

Academic Scientific Journals

Journal for Agricultural Sciences (TJAS)

Tikrit.

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Journal for Agricultural Sciences (TJA

Tikrit .

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ISSN:1813-1646 (Print); 2664-0597 (Online) *Tikrit Journal for Agricultural Sciences* Journal Homepage: <u>http://www.tjas.org</u> E-mail: tjas@tu.edu.ig



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KEY WORDS:

Pomegranate peel, alloxan, diabetes, insulin, glucose, dosing, treatment

ARTICLE HISTORY:

Received: 22/12/2020 Accepted: 14/01/2021 Available online: 31/03/2021

Study of the effect of Ethanolic extract of pomegranate peels on some blood serum biochemical parameters in alloxan induced diabetes male rats

ABSTRACT

This study was conducted in at the Biotechnology Research Center / Nahrain University / Baghdad Governorate, for the period 2/8/2020 to 15/9/2020, the follow of the effects of dosing with ethanolic extract of pomegranate peels on 30 male rats of (2-3) months of age and weights (170-220 g) were included, it was divided into two parts, one of which is intact and the other in which diabetes was introduced by using alloxan at a concentration (90 mg / kg) of body weight, The results showed of the biological study that the development of experimental diabetes showed significant increase (P <0.05) in the concentrations of Glucose, Total cholesterol (TC), Trigleserid (TG), low-density Very-low-density lipoproteins cholesterol (LDL-C), lipoprotein cholesterol (VLDL-C), and the enzymes of aspartate transaminase and alanine transaminase (AST_J ALT), and malonedialdehyde concentration (MDA), Compared with a intact control group, while it led to a significant decrease (P <0.05) in body weight, insulin concentration, high-density lipoprotein cholesterol (HDL), in the affected control group. Dosing in intact rats with ethanolic extract of pomegranate peel at a concentration of 75 and 150 mg / kg of body weight led to a significant decrease in the concentrations of glucose, TC, TG, LDL-C and VLDL-C, as well as AST, ALT, and MDA enzymes, compared with a healthy control group, Significant increase in body weight, insulin and HDL-C concentrations for all treatments compared with the healthy control group. Dosing of diabetic rats with ethanolic extract of pomegranate peels at a concentration of 75 and 150 mg / kg of body weight led to a significant decrease in the concentrations of glucose, TC, TG, LDL-C and VLDL-C, as well as AST, ALT, and MDA enzymes, compared with the infected and untreated control group, a significant increase in body weight, insulin and HDL-C concentrations, for all treatments, compared with the affected control group. © 2021 TJAS. College of Agriculture, Tikrit University

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by high blood glucose levels due to a complete or relative deficiency of the hormone insulin or the presence of anti-insulin agents (Sakahar *et al.*, 2008). The high blood sugar concentration causes disturbances in the metabolism of carbohydrates, fats and proteins, which are caused by insulin deficiency, insulin action, or both (Abd El-Mageid *et al.*, 2016). This leads to symptoms such as thirst, muscle and body wasting, hunger, and blurred vision, frequent urination, high concentration of cholesterol and triglycerides in the blood (Gulfraz *et al.*, 2007; Gao, 2010). Diabetes prevention are high priorities in medical

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research. Fruit extracts have been used extensively in this context because they are natural, readily available and safe in used. One of them is pomegranate (*Punica granatum*), which is ancient edible fruits and they are widely grown in Mediterranean regions including Iraq, Egypt, Iran, and India, but sparsely cultivated in the USA, Japan, Russia and China (Banihani *et al.*,2013; Matthaeus and Ozcan, 2016). Peels of Punica granatum L. also include wide variety of phytochemical compounds, e.g., punicalins, gallotannins, punicalagins, ellagic acid, gallagic acid that exert anti- oxidant activities and prevent oxidative stress (oraki *et al.*, 2011).

Studies indicated that pomegranate peel has a lowering effect on blood sugar, triglycerides, and total cholesterol and harmful, and has a beneficial effect in raising the level of good cholesterol as well as the level of insulin (Osman *et al.*, 2012). In another study, Middha *et al.* (2012) showed that dosing of alloxan-induced diabetic rats with methanolic extract of pomegranate peels at a concentration of 75 and 150 mg / kg of body weight led to a decrease in blood sugar level and increased insulin secretion.

MATERIALS AND METHODS

1 - Preparation of pomegranate peel ethanolic extract (PPE): Pomegranate fruits were obtained from the local variety from the local markets of Salah El-Din Governorate, for sample preparation of pomegranate peel extracts were separated manually from the peel, then the separated peels were cut into small pieces, and then allowed to air-dry in the dark at room temperature until constant weight was achieved. Air-dried peel was then homogenized using a coffee grinder until a fine powder was obtained. A constant amount of pomegranate peel powder (50 g) was used in 1000 ml of 80% ethanol alcohol, the mixture was stirred well, and then placed in a vibrating incubator at 40 ° C for 24 hours, and then filter it using Whatman No. 42 filter paper, Then the filtrate was concentrated using a rotary evaporator and dried in electric oven at a temperature of 40 ° C almost all the solvent was removed. It was placed in dark airtight bottles and kept in the refrigerator until use (Abd El-Mageid *et al.*,2016).

