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Research Article

Effect of *Crataegus aronia* on the Biochemical Parameters in Induced Diabetic Rats

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ABSTRACT

Crataegus aronia is widely known for its antioxidant, anti-inflammatory, and hypolipidemic properties, and it has traditionally been used to treat cardiovascular disorders. This study aimed to evaluate the impact of Crataegus aronia extract on the liver enzyme markers, blood glucose levels, lipid profiles, and kidney function biomarkers as well as hematological parameters in induced diabetic rats. Male Wistar rats were divided into seven groups: normal Control; Diabetic; and Diabetic animals treated with two doses of Crataegus aronia extract (5 and 10mg/kg) (DM + extract), Control treated with the extract (5 and 10mg/kg) and induced diabetic treated with insulin. Streptozotocin (STZ)-induced diabetic rats (50 mg/kg, ip)and normal were orally administrated with Crataegus aronia extract once a day for 4 weeks. At the end of the experiment, the biochemical and hematological parameters were measured in all groups. Also, the phytochemicals and antioxidant activity of the Crataegus aronia extract were evaluated. According to findings, the total phenols, total flavonoid, and flavonol contents were 538.3 mg Galic acid equivalent /g extract, 149.3 mg Rutin equivalent / g extract, and 79.3 mg Rutin equivalent / g extract), respectively. The antioxidant activity according to 2,2-diphenyl-2-picrylhydrazyl (DPPH) IC_{50} and ferric reducing antioxidant power (FRAP) assays were 28.02 µg/ml and in the range of 0.273 – 0.960 µmol Fe^{+2}/g dw, respectively. *Crataegus aronia* extract significantly ($p < 0.05$) affects red blood cells, hemoglobin, hematocrit, white blood cells, lymphocytes, and platelets values. Also, *Crataegus aronia* had a significant ($P < 0.05$) effect on serum biochemical parameters, including glucose, total proteins, albumins, triglycerides, creatinine, bilirubin, and serum aspartate aminotransferase (AST). However, *Crataegus aronia* treatment had no significant effects ($p > 0.05$) on serum alanine aminotransferase, alkaline phosphatase, and cholesterol levels. *Crataegus aronia* exerts antioxidant activity and significantly improves the biochemical and hematological biomarkers in induced diabetic rats.

Key words: *Crataegus aronia*, diabetes mellitus, Insulin, antioxidant, biochemical parameters

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INTRODUCTION

Many studies have been conducted to proof that plants are one of the most important sources of active substances. Plants have therapeutic potential to treat various diseases in humans[1, 2]. The evaluation of pharmacological effects of plants can be used as a valuable tool for discovering new drugs and compounds of plant origin[3, 4]. A world-wide revolution which is mainly focused on the belief that medicinal plants are safer and less damaging to the human body than synthetic drugs[5]. Based on scientific reports from World Health Organization about 80% of the world population depends on traditional medicine for primary health care and more than 30% of the plant species have been used medicinally[6, 7].

The interest of the biological as well as medical research has been focused on natural antioxidant molecules. Human body produces free radicals as byproducts during metabolism because of the oxidative phosphorylation effect. Free radicals such as reactive oxygen species (ROS) are species which contain one or more unpaired electrons, highly reactive chemical species formed in all tissues during normal aerobic cellular metabolism. ROS initiating a chain reaction with the potential to start proteins, DNA damage and cause peroxidation of membrane lipids[8]. ROS are the main reasons for ageing, tumors, arteriosclerosis, hypertension and diabetes. ROS are increasingly formed in diabetes mellitus (DM) by the auto oxidation of glucose and glycosylated proteins. Even though insulin therapy and traditional folk medicine can control many aspects of diabetes complications, still DM is a complex metabolic disorder characterized by high blood glucose levels and is associated with a changed in lipid profiles as well as other metabolic biomarkers[9, 10].

Antioxidants are substances that are efficient to diminish the oxidation rate in proteins, DNA and membrane lipids. Antioxidants molecules can inhibit the process of oxidation include scavenging of the free radicals, chelating of free metals, and inhibition of enzymes responsible to produce the ROS. The antioxidant properties of medicinal plants can be attributed to the polyphenol, Flavonoids, essential oils contents which play an important role in improving DM disorders causes by mediated by oxidative stress[11-13].

