm concentration, total sperm cell, live-dead sperm and significant (P≤0.05) decrease in sperm abnormalities of the diabetic male guinea pigs treated with extract as compared with (-ve) control and another treated groups.

Histological examination showed many pathological changes in pancreas and testis in diabetic group but in those that are treated with ethanolic extract of *Ceratonia siliqua*, the histological changes were near the normal status. It is concluded that good anti-diabetic activity, hypoglycemia effect and spermatogenic activities. Based on these results, we suggested the possible utilization of *Ceratonia siliqua* as a therapy to prevent diabetic and improved the performance of male reproductive system as compared with other treated such as glimephan and metformin drugs.

INTRODUCTION

Diabetes mellitus(DM) is a syndrome of impaired carbohydrate, fat, and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin. Programs by the world health organization (WHO) suggest that the

number of people with diabetes may reach 366 million people in 2030 [1]. There are two general types of diabetes mellitus. Type 1 diabetes, also called insulin-dependent diabetes mellitus, is caused by lack of insulin secretion. Type 2 diabetes, also called non–insulin-dependent diabetes mellitus, is initially caused by decreased sensitivity of target tissues to the metabolic effect of insulin. This reduced sensitivity to insulin is often called insulin resistance.

In both types of diabetes mellitus, metabolism of all the main foodstuffs is altered. The basic effect of insulin deficiency or insulin resistance on glucose metabolism is to prevent the efficient uptake and utilization of glucose by most cells of the body, except those of the brain. As a result, blood glucose concentration increases, cell utilization of glucose falls increasingly lower, and utilization of fats and proteins increases.

The diabetes mellitus leads to damages, impaired and failures of various organs. An important complication of diabetes is the disorder in the male reproductive system. Glucose metabolism is an important event in spermatogenesis. Moreover, glucose metabolism is also important for maintaining basic cell activity, as well as specific functions, such as motility and fertilization ability in mature sperm. Diabetic disease and experimentally induced diabetes both demonstrated that either type 1 diabetes or type 2 diabetes could have detrimental effects on male fertility, especially on sperm quality, such as sperm motility [2]. One of these causes is the alarming elevate of the number of men developing DM during the reproductive age [3] and there is an elevating number of children and adolescents with type 2 DM (T2DM) [4]. When describe the treatment for DM is needed to find the most suitable antidiabetic drug [5]. Several of these compounds, if not all, have the ability to modulate cellular metabolism in a manner that may benefit some organs, but damage others.

Metformin hydrochlorid (Glucophage) is an antidiabetic medicine that belongs to the group of biguanides, describe the first-line treatment for T2DM [6]. It is an insulin-sensitizing drug that exerts its anti-hyperglycemic effects by increasing the skeletal muscle uptake of glucose and reducing the absorption of glucose in the intestinal mucosa [7, 8]. Metformin, the active ingredient in glucophage XR Moreover, it blocks liver gluconeogenesis through regulation of the gluconeogenic flux [9]. Nevertheless, although a common feature has already been attributed to this drug in promoting the activity of AMP-activated protein kinase (AMPK) [10], the

exact molecular mechanisms of metformin action remain to be fully disclosed, despite several decades of research focused on this drug action [9].

Glimephan is a tablets active ingredient glimepiride, its an antidiabetic, it reduces blood glucose levels by stimulating insulin secretion[11].

Carob is medicine plant, native to mediterranean regions and is found in south of Syria, India and most of mediterranean areas as well as in California. The broken pod has a characteristic odor caused by its 1.3% isobutyric acid content [12]. The main application of carob pods in animal feed production [13] and [14]. In fact, it is a suitable replacement for cocoa, because it lacks caffeine and theobromine. Carob germ flour is used as dietic human food [15] or as a potential ingredient in cereal-derived foods for celiac people [16]. *Ceratonia* pulp is prepared for treatment of hypercholesterolemia [17], as well as treating mouth inflammation[18]. Similarly, *Ceratonia siliqua* seeds are useful as antioxidant, anti-ulcer, anti-inflammatory and to treat and improve diabetes symptoms because it has compounds such as fibers, phytosterols and tocopherol [19,20,21]. Carob also contains minerals such as calcium, sodium, potassium and phosphorus, as well as vitamins like D, E, C, B6, niacin, folic acid and polyphenol [22,23,24].

This study aimed to investigate the hypoglycemic effect of ethanol extract of *Ceratonia siliqua* fruit by using an experimental animal model of alloxan-induced damage of β-cell of langerhans islet in male guinea pigs. To determine its effect on semen fluid quality and some biochemical parameters in diabetic male guinea pigs and it could ameliorate the diabetes mellitus and comparison the effects of ethanol extract with glimephan and metformin drugs.

MATERIALS AND METHODS

Drugs and Chemicals.

Alloxan is obtained from Safa co. Diala-Iraq, Glimephan , Metformin

Plant Material.

Ethanolic extract had been extracted from carob fruits (*Ceratonia siliqua*) that were used in this study. The carob was hand-picked from local market. It was washed with tap water. The fruits of the carob were turned to powder with the help of an electric grinder and kept in dark container at 25C°.

Preparation Ethanolic Extract from Carob Fruits(*Ceratonia siliqua*).

Fifty grams of dried carob fruits powder was defatted with (500 ml) of n-hexane for 6hours by soxhlete. The combined n-hexane extract was concentrated below 50°C under reduced pressure in a rotary evaporator to get 7ml of brown oily mass[28].The remain of dried carob powder was (40g) refluxed in (500ml) of ethanol (70%) for 16 hours concentrated by rotary evaporator at 40˚C and dried at room temperature [25].

Experimental Animals.

Thirty adult male guinea pig weight ranged between (800-1000. gm) kept for an adaptation period for 1 month in the animal house of Veterinary Medicine College / University of Basrah. The experimental animals were kept in individual cages, provided with standard ration.

