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Protective Role of Combined *Crataegus Aronia* Ethanol Extract and Phytosomes Against Hyperglycemia and Hyperlipidemia in Streptozotocin-Induced Diabetic Rat

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Abstract: Diabetes is one of the most common chronic diseases worldwide. This study aims to evaluate the hypoglycemic and hypolipidemic effects of *Crataegus aronia* (*C. aronia*) ethanolic extract and *Crataegus aronia* phytosome (*C. aronia*-p) in streptozotocin (STZ)-induced diabetic rats. The phytochemical content of *C. aronia* was identified. The *C. aronia* ethanolic extract and *C. aronia*-p were checked for their antioxidant activity using DPPH assay. Then, *in vivo* evaluation of *C. aronia* ethanolic extract and *C. aronia*-p hypolipidemic and hypoglycemic activity was conducted using STZ-induced diabetic rats. The major phytochemicals component found in *C. aronia* ethanolic extract is phenols, flavonoids, alkaloids, and tannins. Moreover, The IC_{50} of DPPH scavenging activity after the treatment of *C. aronia* leaf ethanolic extract is 69.46±6.4 µl. Furthermore, our study indicated that the *C. aronia* leaves extract and *C. aronia*-p has significant hypolipidemic and hyperglycemic activity in the diabetic rats (P <0.001). This study provides a useful indication for promoting *C. aronia* as a potential novel therapeutic agent against diabetes.

Keywords: diabetes; *C. aronia*; phytosomes; blood glucose level; lipid profile.

1. Introduction

Diabetes is a group of metabolic disorders characterized by chronic hyperglycemia [1]. It is considered one of the most important contemporary diseases with a negative health impact on humans due to its complications, such as kidney failure, vision loss, nerve damage, dyslipidemia, and uterine fibroids [2-6].

Several anti-diabetes and hypolipidemic drugs have unfavorable and dangerous side effects [7,8]. Therefore, searching for natural sources such as plant remedies that do not produce harmful side effects is necessary and inevitable [9,10]. Medicinal plants may provide beneficial effects such as antimicrobial, antioxidant, anti-inflammatory, and anticoagulant [11-20]. Hence, conventional medicines may be a great alternative treatment prescribed to prevent and treat diabetes [21-25].

Crataegus aronia plant, which is endemic in Jordan, is traditionally used to treat many diseases such as cardiovascular diseases, diabetes, and hyperlipidemia [26,27]. In the same context, recent studies demonstrated the efficacy of the nanoparticles in various fields such as data storage [28], cosmetics [29], antimicrobial agents [19], and anticancer [13,30]. Phytosomes are a complex between a natural product and natural phospholipids introduced as herbal formulations that exhibit enhanced absorption and generate higher bioavailability of the active constituents than the conventional phytochemical or botanical extracts [31,32]. In addition, these phyto-phospholipid complexes have improved pharmacokinetic and pharmacological parameters [33]. Thus, more active constituents in the nutraceutical products become available at the site of action at a similar or lower dose than conventional plant extract [34-36].

The role of *C. aronia* extract and *C. aronia*-phytosomes on hyperlipidemic and hyperglycemic conditions induced by diabetes has never been investigated before. Therefore, this study has evaluated the potential hypolipidemic and hypoglycemic effects of *C. aronia* extract and *C. aronia*-p in streptozotocin-induced diabetic rats.

2. Materials and Methods

2.1. C. aronia collection and preparation.

Leaves of *C. aronia* were collected during the fruiting stage in June-July 2019 from plants cultivated in Ajloun, Jordan. *C. aronia* was identified and classified by Professor Sawsan Atallah Oran, Department of Biology, Faculty of Science, University of Jordan, Amman, Jordan. Voucher specimens have been preserved at the herbarium of the Department of Biology, Faculty of Science at Mu'tah University. Then, the powder was packed in glass containers with tight lids and stored in the refrigerator until the time of the experiment [13,37].