As in many countries of the world, traditional medicine particularly herbal medicine is part of Jordanian culture[14]. Jordan is rich in a wide variety of medicinal plants which are not used only for treatment of mild diseases such as colds, headaches or the digestive system, but also in the treatment of long-term illnesses or incurable diseases, such as diabetes, high blood pressure or cancer[15, 16].

Crataegus is indigenous to the Mediterranean Basin. *Crataegus* is a low, dense, spiny tree with a beautiful inflorescence up to 6 m tall and with orange,

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red or yellow fruits[17, 18]. *Crataegus* (Hawthorn) is very important in folk medicine (commonly called Zaarour in Arabic) and has exhibited various biological activities such as cardiovascular, immune systems disorders, anti-inflammatory, cytotoxic, antioxidant, antidiabetic and anti-HIV activities[19-22]. This study aims to determine the total phenol, flavonoids and flavonols contents of the methanolic for *Crataegus aronia* extracts, and to evaluate the antioxidant activity of Crataegus *aronia extracts* by using DPPH and FRAP methods. In addition, the study focuses on the effects of *Crataegus aronia* methanol extract on biochemical and hematological parameters in *streptozotocin* induced diabetic rate, as *in vivo* model.

MATERIALS AND METHODS

Chemicals and equipment

A list of chemicals and equipment used in this study is found in Appendix 1.

Plant Material and Extract Preparation

Crataegus aronia leaves were collected in May 2019 from Wadi Al-Thanya, South of Al-Karak city, Jordan. The plant was identified by Prof. Saleh Al-Qura'n (Department of Biology Mutah Karak, Jordan). After being collected, the leaves were dried for 7-10 days in the shade at room temperature before being ground into powder and stored in plastic containers away from light, heat, and moisture until usage. The extract was prepared according to Odey et al.,(2012)[23] with some modifications. 50 g of plant powder was soaked in 500 ml of methanol in 1000 ml beaker , then kept in a shaker (150 rpm) for 3 days for continues agitation at 150 rpm. The solutions were then filtered and concentrated using a rotary evaporator at 45°C before being stored at -20°C.

Methanolic extract yield (gram/ 50 gram dry leaf extract) was calculated as follows:

Yield (%) = (Wt of dry extract)/(Wt of dry sample) x 100%

Antioxidant activity

2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant activity of *C. aronia leaves extract* was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay and was performed according to a previous method [24] with slight modifications. Different concentrations of the extract were added to DPPH methanolic solution (final absorption of 0.754 at 517 nm). The mixtures were incubated for 30 minutes at room temperature in the dark, and the absorbance at 517 nm was measured against a blank. The extract's percentage inhibition (%) of free radicals was estimated using the following equation:

I (%) = ((A control - A sample)/ A control) X 100

Extract concentrations providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition (%) against extract concentration and compared with the IC_{50} of Gallic acid value as standard.

Ferric reducing antioxidant power (FRAP) assay

The total antioxidant potential of *C. aronia leaves extract* was determined using the ferric reducing ability of plasma FRAP assay by Benzie and Strain, 1999 as a measure of antioxidant power. The FRAP reagent contained 2.5 ml of a 10 mmol/L TPTZ (2, 4, 6-tripyridyl-s-triazine; Sigma) solution in 40 mmol/L HCl plus 2.5 ml of 20 mmol/L FeCl3 and 25 ml of 0.3 mol/L acetate buffer (pH 3.6). The reagent was freshly prepared and warmed at 37 °C. The working FRAP reagent (1.5 ml) was mixed with 50 µl sample or standard in a test tube. After 10 min at 37 °C, the absorbance was determined at 593 nm. FeSO4 at a concentration of 1 mmol/L was used as the standard solution. The result was expressed as the concentration of antioxidant with a ferric reducing ability equivalent to that of 1 mmol/l FeSO4[25].