2 - Animals: In the experiment, 30 male white rats were used, ranging in weight between (170-220) gm. Placed in special cages and supplied with water and animal feed, all the animals subjected to the same conditions of natural light and temperature (23 ± 2) °c.

3 - **Infecting the animals with diabetes:** 15 rats were injected with alloxan at a concentration of 90 mg/kg body weight through subcutaneous injection, it was left for a week, and then all the injected rats were examined for diabetes through a glucose tester and confirmed that they had the disease, as the blood sugar level of the affected rats ranged between (390-483) mg/dl, the remaining 15 rats were left uninjured.

4- **Experimental Design:** The rats were divided into six groups, as each group contained 5 rats, and they were divided as follows:

Group (A): The Intact control group that included 5 intact rats, and they were fed on regular feed and plain water.

Group (B): The control group afflicted with alloxan-induced diabetes that was fed regular diet and plain water.

Group (C): The intact group dosed with (PPE) at a concentration of 75 mg / kg by oral dosing.

Group (D): The intact group dosed with (PPE) at a concentration of 150 mg / kg by oral dosing.

Group (E): The group of diabetics treated with (PPE) at a concentration of 75 mg/kg by oral dosing (Middha *et al.*, 2012).

Group (F): The group of diabetics treated with (PPE) at a concentration of 150 mg/kg by oral dosing (Middha *et al.*, 2012).

5 -Sample collection:

5-1 Body weight: The body weights of all male rats under study were measured before the start of the experiment to calculate the initial weight, after the end of the experiment, their weights were measured to calculate the final weight, then extract the difference in weight, as shown below: Weight difference (g) = final weight (g) - initial weight (g).

5-2 Blood samples: After the trial period ended the animals were starved for 12 hours, then blood samples were withdrawn by heart-stabbing of the anesthetic animal with a 5 ml plastic syringe, test

tubes free of anticoagulants and left for about a quarter of an hour in a water bath at 37° C, then Serum was obtained by centrifugation at 3000 rpm for 15 minutes, it was kept at -20 ° C in new, clean plastic tubes until biochemical tests were performed.

6- **Biochemical Analysis:** Blood glucose concentration was measured using a glucose test kit from the company (Spinreact) of Spain (Trinder, 1969; Tietz, 1995), The insulin level was measured by following the steps provided with the German manufacturer Cobas estimator kit. The concentration of triglycerides in serum was determined using the Spinreact kit (Trinder, 1969; Fossati and Prencipe, 1982), The total serum cholesterol level was determined using the Spinreact kit, in which the cholesterol was converted to the pink Quinonimine pigment (Allain, 1974), The concentration of high-density Lipoprotein in the blood serum of the studied groups was determined using a special kit and according to the instructions of the supplier (Spinreact) (Tietz, 1995). LDL-C was determined in serum of the groups according to the following relationship (Burtis & Ashwood, 1999): LDL-C concentration (mg/dl) = Total cholesterol – (HDL-C) – (VLDL-C), The concentration of (VLDL-C) in serum was determined on the basis of the following relationship (Burtis and Ashwood, 1999): VLDL concentration (mg/dl) = (Triglycerides / 5). The serum malondialdehyde concentration was estimated using the modified method used by researchers (Guidet and Shah, 1989). Liver enzymes (ALT, AST) activities were determined according to Tietz (1995).

7- **Statistical analysis:** The results were analyzed statistically using (SAS, 2001) program and according to one-way analysis of variance. Analysis Of Varience (ANOVA) of the parameters were tested using the Duncun multiple ranges test with a significance level of P \leq 0.05 to determine the significant differences between groups (Duncan and Mahaffey, 1994).

RESULTS AND DISCUSSION

1-1 The effect on body weight:

The statistical results of the study showed that weight changes occurred in a number of groups, Table (1) shows that there was a significant decrease in the weights of the animals of the diabetic and untreated group compared with the intact control group, Where the average weights of animals in the affected group decreased from 181 to 166.33g. This result coincided with El-Hadary, Ramadan (2019) and with Vincent and Desmond (2018) reported in diabetic rats. The causes are that the development of diabetes mellitus in rats Smashing the pancreatic beta cells responsible for the production of the hormone insulin, which works to facilitate the entry of glucose into cells and produce energy, or because of the oxidative stress of the animal, when insulin secretion decreases, the body turns to obtain energy from fat and protein stores (Al-Bayati, 2017; Al-Jafaar, 2020).

induced by anoxan.									
Treatment Body weights	(A)	(B)	(C)	(D)	(E)	(F)			
Initial weight(g)	182	181	189.67	185	183.67	182.33			
final weight (g)	190.67	166.33	197.67	193.33	176	176.67			
Weight difference (g)	9	-14.67	8	8.33	-7.67	-5.67			

Table (1) The effect of pomegranate peel ethanolic extract at a concentration of (75 and 150					
mg/kg) on the body weights of healthy males and those infected with experimental diabetes					
induced by alloyan					

The number of animals are five per group, Different letters mean that there are a significant difference at (P ≤ 0.05), (A) Control (natural) group, (C) intact and dose group (75 mg / kg), (D) intact and dose group (150 mg / kg), (B) diabetic control group, (E) affected and treated group (75 mg / Kg), (F) affected and treated group (150 mg / kg).