2.2. Preparation of the ethanolic extract of C. aronia leaves

The powder was extracted in ethanol/water (80/20, v/v; Hayman, England) in a ratio of 1: 5 (w/v) between the material and solvent using reflux for 48 hours at 40 °C. Afterward, the extract was separated from the material residue by filtration through filter paper Whatman No. 1. The solvent was then evaporated using a rotary evaporator (Rotavapor model: RE-121, Buchi) at 40 °C for 3-4 hours to obtain the plant extract [37,38].

2.3. Phytochemical analysis.

For flavonoid detection, according to previous reports [17, 25]. The plant extract was first mixed with 1N of NaOH solution, followed by the addition of sulphuric acid. A change in color from yellow to colorless upon the addition of acid indicated the presence of flavonoids [39].

According to Banu and Cathrine, the plant extract was mixed with hydrochloric acid, followed by Mayer's reagent for alkaloid measurement. The presence of white precipitate indicates the presence of alkaloids.

For tannins measurement, the plant extract was mixed with 10% ferric chloride. The formation of dark blue or greenish-black indicates the presence of tannins [40].

The plant extract was mixed with 2 ml of distilled water followed by 1% ferric chloride for phenols detection. The appearance of blue or green color confirms the presence of phenols [40].

For glycosides measurement, the plant extract was mixed with glacial acetic acid and ferric chloride and concentrated with sulphuric acid. The formation of greenish-blue color indicates the presence of glycosides [41].

For terpenoids measurement, the plant extract was mixed with chloroform ($CHCl_3$) and concentrated with H_2SO_4. The formation of reddish-brown coloration of the solution at an inert face confirms the presence of terpenes [41].

For phytosterol measurement, the plant extract was mixed with chloroform and with sulphuric acid. The formation of a brown ring indicates the presence of steroids, and the formation of bluish-green color indicates the presence of phytosterols [41].

For quinine measurement, the plant extract was mixed with alcoholic KOH. The appearance of red or blue color proves the presence of quinine [42].

Finally, for anthraquinones measurement, the plant extract was boiled with 10% of HCl for a few minutes in a water bath and filtered. The filtrate was allowed to cool, and an equal volume of $CHCl_3$ was added to the filtrate. A few drops of 10% NH_3 were added to the mixture and heated. The formation of the rose-pink color was taken to indicate the presence of anthraquinones [43].

2.4. In-vitro DPPH assay.

The antioxidant activity of C. *aronia* extract was evaluated using DPPH assay as previously described in [20,44]. In this assay, different concentration of the extract (10 - 130 µl) was mixed with 0.1 mM of 2,2-diphenyl-1-picrylhydrazyl (DPPH) ethanol solution. The control samples were prepared by repeating the above steps without the sample extract, and then the absorbance was measured at 515 nm. Thus, the free radical scavenging activity of the extract was expressed as % inhibition according to the following formula:

Inhibition (%) = [ABS of control – (ABS of the sample/ABS of control)]X100.

2.5. Preparation of C. aronia phytosomes (C. aronia-p).

Preparation of *C. aronia*-p was obtained by using the procedure of Karimi *et al*. In this procedure, one mole of phospholipid (lecithin) is mixed with one mole of *C. aronia* leaf ethanolic extract in an aprotic solvent like acetone. The complex was then isolated by evaporation of the solvent under precipitation with a non-solvent aliphatic hydrocarbon [45].

2.6. Transmission electron microscope analysis.

The shape and size of the phytosomes were determined using a transmission electron microscope, where a drop of phytosomes was placed on a carbon-coated copper to form a thin film and spotted with 2 % uranic acid and left to dry. Afterward, the stained film was examined and snapped in a JOEL (JEM 2100) transmission electron microscope (TEM) [46].

2.7. In vivo study.

All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC). Experiments were performed in the Animals House / Department of Biological Sciences / Mutah university. All animal experiments were conducted according to European Commission Directive 86/609/EEC guidelines for laboratory animal care and use.

2.7.1. Acute toxicity of the ethanolic *C. aronia* leaves extract.

According to Pandey's method (1980) [47], thirty healthy male Wistar albino rats weighing around 300 gm were divided into five groups with 6 rats in each group; the first group received an only saline solution, and the other groups received oral doses of 300 mg/kg, 600 mg/kg, 1200 mg/kg, and 2500 mg/kg of ethanolic *C. aronia* leaves extract. Mortality among rats was monitored for 48 hours with general effects of respiration, response to stimuli, tremors, posture, gait, and depression were monitored.

