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STUDY THE EFFECT OF PHYTOESTEROL OF *Ceratonia siliqua* FRUIT AND INSULIN ON HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN DIABETIC PREGNANT FEMALE RABBITS INDUCED BY ALLOXAN

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Key words: *Ceratonia siliqua*, Insulin ,Alloxan.

ABSTRACT

The our current study was conducted in Collage of Veterinary Medicine\ University of Basrah, to evaluate the effect of phytoesterol extract of *Ceratonia siliqua* fruit and Insulin on haematological and biochemical parameters changes by using pregnant diabetic female rabbits induced by alloxan. The study done was applied on (32) adult female rabbits, their weight ranged between (1500-2000g) and aged between 7-7.5M. The female mated with healthy male before 1st week of it treated. The pregnant diabetic female rabbits divided randomly into three groups, each group consist of eight rabbits as the following:

Group1:-Healthy female rabbits at 1st week of pregnant (Negative controls) administrated normal saline (0.9% of NaCl)(3ml)for 21days.

Group 2:- Female rabbits at 1st week of pregnant given alloxan 150mg\kg B.W. I.P for three days (Positive control) and remain for 21 days.

Group 3:- Female rabbits at 1st week of pregnant initially given alloxan150mg\kg B.W. I.P. for three days, then treated with Insulin for 21 days.

Group 4:- Female rabbits at 1st week of pregnant initially given alloxan 150mg\kg I.P. for three days, then treated with phytoesterol of *Ceratonia siliqua* fruit 1ml\kg B.W. orally administration for 21 days. At the end of treatment period blood samples(10 ml) collected from animals heart and blood sample divided into two parts, first part (2ml) put in tube contain EDTA for measured hematological parameters and



second part (8ml) put in plane tubes then centrifuge for obtained on serum for measurement biochemical parameters.

Results showed that phytoesterol of *Ceratonia siliqua* fruit and insulin caused significant reduction ($P \leq 0.05$) in glucose concentration in serum of diabetic female rabbits compared with (+ve control). It also, showed significant increase ($P \leq 0.05$) in Red Blood Cell (RBC), Hemoglobin (Hb) and Mean Corpuscle hemoglobin concentration(MCHC) in rabbit drenched phytoesterol extract of *Ceratonia siliqua* fruit and Insulin. The results of MCV revealed significant decrease ($P \leq 0.05$) in diabetic female rabbits induced by alloxan (+ve) control compared with (-ve) control and another treated groups while the results showed non-significant ($P \leq 0.05$) in MCV of diabetic female rabbits drenched phytoesterol extract of *Ceratonia siliqua* fruit compared with (-ve) control but the results showed a significant increase ($P \leq 0.05$) in MCV of diabetic female rabbits drenched phytoesterol of *Ceratonia siliqua* fruit compared with insulin group. It showed non-significant changes in White Blood Cell (WBC) of female rabbits drenched phytoesterol of *Ceratonia siliqua* fruit compared with (-ve control). The study revealed significant decrease ($P \leq 0.05$) in total cholesterol, triglyceride, Low Density Lipoprotein(LDL) and very Low Density Lipoprotein(VLDL) of diabetic female rabbits drenched phytoesterol extract of *Ceratonia siliqua* and insulin group compared with positive control group while it showed significant increase ($P \leq 0.05$) in High Density Lipoprotein (HDL) in rabbits treated with phytoesterol of *Ceratonia siliqua* and insulin group compared with (+ve control). It is concluded that good anti-diabetic activity, hypoglycemia effect, amelioration of heamatological parameters and improve dyslipidemia corroborating the folk use of phytoesterol extract of *Ceratonia siliqua* fruit preparations, and contributing for its pharmacological validation

INTRODUCTION

Diabetes mellitus is manifested by hyperglycemia as a result of problems in insulin production, insulin function, or both. Programs by the World Health Organization (WHO) suggest that the number of people with diabetes may reach 366



million people in 2030 [1]. The occurrence of diabetes during pregnancy may be classified into clinical diabetes, in cases previously diagnosed with type 1 or type 2 diabetes and gestational diabetes [2].

In pregnancies complicated by diabetes, hyperglycemia and lipid metabolism were altered and associated with both maternal and fetal complications [2,3] causing reproductive abnormalities that enhance spontaneous abortion, congenital anomalies, and neonatal morbidity and mortality [4,5]. Oxidative stress has been implicated as a Contributor to both the onset and the progression of diabetes and its associated complications. Some of the consequences of an oxidative environment are the development of insulin resistance, β -cell dysfunction, impaired glucose tolerance, and mitochondrial dysfunction, which can lead ultimately to the diabetic disease state. Experimental and clinical data suggest an inverse association between insulin sensitivity and reactive species oxygen (ROS) levels [6]. DM is a risk factor for atherosclerotic diseases, which can be aggravated by the presence of arterial hypertension and dyslipidemia[7]. Maternal diabetes is a predisposing factor for embryonic lethality, congenital abnormalities and placental defects [3, 4, 5]. In rodents, the visceral yolk sac (VYS) acts as an active region for metabolic exchange and nutrition uptake [6, 7, 8], becoming an important tissue that persists active during the days following the placentation period from gestational day 12 (12 g/d) onwards [9].

