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Anti-Obesity and Antihyperglycemic Effects of *Crataegus aronia* Extracts: *In Vitro* and *in Vivo* Evaluations

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ABSTRACT

Hypocholesterolemic activity of *Crataegus aronia* L. (Rosaceae) is therapeutically praised. Its potent antiobesity ($P < 0.001$, $n = 6 - 8$) as well as marked triacylglycerol-reducing efficacies ($P < 0.001$, $n = 6 - 8$) in 10 weeks-high cholesterol diet (HCD) fed rats are demonstrated. Pancreatic triacylglycerol lipase (PL), α-amylase and α-glucosidase are an interesting pharmacological target for the management of dyslipidemia, atherosclerosis, diabetes and obesity. Comparable to acarbose, acute starch induced postprandial hyperglycaemia as well glycemic excursions in normoglycemic overnight fasting rats was highly significantly ($P < 0.001$) dampened by *C. aronia* 100, 200 and 400 mg/Kg b.wt aqueous extracts (AE), but not acute glucose evoked postprandial hyperglycaemia increments, unlike diabetes pharmacotherapeutics metformin and glipizide. *C. aronia* aerial parts as well as fruits AEs (0.1 - 10 mg/mL) were identified as *in vitro* dual inhibitors of α-amylase and α-glucosidase with respective IC_{50} (mg/mL) of 2.1 ± 0.3 and 3.5 ± 0.7. Still, it lacked on *in vitro* hindrance of glucose movement, dissimilar to guar gum. Equivalent to orlistat (PL IC_{50} of 0.1 ± 0.0 µg/mL), *C. aronia* tested AEs and its purified bioactive phytoconstituents; quercetin and rutin, inhibited highly substantially in a dose dependent trend PL *in vitro* ($n = 3$), in an ascending order of obtained PL-IC_{50} (µg/mL): quercetin; 30.1 ± 2.8, rutin; 77.3 ± 11.7, *C. aronia* aerial parts; 225.2 ± 33.4 and *C. aronia* fruits; 286.1 ± 37.4. Flavonoid-rich *C. aronia*, as a functional food and a nutraceutical, modulating gastrointestinal carbohydrate and lipid digestion and absorption, maybe be advocated as an exquisite and potential candidate for combinatorial obesity-diabetes prevention and phytotherapy.

Keywords: *Crataegus aronia*; Rosaceae; Pancreatic Lipase; Enzymatic Starch Digestion; High Cholesterol Diet; Flavonoids

1. Introduction

With the rising prevalence of diabetes mellitus (DM) worldwide, several studies indicated that the incidence of type 2 DM (T2DM), impaired fasting glycaemia and obesity in Jordan is increasing [1-3]. These observations have been confirmed by the International Diabetes Federation (IDF) data stating that the current prevalence of DM in Jordan was at 10.1%. Among Middle Eastern and North Africa (MENA) countries; this percentage indicates the ninth highest prevalence [4]. Treatment goals of T2DM have centred on using oral agents that promote insulin secretion, improving tissues' sensitivity to insulin, or reducing the rate of carbohydrate absorption from the gastrointestinal tract and retarding the development of diabetic complications [5]. Due to the good acceptance of herbal drugs among population, phytopharmaceuticals with demonstrated clinical efficacy could become a suitable alternative/complementary therapy to current medication for specific indications like the adjuvant treatment of diabetes [6]. There is still, however, an unmet need for the medicinal plants and phytopharmaceuticals with scientifically proven antidiabetic efficacy comparable to orthodox medicine [7-9]. In parallel realms, possible antiobesity therapeutics from nature were closely investigated as obesity was reaching alarming epidemic proportions globally [7-10]. Locally, ethnopharmacological studies and surveys confirmed the appreciable prevalence of herbal use among patients with diabetes in Jordan [11,12]. Comprehensively, multiple reports were investigating diverse biological efficacies, cardiovascular therapeutic benefits and phytochemical analyses of *C. aronia* (Rosaceae), among the other *Crataegus* spp. [13-25]. Diverse studies were conducted to explore medicinal plants as potential therapeutic agents