The results also showed a significant increase in the body weights of the animals of the intact group, which were dosed with pomegranate peel extract at concentrations of (75 and 150 mg / kg) compared to the infected control group, the results showed that there was a significant increase in the body weights of the animals of the diabetic treated group with pomegranate peel extract at concentrations of (75 and 150 mg / kg) compared with the infected control group, This result is agreeted with Salwe et al. (2015) that aqueous and alcoholic extract of pomegranate peel increases

body weight in diabetic rats due to its anti-diabetic activity. The cause for the increase in body weight is that pomegranate peels are a rich source of fiber, polyphenols, ellagic acid and tannic acid, which lead to an increase in total protein production in the serum and protein synthesis (Ramzy, 2019), The response is also due to the alcoholic and aqueous extract of pomegranate peels containing phenolic and flavonoid compounds in its composition, which play an effective role in lowering the blood sugar level (El-Hadary and Ramadan, 2019).

1-2 The effect on some biochemical parameters of healthy and diabetic rats: **1-2-1** The concentration of glucose in the blood serum:

The results shown in Table (2) that there was a significant increase ($P \le 0.05$) in the concentration of glucose in the blood serum of animals with novel alloxan diabetes in comparison with the intact control group, that the creation of diabetes mellitus with alloxan caused an increase in the level of glucose in the blood serum in rats, This result was consistent with the findings of the Al-Badya (2018) in diabetic rats, The caused elevated serum glucose concentration in infected rats was attributed to the damage to pancreatic beta cells by alloxan (Ali et al., 2011), It could have caused a development of insulin resistance and a disruption of the cellular receptor functions of insulin, thereby stopping the cells' reception of glucose and activating the processes of glycogenolysis and the formation of glucose from non-carbohydrate sources (Alhazza, 2007). The table showed that there were no significant differences in the concentration of glucose in the blood serum of intact rats when treated with the ethanolic extract of pomegranate peel at concentrations (75 and 150) mg / kg of body weight for the first day, as it reached (84.67 and 84.67) mg/dl respectively, compared with a group the intact control for the first day of 85.00 mg / dl. Whereas, the glucose concentration in blood serum for day 14 was at (75 and 150) mg/kg of body weight from the ethanolic extract of pomegranate peel (84.33 and 83.67 mg/dl) respectively, compared with the intact control group for day 14 which reached 85.33 mg/dl. The concentration of glucose in blood serum for day 28 (84.00 and 81.33 mg/dl) at a concentration of (75 and 150) mg/kg of body weight from ethanolic extract of pomegranate peel respectively compared with the control group for the same day, which amounted to 85.00 mg/dl. The same table shows that there were no significant differences in the concentration of glucose in the blood serum of infected rats when treated with the ethanolic extract of pomegranate peelings of the two specified concentrations, it reached (421.67 and 413.33) mg/dl respectively for the first day compared with the control group with diabetes of 443.67 mg/dl for the same day, While the results showed a significant decrease in the concentration of glucose in the blood serum of infected rats and treated with ethanolic extract of pomegranate peels at concentrations of (75 and 150) mg/kg of body weight, for a period of 14 days, reaching (315.67 and 301.33 mg/dl) respectively compared to a group of diabetic control for the same period which was 451.67 mg/dl, The results also showed a significant decrease in the concentration of glucose in the blood serum of the infected and treated rats at a concentration of (75 and 150) mg/kg of body weight, with ethanolic extract of pomegranate peel for 28 days, reaching (190.00 and 174.67) mg/dl respectively compared with the control group Infected for the same period.

The results of study are in agreement with the findings of Middha *et al.* (2012) who indicated a decrease in the blood glucose concentration in the blood serum of rats with alloxan-induced experimental diabetes when dosed with methanolic extract of pomegranate peel at a concentration of (75 and 150) mg/kg of body weight for 45 days, The antidiabetic activity was attributed to punicalagin, ellagic acid, gallic acid, and other phenolic compounds in pomegranate peels that show a decrease in serum glucose levels (El-Hadary and Ramadan, 2019), or it is attributed to the presence of ellagic acid, gallic acid and ursolic acid compounds in pomegranate peels, which have an anti-diabetic effect, this is indicated by Hidayat *et al.* (2014).

Table (2) The effect of the ethanolic extract of pomegranate peels with different concentrations on the level of glucose and the concentration of insulin in the blood serum of intact male rats and the diabetes induced by alloxan.

Groups	Transactions	Gluco	se concent (mg/dl)	tration	Insulin concentration (uU/ml)		
sdn		Day 1	Day 14	Day28	Day 1	Day14	Day28
Α	Control (natural)	85.00 b	85.33 c	85.00c	5.77a	5.58 a	5.08 ab
В	Diabetic control	443.67a	451.67a	409.67a	2.27 b	2.13 c	1.83 c
С	Intact and dose (75 mg/kg)	84.67 b	84.33c	84.00c	5.00 a	5.13 a	5.41 ab
D	Intact and dose (150 mg/kg)	84.67b	83.67 c	81.33c	5.20 a	5.38 a	5.83 a
Е	Diabetic and treatments (75 mg/kg)	421.67a	315.67b	190.0b	2.24 b	3.81 b	4.36 b
F	Diabetic and treatments(150 mg/kg)	413.33a	301.33b	174.67b	2.48 b	3.96 b	4.48 ab

The number of animals is five per group, Different letters mean that there is a significant difference at (P ≤ 0.05), (A) Control (natural) group, (C) intact and dose group (75 mg/kg), (D) intact and dose group (150 mg/kg), (B) diabetic control group, (E) affected and treated group (75 mg/Kg), (F) affected and treated group (150 mg/kg).