Carob is native to Mediterranean regions and is found in south of Syria, India and most of Mediterranean areas as well as in California. It grows wildly in shapoor, Fars, Iran [10,11,12]. The pod is light to dark brown, oblong, flattened, straight or slightly curved, with a thickened margin, and ranges from 10 to 20 cm in length and 1.5–2 cm in width. The unripe pod is green, moist and very astringent, but the ripe pod is sweet. The broken pod has a characteristic odor caused by its 1.3% isobutyric acid content [13]. Current world production of carob pod has been estimated at about 310,000 tons per year, produced from about 200,000 hectares with very variable yields depending on the cultivar, region, and farming practices [14]. The main application of carob pods in animal feed production, but in some area, carob seeds is used like tea and coffee [15 and [16]. In fact, it is a suitable replacement for cocoa, because it lacks



caffeine and the obromine. Carob powder is a natural sweetener with flour and appearance similar to chocolate; therefore it is often used as cocoa substitute. Carob germ flour is used as dieted human food [17] or as a potential ingredient in cereal-derived foods for celiac people [18]. Carob powder is also used as a food stabilizer and as a darkening agent. *Ceratonia* pulp is prepared for treatment of hypercholesterolemia [19], as well as treating mouth inflammation[20]. Similarly, *Ceratonia siliqua* seeds is useful to treat and improve diabetes symptoms because it has compounds such as fibers, phytosterols and tocopherol [21].

The present study aimed to investigate the hypoglycemic effect of phytoestrol extract of *Ceratonia siliqua* fruit by using an experimental animal model of alloxan-induced damage of β -cell of langerhans islet in pregnant rabbits. To determine its effect on some physiological parameters in diabetic pregnant rabbits and it could ameliorate the diabetes mellitus and comparison of effects of phytosterol extract with insulin.

MATERIALS AND METHODS

Drugs and Chemicals.

Alloxan obtained from Safa co. Diala-Iraq, and insulin provided from GlaxoSmithKline, S.A. Aranda de Duero.

Plant Material.

Phytoesterol 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 phytoesteroil 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 [22].



Experimental Animals.

Thirty two adult female rabbits weight ranged between (1500-2000.0mg) kept for an adaptation period for 1 month in the animal house of Veterinary Medicine College / Basrah University. The experimental animals were kept in individual cages, provided with standard ration in addition to green alfalfa (*Medicago sativa*) and tap water *ad libitum* and given a prophylaxis drug against coccidiosis (Amprolium 1g/L of drinking water).

Experimental design.

The rabbit divided into four groups of comprising 8 animals in each group as the following:

Group1:- Healthy female rabbits at 1st week of pregnant (negative control group) administrated normal saline (0.9% of NaCl)(3ml)for 21days.

Group 2:- Female rabbits at 1st week of pregnant initially given alloxan 150mg/kg B.W. dissolved in 3ml of normal saline by I.P for three days (positive control group) and remain for 21 days.

Group 3:- Female rabbits at 1st week of pregnant initially given alloxan150mg/kg B.W. dissolved in 3ml of normal saline by I.P. for three days and then treated with Insulin for 21 days.

Group 4:- Female rabbits at 1st week of pregnant initially given alloxan150mg/kg B.W. dissolved in 3ml of normal saline by I.P. for three days and then treated with phytoesteriol extract of *Ceratonia siliqua* fruits 1ml/kg B.W. for 21days.

Induction of Diabetes Mellitus.

Diabetes mellitus were induced in twenty four starved pregnant female rabbits 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). The (8ml) of 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 (-20°C), which then used to study Biochemical Parameters (Serum Glucose, Total Cholesterol, Triglyceride, HDL, LDL and VLDL). The remaining (2ml) of blood was deposited into tube with anticoagulant which used for hematological analysis (RBC, WBC, Hb, MCV, MCH, MCHC, PCV, differential WBC, PLT, PCT, MPV, PDW).

Study parameter:-

-Hematological analysis.

RBC, WBC, Hb, MCV, MCH, MCHC, PCV, differential WBC were measured by count 60 (Genex laboratories, Germany) apparatus.

-Measurement of total cholesterol:

Total cholesterol measured by using method CHOD-POP, France.

-Measurement of triglycerides:

Triglycerides measured by using Triglycerides-liquizyme\GPO-POP, Germany.

-Measurement of High Density lipoprotein-cholesterol (HDL-c)

HDL-C measured by CHOLESTEROL liquicolor test kit.

-Measurement of serum Low-density Lipoprotein Cholesterol (LDL):-

Serum LDL-C concentration was calculated according to [23].

$LDL-C = \text{Total cholesterol} - [(\text{HDL-C}) + \text{Triglyceride} / 5]$.

-Measurement of serum very low- density Lipoprotein (VLDL):-

The serum very low-density lipoprotein concentration was calculated by dividing serum triglyceride by five [24].

$VLDL = \text{Triglyceride} / 5$.



-Statistical Analysis:

The results of the present study were analyzed by using two-way covariance (ANOVA) test in all study. All statistical calculations were carried out by the aid of the statistical package SPSS V. 17 (SPSS Inc.). The data were expressed as means $\pm$ standard deviation ($X \pm SD$). Least significant different test (LSD) was calculated to test difference between means of groups and subgroups [25].

RESULT

1-Effect of Phytoestrol Extract of *Ceratonia siliqua* Fruit and Insulin on Glucose Level in serum of Pregnant Diabetic Female Rabbits Induced by Alloxan.

The obtained results in Fig (1) revealed significant increase ($P \leq 0.05$) in glucose level in serum of diabetic female rabbits induced by alloxan((+ve) control group) compared with (-ve) control group and another treated groups while the results showed non-significant change ($P \leq 0.05$) glucose level in serum of diabetic female rabbits treated with phytoesterol of *Ceratonia siliqua* fruit compared with (-ve) control group and insulin treated group.