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for dual management of diabetes and hyperlipidemia via digestive enzymes' inhibition namely pancreatic alpha-amylase, intestinal alpha-glucosidase and pancreatic lipase [26,27]; hence, the purpose of present study was to investigate the inhibitory effects of crude aqueous extracts of *C. aronia* L. (Rosaceae) on these extra-pancreatic digestive enzymes *in vitro*. Thus, more detailed investigations to elucidate *C. aronia* dual antidiabetic-antiobesity pharmacotherapeutic effects on cell-free *in vitro* systems of carbohydrate and lipid extrapancreatic enzymatic digestion and absorption were undertaken. Additionally, acute and chronic *in vivo* effects were exhaustively investigated.

2. Materials and Methods

2.1. Chemicals and Biochemicals

Unless stated otherwise, all reference drugs (orlistat, metformin, glipizide, acarbose and atorvastatin with purities > 95%), reference flavonoids (rutin and quercetin with purities > 94%), reagents and chemicals were from Sigma (Dorset, UK). Dialysis tubing Spectra/Por® 7 Biotech Regenerated Cellulose (RC) membranes, MWCO 2000 was purchased from Spectrum Europe B.V, Breda, Netherlands. Shaking incubator was from LabTech®, Daihan LabTech Co., LTD. (Korea). Glucose GOD-PAP kit was obtained from BioLabo Reagents, France. In UV determinations; UV-VIS spectrophotometer from SpectroScan 80D (UK) was used. Sonicator (Bandelin Sonorex, Bandelin electronics, Germany) and rotary evaporator (Laborota 4000-efficient, Heidolph, Germany) were also used.

2.2. Plant Material

Dried whole flowers and leaves of *C. aronia* L. were purchased from herbalist shops in downtown, Amman; while fruits were collected during June-July from different places of the country (Ajloun, Al-Salt and Shafa Badran). The identification of the plants material was kindly confirmed by Professor D.M. Al-Eisawi (Department of Biological Sciences; The University of Jordan). Voucher specimens of the plant material were deposited at the Herbarium of the Department of Biological Sciences, and at the Department of Pharmaceutical Sciences, (Reference No. 27). All collected fresh plant samples were air dried at room temperature and coarsely powdered.

2.3. Preparation of the *C. aronia* Aqueous Extracts (AEs)

AEs were prepared by refluxing each 10 g of the dried coarsely powdered plant material with 100 mL tap water for 15 min. The overnight kept extracts were filtered twice through filter paper and the volume of the filtered solution was increased to 100 mL with tap water to obtain 10% (equivalent to 100 mg/1 mL) crude aqueous solutions. Sonication of stock crude extract or testing concentrations was performed before implementation of investigations. For pancreatic lipase experimentation; water was evaporated under vacuum at 40˚C using a rotary evaporator. The solid residues were collected and stored in dry conditions until analysis.

2.4. Preparation of *C. aronia* Crude Aqueous Extract (Aerial Parts as Well as Fruits) and Its Phytoconstituents for *in Vitro* Pancreatic Triacylglycerol Lipase Activity Assay

The tested aqueous extracts were initially dissolved in Tris-HCl buffer (2.5 mM (Promega, USA), pH 7.4 with 2.5 mM NaCl) to give five different stock solutions with a concentration range of 6.25 - 100 mg/mL (6.25, 12.5, 25, 50 and 100 mg/mL). Subsequently, 20 µL aliquot of each stock solution was used in the reaction mixture to give a final concentration range of 125 - 2000 µg/mL (125, 250, 500, 1000 and 2000 µg/mL). Extracts were prepared according to the traditional indications of use, thus DMSO or any other organic solvent, even to the minimum concentration was avoided [28]. Also, quercetin and rutin, isolated from *C. aronia* (dissolved in DMSO), were prepared into five different stock solutions with an initial concentration range of 0.625 - 10 mg/mL (0.625, 1.25, 2.5, 5 and 10 mg/mL) [13]. Thereafter, 20 µL aliquot of each stock solution was used in the reaction mixture to give a final concentration range of 12.5 - 200 µg/mL (12.5, 25, 50, 100 and 200 µg/mL). Finally, orlistat (Sigma, USA), the reference drug (dissolved in DMSO; 1 mg/mL), was prepared into six different stock solutions with a concentration range of 0.625 - 20 µg/mL [29]. Thereafter, 20 µL aliquot of each stock solution was used in the reaction mixture to give a final concentration range of 0.0125 - 0.4 µg/mL.