Total Phenol Content

The total phenol content in the *C. aronia* leaves methanolic extract was determined according to Sakat et al. (2010). The extracted sample (0.2 ml) of the plant extract (0.5 mg/ml) was mixed with 1ml of 10% Folin Ciocalteu solution and 0.8 ml of 7.5% sodium carbonate solution[26]. The mixture was incubated for 1 h at room temperature. The absorbance at 725 nm was measured and converted to phenolic contents according to the calibration curve of Gallic acid[27]. All determinations were performed in triplicate. Total content of phenolic compounds in leaves methanolic extracts in Gallic acid equivalents was calculated by the following formula:

C= c.V/m

Where: C-total content of phenolic compounds, mg/g leaves extract, in GAE; c-the concentration of Gallic acid established from the calibration curve, mg/ml. V- the volume of extract. m-the weight of pure leaves extract.

Total Flavonoid Content

Total flavonoid content of the extract was determined according to (Sharma and Agarwal, 2015) using the aluminum chloride colorimetric method with slight modification using rutin as standard and the results were expressed as mg of rutin equivalents per g dry weight of the plant (mg RE/g dw)[28]. Briefly, the extract solution, 0.5 ml of (10 mg/ml), 2 ml distilled water and 0.15 ml 5% $NaNO_2$ solution were added. After 6 min, 0.15 ml 10% $AlCl_3$ solution was added and kept for another 6 min. To this reaction mixture, 2 ml 4% NaOH solution and 0.2 ml water were added to make up the final volume 5 ml. The reaction mixture was mixed well and allowed to stand for 15 min after which absorbance was recorded at 510 nm. Total flavonoid content was expressed as mg rutin equivalent (RE)/g plant sample. Total content of flavonoids compounds in plant methanolic extracts in RE was calculated by the following formula:

C=c.V/m

Where: C-total content of flavonoids compounds, mg/g plant extract, in RE. c-the concentration of Rutin established from the calibration curve, mg/ml. V- the volume of extract, ml. m-the weight of plant methanolic extract.

Total Flavonol Content

Total flavonol content was estimated using the AlCl3 colorimetric method as described by Sakat et al.,2010 with some modifications. 2 ml of plant extract (10 mg/ml) were mixed with 2 ml (20 mg/L) AlCl3 and 6 ml (50 mg/L) sodium acetate. The absorbance was measured at 440 nm after 2 h. The total Flavonol concentration was given in mg Rutin equivalent (RE)/g extract. All measurements were made in triplicate. The content of Flavonols, in RE was calculated by the following formula:

X=C.V/m

Where: X- Flavonol content, mg/g plant extract in RE. C-the concentration of Rutin solution, established from the calibration curve, mg/ml. V- the volume of extract, ml. m-the weight of pure plant methanolic extract.

Animals

Male albino Wister rats, weighing 180 -225 g was used in this study. The chosen animals were housed in plastic well aerated cages at normal atmospheric temperature (25 ± 5 °C) and normal 12 hour light/dark cycle. Moreover, they had free access to water and were supplied daily with a standard diet of known composition *ad libitum*.

Induction of Diabetes in Rats.

Albino Wister male rats were fasted overnight, and diabetes was induced by a single dose of streptozotocin (STZ) (65 mg / kg body weight) in a 0.1M citrate solution (pH 4.5) subcutaneously[29]. Control Rats were injected with normal saline only. Rats having serum glucose ≥ 300 mg/dl, after 3 days of the STZ injection, were considered diabetic and selected for further pharmacological studies. The rats were allowed to continue to feed on their respective diets until the end of the study. Treatment began on the fourth day after STZ injection and is considered the first day of treatment and continued for 4 weeks[30].

Determinations acute toxicity of LD50 for Crataegus -aronia methanol extract

For this purpose, 10 diabetic rats were divided into 5 groups (2 rats in each group), then each group was injected subcutaneously with a single dose of extract. Group 1 was given 50 mg/kg of body weight, while groups 2,3,4 and 5 were given 100,200,500 and 1000 mg/kg respectively. After that, animals were monitored for 72 hour and so dead rats from each group were recorded.