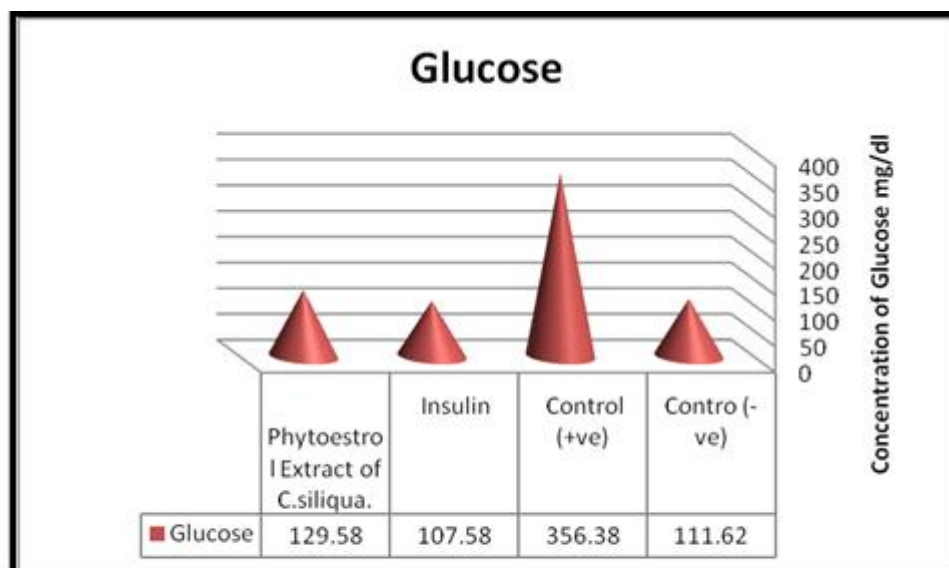


Fig.1:-Represent the effect of phytoestrol extract of *Ceratonia siliqua* fruit and insulin on glucose level in serum of pregnant diabetic female rabbits induced by alloxan.



2-Effect of Phytoestrol Extract of *Ceratonia siliqua* Fruit and Insulin on RBC Counts and RBC Index in Pregnant Diabetic Female Rabbits Induced by Alloxan.

The obtained results in Fig(2) revealed significant decrease ($P \leq 0.05$) in RBC of pregnant diabetic female rabbits induced by alloxan((+ve) control group) compared with (-ve) control group and in pregnant diabetic treated with insulin while the results showed significant increase ($P \leq 0.05$) in RBC of pregnant diabetic female rabbits treated with phytoesterol of *Ceratonia siliqua* compared with (-ve) control group and another treated group.

The results of Hb revealed significant decrease ($P \leq 0.05$) in diabetic female rabbits induced by alloxan((+ve) control group) and in pregnant diabetic treated with insulin compared with (-ve) control group and pregnant diabetic female rabbits treated with phytoesterol of *Ceratonia siliqua* while the results showed non-significant ($P \leq 0.05$) in Hb of pregnant diabetic female rabbits treated with phytoesterol of *Ceratonia siliqua* compared with (-ve) control group.

The results of PCV% revealed significant decrease ($P \leq 0.05$) in diabetic female rabbits induced by alloxan((+ve) control group) compared with (-ve) control group and another treated groups while the results showed non-significant ($P \leq 0.05$) in PCV% of diabetic pregnant female rabbits treated with phytoesterol extract of *Ceratonia siliqua* fruit compared with (-ve) control group while PCV% of diabetic pregnant female rabbits treated phytoesterol extract of *Ceratonia siliqua* fruit significant increase ($P \leq 0.05$) compared with insulin treated group.

The results of MCV revealed significant decrease ($P \leq 0.05$) in diabetic female rabbits induced by alloxan ((+ve) control group) compared with (-ve) control group and another treated groups while the results showed non-significant ($P \leq 0.05$) in MCV of diabetic female rabbits treated with phytoesterol extract of *Ceratonia siliqua* compared with (-ve) control group but the results showed significant increase ($P \leq 0.05$) in MCV of diabetic female rabbits treated with phytoesterol extract of *Ceratonia siliqua* compared with insulin group.



The results of MCH revealed non-significant change ($P \leq 0.05$) in diabetic female rabbits induced by alloxan ((+ve) control group) compared with (-ve) control group and another treated groups while the results showed significant increase ($P \leq 0.05$) in MCH of diabetic female rabbits treated with phytoesterol extract of *Ceratonia siliqua* compared with (-ve) control group and another treated groups.

The results of MCHC% revealed significant decrease ($P \leq 0.05$) in diabetic female rabbits induced by alloxan ((+ve) control group) compared with (-ve) control group and another treated groups while the results showed significant increase ($P \leq 0.05$) in MCHC% of diabetic female rabbits treated with insulin compared with (-ve) control group and another treated groups but the results showed non-significant change ($P \leq 0.05$) in MCHC% of diabetic female rabbits treated with phytoesterol extract of *Ceratonia siliqua* compared with (-ve) control group and (+ve) control group.