2.7.2. Chronic toxicity of the ethanolic *C. aronia* leaves extract.

To determine the chronic toxicity effects of ethanolic *C. aronia* leaves extract, similar dosages were administered to the rats as those used for the acute toxicity experiment. The gross observational effects, the liver and kidney function tests, and mortality were monitored for 3 weeks. The gross observational effects were observed for respiration, response to stimuli, tremor, posture, and gait.

2.7.3. Induction of diabetes.

In this experiment, a method of concurrent administration of streptozotocin (Sigma, STZ) with nicotinamide was used. Nicotinamide (Sigma) was dissolved in 0.9% (w/v) normal saline solution to a concentration of 110 mg/ml. STZ dissolved in the cold fresh 0.05 M sodium citrate buffer (pH 4.5) immediately before injection. The rats were injected (i.p) with 110 mg/kg of nicotinamide, and after 15 minutes they were injected (i.v) with 50 mg/kg of STZ for the experimental group. After the STZ injection, the rats were returned to their cages and given 5% D-glucose for 4 days as well as providing them with routine food and drinking water. After four days, the fasting rats were measured for diabetes by testing their blood glucose concentration. The control animals received only an equal volume of citrate buffer (pH 4.5). A rat is considered diabetic if the blood sugar level is greater than 200 mg / dL [48].

2.7.4. Study design.

In the experiment, a total of 42 rats were used. The rats were divided into 7 groups (6 for each group). Group 1, healthy control rats (HCR); Group 2, diabetic control rats (DCR); Group 3, diabetic rats administered metformin orally at a dose of 100 mg/kg (DMT); Group 4, diabetic rats administered orally only ethanolic *C. aronia* leaves extract at a dose of 150 mg/kg (*C. aronia* 150); Group 5, diabetic rats administered orally single ethanolic *C. aronia* leaves extract at a dose of 250 mg/kg (*C. aronia* 250); Group 6, diabetic rats administered orally *C. aronia*-p at a dose of 150 mg/kg (*C. aronia*-p 150); Group 7, diabetic rats were administered orally *C. aronia*-p at a dose of 250 mg/kg (*C. aronia*-p 250).

2.7.5. Measurement of body weight and biochemical analysis.

Bodyweight, blood glucose levels, and lipid profile, including (Total Cholesterol (TC), Triglycerides (TG), High-density lipoprotein (HDL-C), and Low-density lipoprotein (LDL-C)) were estimated on 0, 7, 14, and 21st day after the administration of the extract orally. Blood samples were collected from rats by using retro-orbital puncture in plain gel glass tubes (BD Diagnostics, Ireland). Serum was separated using a centrifuge (Druckerdiagnostics, USA) to perform blood glucose and lipid profile measurements using automated blood Biomaxima analyzer and its Reagents (Biomaxima, Poland)[47].

2.8. Statistical analysis.

The result is expressed as means ± S.E.M. (standard Error of Mean) for six rats in each group. To investigate any significant differences between groups, a one-way analysis of variance (ANOVA) was used, followed by the Tukey test. Size of effect calculated by partial eta square test.

3. Results and Discussion

3.1. Phytochemicals analysis.

Phytochemical analysis of *C. aronia* leaves revealed the presence of phenols, flavonoids, alkaloids, and tannins (Table 1). In plants, these compounds afford protection against ultraviolet UV-B radiation, pathogens, and herbivores [49]. In addition, it has been proved that these compounds exhibit beneficial health effects by decreasing the risk of cardiovascular diseases and providing protection against cancer, diabetes, osteoporosis, and neurodegenerative diseases [50]. Moreover, they effectively diminish free radicals due to their high antioxidant potential [51].

Table 1. Qualitative results of ethanolic *C. aronia* leaf extract.