1-2-2 Insulin concentration in blood serum:

Table (2) showed that there was a significant decrease ($P \le 0.05$) in the concentration of insulin hormone in the blood serum of rats with experimental diabetes compared with the Control (natural) group, the insulin concentration of diabetic rats reached (2.27, 2.13 and 1.83) uU/ml for (1, 14 and 28) days respectively compared with the intact control group of (5.77, 5.58 and 5.08) uU/ml for the same period. The results of the study showed that the development of diabetes mellitus with alloxan caused a decrease in the level of the insulin hormone in the blood serum of the affected rats, this finding was in agreement with El-Missiry and El-Gindy (2000). The decrease in the level of the insulin hormone may be attributed to the damage to the beta cells that secrete insulin in the pancreas, which leads to necrosis in them after injecting them with alloxan (Singh and Gupta, 2007), insulin deficiency caused by the destruction of beta cells causes additional changes in the liver of affected animals due to high oxidative stress resulting from the accumulation of free radicals, which leads to the collapse of liver cells and the accumulation of lipid peroxidation in the cell membranes or mitochondrial membranes and this leads to impaired venous flow at the level of the hepatic vein or Inferior vena cava and expansion of sinusitis and formation of inflammatory areas (Mukhlif et al., 2020). The results (Table 2) showed no significant differences at (P≤0.05) in the concentration of the insulin hormone in the blood serum of healthy rats dosed with ethanolic extract of pomegranate peel when compared with the control (natural) group, the insulin hormone concentration in the blood of healthy rats dosed with the extract at a concentration of 75 mg/kg of body weight was (5.00, 5.13 and 5.41) uU/ml for a period of (1, 14 and 28) days respectively, while it reached (5.20, 5.38 and 5.83) uU/ml for the same periods respectively when dosed with the extract at a concentration of 150 mg/kg of body weight. The results showed that treating diabetic rats with ethanolic extract of pomegranate peels at a dose of 75 and 150 mg/kg of body weight at a rate of one dose per day for 28 days caused a significant increase in the level of insulin hormone in the blood compared to the affected control group, as the concentration of insulin hormone in blood serum in infected rats and treated with ethanolic extract of pomegranate peel at a concentration of 75 mg / kg of body weight for the period (1, 14 and 28) days was (2.24, 3.81 and 4.36) uU/ml respectively, While, the hormone concentration in the blood serum of infected rats and treated with the extract at a concentration of 150 mg/kg of body weight was (2.48, 3.96 and 4.48) uU/ml for the same periods respectively. Daily dosing with ethanolic extract of pomegranate peels at doses of 75 and 150 mg / kg of body weight for a period of 28 days caused an increase in the level of the insulin hormone in the blood of infected and healthy rats, the results are in agreement with Punasiya et al. (2010) who indicated an increase in the level of the hormone insulin in the blood serum of male rats with diabetes induced by Alloxan and treated with aqueous extract of pomegranate peel. The

attribute reasons for the increase in the hormone insulin is to the stimulation of its formation by pancreatic beta cells, and this was indicated by Suba *et al.*, (2004), Pomegranate peel also has a natural antioxidant property and so protects beta cells from damage through its action of scavenging free radicals, as it increases the efficiency of insulin receptors (Osman *et al.*, 2012).

1-3 Changes in the lipid profile:

1-3-1 The concentration of triglycerides in the blood serum:

The results indicate in Table (3) there is a significant increase ($p \le 0.05$) in the concentration of triglycerides in the serum of animals with experimental diabetes (B), It was 98.67 mg/dl compared to the control (natural) group (A) which was 84.00 mg/dl, this result was in agreement with the findings of Afify et al. (2018) in diabetic rats. The cause for this increase is due to diabetes, which leads to the breakdown of pancreatic beta cells (B-Cells) and thus a decrease in insulin secretion (Xiao, 2003), Also, insulin deficiency leads to a decrease in the activity of the enzyme (LPL) lipoprotein lipase, which has a major role in the breakdown of triglycerides into fatty acids and glycerol (Nelson and Cox, 2005), and the treatment of hyperlipidemia in persons with diabetes is through controlling the level of glucose in the blood, as it has an effective role in controlling lipid problems, especially triglycerides, as indicated by Dewanjee (2008).

The results show that intact animals were dosed with ethanolic extract of pomegranate peels at a concentration of 75 and 150 mg/kg of body weight (C and D), It led to a significant decrease in the triglyceride concentration (78.67 and 75.00) mg/dl respectively, compared with the intact control group which was 84.00 mg/dl. These results are consistent with the findings of Gabr (2017) who pointed out a decrease in the concentration of triglycerides in the blood of male intact rats when dosed with aqueous extract of pomegranate peel at a concentration of 500 mg/kg of body weight for 4 weeks compared with the intact control group. The results showed also that treating diabetic animals with ethanolic extract of pomegranate peels at a concentration of 75 and 150 mg/kg of body weight (E and F), It led to a significant decrease in the concentration of triglycerides, reaching (83.00 and 80.67) mg/dl respectively compared to the control group with diabetes that was 98.67 mg/dl, These results are in agreement with the findings of Gabr (2017) who observed a significant decrease in the concentration of triglycerides in rats with diabetes induced by Alloxan treated with aqueous extract of pomegranate peel at a concentration of 500 mg/kg of body weight. The attributed Sadeghipour et al., (2014) the cause for the low concentration of triglycerides in the blood serum of male rats to the experiment to the phenolic compounds and flavonoids present in the pomegranate peel extract in addition to their high antioxidant activity, and this was also indicated by Al-Muslehi (2013).