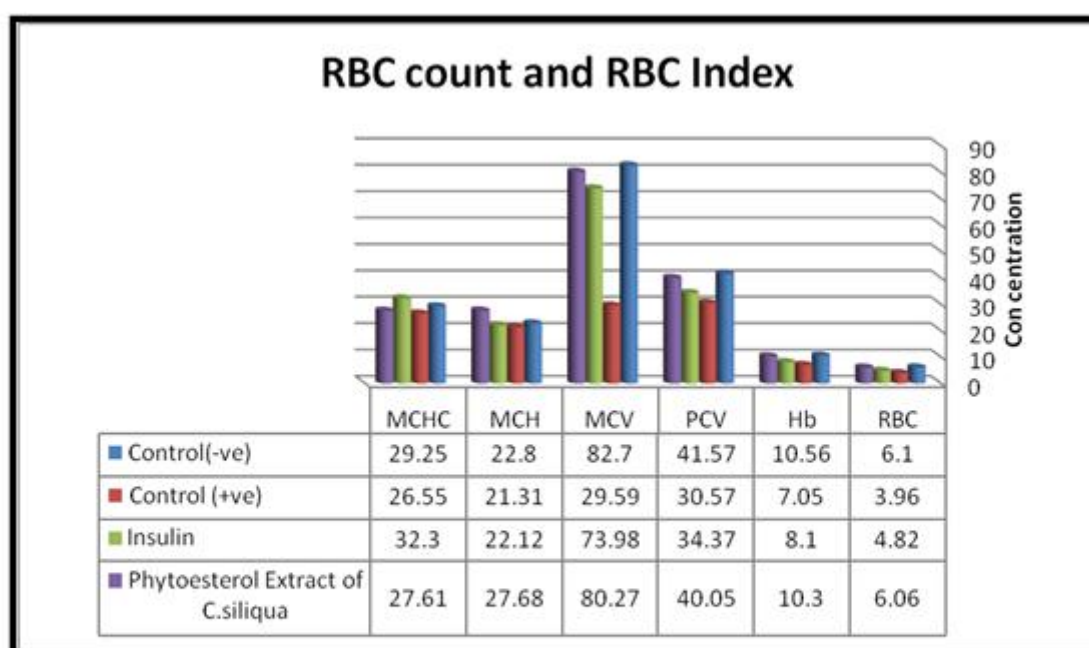


Fig.2:-Represent the effect of phytoesterol extract of *Ceratonia siliqua* fruit and insulin on RBC counts and RBC index in pregnant diabetic female rabbits induced by alloxan.



3-Effect of Phytoestrol Extract of *Ceratonia siliqua* fruit and Insulin on WBC Counts and Percentage of Differential Count of WBC in Diabetic Pregnant Female Rabbits Induced by Alloxan.

The obtained results in Fig (3) revealed significant decrease ($P \leq 0.05$) in WBC of diabetic female rabbits induced by alloxan ((+ve) control group) and in diabetic treated with insulin compared with (-ve) control group and phytoesterol extract of *Ceratonia siliqua* while the results showed non-significant changes ($P \leq 0.05$) in WBC of diabetic female rabbits treated with phytoesterol extract of *Ceratonia siliqua* compared with (-ve) control group.

The results of neutrophils% showed significant decrease ($P \leq 0.05$) in diabetic female rabbits induced by alloxan((+ve) control group) compared with (-ve) control group and another treated groups while the result of neutrophils% showed significant decrease ($P \leq 0.05$) in diabetic female rabbits treated with insulin and phytoesterol extract of *Ceratonia siliqua* compared with (-ve) control group.

The results of eosinophile % showed significant increase ($P \leq 0.05$) in diabetic female rabbits induced by alloxan ((+ve) control group) compared with another treated groups and non-significant changes($P \leq 0.05$) compared with (-ve) control group while the result of eosinophile % showed non-significant change ($P \leq 0.05$) in diabetic female rabbits treated with insulin and phytoesterol extract of *Ceratonia siliqua* group compared with (-ve) control group.

The results of basophile % showed significant increase ($P \leq 0.05$) in diabetic female rabbits induced by alloxan ((+ve) control group) compared with another treated groups and non-significant changes ($P \leq 0.05$) compared with (-ve) control group while the result of basophile % showed non-significant change ($P \leq 0.05$) in diabetic female rabbits treated with insulin and phytoesterol extract of *Ceratonia siliqua* group compared with (-ve) control group.

The results of lymphocyte % showed significant decrease ($P \leq 0.05$) in diabetic pregnant female rabbits induced by alloxan ((+ve) control) compared with (-ve) control group, group treated with insulin and group treated with phytoesterol extract of *Ceratonia siliqua*.



The results of monocyte % showed significant decrease ($P \leq 0.05$) in diabetic female rabbits induced by alloxan((+ve) control group) compared with (-ve) control group, insulin group and phytoesterol extract of *Ceratonia siliqua* while The results of monocyte % showed non-significant change ($P \leq 0.05$) in and diabetic female rabbits treated with Phytoesterol extract of *Ceratonia siliqua* compared with (-ve) control group and insulin group.