Test Name	Result
Flavonoids	++
Alkaloids	+++
Saponins	+
Tannins	+++
Phenols	++
Glycosides	++
Trepenoids	+
Phytosterol	+
Quinine	+
Anthraquinones	-

Strongly Present +++, Present ++ , weekly present + , Absent

3.2. Antioxidant assay.

The Antioxidant activity of *C. aronia* extract was determined by using free radical scavenging capacity using DPPH assay. The IC50 of DPPH scavenging activity under the influence of *C. aronia* leaf ethanolic extract is 69.46±6.4 µl (Figure 1). Generally, it was found that ethanolic plant extracts are the most effective scavenger of DPPH radical compared with other solvents [52]. Despite that, it was proposed that ethanol is a more functional solvent for degrading the cell walls and seeds because of having nonpolar nature leading to the release of active phyto-contents outside the cells. Therefore, we suggested that the flavonoids, alkaloids, and tannins contents are responsible for the antioxidant activity of *C. aronia* extract; such

observation is in agreement with several previous findings [53-56]. The potent antioxidant activity of *C. aronia* leaves could be attributed to the presence of different phytochemicals, which have a therapeutic implication in protecting cells and tissues against oxidative damage [57].

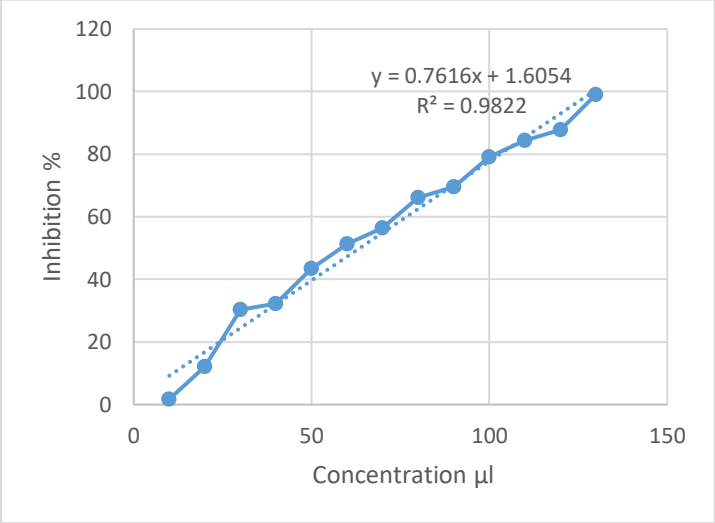

Figure 1. DPPH radical scavenging activity of *C.aronia* leaf.

3.3. Phytosome analysis.

Phytosomes are appeared as regular circular bodies of multiple sizes and distributed separately. The surfaces of these nanobodies were smooth, as shown in Figure 2.

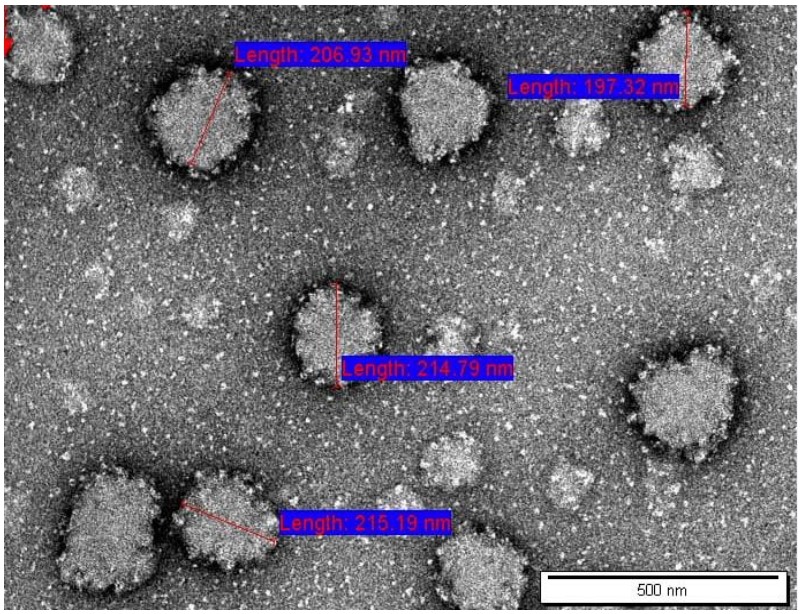

Figure 2. Topographic view of *C. aronia* phytosomes using Transmission Electron Microscope (TEM).