Т	able (3) The effect of dosing with ethanolic extract of pomegranate peels at a concentration							
	of (75 and 150 mg / kg of body weight) for 28 days on lipid profile and malondialdehyde							
	concentration in the serum of intact male rats with alloxan-induced diabetes.							

Treatment Estimates	(A)	(B)	(C)	(D)	(E)	(F)
Triglycerides (mg/dl)	84.00 b	98.67 a	78.67 bc	75.00 c	83.00 b	80.67 cb
Total cholesterol (mg/dl)	80.67 bc	96.33 a	76.67 cd	72.33 d	83.33 b	78.67 cb
HDL-C (mg/dl)	40.33 ab	30.33 c	42.67 ab	45.00 a	38.67 b	41.00 ab
LDL-C (mg/dl)	23.54 c	46.27 a	18.27 d	12.33 e	28.07 b	21.53 d
VLDL-C (mg/dl)	16.80 b	19.73 a	15.73 cb	15.00 c	16.60 b	16.13 cb
Malondialdehyde (micromol/l)	2.23 c	5.47 a	2.3 c	1.96 c	3.75 b	3.18 b

The number of animals are five per group, Different letters mean that there is a significant difference at a significant level (P ≤ 0.05), (A) Control (natural) group, (C) intact and dose group (75 mg / kg), (D) intact and dose group (150 mg / kg), (B) diabetic control group, (E) affected and treated group (75 mg / Kg), (F) affected and treated group (150 mg / kg).

1-3-2 Total cholesterol concentration in blood serum:

The results showed in Table (3) that the infection of male rats with diabetes led to a significant increase in the level of total cholesterol (TC) in the blood of the affected control group (B) reaching 96.33 mg/dl compared with the intact control group (A) that was 80.67 mg/dl, the results are

consistent with his findings (Mans and Aburjai, 2019) in diabetic rats. The cause return for the high concentration of total cholesterol in the blood serum is that when the body has diabetes it does not have the ability to consume glucose as a source of energy, this will stimulate the decomposition of fats in the adipose tissue in the body and the release of free fatty acids (FFA) that lead to an increase in the concentration of cholesterol in the blood serum (Salmenniemi, 2004). The results show that intact animals were dosed with ethanolic extract of pomegranate peels at a concentration of 75 and 150 mg/kg of body weight (C and D), It led to a significant decrease in the cholesterol concentration reaching (76.67 and 72.33) mg/dl respectively, compared with the intact control group which was 80.67 mg/dl, these results are in agreement with Gabr (2017), El-Hadary, and Ramadan (2019). The results also showed that treating diabetic animals with ethanolic extract of pomegranate peels at a concentration of 75 and 150 mg/kg of body weight (E and F). It led to a significant decrease in cholesterol concentration reaching (83.33 and 78.67) mg/dl respectively, after the diabetic control group 96.33 mg/dl These results are in agreement with Osman et al., (2012) when they indicated a decrease in the serum of cholesterol in the blood of female albino rats with diabetes induced by Alloxan and treated with pomegranate peel extract at a concentration of 250 mg/kg of body weight for a period of four weeks, reaching 135.94 mg/dl versus 162.43 for the control group with diabetes. All parts of pomegranate fruit such as peels, leaves, flowers and juice contain in their composition many bioactive compounds such as punicalagin, calic acid, ellagic acid, ursolic acid, punicalin and oleinolic acid, which are responsible for lowering cholesterol levels in the blood, also, hypercholesterolemia is treated by eating pomegranate fruit, which is characterized by its antioxidant properties because it contains biologically active ingredients that inhibit lipid peroxidation (Liu, 2005; Lei et al. 2007; Li et al. 2008; Al-Muammar and Khan, 2012).

1-3-3 Concentration of high-density lipoprotein cholesterol (HDL-C) in serum:

The results of the current study shown in Table (3) indicated that there was a significant decrease in the concentration of (HDL-C) in the blood of the control group with diabetes (B), reaching 30.33 mg/dl, compared with the intact control group (A) (40.33 mg/dl), This result was in agreement with Salwe et al. (2015) in diabetic rats, and also agreed with the result of Achi et al., (2017) in diabetic rats. The reason for the decrease in HDL cholesterol in diabetes is due to the increase in the effectiveness of the enzyme Cholesterol ester transferase, which works to transfer cholesterol esters from high-density lipoproteins for cholesterol (HDL-C) to very low-density lipoproteins for cholesterol (VLDL-C) leaving HDL-C is rich in triglycerides and less familiarized with Apo-A, so it remains free and becomes easier to filter through the kidney (Al-Jubouri, 2008). The results of the study show that dosing in intact animals with ethanolic extract of pomegranate peels at a concentration of (75 and 150) mg / kg of body weight (C and D), it gave an insignificant increase in high-density lipoproteins for cholesterol in the blood serum of male rats, reaching (42.67 and 45.00) mg/dl, respectively, compared with the intact control group of 40.33mg/dl. These results are consistent with the findings of Gabr (2017) and with those reported by El-Hadary and Ramadan (2019). The results showed that treating diabetic animals with ethanolic extract of pomegranate peels at a concentration of 75 and 150 mg / kg of body weight (E and F), it led to a significant increase in the concentration of HDL cholesterol, which became (38.67 and 41.00) mg/dl, respectively, after it was in the affected control group (B) 30.33 mg/dl, These results are agreeted with El-Mageid et al., (2016) who indicated an increase in HDL cholesterol concentrations in the blood serum of male rats with alloxan-induced diabetes treated with ethanolic extract of pomegranate peel at a concentration of 300 mg / kg of body weight for a period of four weeks, it was 43.30 mg/dl versus 30.87 mg/dl for the diabetic control group. It is also evident that the results indicated an increase in the concentration of HDL-C in the infected and intact group of animals dosed with the ethanolic extract of pomegranate peel, which may be attributed to the containment of pomegranate fruits and peels containing flavonoids, polyphenols and vitamins known for their antioxidant efficacy, regulation of lipid metabolism and an increase in the concentration of highdensity lipoproteins for cholesterol. (Ahmed, 2012).

1-3-4 The concentration of low-density lipoproteins (LDL-C) in blood serum:

The results shown in Table (3) showed that there was a significant increase (P ≤ 0.05) in the concentration of (LDL-C) in the blood serum of animals with experimental diabetes (B), which reached 46.27 mg/dl compared with the intact control group (A) it was 23.54 mg/dl. This result is consistent with Al Jafar (2020) in diabetic rats, the cause may be due to the free radicals generated as a result of the use of alloxan and damage to B-cells of the pancreas, which causes an increase in the level of oxidative stress, which leads to a defect in the low-density lipoprotein receptors for cholesterol (LDL-C) in the liver (Brewer, 2004), The increased concentration of free radicals in the body causes damage to beta cells insulin deficiency and the breakdown of fat cells, releasing free fatty acids, which the liver uses frequently to form VLDL-C cholesterol-lowering lipoproteins then it turns into low-density lipoproteins for cholesterol (LDL-C) leading to an increase in its concentration in the body, causing serious complications such as atherosclerosis and heart disease (Murray et al., 2000: Zhang, 2010). The present results show that dosing in intact animals with ethanolic extract of pomegranate peels at a concentration of 75 and 150 mg / kg of body weight (C and D), it gave a significant decrease in the concentration of low-density lipoproteins of cholesterol in the blood serum of male rats (A), directly proportional to the concentration of the extracted extract, reaching (18.27 and 12.33) mg/dl respectively, compared with the control group (A) (23.54 mg / dl). These results were in agreement with El-Hadary and Ramadan (2019), when they observed a significant decrease in the low-density lipoproteins of cholesterol in the blood serum of healthy rats dosed with methanolic extract of pomegranate peel at a concentration of 200 mg / kg of body weight compared to the control group. The results showed that treating diabetic animals with ethanolic extract of pomegranate peels at a concentration of 75 and 150 mg / kg of body weight (E and F). It led to a significant decrease in the concentration of low-density lipoproteins for cholesterol, reaching (28.07 and 21.53) mg/dl respectively, compared with the affected control group (B), which was 46.27 mg/dl. These results are in agreement with the findings of Gabr (2017). The results showed a significant decrease in the LDL-C concentration in the diabetic group, treated, and the intact group dosed with ethanolic extract of pomegranate peel, and the reason is due to the presence of active compounds in pomegranate peels such as punicalagin, gallic acid and ellagic acid, which are natural antioxidants that have the ability to inhibit protein oxidation low-density lipoprotein cholesterol with a significant decrease in arterial foam cells and thus reduces the risk of developing atherosclerosis (Rosenblat and Aviram, 2009; Khateeb et al., 2010).

1-3-5 Concentration of very low-density lipoprotein cholesterol (VLDL-C) in serum:

The results in Table (3) showed that the infection of experimental diabetes in rats led to a significant increase (P <0.05) in the concentration of very low-density lipoproteins of VLDL-C cholesterol compared with the intact control group and this result is consistent with what Ismaiil et al. (2019) In diabetic rats. The cause for the increase in the concentration of very low-density lipoproteins of VLDL-C cholesterol is due to the decrease in the activity of the enzyme lipoprotein lipase, which works to raise the concentration of triglycerides that are included in the formation of VLDL-C leading to an increase in the blood serum (Betteridge, 2000), Or it may be due to increased oxidative stress resulting from high concentrations of free radicals that reduce the activity of the enzyme Lipoprotein Lipase present in the body tissue and this decrease causes an imbalance in the concentrations of fats and an increase in the concentration of triglycerides in the blood serum, which is the main component of very low-density lipoproteins of cholesterol leading to increase its concentration in the blood(Betteridge, 2000: Salmenniemi et al., 2004). The current results show that dosing in intact animals with ethanolic extract of pomegranate peels at a concentration of 75 and 150 mg/kg of body weight (C and D), caused a significant decrease in the concentration of very low-density lipoproteins of cholesterol in the blood serum of healthy male rats, reaching (15.73 and 15.00) mg/dl respectively, compared to intact control group (A) of 16.80 mg/dl. The results are agreeted with El-Hadary and Ramadan (2019), as they observed a significant decrease in the concentration of very low-density lipoproteins of cholesterol in the serum of intact rats dosed with methanolic extract of pomegranate peel at a concentration of 200 mg/kg of body weight compared to the control group. The results also show that treating diabetic rats with ethanol extract of pomegranate peels at a concentration of 75 and 150 mg/kg of body weight (E and F), led to a significant decrease in the concentration of very low-density lipoproteins of cholesterol, reaching (16.60 and 16.13) mg/dl Respectively, compared with the control group with diabetes (B), which was 19.73 mg/dl, this result is consistent with El-Hadary and Ramadan (2019) in diabetic rats.