2.5. Spectrophotometric Quantification of Pancreatic Lipase (PL) Activity and Assaying PL Inhibition by Test Extracts and Compounds

According to Bustanji *et al.*, *in vitro* enzymatic PL activity was assayed. Subsequent determinations were undertaken for the tested extracts of different parts/phytoconstitutive compounds in comparison to control evaluations, to calculate the concentration required for PL 50% inhibition (IC_{50}) [30].

2.6. *In Vitro* Enzymatic Starch Digestion Assay

In vitro enzymatic starch digestion was assayed with acarbose, as the reference drug [31]. The extent of polysaccharide breakdown into glucose was evaluated in

a concentration range of different parts of plant aqueous extract 0.1, 0.5, 1.0, 1.25, 2.5, 5.0 and 10.0 mg/mL. The effects of acarbose at 100 µg/mL concentration were evaluated as well. Control (tap water only) samples contained neither acarbose nor plant extract.

2.7. Glucose Movement *in Vitro* Assay

In vitro glucose movement was assayed according to Kasabri *et al.* [32]. To imitate the viscosity-based diffusion hindrance of gel-forming dietary fibres, and hence, their postprandial glucose lowering efficacies *in vitro*, guar gum 50 mg/mL was used as a classical positive control, and 10, 20 and 40 mg/mL of *C. aronia* AEs in 0.22 M glucose in triplicates were dialysed against 0.15 M NaCl overnight at 37˚C with gentle shaking and a parallel plant-free (negative) control was included [33, 34].

2.8. *In Vivo* Confirmatory Studies

2.8.1. Oral Starch Tolerance Test (OSTT) and Oral Glucose Tolerance Test (OGTT)

With treatment plant administered in doses 100, 200 and 400 mg/Kg body weight (b.wt); OSTT and OGTT were conducted according to Kasabri *et al.* in the Experimental Animal Laboratory of the Faculty of Medicine, University of Jordan [31]. All animals were housed, fed and treated in accordance with the University of Jordan ethical guidelines for animal protection and experimental approval (registration number 218/2007-2008) was obtained from the Scientific Research Council at the Deanship of Academic Research and the Faculty of Pharmacy.

2.8.2. Body Weight and Triglycerides Determination

1) Experimental Animals

The study was conducted at the Experimental Animal Laboratory of the Department of Biological Science, Faculty of Science; The University of Jordan. All animals were housed, fed and treated in accordance with the University of Jordan ethical guidelines for animal protection and experimental approval. Throughout the experimental period, animals were kept in single cages. Locally inbred male Wistar rats of 212.5 ± 1.9 g average body weight (b.wt) were used in the experiments. Rats were provided with normal diet chow (called basal diet hereafter) and water *ad libitum* for the duration of the experiment except during the 12 - 13 hrs fasting period preceding cholesterol administration and blood collection. Rats were divided into the followings groups (n = 6 - 8) as follows: Group 1: The control group was given only the standard diet *ad libitum* for 10 weeks. Group 2: The hypercholesterolemic control group was given the standard diet *ad libitum* and HCD once daily for 10 weeks [19]. Group 3: The preventive group was given the standard diet *ad libitum*. HCD and *C. aronia* AE (200 mg/Kg b.wt) were administered once daily for 10 weeks. Group 4: This group took standard diet + *C. aronia* AE (400 mg/Kg b.wt) for 10 weeks daily. Group 5: This group was given the standard diet *ad libitum*. HCD and *atorvastatin* (10 mg/Kg b.wt) were administered once daily for 10 weeks.