Experimental design

The experimental animals were divided into seven groups, each group comprising five rats as detailed follows in Table 1. After 4 weeks of treatment, the rats were sedated, then blood samples were collected for further biochemical analysis.

Insulin treatment

Insulin was used in the present study, based on the desired pharmacological effect First, insulin (Novo Nordisk A/S; Denmark; product number HS67C87) and second, insulin-releasing implants that release a basal dose of insulin (5 IU/implant) These implants were inserted the subcutaneous of the rats under short-acting anesthetic conditions, The injection was performed subcutaneously by concentrated 200 µL insulin + 800 µL normal saline solution per rat.

Determination of blood glucose levels

Blood glucose concentration (mg/dl) was determined weekly using a blood glucose test meter (glucolab Auto-coding) manufactured by (Infopia co., korea). Animals were fasted overnight then blood samples were collected from the tip of tail[31].

Hematological assay

Blood samples were obtained from anaesthetized animals by cardiac puncture in vacutainer tubes. The hematological parameters, such as Hb, hematocrit (Hct), platelets, RBCs, WBCs, and lymphocytes were determined using an automated hematology analyzer (Orphee, mythic-18, Swiss). Also, biochemical parameters such alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities, glucose, albumin, total protein, blood urea nitrogen, creatinine, total bilirubin, triglycerides, cholesterol were determined using an automated biochemical analyzer (Cormay accent-200, Poland) in the Integrated Laboratory, Private medical lab company; according to the manufacturer's protocol[31].

RESULTS

Percentage yield determination

Extract yield of *Crataegus aronia* leaves prepared by soaking with continuous agitation methods using methanol is summarized in Table (3).

Quantitative analysis: Total phenols, flavonoid and phenolic compounds.

As shown in Table (3) the total phenol, flavonoid and flavonol concentrations in *Crataegus aronia* are 538.5 mg GAE/g extract, 149.3 mg RE/g extract, and 79.3 mg RE / g extract, respectively.

Antioxidant activity.

DPPH assay (Free radical scavenging activity)

DPPH and FRAP assays were used for the determination of antioxidant activity of the extract. Antioxidant capacities of the extract was expressed in terms of IC_{50} value of the extracts and low IC50 value corresponds to a high antioxidant capacity (Table 3; Figure 1 and 2). Figure (1) illustrated an increase of DPPH % inhibition due to the scavenging ability of methanolic leaves extract. The DPPH % inhibition (IC_{50}) value of *C. aronia* leave extracts is 28.02 µg/ml.

Crataegus aronia LD_{50}

Crataegus aronia is a well-tolerated plant The results of this study are shown in Table (4), in this study the injection administration of the aqueous extract of *Crataegus aronia* at all given doses (50 to 1000 mg kg^{-1}) did not produce any visible sign of acute toxicity or instant death in rats tested during the period of observation.

Blood glucose levels

The average blood glucose level for each group was estimated weekly for up to 4 weeks Table (5). The glucose level in the diabetic rats was significantly higher compared to the normal groups. Moreover, the

Table 1: Experimental design

Group number	description	Extract	Injection type	Amount of the Extract/ kg
1	Normal rats (N)	-	-	-
2	Diabetic rats (DN)	-	-	-
3	Diabetic rats (D5)	*Crataegus aronia*	subcutaneous	5 mg/kg
4	Diabetic rats (D10)	*Crataegus aronia*	subcutaneous	10 mg/kg
5	Diabetic rats (DI)	Insulin	subcutaneous	5 IU/implant
6	Normal rats	*Crataegus aronia*	subcutaneous	5 mg/kg
7	Normal rats	*Crataegus aronia*	subcutaneous	10 mg/kg

Table 2: Biochemical tests used in this study.

	Biochemical Markers
Hepatotoxicity	Total bilirubin; Alanine aminotransferase; Aspartate aminotransferase; Alkaline phosphatase
Nephrotoxicity	Creatinine; Total protein; Albumin; Blood Urea
Glucose and lipid profile	Glucose; Triglycerides; Cholesterol

Table 3: Yield, total phenols, flavonoids, Flavonol contents and DPPH IC_{50} values of the methanol extract of Crataegus aronia.