Experimental design.

The guinea pigs were divided into five groups of comprising 6 animals in each group as the following:

Group1:-Male guinea pigs (negative control group) administrated normal saline (0.9% of NaCl) (3ml) for 30days.

Group 2:-Male guinea pigs were given alloxan (150mg/kg B.W. I.P) dissolved in 3ml of normal saline for three days (positive control group) and remain for 30 days.

Group 3:-Male guinea pigs were given alloxan(150mg/kg B.W.) dissolved in 3ml of normal saline by I.P. for three days and then treated with glimephan (0.1 mg/kg B.W.) orally for 30days.

Group 4:-Male guinea pigs were given alloxan (150mg/kg I.P.) for three days, then treated with metformin (15mg\kg B.W.) orally for 30 days.

Group 5:- Male guinea pigs were given alloxan (150mg/kg I.P.) for three days, then treated with ethanolic extract of *Ceratonia siliqua* fruit (500mg/kg B.W.) orally administration for 30days.

Induction of Diabetes Mellitus.

Diabetes mellitus were induced in twenty four starved male guinea pig by giving alloxan injected by one ml size syringe and in dose 150mg/kg for three days.

Collection of Blood Samples.

Blood samples (10ml) were collected from each animals at end of experiment by the heart (cardiac puncture). Blood was deposited into tube without anticoagulant and

then the blood samples were centrifuged at (3000 rpm) for 15 minutes and serum samples stored in polyethylene eppendorff tubes at (-20ºC),which are used to study biochemical parameters (serum glucose and hormonal assay such as FSH,LH and testosterone).

Study parameter:-

١-Measurement of Body weight and body weight gain.

The animals were weighed in the 0 day, 15th day and at the end period of the experiment.

2- Estimation of Glucose concentration.

Serum glucose was enzymatically measured by using a linear chemical kit (RANDOX/GLUC-PAP, United Kingdom) [26].

3-Hormonal Assay.

Serum samples and plasma semen were estimated for FSH, LH and testosterone concentrations, using ELISA technique .

4-Semen Collection.

The testis were removed along with the epididymides according method[27]

4.1- Semen Analysis.

Progressive sperm motility according method to[28].

4.2- Sperm viability (Live/dead ratio): The percentage was calculated by [28].

$$\text{Live sperm \%} = \frac{\text{Live sperm}}{\text{Total sperm count}} \times 100$$

$$\text{Dead sperm \%} = \frac{\text{Dead sperm}}{\text{Total sperm count}} \times 100$$

4.3- Sperm maturation by aniline-blue:-

The nuclear maturation was to evaluate by aniline-blue stain, according to [28].

4.4- Sperm Morphology:-

The changes in sperm morphology were to evaluate according to the method of [29].

4.5- Sperm Count: The sperm count according to [30].

Histology Examination.

At the end of experimental period the animals were sacrificed and the samples removing such as pancreas and testes were taken and fixed in 10% formalin according to [31].

Statistical Analysis:

The result were tabulated as mean values ± SD. and statistical analyze by using one-way analysis of variance. All statistical analyzes were performed using SPSS statistical version 23 software package [32].

RESULT

1-Body Weight and Body Weight Gain in Diabetic Male Guinea Pig Treated of *Ceratonia siliqua* Fruit, Glimephan and Metformin.

The results in Table (1) revealed a significant reduce ($P \leq 0.05$) in body weight and body weight gain of diabetic male pigs as compared with (-ve) the control group and the treated group with ethanolic extract of *Ceratonia siliqua* while the results showed non-significant change ($P > 0.05$) body weight of diabetic male pigs treated with ethanolic extract of *Ceratonia siliqua* as compared with control (-ve) group.

Table(1) Mean of Body Weight and Body Weight Gain in Diabetic Male Guinea Pig Treated with *Ceratonia siliqua* Fruit, Glimephan and Metformin. (n=6)

Parameters / Groups	Body Weight (g)			Body Weight Gain (g)
	0Days	15Days	30Days	
Control (-ve) Normal Saline(0.9% NaCl)	930.78±46.19 a	933.70±79.23 a	935.53±39.25 a	4.75±0.11 b
Control (+ve)	940.83±70.65	885.81±59.37	860.40±89.19	-80.43±5.02

Alloxan (150mg/kg)	**a**	**a**	**b**	**c**
Alloxan + Glimephan	**908.39±33.08** **a**	**800.94±23.62** **b**	**913.89±51.11** **a**	**5.50±0.49** **b**
Alloxan + Metformin	**905.45±26.89** **a**	**890.35±78.21** **b**	**909.65±49.08** **a**	**4.20±0.85** **b**
Alloxan + Ehanolic Extract of *Ceratonia siliqua* (500mg/kg/B.W.)	**910.18±24.52** **a**	**886.69±92.18** **a**	**933.39±68.73** **a**	**23.21±5.67** **a**

N=number of animals., Small letters denote differences between groups,P≤0.05 vs. control.

2- Glucose Con centration in Serum of Diabetic Male Guinea Pig Treated with *Ceratonia siliqua* Fruit, Glimphan and Metformin.

The results in Table (2) showed a significant rise (P≤0.05) in glucose concentration in serum of diabetic male pigs as compared with the control group(-ve.) and another treated groups while the results showed non-significant change (P>0.05) glucose concentration in serum of diabetic male pigs treated with ethanolic extract of *Ceratonia siliqua* compared with control(-ve) group.