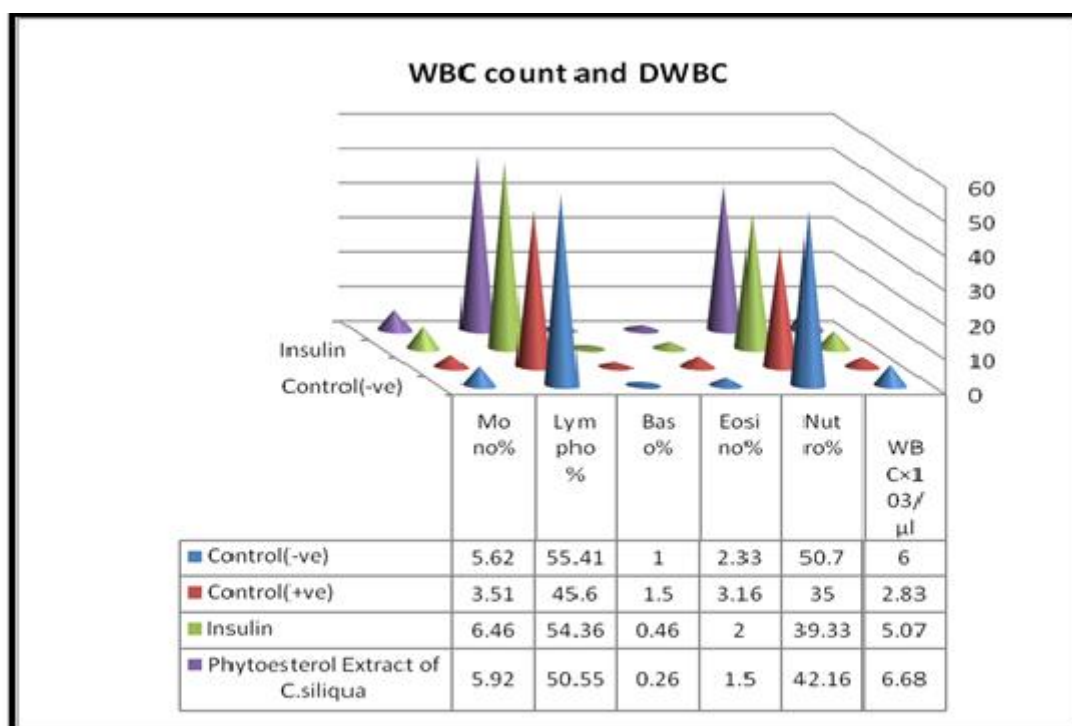


Fig.3:-Represent the effect of Phytoesterol Extract of *Ceratonia siliqua* Fruit and Insulin on WBC and DWBC in Pregnant Diabetic Female Rabbits

4-Effect of Phytoesterol Extract of *Ceratonia siliqua* Fruit and Insulin on Platelet count and Platelet Morphological Parameters in Pregnant Diabetic Female Rabbits Induced by Alloxan.

The obtained results in Fig.(4) revealed significant decrease ($P \leq 0.05$) in PLT(platelet count) of diabetic female rabbits induced by alloxan((+ve) control group) and in diabetic treated with insulin compared with (-ve) control group and group treated with phytoesterol extract of *Ceratonia siliqua* while the results showed



non-significant change ($P \leq 0.05$) in PLT of diabetic female rabbits treated with phytoesterol extract of *Ceratonia siliqua* compared with (-ve) control group.

The results of MPV(Mean Platelet Volume) revealed significant increase ($P \leq 0.05$) in diabetic female rabbits induced by alloxan((+ve) control group) compared with (-ve) control group and another treated groups while the results showed decrease significant ($P \leq 0.05$) in MPV of diabetic female rabbits treated with insulin and treated phytoesterol extract of *Ceratonia siliqua* compared with (-ve) control group.

The results of PDW(Platelet Distribution Width) revealed significant increase ($P \leq 0.05$) in diabetic female rabbits treated with phytoesterol extract of *Ceratonia siliqua* compared with (-ve) control group and another treated groups while the results showed decrease significant ($P \leq 0.05$) in PDW of (-ve) control group compared with and another treated groups.

The results of PCT(Plateletcrit)% revealed significant increase ($P \leq 0.05$) in diabetic female rabbits induced by alloxan ((+ve) control group)compared with (-ve) control group and another treated groups while the results showed non-significant change ($P \leq 0.05$) in PCT of diabetic female rabbits treated with insulin and treated phytoesterol extract of *Ceratonia siliqua* compared with (-ve) control group.

The results of P-LCR(Platelet Large Cell Ratio) revealed significant increase ($P \leq 0.05$) in diabetic female rabbits induced by alloxan ((+ve) control group) compared with (-ve) control group and another treated groups while the results showed significant decrease ($P \leq 0.05$) in P-LCR of diabetic female rabbits treated with insulin and diabetic female rabbits treated with phytoesterol extract of *Ceratonia siliqua* compared with (-ve) control group and (+ve) control group.



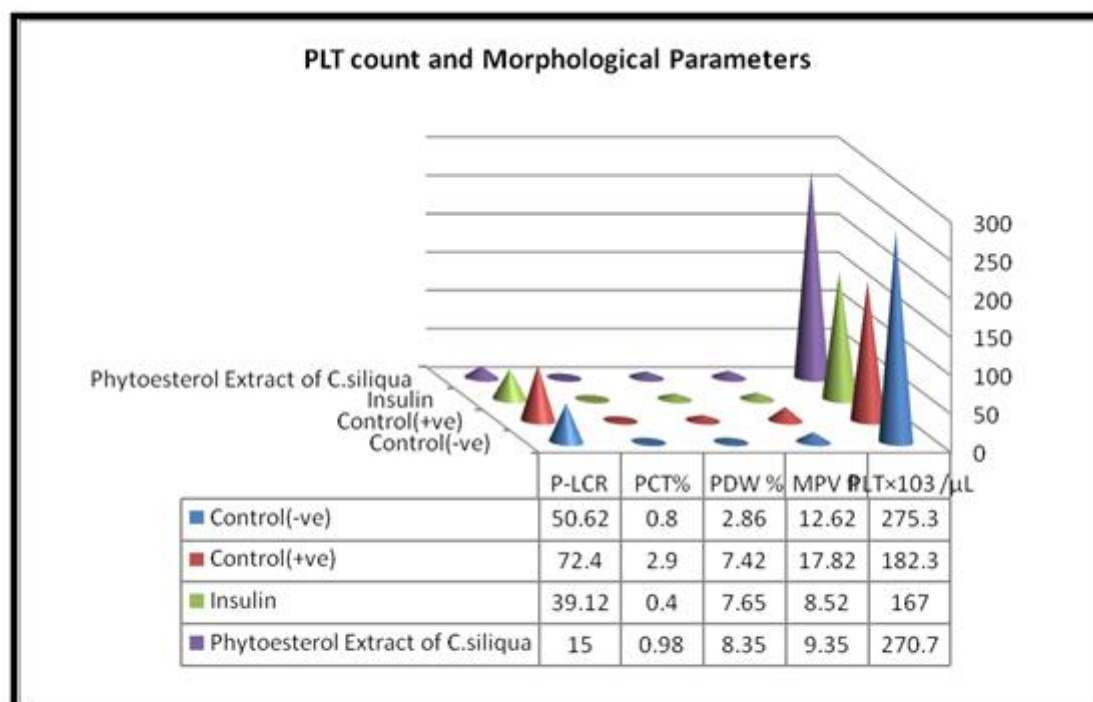


Fig.4:-Represent the effect of phytoesterol extract of *Ceratonia siliqua* fruit and insulin on platelet count and platelets morphological parameters in pregnant diabetic female rabbits induced by alloxan.