3.4. Lethal dose (LD50) and acute toxicity.

The acute toxicity study showed that the *C. aronia* leaves ethanolic extract did not cause any death in the animal groups during a 48-hour period. The LD_{50} was higher than 2500 mg / kg as shown in Table 2, whereas the median effective dose (ED_{50}) = 250 mg / kg. Both were calculated by using these equations:

LD50 = higher dose ─ Σ (a x b)/ n where n = No. of animals in each group

ED50 = LD50 / 10

Table 2. Results of acute toxicity studies of *C.aronia* extract

Group	Dose (mg/kg)	No. of animals	Dose Difference (a)	Animals Died (b)	Mean	a x b
1.	300	6	30	0	-	0
2.	600	6	45	0	-	0
3.	1200	6	60	0	-	0
4.	2500	6	90	0	-	0

3.5. Chronic toxicity.

To determine the chronic toxicity of *C.aronia* ethanolic extract, blood urea and creatinine concentrations were measured and the level of the liver enzymes, AST and ALT, in both treated and control groups. As demonstrated in Table 3, no changes were observed in the level of these parameters in the tested groups compared to the control group.

Table 3. The effect of 250 mg/kg of *C.aronia* ethanolic Extract on liver and kidney rats' functions.

	ALT (U/l)		AST (U/l)		Creatinin (mg/dl)		Urea (mg/dl)	
	Cont	Exp	Cont	Exp	Cont	Exp	Cont	Exp
Average value	76.5	76.1	107.7	106.6	0.815	0.798	35.81	35.72
Max. value	95	100.1	123.33	120	0.92	0.86	45.5	44.6
Min. value	68.1	66.7	88	87.7	0.71	0.76	31.33	33.1

3.6. Effect of C. aronia-p and ethanolic extract of C. aronia on blood glucose level.

STZ treatment induces T2DM in rats, representing a model of insulin-deficient but not insulin resistant T2DM characterized by stable, moderate hyperglycemia associated with 60% loss of β-cell function [48,58]. The administration of STZ was combined with nicotinamide to protect β-cells against STZ cytotoxicity [58]. In this experiment, adult rats were given a dose of STZ (50 mg/kg) to stimulate the development of diabetes, which was confirmed by the presence of polyuria and hyperglycemia in animals treated with STZ.

Table 4. The measured blood glucose level in induced diabetic rats.

#	Group Type	Day0	Day7	Day14	Day21
1	HCR	99.39 ± 0.58	101.09 ± 0.65	98.61 ± 1.55	97.92 ± 1.29
2	DCR	213.02±1.6[a]	224.39 ± 1.18[a]	245.2 ± 7.2[a]	242.53 ± 1.02[a]
3	DMT	239.62± 0.5[a]	206.01± 0.78[a]	159.6±0.53[a]	113.91±1.7[a]
4	*C. aronia* 150	229.29±1.1[a]	222.4±2.7(N.S)	207.2± 3.2[c]	189.09 ± 3.07[c]
5	*C. aronia* 250	230. ± 1.1[a]	223.40±2.46 (N.S)	204.99±3.94[b]	187.02±2.77[b]
6	*C. aronia*-p 150	228.8 ± 2.0[a]	221.28±2.61 (N.S)	201.45±2.48[b]	180.69±0.36[b]
7	*C. aronia*-p 250	232.2±1.02[a]	224.41±1.81 (N.S)	197.71±1.88[a]	171.09±1.81[a]

Values are mean ± SEM; n=6 in each group; HCR: healthy control rats; DCR: diabetic control rats; DMT: diabetic rats administered metformin orally at a dose of 100 mg/kg; Groups 3-7 were compared with group 2, and group 2 was compared with group 1. Values of significance a=p<0.001, b=p<0.01, c =p<0.05**.**

Our results shown in Table 4 demonstrated that fasting blood glucose level in the diabetic rats was significantly higher than in the control group ($P < 0.001$).