Yield[a] %	*Total phenols (mg GAE/g extract)	#Total flavonoids (mg RE/g extract)	#Total Flavonol (mg RE/g extract)	DPPH IC_{50} (µg/ml)
25.68	538.5 ± 0.095	149.3 ± 0.0847	79.3 ± 0.061	28.02 ± 2.53

*GAE: gallic acid equivalent; # RE: Rutin equivalent. [a](g extract / 100 g dry leaves)

Table 4: Crataegus aronia extract LD5o

Group number	Number of animals	Dose mg/kg*	No. of animals dead
1	2	50 mg kg^{1}	---
2	2	100 mg kg^{-1}	---
3	2	200 mg kg^{-1}	---
4	2	500 mg kg^{-1}	---
5	2	1000 mg kg^{-1}	---

*Injection dose of *Crataegus aronia* extract.

Table 5: Effect of Crataegus aronia leaves extract on blood glucose

Group	Fasting blood glucose (mg/dl)			
	1st week	2nd week	3rd week	4th week
N	98.0±15	115.0±15	121.0±37	135.0±18
D	401.0±45	616.0±57	770.0±62	878.0±81
D5	435.0±30	459±51	430.0±43	410.0±59
D10	446.0±42	542.0±95	701.0±88	845.0±58
DI	453.0±47	320.0±14	285.0±10	236.0±12
N5	95.0±37	130.0±45	170.0±24	192.0±15
N10	135.0±15	168.0±49	190.0±56	221.0±51

N, N5, N10: normal and normal treated by 5 and 10 mg/kg, respectively. D, D5, D10, DI: diabetic, diabetic treated by 5 and 10 mg/kg and insulin; respectively
Data means ± SD of rats/group. FBL: fasting blood sugar

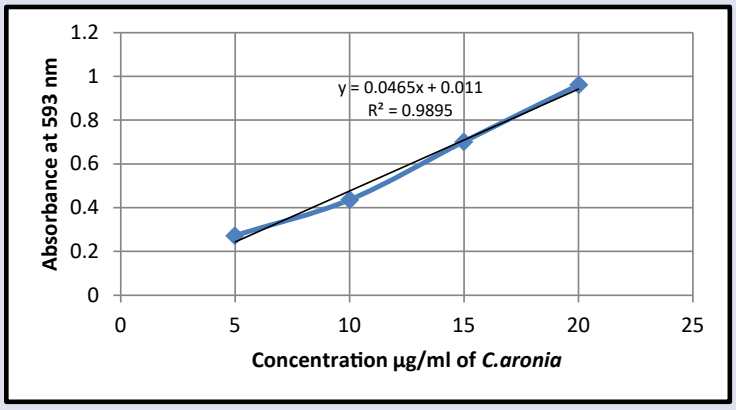

Figure 1: DPPH % inhibition of *C. aronia.*

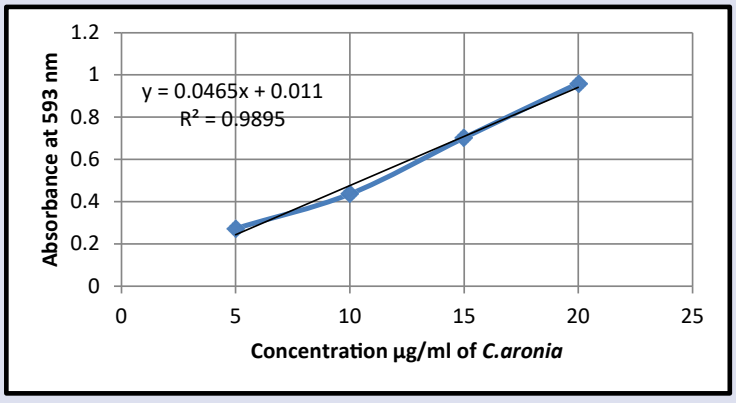

Figure 2: FRAP test absorption value of *C. aronia.*

Table 6: Effect of Crataegus aronia leaves extract on the blood parameters in induced diabetic rats