5-Effect of Phytoesterol Extract of *Ceratonia siliqua* Fruit and Insulin on Serum of Lipid profile in Pregnant Diabetic Female Rabbits Induced by Alloxan.

The obtained results in Fig. (5) revealed significant increase ($P \leq 0.05$) total cholesterol in serum of diabetic female rabbits induced by alloxan((+ve) control group) compared with (-ve) control group and another treated groups while the results showed non-significant change ($P \leq 0.05$) total cholesterol in serum of diabetic female rabbits treated with phytoesterol extract of *Ceratonia siliqua* and group treated with insulin compared with (-ve) control group.

The results of triglyceride revealed significant increase ($P \leq 0.05$) in diabetic female rabbits induced by alloxan((+ve) control group) compared with (-ve) control group and another treated groups while the results showed increase significant ($P \leq 0.05$) triglyceride in serum of diabetic female rabbits treated with insulin compared with (-ve) control group and group treated with phytoesterol extract of *Ceratonia siliqua* but the results of revealed non-significant change ($P \leq 0.05$) triglyceride in serum of diabetic female rabbits treated with phytoesterol extract of *Ceratonia siliqua* compared with (-ve) control group.



The results of HDL revealed significant decrease ($P \leq 0.05$) in diabetic female rabbits induced by alloxan ((+ve) control group) compared with (-ve) control group and another treated groups while the results showed non-significant change ($P \leq 0.05$) HDL in serum of diabetic female rabbits treated with insulin but the results revealed significant increase ($P \leq 0.05$) HDL in serum of diabetic female rabbits treated with Phytoesterol extract of *Ceratonia siliqua* compared with (-ve) control group and another treated groups.

The results of LDL revealed significant increase ($P \leq 0.05$) in diabetic female rabbits induced by alloxan ((+ve) control group) compared with (-ve) control group and another treated groups while the results showed significant increase ($P \leq 0.05$) LDL in serum of diabetic female rabbits treated with insulin compared with (-ve) control group and diabetic female rabbits treated with Phytoesterol extract of *Ceratonia siliqua* but the results showed non-significant change ($P \leq 0.05$) LDL in serum of diabetic female rabbits treated with Phytoesterol extract of *Ceratonia siliqua* compared with (-ve) control group.

The results of VLDL revealed significant increase ($P \leq 0.05$) in diabetic female rabbits induced by alloxan ((+ve) control group) compared with (-ve) control group and another treated groups while the results showed significant increase ($P \leq 0.05$) VLDL in serum of diabetic female rabbits treated with insulin compared with (-ve) control group and diabetic female rabbits treated with Phytoesterol extract of *Ceratonia siliqua* but the results showed non-significant change ($P \leq 0.05$) VLDL in serum of diabetic female rabbits treated with Phytoesterol extract of *Ceratonia siliqua* compared with (-ve) control group.



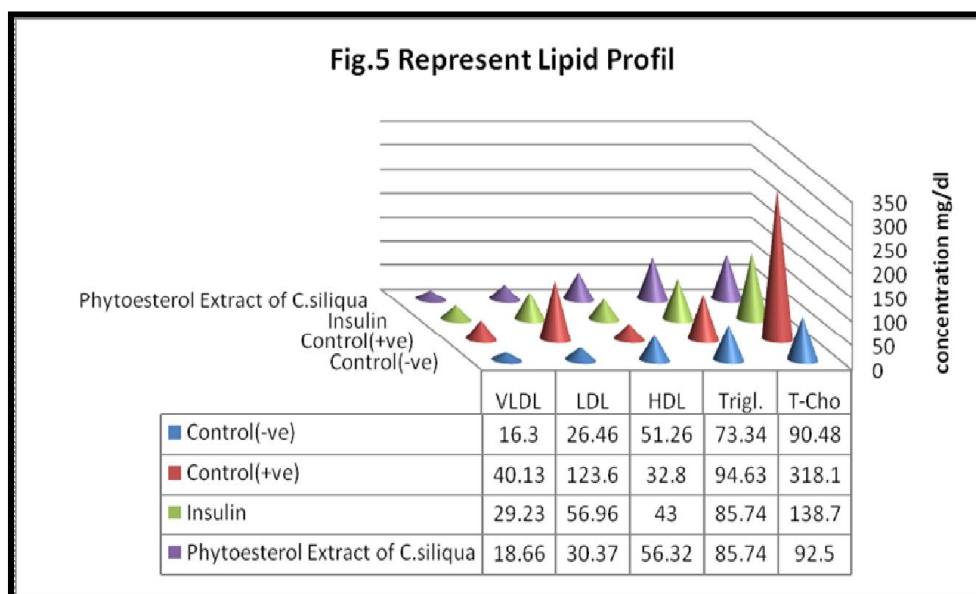


Fig.5:Represent the effect of phytoestrol extract of *Ceratonia siliqua* fruit and insulin on serum of lipid profile in pregnant diabetic female rabbits induced by alloxan

DISCUSSION

Most pharmacological treatments of DM are based on either oral hypoglycemics and/or insulin therapy, which has many side effects such as weight gain, hypoglycemia, gastrointestinal disturbances, and hypersensitivity reactions[26]. These have been of tremendous interest to health practitioners. Alternative therapies, especially those involving medicinal plants, for management of DM has long been in practice[27]. These plants are rich sources of phytochemicals and dietary fibers, which serve as nutraceuticals and ingredients for functional foods. Therefore, the need to document agents/compounds including medicinal plants that can ameliorate, attenuate or mitigate these alloxan toxicity cannot be overemphasized. The use of plants for various purposes such as providing shelter, as food, source of income, and in folkloric management of diseases dates back to millennia. In addition, plants are now been explored to mitigate/attenuate and/or reverse chemical compound-induced toxicity in animals.