Impressively, the *C. aronia* leaves extract and *C. aronia*-p significantly and dose-dependently decreased the blood glucose level in the diabetic rats ($P < 0.001$). Nevertheless, *C. aronia* leaves extract, and *C. aronia*-p were not equipotent to metformin.

It is well known that the biological membrane has a double-layered lipoidal nature. Therefore, the components present in the phytosomes have the ability to penetrate the lipoidal bilayer and hence, exhibited higher uptake and improved bioavailability than conventional

herbal extracts, which could explain the restricted efficacy of the *C. aronia* leaves extract alone compared to *C. aronia*-p as shown in Table 4.

We suggested that the effect of *C. aronia* leaves extract and *C. aronia*-p in reducing the elevation of blood glucose induced by STZ is presumably mediated through enhancing insulin secretion as well as reducing the oxidation stress condition in beta cells [59] or through the hindering glucose absorption by inhibiting carbohydrate-hydrolyzing enzymes, such as α-amylase and glucosidase in the intestine [60-62].

3.7. Effect of C. aronia-p and ethanolic extract of C. aronia on body weight.

Several studies revealed a link between obesity and insulin resistance with the increased risk of developing T2DM, cardiovascular disease as well as certain types of cancer such as colorectal, breast, and pancreatic cancer [63]. In this context, our data demonstrated that both *C. aronia*-p ($P < 0.001$) and ethanolic extract of *C. aronia (*$P < 0.05$) can significantly improve the bodyweight of diabetic rats compared to the untreated rats (see Table 5). Yet, none of the investigated treatments could be identified as equally effective as metformin. A plausible explanation for this effect could be that *C. aronia*-p and ethanolic extract of *C. aronia* decrease lipase enzyme activity, leading to enhanced body weight in diabetic rats [64]. Furthermore, weight reduction is closely linked to improving insulin efficacy and lowering blood sugar levels [65].

Table 5. The bodyweight of diabetic and treated rats.

#	Groups	Day 0	Day 7	Day 14	Day 21
1	HCR	216.66±1.02	218.50±0.56	218.83±0.5	223.0±1.15
2	DCR	211.83±0.6	214.5±1.1$^{(N.S)}$	217.3±0.76$^{(N.S)}$	220.16±0.47$^{(N.S)}$
3	DMT	219.33 ± 0.3	219.16±0.3$^{(N.S)}$	219.0±0.9$^{(N.S)}$	218.8±1.19$^{(N.S)}$
4	*C. aronia* 150	235±5.25$^{(N.S)}$	234.0±4.76 $^{(N.S)}$	232.2±5.3$^{(N.S)}$	232.50±4.4^{c}
5	*C. aronia* 250	239.33±9$^{(N.S)}$	240.83±8.3$^{(N.S)}$	238.3±8.5$^{(N.S)}$	236.0±8.46^{c}
6	*C. aronia*-p 150	235±1.36$^{(N.S)}$	235.0±1.39$^{(N.S)}$	230.8±2.3^{b}	229.8±0.74^{b}
7	*C. aronia*-p 250	234.83±1.44	235.83±1.19^{a}	231.2±1.7^{a}	226.0±1.12^{a}

Values are mean ± SEM; n=6 in each group; HCR: healthy control rats; DCR: diabetic control rats; DMT: diabetic rats administered metformin orally at a dose of 100 mg/kg; Groups 3-7 were compared with group 2, and group 2 was compared with group 1. Values of significance a=p<0.001, b=p<0.01, c=p<0.05; N.S =Not significant.

3.7. Effect of C. aronia-p and ethanolic extract of C. aronia on lipid profile.

Hyperlipidemia is caused not only by increased exogenous lipid absorption through the gut but also by enhanced endogenous lipid synthesis [66]. It is a recognized complication of T2DM and is characterized by elevated levels of cholesterol, TG, and LDL-C. Many Studies demonstrated that hyperlipidemia reduces the antioxidant defense system [67,68]. Thus, an antioxidant capacity can be considered an important property of the hypolipidemic agent. Furthermore, antioxidants can suppress lipid accumulation in the wall of blood vessels and the buildup of atheromatous plaques in the animal models of atherosclerosis [68]. Accordingly, we designed an experiment to investigate the potential hypolipidaemic effect of *C. aronia*-p and the ethanolic extract of *C. aronia* in STZ-induced diabetic rats.