	Control	Diabetic	Diabetic + 5 mg extract	Diabetic + 10 mg extract	Diabetic + Insulin	Control+ 5 mg extract	Control+ 10 mg extract
RBCs (10^6/µL)	6.352±1.036	5.590±0.701	6.815±0.573	5.715±1.775	8.325±1.387[a]	5.768±0.616	6.464±0.523
Hb (g/dL)	14.080±2.054	12.620±1.112[a]	14.750±1.202	12.425±3.409	6.760±0.665[a]	13.440±1.383	14.420±1.130
WBCs (10^3/µL)	3.720 ± 0.993	5.840 ± 2.365[a]	7.450 ±2.758[a]	6.150±2.461[a]	2.500±1.291	7.060±1.205[a]	7.500±1.091[a]
Lymphocytes (10^3/µL)	3.040±0.891	4.840±2.223[a]	6.050±1.485[a]	4.525±2.243[a]	6.250±0.926[a]	5.920±1.018[a]	6.160±0.720[a]
Hematocrits (%)	39.700±4.445	35.760±3.186	43.050±0.919[a]	33.900±10.576[a]	15.075±2.030[a]	36.360±3.917	40.300±3.081
Platelets (10^3/µL)	579.800±94.487	419.600±204.947	589.000±200.818	315.000±198.335[a]	16.525±2.035[a]	671.200±198.750	724.200±73.727[a]

Data are means ± SD of (3-5) rats/group. [a]$p < 0.05$ vs. control.

Table 7:Effect of Crataegus aronia leaves extract on the Liver biomarkers enzymes in induced diabetic rats

	Control	Diabetic	Diabetic + 5 mg extract	Diabetic + 10 mg extract	Diabetic + Insulin	Control+ 5 mg extract	Control+ 10 mg extract
Alanine aminotransferase (U/L)	146.460±63.786	122.120±75.406	181.600±41.581	104.000±21.737[a]	371.250±65.589[a]	121.360±97.022	86.400±39.129[a]
Aspartate aminotransferase (U/L)	212.120±45.380	228.800±89.893	513.733±72.707[a]	319.800±178.359	348.200±188.704	208.000±93.365	129.180±26.799
Alkaline Phosphatase (U/L)	132.200±11.606	324.200±131.732[a]	324.333±115.678[a]	290.500±114.884[a]	451.000±50.516[a]	171.000±130.704	91.800±31.673

Data are means ± SD of (3-5) rats/group. [a]$p < 0.05$ vs. control.

Table 8 Effect of Crataegus aronia leaves extract on the blood biochemical marker in induced diabetic rats

	Control	Diabetic	Diabetic + 5 mg extract	Diabetic + 10 mg extract	Diabetic + Insulin	Control+ 5 mg extract	Control+ 10 mg extract
glucose (mg/dl	156.200±38.402	962.600±23.093[a]	421.333±187.897[a]	897.750±141.474[a]	394.500±117.323[a]	202.400±29.407	221.800±81.211
Creatinine (mg/dL)	0.384±0.044	0.332±0.068[a]	0.197±0.147[a]	0.308±0.039	0.318±0.051[a]	0.348±0.043	0.338±0.058
Total bilirubin (mg/dL)	0.326±0.072	0.524±0.343[a]	0.820±0.538	0.413±0.114	0.393±0.178 [a]	0.424±0.233	0.290±0.084
Blood Urea Nitrogen (mg/dL)	23.680±5.077	35.960±3.071[a]	50.667±11.809[a]	40.725±9.163[a]	22.725±3.021	22.080±1.242	20.060±2.217
Albumin (g/dL)	3.442±0.164	1.116±1.528[a]	2.807±0.502	2.125±1.428	3.373±0.092	3.612±0.126	3.614±0.325
Triglycerides (mg/dL)	31.066±4.476	104.632±25.261[a]	66.327±31.215	82.563±33.732[a]	54.020±29.879	36.786±5.368	39.110±6.932
Cholesterol (mg/dL)	65.000±0.707	68.400±5.273	70.000±30.050	71.000±10.100	68.250±6.602	82.200±8.228[a]	82.600±12.422[a]
Total protein (g/dL)	6.152±0.118	5.056±0.447[a]	6.253±0.216	5.493±0.418	6.295±0.261	6.418±0.238	6.716±0.537

Data are means ± SD of (3-5) rats/group. [a]$p < 0.05$ vs. control.

glucose concentration was significantly decreased in the diabetic mice treated with *Crataegus aronia*.