In current, the phytoesterol extract of *Ceratonia siliqua* fruit significantly reduced the blood glucose level in alloxan induced diabetic rabbits. This result agreement with [28] whom mention that the presents of phytosterols, such as β -sitosterol, campesterol and stigmasterol have been reported in the seeds of *Ceratonia siliqua*. The administration of extract containing β -sitosterol to diabetic and healthy rats; they observed a decrease in blood glucose level in diabetic. In another study, it was shown that plasma insulin level increases in normal and hyperglycemic rats receiving phytosterols orally. These compounds can reduce glucose concentration by stimulating pancreatic β -cells to secret more insulin in blood circulation; in this way, blood glucose level is controlled better [29]. It is possible that the plant may increase glucose removal from blood, decrease the release of glucagon or increase that of insulin, stimulate directly glycolysis in peripheral tissues, or reduce glucose absorption from the gastrointestinal tract.

Inclusion of dietary active compounds in human and animals nutrition has been demonstrated to have tremendous health benefits and reduce the risk of chronic diseases such as colon cancer, obesity and diabetes [30 and 31].

Medicinal plants have various mechanisms of action through which their effects are exhibited that include promoting regeneration of β -cells of islets of langerhans in the pancreas as exhibited by phytoesterol extract of *Ceratonia siliqua* fruit, enhancement of insulin release and activity on the cells as exhibited by phytoesterol extract of *Ceratonia siliqua* fruit, decrease peripheral glucose uptake at the duodenal cellular level and other aspects of small intestine exhibited by phytoesterol extract of *Ceratonia siliqua* fruit and by restricting the rise of blood glucose levels caused by pituitary hormones responsible for inhibiting peripheral utilization of glucose as well as glycogenolysis exhibited by phytoesterol extract of *Ceratonia siliqua* and the presence of high level of fiber in plants which interferes with carbohydrate absorption [32].

Diabetic agent (alloxan) may be hematotoxic effects associated with toxic substances on bone marrow depression caused by damage to multiple classes of hematopoietic cells and a variety of hematopoietic functions [33]. The alterations in



the RBC, Hb, and PCV% of the diabetic rabbits suggest occurrence of anemia. The degree of anemia in diabetes animals can be associated with a number of factors, including glomerular filtration rate, urinary albumin excretion rate, and glycated h (HbA1c) levels[34]. Anemia has been reported to be due to diminished erythropoietin production by renal failure and increased non-enzymatic glycosylation of RBC membrane proteins[35]. It reported that phytoesterol extract of *Ceratonia siliqua* fruit treatment improved RBC count from diabetic reduced state.

The observed increase in these parameters in treating the diabetic rabbits with the phytoesterol extract of *Ceratonia siliqua* fruit suggests its potency in the management of the ailment. This could be attributed to its phytochemical and mineral contents. The antioxidant properties of these phytoesterol extract of *Ceratonia siliqua* have been reported by [36]. Thus, preventing oxidation of RBCs and Hb that often lead to hemolysis [37]. It may also stimulate formation or secretion of erythropoietin in the stem cells of the animals, as evidenced by the increased level of RBC[38] and to evaluate normal erythropoiesis.

The reductions in the levels of the haematological parameters by alloxan may be due to imbalance or absence of equilibrium in or between the rate of synthesis/production of the haematological parameters and their catabolism/destruction [39], as a consequence of oxidative products or reactive species generated by alloxan. The extract however mitigated/attenuated the toxic effects of alloxan on the blood parameters and this might lead to restoration of the functional capacity of the blood.

Alloxan diabetogenesis may cause perturbation in the bone marrow stem cells [40]. The significant increase ($P<0.05$) in WBC and Lymphocytes levels of diabetic rabbits treated with phytoestrol extract of *Ceratonia siliqua* fruit (1ml/kg) when compared with the control group gave credence to the abilities of the above treatment groups in curtailing hematological abuses in the defense system of the diabetic rabbits.

phytoestrol extract of *Ceratonia siliqua* fruit is one such active compound [27 and 28]. In this study, phytoestrol extract of *Ceratonia siliqua* fruit used to prepare a drug



against diabetes. The reduced levels of WBC in diabetic rabbits indicate a suppression of the immune system[41 and 42].

Platelets are fragment of cells that participates in blood clotting, they initiate repair of blood vessels walls and are also considered as an acute phase reactant to infection or inflammation; plateletcrits show cases the precise method of determining the degree of acute blood loss while mean platelet volume (MPV) is used to investigate the ability of a drug to enhance blood clotting [43]. The implication of the significant increases in PLT levels in phytoesterol of *Ceratonia siliqua* treated group as well as in PCT levels and MPV levels in group treated with phytoesterol extract of *Ceratonia siliqua* fruit when compared with diabetic control group. This was consistent with the report of [44] on the ability of medicinal compounds or drugs in altering the normal range of hematological parameters. Moreover, there was no significant change in PLT levels of group treated with insulin, phytoesterol extract of *Ceratonia siliqua* fruit as well as in PCT levels of group treated with insulin, phytoesterol extract of *Ceratonia siliqua* when compared of the diabetic control, suggesting that the plant may not cause thrombosis.