Table (2) Mean of Glucose Concentration in Serum of Diabetic Male Guinea Pig Induced by Alloxan treated with *Ceratonia siliqua* Fruit and Glimephan and Metformin. (n=6)

Parameters **Treatments**	**Glucose** **mg/dl**
Control (-ve) **Normal Saline(0.9% NaCl)**	**100.31±9.75** **c**
Control (+ve) **Alloxan (150mg/kg)**	**389.14±20.05** **a**

Alloxan + Glimephan	**137.25±13.68 b**
Alloxan + Metformin	**168.12±36.49 b**
Alloxan + Ehanolic Extract of *Ceratonia siliqua*(500mg/kg/B.W.)	**117.58±24.52 c**

n=number of animals., Small letters denote differences between groups,P≤0.05 vs. control,

3-Physical Properties of Semen Analysis in Diabetic Male Guinea Pig Treated With *Ceratonia siliqua* Fruit, Glimephan and Metformin.

The results in Table (3) revealed that the physical properties of semen analysis in diabetic male pigs treated with *Ceratonia siliqua* fruit, glimephan and metformin drugs. The results were revealed a significant ($p \leq 0.05$) decline in sperm motility, sperm concentration, live-dead sperm and a significant ($p \leq 0.05$) increase in sperm abnormalities in diabetic male pigs(+ve) when compared with the control(-ve) and another treated groups. While the results were showed a significant ($p \leq 0.05$) exceed in sperm motility, sperm concentration, live-dead sperm and a significant ($p \leq 0.05$) decrease in sperm abnormalities in diabetic male pig treated with ethanolic extract of *Ceratonia siliqua* compared with control and another treated groups but its non-significant ($p > .05$) in sperm motility, sperm concentration in diabetic male pig treated with glimephan and metformin drugs as compared with the control(-ve).

Table (3) Physical Properties of Semen Analysis in Diabetic Male Guinea Pig Treated with *Ceratonia siliqua* Fruit, Glimephan and Metformin Drugs. (n=6)

Groups / Parameters	Control (-ve) Normal Saline (0.9% NaCl)	Control (+ve) Alloxan (150mg/kg)	Alloxan + Glimephan	Alloxan + Metformin	Alloxan + Ehanolic Extract of *Ceratonia siliqua*(500mg/kg /B.W.)
Sperm motility%	75.17 ±10.21b	40.10 ±3.43c	60.22 ±11.47b	55.23 ±8.63b	85.40 ±19.12a
Sperm concentration ($\times10^6$/ml)	7.25 ± 1.04b	3.75±0.19c	6.91±0.53ab	6.32 ± 0.12b	7.74±0.67a
Total sperm cell / ejaculate($\times10^6$/ml)	4.74±0.36b	1.98 ± 0.14c	5.35 ± 0.13b	4.74±0.36b	6.79 ± 0.21a
Live-dead sperm ratio	70:30 ± 5.98b	35:65 ± 6.17c	82:18 ± 3.04a	70:30 ± 5.98b	87:13 ± 2.27a
Sperm abnormalities	17.56 ±2.5b	24.38 ± 5.23a	19.25±2.12ab	21.52±3.24ab	8.59 ± 0.28c

N=number of animals., Small letters denote differences between groups,P≤0.05 vs. control.

4- Sperm morphology in Diabetic Male Guinea Pig Treated with *Ceratonia siliqua* Fruit, Glimephan and Metformin Drugs.

Bent tail:- The pigs in (+ve control) induced diabetic were observed to have significantly (P≤0.05) elevate spermatozoa bent tail abnormality when compared with control pigs and another groups.

Curved mid-piece and tail:- Fewer spermatozoa of pigs in the control group and another groups (P≤0.05) had curved tail sperm abnormality than those of the diabetic pigs (+ve control).

Normal head without tail/tailless head:- The pigs in the control group and treated with extract had low number of spermatozoa with normal head without tail abnormality when compared with pigs in another treated groups. The percentage of this abnormality in the male guinea pigs (+ve control group) was higher than that of the control pigs and another groups.

Normal tail without head/headless tail:- The spermatozoa of diabetic pigs in (+ve control group) were observed much of the normal tail without head/ headless tail abnormality than those of the control guinea pigs and another treated groups.

Big head: distinct changes have been observed in sperm structure like big head of diabetic pigs as compared with control (-ve) and treated with ethanolic extract of *Ceratonia siliqua* group.

Table (4):- Sperm morphology Diabetic Male Guinea Pig Treated with *Ceratonia siliqua* Fruit, Glimephan and Metformin Drugs. (n= 6)

Groups / Parameters	Control (-ve) Normal Saline (0.9% NaCl)	Control (+ve) Alloxan (150mg/kg)	Alloxan + Glimephan	Alloxan + Metformin	Alloxan + Ehanolic Extract of *Ceratonia siliqua* (500mg/kg/B.W.)
Bent tail	1.31 ±0.012 c	10.08±0.21 a	3.14±0.01 b	4.36 ±0.005 b	2.26±0.002 c
Curved mid-piece and tail	1.23 ± 0.011 c	20.21±0.01 a	4.59±0.001 b	5.92±0.004 b	1.78±0.006 c
Normal head without tail	2.02± 0.014 c	15.3±0.011 a	4.07±0.002 b	5.54±0.032 b	2.65±0.005 c
Tailless head	1.28± 0.001 c	18.09±0.04 a	6.04±0.006 b	4.11±0.001 b	2.06±0.001 c
Normal tail without head	3.76± 0.005 c	17.02±0.02 a	5.92±0.102 b	6.24±0.023 b	2.90±0.10 c
Headless tail	2.15±0.004 c	15.13±0.10 a	5.68±0.003 b	5.31±0.020 b	1.69±0.002 c
Big head	0.0±0.0 c	5.13±0.10 a	1.29±0.001 b	2.63±0.010 b	0.0±0.0 c

N=number of animals., Small letters denote differences between groups,P≤0.05 vs. control,

N=number of animals., Small letters denote differences between groups,P≤0.05 vs. control.