1-4 The malondialdehyde concentration:

The results in Table (3) show that the development of diabetes in rats led to a significant increase $(P \le 0.05)$ in the MDA values in the serum when compared with the intact control group, this result is consistent with Al-Badya (2018). The results showed an insignificant decrease in the MDA concentration in the intact group of rats dosed with the ethanolic extract of pomegranate peels at a concentration of 75 and 150 mg/kg of body weight, reaching 2.23 and 1.96 (micromol/l) respectively, when compared with the healthy group, which was 2.3 (µ mol /l), These results are agreeted with Gabr (2017) which indicated a decrease in MDA concentration in healthy male rats dosed with aqueous extract of pomegranate peel. The results showed a significant decrease in the MDA concentration in diabetic rats treated with the ethanolic extract of pomegranate peels at a concentration of 75 and 150 mg/kg of body weight, reaching 3.75 and 3.18 (micromol /l) respectively, when compared with the diabetic group, which was 5.47 (micromol /l), These results are in agreement with Gabr (2017). The cause is attributed to the role of active substances in pomegranate peels, such as phenolic compounds, gallic acid, ellagic acid, and ellagitannins, which act as natural antioxidants, and thus play an important role in reducing oxidative stress and enhancing the activity of antioxidant enzymes that work to suppress free radicals and reduce their activity (Moneim, 2012).

1-5 The effect on the serum activity of aminotransferase enzymes of intact and diabetic rats: 1-5-1 Aspartate aminotranseferase (AST) activity:

The results of Table (4) show a significant increase ($P \le 0.05$) in the activity of AST enzyme in the group of diabetic rats (B) compared to the control group (A), reaching 43.00 and 27.67 IU/l respectively. This result was consistent with results Al-Bayati (2017) in laboratory mice affected with diabetes. The cause may be that the elevation of AST due to cellular liver damage is usually associated with an increase in the enzyme ALT (Kaleem et al., 2008), the increase in the level of AST enzyme may also be due to the hepatocyte enlargement and the stimulation of the endoplasmic reticulum to produce a larger amount of the enzyme to match the cell size (Ene et al., 2006). The results also showed that there was an insignificant decrease in the AST level of the intact groups of rats dosed with ethanolic extract of pomegranate peels at a concentration of 75 and 150 mg / kg of body weight (C and D), reaching 24.67 and 22.67 IU/l respectively, compared with the intact control group, that amounted to 27.67 IU/l, this finding is in agreement with the findings of El-Hadary and Ramadan (2019). The results also indicate a significant decrease in the AST level of groups of diabetic rats treated with ethanolic extract of pomegranate peels at a concentration of 75 and 150 mg / kg of body weight (E and F), reaching 30.33 and 29.00 IU/l respectively, when compared with the affected group diabetes that was 43.00 IU/l, this result is consistent withEl-Hadary and Ramadan (2019). The reason for this decrease may be attributed to the active ingredients in pomegranate peels that act as natural antioxidants such as flavonoids (epicatechin, epigallocatechin gallate, quercetin, and luteolin), phenolic acids (chlorogenic and caffeic acid) and ellagitannin that work to break down free radicals and prevent cell membranes from breaking down, thus preserving cell membranes preventing liver tissue damage and preventing the exit of enzymes into the bloodstream (Shaban et al., 2013).

1-5-2 Alanine aminotranseferase (ALT) activity:

The results of Table (4) showed a significant increase ($P \le 0.05$) in the activity of the enzyme aminotransferase enzyme in the group of experimental diabetic rats (B), which reached 46.33 IU/l, compared with the intact control group (A), which was 21.67 IU/l, these results were consistent with what was mentioned by Mohammed *et al.*, (2011) when they indicated a significant increase in the activity of the ALT enzyme compared to the intact control group. The cause of the increase in diabetic rats may be attributed to the damage to pancreatic beta cells caused by Alloxan (Ali *et al.*, 2011). The results showed that there was an insignificant decrease in the level of ALT values for the healthy groups of rats dosed with ethanolic extract of pomegranate peels at a concentration of 75

and 150 mg/kg of body weight (C and D), reaching 21.33 and 20.67 (IU / L) respectively, when compared with the group intact control of 21.67 IU/L, this result is agreeted with El-Hadary and Ramadan (2019). The table shows a significant decrease in the level of ALT values for groups of diabetic rats treated with ethanolic extract of pomegranate peels at a concentration of 75 and 150 mg/kg of body weight (E and F) with an increase in the decrease with the increase in the concentration of the extracted extract, reaching 29.00 and 26.67 IU/l respectively compared to the diabetic control group which was 46.33 IU/L, this result is consistent with the findings of Gabr (2017). This may be due to the fact that pomegranate peels are rich in various flavonoids and thus reduce fat oxidation in the liver tissues and eliminate the risk of free radicals (Moneim *et al.*, 2011; Sadia *et al.*, 2016), The use of pomegranate peel extract rich compounds phenolic antioxidants which are a natural antioxidant that can keep the ALT values and this is indicated by Osman *et al.* (2012)

Table (4) The effect of dosing with ethanolic extract of pomegranate peels at a concentration of (75 and 150 mg / kg of body weight) for 28 days on the concentrations of AST and ALT enzyme in the blood serum of male rats with normal and diabetic induced by Alloxan.