Table 6. Lipid profile of diabetic and treated rats.

		TC level			
#	**Groups**	**Day 0**	**Day 7**	**Day 14**	**Day 21**
1	HCR	106.1 ± 1.5	105.3 ± 1.4	104.12 ± 1	102.5 ± 1
2	DCR	117.6± 0.7^{b}	118.1± 0.8^{b}	118.8 ± 1.8^{a}	119.7 ± 2.2^{a}
3	DMT	105.4± 3.1^{a}	105.6 ± 3.5^{a}	106.5 ± 7.2^{a}	107.06±5.5^{a}
4	*C. aronia*150	107.35±2.4^{b}	102.6± 1.7^{b}	99.47±7.5^{b}	95.24±6.7^{b}

	TC level				
#	**Groups**	**Day 0**	**Day 7**	**Day 14**	**Day 21**
5	*C. aronia* 250	111.2±0.64^{c}	110.62±0.6^{c}	106.21±5.02^{b}	102.5±2.91^{b}
6	*C. aronia*-p 150	106.07±0.14^{c}	107.43±0.4^{c}	102.03±0.96^{a}	97.1± 3.14 ^{a}
7	*C. aronia*-p 250	109.96±2.42	110.6±0.92^{a}	102.26± 4.1^{a}	95.51 ± 2.1^{a}
	TG level				
#	**Groups**	**Day 0**	**Day 7**	**Day 14**	**Day 21**
1	HCR	63.66 ± 0.4	62.14 ± 0.5	61.82 ± 0.2	61.69 ± 0.15
2	DCR	66.29 ± 2.7	69.11 ± 3.7	69.74 ± 3.8	70.26 ± 3.7
3	DMT	70.26 ± 1.8	71.29 ± 1.7	72.16 ± 2.3	73.07 ± 2.5
4	*C. aronia* 150	65.65 ± 0.4	65.13 ± 0.3	63.95 ± 1.1	61.96 ± 0.76^{c}
5	*C. aronia* 250	67.76 ± 0.2	67.09 ± 0.3	66.65 ± 0.2	65.74 ± 0.2^{c}
6	*C. aronia*-p 150	69.24 ± 0.2	67.40 ± 0.3	65.41 ± 0.4	63.29 ± 0.8^{c}
7	*C. aronia*-p 250	71.16 ± 0.2	69.72 ± 1.0	63.14 ± 0.7	56.47 ± 1.44^{b}
	HDL-C level				
#	**Groups**	**Day 0**	**Day 7**	**Day 14**	**Day 21**
1	HCR	34.62±0.44	35.26 ± 0.83	42.87 ± 0.51	48.79 ± 0.23
2	DCR	28.09±0.3^{a}	27.29± 0.4^{a}	26.75±0.42^{a}	26.25±0.37^{a}
3	DMT	26.65±0.25	26.51± 0.17	25.68±0.21	24.85± 0.45
4	*C. aronia* 150	44.14±0.26^{a}	45.14±0.32^{a}	45.57 ± 0.4^{a}	46.58±0.24^{a}
5	*C. aronia* 250	47.21±0.32^{a}	47.32±0.32^{a}	48.30±0.33^{a}	48.92±0.45^{a}
6	*C. aronia*-p 150	52.37±0.67^{a}	52.41±0.70^{a}	54.12±1.04^{a}	55.76±0.36^{a}
7	*C. aronia*-p 250	56.18±0.90^{a}	56.89±0.42^{a}	60.3±0.31^{a}	63.59±0.25^{a}
	LDL-C level				
#	**Groups**	**Day 0**	**Day 7**	**Day 14**	**Day 21**
1	HCR	58.53± 1.89	58.3 ± 1.09	48.89±0.88	41.40 ± 0.51
2	DCR	76.73±0.53^{a}	76.88±0.88^{a}	77.65±1.13^{a}	79.55±0.94^{a}
3	DMT	71.6± 0.83	69.62±0.66^{b}	70.49± 0.95	71.4± 1.22^{b}
4	*C. aronia* 150	50.08±2.19^{a}	44.51±1.65^{a}	41.11 3.2^{a}	36.2±2.81^{a}
5	*C. aronia* 250	50.1± 0.25^{a}	49.88±0.33^{a}	44.58±2.21^{a}	40.42±1.42^{a}
6	*C. aronia*-p 150	41.84±0.76^{a}	41.52±0.94^{a}	34.82±1.35^{a}	28.67±1.48^{a}
7	*C. aronia*-p 250	42.71±0.79^{a}	39.73±0.27^{a}	29.31±1.90^{a}	21.51±0.47^{a}