5- FSH, LH and Testosterone Concentrations in Serum and Semen Male Guinea Pig with Treated *Ceratonia siliqua* Fruit, Glimphan and Metformin Drugs.

The results in Table (5) revealed that the FSH, LH and testosterone concentrations in serum and semen diabetic male pigs treated with *Ceratonia siliqua* fruit, gimephan

and metformin drugs. The results indicated a significant (P≤0.05) decline of FSH,LH and testosterone concentrations in serum and semen diabetic male pigs (+ve) as compared with the control negative group and other groups while the results showed non-significant (P>0.05) changes of FSH and LH concentrations in serum of diabetic male pigs treated with ethanolic extract of *Ceratonia siliqua* fruit as compared with control (-ve) group while the results were showed a significant(P≤0.05) rise of testosterone serum and semen diabetic male pig treated with ethanolic extract of *Ceratonia siliqua* fruit whencompared with the control (–ve) group and other groups.

Table (5):- Mean of FSH, LH and Testosterone Concentrations in Serum and Semen Male Guinea Pig treated with *Ceratonia siliqua* Fruit, Glimphan and Metformin Drugs on Induced by Alloxan. **(n=6)**

Parameters / Groups	FSH (mlU/ml)	LH (mlU/ml)	Testosterone in Serum ng/ml	Testosterone in Semen ng/ml
Control (-ve) Normal Saline(0.9% NaCl)	2.73±0.021 a	4.97±0.002 a	0.76±0.011 b	0.98±0.014 b
Control (+ve) Alloxan (150mg/kg)	2.05±0.014 d	1.09±0.025 d	0.24±0.010 d	0.37±0.012 d
Alloxan + Glimephan	2.35±0.016 c	4.12±0.0012 b	0.49±0.013 c	0.53±0.017 c
Alloxan + Metformin	2.42±0.012 b	4.01±0.001 b	0.40±0.007 c	0.48±0.008 c
Alloxan + Ehanolic Extract of *Ceratonia siliqua*(500mg/kg/B.W.)	2.89±0.011 a	5.30±0.006 a	1.23±0.019 a	1.64±0.015 a

N=number of animals., Small letters denote differences between groups,P≤0.05 vs. control.

-Histological Examination:-
-Pancreas:-

The section of pancrease of **negative control group** guinea pig is composed of two major types of tissues, the *acini,* which secrete digestive juices into the duodenum, and, the *islets of Langerhans,* which secrete insulin and glucagon directly into the blood. Fig.(1) normal of langerhan's islets. The section of control group of guinea pigs' pancreas showed closely packed lobules of pancreatic acini. While the diabetic group of pig by alloxan positive control group revealed histopathological changes of both exocrine and endocrine part of the pancreas represented by vacuolation and

degeneration marked decrease of β-cells. Some exocrine acini revealed focal acinar damage represented by cytoplasmic vacuolation and pyknotic nuclei of some acinar cells obvious as shown in Fig. (2). Moreover the pancrease of the pigs treated with glimephan drug showed clear revealed histopathological changes. The changes included vacuolation of langerhan's islets as shown in Fig. (3) and the pancrease of guinea pig treated with metformin drug showed clear revealed histopathological changes. The changes included vacuolation of langerhan's islets, hyperplasia of β-cells of islets of langerhans associated with pyknosis of their nuclei as shown in Fig. (4). But the pancrease of guinea pig treated with *Ceratonia siliqua* showed amelioration of architecture of islets langerhan's compared to pancreas treated with alloxan alone as shown in Fig. (5).

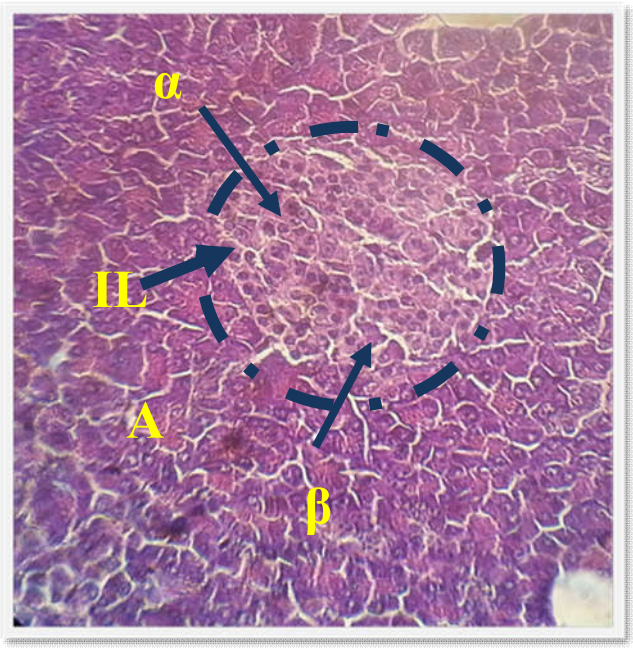


Fig.(1):-Section of pancreas of guinea pig ((-ve) control group). Showing present normal islet of langerhans (IL) cells granulated cytoplasm of islet cell with light and large nuclei (β-cell) or with small, dark nuclei on periphery (α-cell)and normal acini (A), stained with H&E. (400X).