The Previous tables indicate that the probability value is statistically significant at p= 0.05. Whereas results according to the ANOVA score showed a difference between the seven groups of blood content variables. Probability values check was (0.002) for WBC; (0.025) for LYM; (0.013) for RBC; (0.000) for HGB; (0.000) for PLT (Table 6). By comparing the observed likelihood values with the level of margin of error of type I error (α = 0.05), it can be seen that all the presented convertibility values were <0.05 indicating statistically significant differences in the averages between the seven research groups on blood content variables (. To determine which groups may differ significantly from each other over the variables that suggested significant statistical differences; the Bonferroni post hoc test was used (Appendix 2).

Liver and kidney function biomarkers (ALT, AST, and ALP) were statistically analyzed and indicate that the probability value is statistically significant at 0.05 (Tables 7 and 8). Whereas results according to the ANOVA score showed a difference between the seven groups of blood serums' variables. Comparing the observed probability values to the level of error margin of type 1 error (α=0.05), it could be noted that some probability values were > 0.05, suggesting that no significant statistical means differences among the seven research groups over these blood serum variables. These variables include total bilirubin, (0.138), alanine aminotransferase (0.119), alkaline phosphatase (0.086), and cholesterol (0.146), respectively. On the other hand, the rest of the blood serum variables reflected a probability value that was < 0.05 suggesting significant statistical differences among the seven research groups over these specified variables.

DISCUSSION

In this study, streptozotocin was used to induce diabetes in rats. It produced hyperglycemia, as well as elevated plasma TG. It is known

that alkylating agents such as streptozotocin are toxic to cells by causing damage to the DNA, the release of nitric oxide, increased pancreatic proteins, glycation, and increased production of ROS. These will induce pancreatic β-cell damage[32]. *C. aronia* reduced the hyperglycemia of streptozotocin-induced diabetic rats but had a slight effect on the plasma glucose level of normal rats. This effect of *C. aronia* is consistent with many studies indicating a glucose-lowering effect of plant phytochemicals such as polyphenols and flavonoids. *Aronia melanocarpa* leaves extracts Stimulate glucose uptake in PC12 pheochromocytoma cells and L 929 fibroblasts (PC 12 and L 929 cells)[33, 34].

Cignarella et al. (1996) showed that blueberry leaves are traditionally used as a folk medicine treatment for diabetes and reduced plasma glucose levels[34]. *Vaccinium myrtillus* L. leaf caused a significant reduction in blood glucose levels compared with control diabetic rats. Bilberry supplementation resulted in a significant reduction of glucose compared with the diabetic control[35, 36]. Also, anthocyanin from grapes, red wine polyphenolic, grape seed-derived procyanidins, myricetin, and rutin reduced glucose levels in streptozotocin-induced diabetic rats[37-39].

The phytochemical analysis of *C. aronia* leaves extracts showed that they contained a high concentration of polyphenols 538.5 mg GAE, followed by flavonoids 149.3 mg RE, and flavonols 79.3 mg RE. *C. aronia* is very rich in phenolic antioxidants. *C. aronia* extract has an antioxidant capacity of DPPH (IC50) value of 28.02 µg/ml for the radical scavenging activity compared to gallic acid. According to the FRAP assay, *C. aronia is* a reducing powers capacity and it was increased in concentration and so in DPPH-dependent matter. Therefore, Probably, by acting as an antioxidant *C. aronia* might protect pancreatic β-cells from streptozotocin-induced increased production of ROS.