One of the probable mechanisms by which diabetes mellitus is involved in hyperglycemia and hypercholesterolemia is oxidative stress exhibiting effects which leads to tissue destruction and dysfunction [43]. Diabetes is associated with elevated levels of total cholesterol, triglycerides, and LDL, which are also risk factors of cardiovascular diseases. [44]. These elevated levels observed in diabetics have been linked to increased mobilization of free fatty acids from the peripheral fat depots.[45]. The observed reductions [46] characterized by elevated serum levels of TG and LDL-C [47] and TC [48]. The higher level of serum lipid is mainly due to the decrease in the action of lipolytic hormones in fat depots due to insulin action. Under normal circumstances, insulin activates the enzyme lipoprotein lipase which hydrolysis triglycerides. In diabetes, lipoprotein lipase is not activating due to insulin deficiency resulting in hypertriglyceridemia and hypercholestermia [49]. In diabetic rabbits, the impaired carbohydrate utilization leads to accelerate lipolysis, resulted in hyperlipidaemia and increased lipid peroxidation which is associated with



hyperlipidaemia [50]. Increased generation of free radicals due to oxidative stress may develop several adverse effects in diabetes mellitus such as hepatopathy and nephropathy disorders [51]. Phloesterol extract of *Ceratonia siliqua* reduce blood cholesterol level. *Ceratonia* pulp is prepared for treatment of hypercholesterolemia[52].

دراسة تأثير مستخلص الفاييتواسترول من ثمرة نبات الخروب *Ceratonia siliqua* fruit على المعايير الدمية والكيموحيوية في إناث الأرانب الحوامل المستحدثة داء السكري بواسطة الالوكسان

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الخلاصة

تمت الدراسة في كلية الطب البيطري- جامعة البصرة وكان الغرض منها هو تقييم تأثير داء السكر المستحدث باستخدام عقار الالوكسان على بعض المعايير الدمية والكيموحيوية في إناث الأرانب الحوامل فضلاً عن دراسة التأثير العلاجي لمستخلص الفاييتوستيرول لثمار نبات الخروب بجرعة (1مل / كغم من وزن الجسم) بالمقارنة مع عقار الأنسولين(المضاد لداء السكري 0.1 مل/كغم من وزن الجسم) في إناث الأرانب المستحدث فيها داء السكر ومدى فاعلية هذا المستخلص في تخفيض السكر وتخفيف الآثار الضارة الناجمة عن داء السكر.

وتضمنت الدراسة استخدام عينة مكونة من 32 من إناث الأرانب البالغة المحلية، تتراوح أوزانها ما بين (1500-2000غم) وبأعمار تتراوح (7- 7.5 شهر) قسمت عشوائياً بالتساوي إلى أربعة مجاميع (8 أرنب/ مجموعة). تم مزاجاة الإناث قبل المعاملة بأسبوع بذكور سليمة غير معاملة.

الأولى:- مجموعة السيطرة(السالبة) جرعت إناث الأرانب الحوامل (0.9) من المحلول الفسيولوجي (3مل)لمدة 21 يوم والمجموعة الثانية:- حقنت إناث الأرانب الحوامل في غشاء البروتون عقار الالوكسان 150 ملغم/كغم بعد أذنته ب(3مل) من المحلول الفسيولوجي لمدة 3 يوم لاستحداث داء السكري وتركت لمدة 21 يوم بدون إي معاملة(وعدت مجموعة سيطرة موجبة). إما المجموعة الثالثة:- حقنت إناث الأرانب الحوامل في البروتون عقار الالوكسان 150 ملغم/كغم بعد أذنته ب(3مل) من المحلول الفسيولوجي لمدة 3 أيام + (0.1 مل/ كغم) هرمون الأنسولين لمدة 21 يوم، المجموعة الرابعة:- حقنت إناث الأرانب الحوامل في البروتون عقار الالوكسان



150 ملغم/كغم بعد أذنبته ب(3مل) من المحلول الفسيولوجي لمدة 3 أيام+ (1 مل/ كغم) مستخلص الفايستول لثمار نبات الخروب لمدة 21 يوم.

بعد انتهاء فترة المعاملة تم سحب عينات الدم (10مل) من قلب الحيوانات حيث قسمت عينة الدم إلى جزأين. الجزء الأول (2 مل) وضعت في أنابيب تحتوي على مانع التخثر (EDTA) لغرض إجراء الفحوصات الدمية إما الجزء الثاني (8 مل) يوضع في أنابيب غير حاوية على مانع للتخثر وطردت (5000 دورة) لمدة 10 دقائق لغرض الحصول على مصل الدم لإجراء بعض قياسات المعايير الكيموحيوية كقياس تركيز السكر في مصل الدم ونمط الدهون. وتوصلت الدراسة إلى النتائج الآتية:

تأثرت المعايير الدمية والكيموحيوية إذ سبب داء السكري إلى نقص في المعايير الدمية إذ لوحظ انخفاضاً معنوياً ($p \leq 0.05$) للعد الكلي لكريات الدم الحمراء والهيموكلوبين والنسبة المئوية لكريات الدم المضغوطة لكريات الدم البيضاء والنسبة المئوية لكريات الدم البيضاء الحبيبية (العدلة)، اللعافية ووحيدة النواة والصفائح الدموية بينما لوحظ عدم وجود فرق معنوي للعد التفريقي لكريات الدم البيضاء الحامضية والقاعدية.

وبينت الدراسة لمعايير الكيموحيوية وجود ارتفاع معنوي ($p \leq 0.05$) في تركيز مستوى السكر ونمط الدهون لمصل دم إناث الأرانب الحوامل المصابة بداء السكري ماعدا بروتينات الدهنية عالية الكثافة انخفضت معنوياً ($p \leq 0.05$).

نستنتج أن مستخلص الفايستول لثمار نبات الخروب له فعالية مضادة لداء السكري جيدة إي له تأثير مخفض للسكر ومحسن للمعايير الدمية وكذلك نمط الدهون ويوصى باستخدامه كعقار.

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