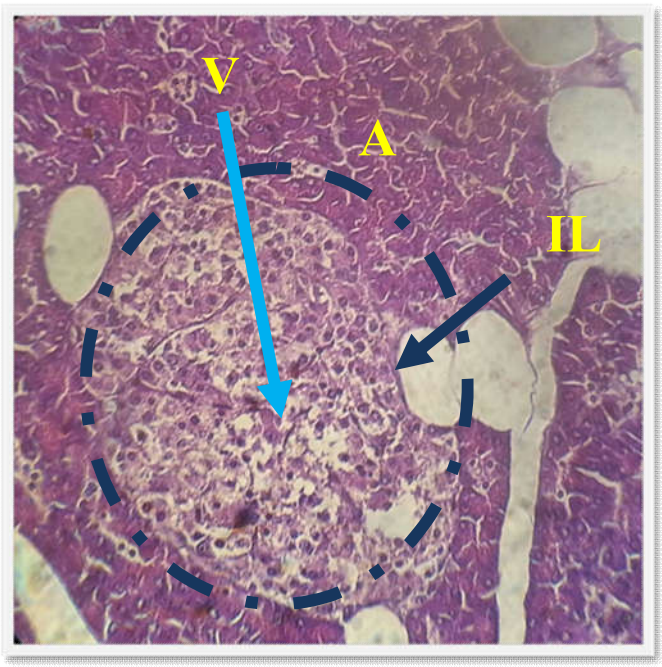
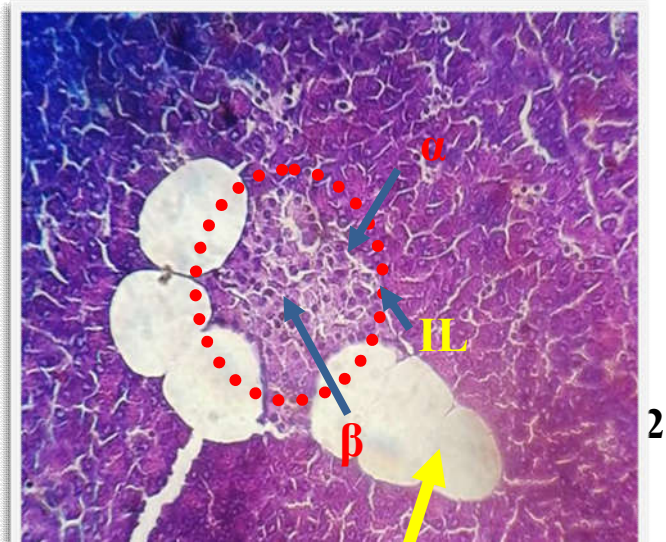
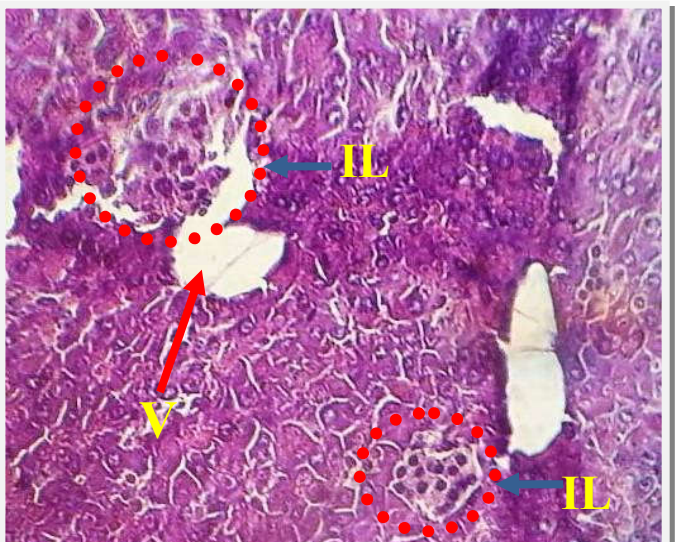


Fig.(2):-Section of pancreas of diabetic guinea pig ((+ve) control group). Showing hypertrophy and hyperplasia of β-cells of islets of langerhans associated with pyknosis of their nuclei,vacuolated(V) islet of langerhans (IL), Stained with H& E. (400X).





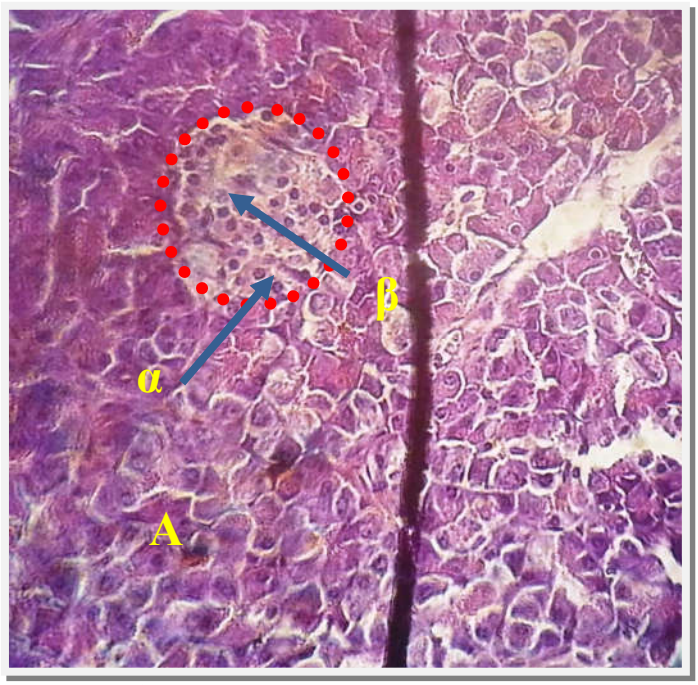
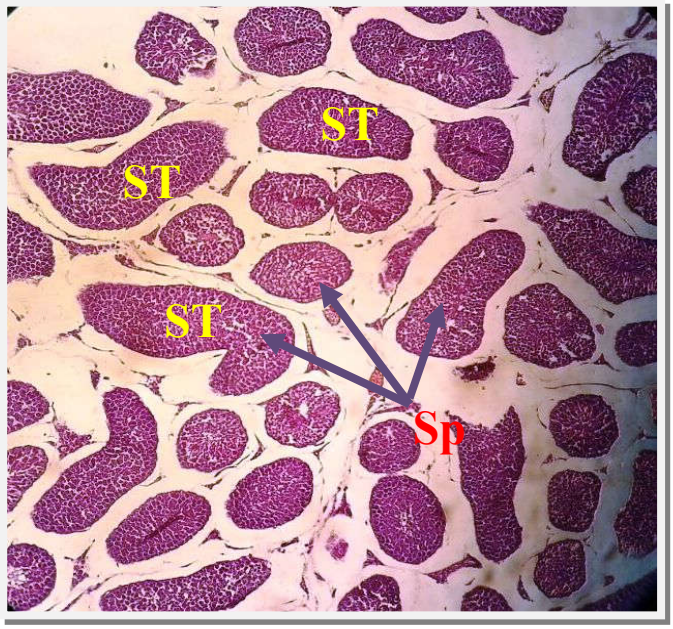