Treatment Estimates	Group(A)	Group (B)	Group (C)	Group (D)	Group (E)	Group (F)
AST (U/L)	27.67 cbd	43.00 a	24.67 cd	22.67 d	30.33 b	29.00 cb
ALT (U/L)	21.67cd	46.33 a	21.33 d	20.67 d	29.00 b	26.67 cb

The number of animals is five per group, Different letters mean that there is a significant difference at a significant level (P \leq 0.05), (A) Control (natural) group, (C) intact and dose group (75 mg / kg), (D) intact and dose group (150 mg / kg), (B) diabetic control group, (E) affected and treated group (75 mg / Kg), (F) affected and treated group (150 mg / kg).

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دراسة تأثير المستخلص الايثانولي لقشور الرمان على بعض المتغيرات الكيموحيوية في مصل دم ذكور الجرذان المصابة بداء السكري المستحث بالالوكسان

> **مهند مهدي جمعه جندل وإيثار زكي ناجي** قسم علوم الأغذية – كلية الزراعة – جامعة تكريت – العراق.

المستخلص

أجريت التجربة في مركز بحوث التقنيات الإحيائية/ جامعة النهرين/ محافظة بغداد، وللفترة 2/2020/8/2 ولغاية 2020/9/3 وشملت متابعة تأثيرات التجريع بالمستخلص الايثانولي لقشور الرمان على 30 جرذا ذكرا بعمر (2-3) أشهر وبأوزان (170 – (220) غم، قسمت إلى قسمين احداها سليم والأخرى استحدث فيها مرض السكري باستخدام الالوكسان بتركيز (90 ملغم/كغم) من وزن الجسم، وأوضحت نتائج الدراسة البايولوجية أنّ إستحداث داء السكر التجريبي بالالوكسان أدى إلى إرتفاع معنوي (20.0 في تراكيز الكلوكوز (Glucose)، والكوليسترول الكلي (TC)، والكلسيريدات الثلاثية (TG)، والبروتين الدهني واطئ الكثافة للكوليسترول (DL–C)، والبروتين الدهني واطئة الكثافة جدا للكوليسترول (D–ULD)، وإنزيمات انزيم ناقل أمين الأسبارتيت و أنزيم ناقل أمين الألنين (AST وALT)، والكرويتين الدهني واطئة الكثافة جدا للكوليسترول (D–ULD)، وإنزيمات انزيم ناقل أمين الأسبارتيت و الكوليسترول (OLD–C)، والبروتين الدهني واطئة الكثافة جدا للكوليسترول (D–D–X)، وإنزيمات انزيم ناقل أمين الأسبارتيت و إلى انخفاض معنوي (OLD–C)، والبروتين الدهني واطئة الكثافة جدا للكوليسترول (D–D–X)، وإنزيمات انزيم ناقل أمين الأسبارتيت و المي انخفاض معنوي (OLD–C)، والبروتين الدهني واطئة الكثافة جدا للكوليسترول (D–D–X)، مقارنية مع مجموعة السيطرة السليمة، بينما أدت إلى انخفاض معنوي (OLD–C)، والبروتين الدهني واطئة الكثافة جدا للكوليسترول (D–D–X)، مقارنية مع مجموعة السيطرة السليمة، بينما أدت إلى انخفاض معنوي (HD–C)، في وزن الجسم، وتركيز البرادولين، وتراكيز البروتينات الدهنية عالية الكثافة للكوليسترول معام/كغم من وزن الجسم أدت إلى انخفاض معنوي في تراكيز المولكوز، و TC، و TC، و D–D ل 20–100، وانزيمات 30، المعام/كنع من وزن الجسم أدت إلى انخفاض معنوي في تراكيز المولكوز، و TC، و TC، و TC، و TC، و TL، والزيمات بتركيز المعاملات مقارنة مع مجموعة السيطرة السليمة، وإرتفاع معنوي في وزن الجسم وتراكيز الأسولين و 20–100، المعاملات مقارنة مع مجموعة السيطرة السليمة، وإرتفاع معنوي في وزن الجسم وتراكيز الأسولين و 20–100، المعاملات مقارنة مع مجموعة السيطرة السليمة، وإرتفاع معنوي في وزن الجسم وتراكيز ولرمان بتركيز وازيمات ACT ور ACT، ور ACM، مقارنة المعامية، وإرتفاع معنوي في تراكيز الجلوكوز، و CT، و TC، و CT، و CD–100، و 4. والدم ورن الجسم أدت إلى

الكلمات المفتاحية: قشور الرمان، الالوكسان، السكري، الانسولين، الكلوكوز، التجريع، المعالجة.