Values are mean ± SEM; n=6 in each group; HCR: healthy control rats; DCR: diabetic control rats; DMT: diabetic rats administered metformin orally at a dose of 100 mg/kg; Groups 3-7 were compared with group 2, and group 2 was compared with group 1. Values of significance a=p<0.001, b=p<0.01, c=p<0.05.

The data presented in Table 6 demonstrated that diabetes markedly increases the plasma TG, TC, and LDL-C but decreases HDL-C concentration. In addition, our data showed that metformin significantly suppresses the elevated plasma level of TG, TC, and LDL-C and increases HDL-C plasma levels. Besides, *C. aronia*-p and ethanolic extract of *C. aronia* significantly decrease TG, TC, and LDL-C levels in a diabetic rat model. The hypolipidemic effect of *C. aronia*-p causes a reduction in blood TC to 95.5 mg/dl at a percentage of 13.08% (P-Value <0.001). Further, the ethanolic extract of *C. aronia* alone causes even a lower percentage (8.7%) (P-value <0.001). As well as, *C. aronia*-p produces a significant reduction in TG level (P <0.01), which is the lowest among all treatments that we used. Also, our results indicated that all the treated rats had an increased HDL-C level. Moreover, *C. aronia*-p and ethanolic extract of *C. aronia* exhibited a significant reduction in LDL-C level (P-value <0.001). It appears that *C. aronia*-p (150 & 250 mg/kg) led to decreased cholesterol levels, possibly by increasing the oxidation of fatty acids and inhibiting their synthesis while increasing bile's effectiveness in eliminating cholesterol [69]. In supporting fashion, it is well known that *C. aronia* contains terpenoids (see Table 1) that suppress the excretion of triglycerides in liver cells [70].

In the same context, it has been proved that insulin plays an important role in regulating lipid metabolism by suppressing the production of TGs and VLDL by hepatocytes both *in vitro* and *in vivo* and by promoting LDL-C clearance [71-73]. Moreover, insulin promotes Apolipoprotein A and HDL-C biosynthesis by hepatocytes *in vitro* [74].

We thus suggested that the insulinotropic effect of *C. aronia*-p and ethanolic extract of *C. aronia* could provide a reasonable explanation for such improvement in lipid profile in STZ-induced diabetic rats.

4. Conclusions

Collectively, these results demonstrate that *C. aronia*-p and ethanolic extract of *C. aronia* can significantly improve glucose and lipid homeostasis as well as possess strong antioxidant activity. Therefore, they represent a potentially useful guard from diabetes. Moreover, C. *aronia*-p was successfully formed to be a novel delivery system that can overcome the hurdles associated with using plants extracts, such as poor stability in the gastric environment, herbal toxicity, poor pharmacological activity, physical and chemical degradation, and high doses of ingredients that could affect the safety and efficacy of the extracts.

These multiple pharmacokinetic profiles may result from the synergistic effect of the active ingredients, high absorption of these components, and hence the bioavailability of these compounds. This presented scientific study provides some scientific and indicative support for the design and role of phytosomes.

Values are mean ± SEM; n=6 in each group; HCR: healthy control rats; DCR: diabetic control rats; DMT: diabetic rats administered metformin orally at a dose of 100 mg/kg; Groups 3-7 were compared with group 2, and group 2 was compared with group 1. Values of significance a=p<0.001, b=p<0.01, c=p<0.05.

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Conflicts of Interest

The authors declare no conflict of interest

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