Fig.(5):-Section of Pancreas of diabetic guinea pig treated with ethanolic extract of *Ceratonia siliquia*. Showing regeneration proliferation of islet of langerhans (IL), showing small islet(IL) appear as newly formed cells(N), normal acini (A), Stained with H&E.(400X).

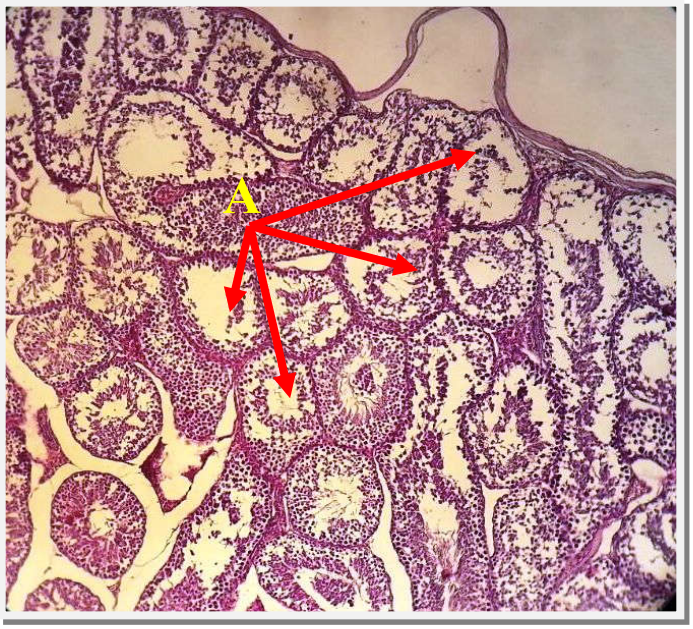
Testis:-

The section of testis of control group pigs showed normal architecture of seminiferous tubules with different stage of spermatogenesis, primary spermatocyte and normal sertoli cells as shown in Fig.(6). While the section of testis untreated diabetic pigs

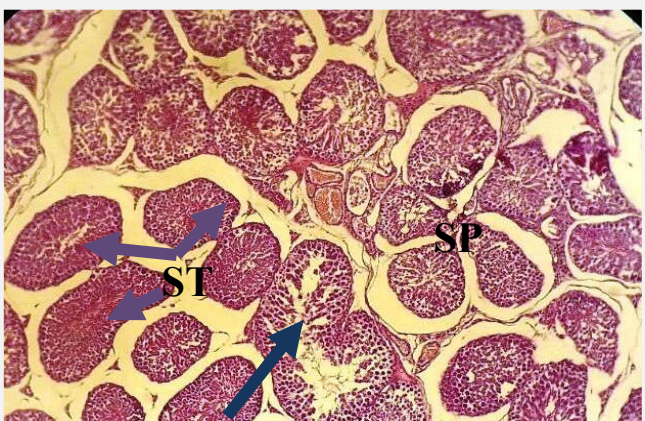
showed vacuolation of spermatognia and suppression of spermatogenesis, widening of inter seminiferous tubules, arrested of spermatogenesis and reduce in the number of interstitial leydig cells as shown in Fig. (7). Moreover, section of testis of pig treated with **glimephan drug.** The seminiferous tubules have mild vacuolation and shows normal association of spermatogenesis. as shown in Fig. (8). Also, section of testis of guinea pig treated with **metformin** drug showed normal seminiferous tubules and spermatogenesis, interstitial leydig cells and present sertoli cells as shown in Fig.(9). But the testis of pig treated with **ethanolic extract of *Ceratonia siliqua*** showednormal seminiferous tubules, spermatogenesis, interstitial leydig cells between seminiferous tubules and present primary spermatocyte as shown in Fig.(10).