In this study, *C. aronia* significantly reduced the TG levels in treated diabetic rats when compared with untreated diabetic rats. This lipid-lowering effect might be due to the improvement of the diabetic condition in *C. aronia* -treated rats. A similar TG-lowering effect in streptozotocin-diabetic rats have been demonstrated for anthocyanins from blueberry leaves[34]. In this study, streptozotocin did not induce significant changes in the levels of TC.

Crataegus Aronia reduced serum levels of oxidized LDL and protects and reverses vascular inflammation in a high-fat diet rat as described previously[40]. Many studies have examined the probable mechanisms of the hypolipidemic effect of *C. aronia* and showed that *C. aronia* could inhibit the activity of intestinal acyl-CoA cholesterol acyltransferase[20], suppressing lipogenesis in the rat's liver[41] and reducing TGs synthesis by *C. aronia* flavonoids[42]. Previous work described the administration of *C. aronia* associated with a significant increase in the antioxidant potential of the aortic cells through the decrease of MDA levels and increases of GSH levels and SOD activity[43].

Crategous extract can effectively inhibit oxidative processes in the laboratory and the extract of this plant is a highly effective radical scavenger and agree with the previously described. Treatment of diabetic rats with two different concentrations of *C. aronia* extract caused a concentration-dependent drop in plasma blood sugar levels and this will improve the antioxidant protective mechanism against the oxidative stress that worsens in diabetes[44, 45].

Diabetic rats given the supplementation of *Crataegus* for four weeks significantly restored the WBC count and lymphocytes and hemoglobin and platelets near the control level. This was noted by One Way ANOVA score concerning the differences between the seven groups of blood content variables. Probability values check was (0.002) for WBC; (0.025) for LYM; (0.013) for RBC; (0.000) for Hb; (0.000). (0.000) for PLT. By comparing the observed likelihood values with the level of margin of error of type I error ($\alpha = 0.05$).

According to results of total protein, albumin, and liver enzyme values in this study clearly show the positive effects of *C. aronia* on the improvement of liver function through increasing antioxidant capacity of *C. aronia*, which is, agrees with previous research[46, 47]. According to previous work, Eshrat (2003) shows that treatment of Streptozotocin-induced albino rats with water extract of *A. augusta* plus *C. indica* at a dose of 300 mg/kg body wt brought down fasting blood glucose to a normal value, while in the untreated group it is increased. Also *A. augusta* plus *C. indica* affect the fasting blood sugar, glucose tolerance, and lipid profile[44].

It has been noted that *A. Indica* leaf extract significantly decreased total cholesterol, LDL-and VLDL-cholesterol, triglycerides, and total serum lipids in streptozotocin-induced diabetic rats, but HDL-cholesterol levels remained unchanged when compared to streptozotocin-induced diabetic control animals[45]. The combined extracts of *Moringa oleifera* and *Vernonia amygdalina* decreased significantly ($P<0.05$) in the alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and total protein of induced diabetic albino Wistar rats[48].

CONCLUSIONS

This study indicates the positive effect of *C. aronia* in reducing glucose levels and hyperlipidemia, improving the antioxidant in control and diabetic rats, and the following conclusions were reached. *Crataegus aronia* reduced hyperglycemia in diabetic rats and significantly decreased TG levels in diabetic mice. In addition, Diabetic rats given the supplementation of *Crataegus* significantly restored the WBC count and lymphocytes and hemoglobin, and platelets near the control level. The improvement of liver function through the increased antioxidant capacity of *C. aronia* also was noted.

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GRAPHICAL ABSTRACT

Table (6) Blood glucose concentration

Group	FBG (mg/dl)	1st week	2nd week	3rd week	4th week	
N	98.0±15	115.0±15	121.0±37	135.0±18		NS
D	401.0±45	616.0±57	770.0±62	878.0±81		NS
D5	435.0±30	459±51	430.0±43	410.0±59		NS
D10	446.0±42	542.0±95	701.0±88	845.0±58		sig
DI	453.0±47	320.0±14	285.0±10	236.0±12		NS
N5	95.0±37	130.0±45	170.0±24	192.0±15		NS
N10	135.0±15	168.0±49	190.0±56	221.0±51		NS

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