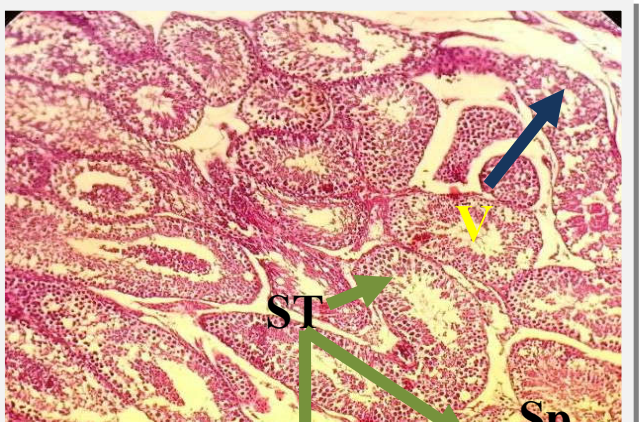


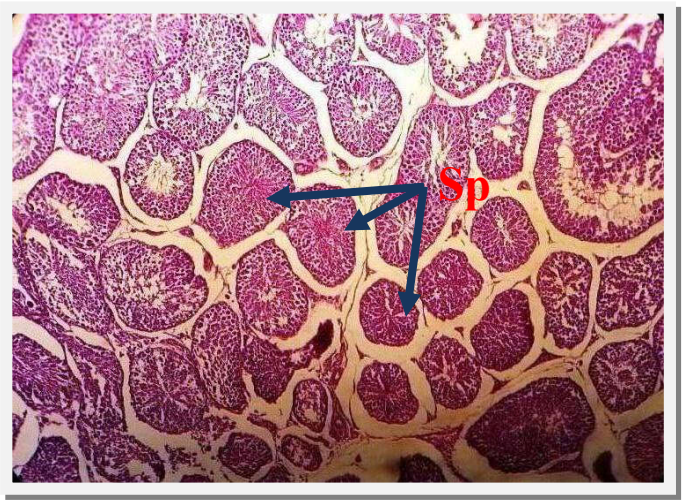
Fig(6):- Section of testicular tissue of guinea pig belongs to the control group. Shows normal association of spermatogenesis (Sp). (H&E stain,100X)



Fig(7):- Section of testis of guinea pig treated alloxan (DM). Showing (A) sever vacuolation and widening of inter seminiferous tubules, suppression of spermatogenesis(Sp), . (H&E,stain,100X).







Fig(10):- Section of testis of diabetic guinea pig treated with ethanolic extract of *Ceratonia siliqua*. Showing normal seminiferous tubules and spermatogenesis(Sp).(H&E stain,100X)

DISCUSSION

Hyperglycemia leads to dysfunction in target organs of diabetic animals [2]. Partial or complete insulin deficiency in diabetic animals appears to have adverse

effects on all organs, including reproductive systems[33] [34] [35]. This study confirms that diabetes have an adverse effect on male reproductive function as proposed by others[36] also this study demonstrates a protective effect of the ethanolic extract of *Ceratoina siliqua* recovery of diabetes and testicular dysfunctions eminent in diabetic state.

The body weight of diabetic guinea pigs was declined. This is associated with dehydration and catabolism of fats and proteins due to proteolysis on muscular tissues occurred in insulin deficiency states[37] [38], furthermore, our results also showed that treated with ethanolic extract of *Ceratoina siliqua* lead to improve the body weight gain in male guinea pigs, Through its contents which were flavonoids several flavonoid subclasses have shown decrease energy intake, increase glucose uptake in muscle in vivo, and decrease glucose uptake in adipose tissue in vitro.

In this study, glucose concentration of serum diabetic guinea pigs elevated above the normal concentration, and this elevation in glucose concentration was approximately constant through the course of diabetes. Reduction in pancreatic β-cell mass is associated with development of diabetes [39]. In the present study, alloxan was used for induction of diabetes. This alkylating agent causes pancreatic β-cell damage and death lead to insulin deficiency. Treatment with the ethanolic extract of *Ceratonia siliqua* revealed reduction in blood glucose concentration diabetic guinea pigs, due to the presence of flavonoid in *Ceratonia siliqua* fruit. These compounds can reduce glucose concentration by stimulating pancreatic β-cells to secret more insulin in blood circulation, in this way, blood glucose concentration is controlled better [40,41]. It is possible that the ethanolic extract of *Ceratonia siliqua* may increase glucose removal from blood, through decrease the release of glucagon or increase that of insulin, and stimulate directly glycolysis in peripheral tissues, or reduce glucose absorption from the gastrointestinal tract.

The changes of pancreas in diabetic pigs showed the reduced number of islet, degeneration of β cells, hydropic degeneration and pyknosis. While the pancreas of pigs treated with ethanolic extract of *Ceratoina siliqua* showed improvement of architecture of islets langerhan's compared to pancreas treated with glimephan and metformin drugs. The pancreas of pigs treated with the ethanolic extract of *Ceratoina siliqua* showing nearly normal structure of islets of langerhans. The protective effects of *Ceratoina siliqua* as antioxidant on alloxan induced β cells destruction, increasing

islets size, and β cell population were presented. β cell apoptosis and replication rates and islets neogenesis are the major determinants of pancreatic endocrine capability for insulin secretion and glucose homeostasis [49].

Also in this study the effect of diabetes on semen quality was investigatedand this is in agreement with the previous observations[42] [43] [44]. An induction of reduced fertility in the subjects[45]. This was attributed to damage of the secretory epithelia cells of the seminiferous tubules[46], probably due to oxidative damage from glucose auto- oxidation and excessive production of superoxide radicals and formation of advanced glycation end products associated with DM [33]. Our results showed that administration of ethanolic extract of *Ceratoina siliqua* enhanced the reproductive parameters of diabetic male guinea pigs. The extract was more prominent on the percentage motility and the live /dead ratio of the spermatozoa than the effects of the glimephan and metformin drugs, this result reported that the presence of flavonoid in the extract is potent antioxidant and able to scavenge free radicals, flavonoid also has an anti-hyperglycaemic activity, which may suppress glucose release from the liver as well as improving glucose uptake in peripheral tissues[43] .

In present study, the diabetic caused germ cells depletion in pigs. The spermatide and spermatozoa cells affected. The majority changes that occur on testicle tissue and spermatogenesis procedure following diabetic are probably due to the changes in testosterone concentration [44] while its treated with ethanolic extract of *Ceratonia siliqua* resulted stimulation on testicle tissue and spermatogenesis procedure, it increased the concentration testosterone by stimulating the gonad regulating cycle including the hypothalamo-pituitary axis. This in turn regulates the amount of testosterone in the organism if the testosterone concentration in the blood is low and the tests will signal the hypothalamus to release LHRH thus the hypophysis releases gonadotropin, LH and FSH consequently, the interstitial cell of leydig activate the production of testosterone [48,49].

The *Ceratonia siliqua* can ameliorative the spermatogenesis and steroidogenesis processes of leydic cells through the observation in this study we concluded that the ethanolic extract of *Ceratoina siliqua* induces anti-hyperglycemia and improves the male reproductive system was more than treated to glimephan and metformin drugs. In addition, these findings suggest the possible utilization of the ethanolic extract of *Ceratoina siliqua* as a therapy.

دراسة تأثير المستخلص الايثانولي لنبات الخروب *Ceratoina siliqua* fruit وعقار Glimephan و Metformin على كفاءة السائل المنوي في ذكور خنازير غنيا المستحثة داء السكري بوساطة الالوكسان

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الخلاصة

تمت الدراسة في البيت الحيواني التابع الى كلية الطب البيطري- جامعة البصرة لمحاولة استحداث داء السكري باستخدام الالوكسان ولمعرفة ما يعكسه داء السكري على كفاءة السائل المنوي في ذكور خنازير غنيا فضلا عن دراسة التأثير العلاجي لمستخلص الايثانولي لثمار نبات الخروب بجرعة (٥٠٠ملغم / كغم من وزن الجسم) بالمقارنة مع عقار **Glimephan** (المخفض لمستوى السكر في الدم بجرعة ٠.١ ملغم/كغم من وزن الجسم) و عقار(**Metformin** ١٥ ملغم/كغم) ومدى فاعلية هذا المستخلص في تخفيض السكر و اثاره الضارة .

وتضمنت الدراسة استخدم ٣٠ ذكر من خنازير غنيا البالغة المحلي ، تتراوح أوزانها ما بين (٨٠٠ - ١٠٠٠غم) وباعمار تتراوح (٦- ٦.٥ شهر) قسمت عشوائيا بالتساوي إلى خمس مجاميع (٦ خنزير غنيا/ مجموعة)

الأولى:- مجموعة السيطرة(السالبة) جرعت ذكور خنازير غنيا (٠.٩ %) من المحلول الفسيولوجي (٣مل)لمدة ٣٠ يوم.

والمجموعة الثانية:- مجموعة سيطرة (موجبة) حقنت ذكور خنازير غنيا في غشاء البروتون بالالوكسان ١٥٠(ملغم/كغم)بعد أذابته ب(٣مل)من المحلول الفسيولوجي لمدة ٣ يوم لاستحداث داء السكري وتركت لمدة ٣٠ يوم بدون إي معاملة.

المجموعة الثالثة:- حقنت ذكور خنازير غنيا في البروتون بالالوكسان (١٥٠ ملغم/كغم)بعد أذابته ب(٣مل) من المحلول الفسيولوجي لمدة ٣ أيام + عقار **Glimephan** لمدة٣٠ يوم.

المجموعة إلرابعة:- حقنت ذكور خنازير غنيا في البروتون بالالوكسان ١٥٠ ملغم/كغم بعد أذابته ب(٣مل)من المحلول الفسيولوجي لمدة ٣ أيام+ (١٥ ملغم/ كغم) عقار **Metformin** لمدة٣٠ يوم،

المجموعة إلخامسة :- حقنت ذكور خنازير غنيا في البروتون بالالوكسان ١٥٠ ملغم/كغم بعد أذابته ب (٣مل)من المحلول الفسيولوجي لمدة ٣ أيام+ (٥٠٠ ملغم/ كغم) من المستخلص الايثانولي لثمار نبات الخروب لمدة ٣٠ يوم المجموعة .

بعد انتهاء فترة التجربة تم سحب عينات الدم(١٠مل) من قلب الحيوانات حيث وضعت في أنابيب غير حاوية على مانع للتخثر لغرض الحصول على مصل الدم لإجراء بعض قياسات المعايير الكيموحيوية كقياس تركيز

السكر في مصل الدم وقياس تراكيز بعض الهرمونات مثل **FSH,LH and testosterone** وتوصلت الدراسة إلى النتائج التالية :

لوحظ هناك انخفاض معنوي (**p≤0.05**) في اوزان الحيوانات وفي تركيز هرموني **FSH,LH** و هرمون التيستوسترون في مصل ومني الحيوانات كذلك تأثرت كفاءة السائل المنوي حيث لوحظ قلة في حركة الحيامن وزيادة في اعداد الحيامن المشوهة والميتة ووجود ارتفاع معنوي(**p≤0.05**) في معدل مستويات السكر في مصل الدم في مجموعة السيطرة المصابة بداء السكر(مجموعة السيطرة الموجبة) مقارنة مع مجموعة السيطرة السالبة . بينما المجموعة التي عوملت بالمستخلص الكحولي للخروب لوحظ ان هنالك ارتفاع معنوي (**p≤0.05**) في اوزان الحيوانات بالإضافة الى وجود ارتفاع معنوي(**p≤0.05**) في تركيز هرمون **FSH,LH** والتيستوسترون في مصل ومني الحيوانات المعالجة بالإضافة الى زيادة في حركة الحيامن و انخفاض معنوي في اعداد الحيامن المشوهة والميتة في الحيوانات المعالجة في بالمستخلص الايثانولي لنبات الخروب مقارنة مع مجموعة السيطرة والمجاميع المعالجة الاخرى .

لوحظ ايضا وجود تغيرات نسجية في البنكرياس والخصى لذكور خنازير غنيا المصابة بالسكري ولكن هذه التغيرات النسجية اختفت في الحيوانات المعاملة بالمستخلص الايثانولي لنبات الخروب وكانت اقرب الى الطبيعي.

نستنتج أن مستخلص الايثانولي لثمار نبات الخروب له فعالية مضادة لداء السكري ممتازة وامن مقارنة بالعلاجات الاخرى المستخدمة مثل عقار **glimephan** وعقار **metformin**. إي له تأثير مخفض للسكر ويحسن كفاءة وصفات السائل المنوي لذا يمكن ان يوصى باستخدامه كعقار.

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