2) Crude Extract Administration

C. aronia AE was administered to animals by gavage at 6:30 am daily. *C. aronia* AE doses for groups 3 and 4 were selected based on the preliminary LD_{50} screening The selected dose-400 mg/Kg/day-was 10× more diluted than the maximum soluble concentration of the extract, which did not cause any mortalities during LD_{50} experiments [13]. Blood samples were collected weekly from the retro orbital plexus of rats using a 10 µL glass capillary [35]. A blood sample was also collected just before the start of the experiment to measure the control values for each parameter for each animal group. All blood samples were collected after 12 - 13 h of fasting. Serum was collected from blood by centrifugation for 10 min at 3500 rpm and then stored at −80˚C until analysis. Analysis was conducted in duplicate and was completed within 1 - 3 days of collection. As study was on HFD rats, therefore quantitative determination of lipid profiles (cholesterol, HDL, LDL and VLDL) were achieved using enzymatically commercial available kits (Lab kits, Barcelona, Spain).

3) Body Weight

Body weight was measured daily; doses of different treatment and plant extract were calculated and given according to body weight.

4) Triglycerides

Serum triglyceride concentration was tested following the same above procedure.

5) Atherogenic Index

Due to the fact that obesity is positively correlated with coronary heart diseases (CHD), it was valuable to check TC/HDL and HDL/LDL ratios as indicators as CHD predictors.

2.9. Statistical Analysis

The values are presented as mean ± S.E.M. (Standard Error of the Mean) of 3 - 4 independent experiments. Statistical differences between control and different treatment groups and A.U.Cs (incremental Area Under 24-h glucose Curve) were determined using Graphpad Prism one way analysis of variance (ANOVA) followed by Dunnett post test whenever appropriate (version 3.02 for windows; GraphPad Software, San Diego, CA, USA). Values were considered significantly different if $P < 0.05$ and highly significantly different if $P < 0.01$ and $P < 0.001$.

3. Results and Discussion

Pancreatic Lipase inhibition is one of the most widely studied mechanisms to determine the potential efficacy of natural products and ethnomedicinal botanicals as obesity modulating agents [29,36]. In this current study, the pancreatic triacylglycerol antilipase activity profiles of the crude aqueous extracts of *C. aronia* and their isolated quercetin and rutin are shown in **Figure 1** [13]. Orlistat's PL IC_{50} of 114.0 ± 4.0 ng/mL, equivalent to 0.2 ± 0.0 μM, is comparable to reported PL IC_{50} values elsewhere [29], thus promoting the pronounced sensitiveity and reliability of recruited PL activity assay (**Table 1**). Comparable to orlistat performance, a marked concentration-dependent PL inhibition trend was obtained for the tested extracts (the same figure) and their pure phytocomponents. PL IC_{50} values obtained for triple separate determinations are also illustrated (**Table 1**).

Most distinctively, Hawthorn flavonoids could modulate lipoprotein lipase expression in mice, comparable to therapeutic pioglitazone [37]. Pharmacological lipid lowering efficacies of *Crataegus* spp fruits are extensively elaborated elsewhere [38]. Additionally, Hawthorn combination with simvastatin exhibited a substantial lipid lowering effectiveness in hyperlipidemic albino rats [39]. These significant effects may be solidly related to the effect of the major compounds identified in the crude extract [29]. Unequivocally, Hawthorn fruit active principles, inhibiting synergistically HMG-CoA reductase and cholesterol absorption could manifest significant hypolipidemic benefits, further validating the hypocholesterolemic effect of *C. aronia* in high cholesterol diet fed rats [13,40-42]. In effect, the results indicate that the pancreatic triacylglycerol lipase inhibitory efficacy of *C. aronia* may be attributable to its multiple components acting additively or synergistically in optimal ratio. The discriminative inhibitory power of antilipolytic *C. aronia* for circulatory lipoprotein lipase, intracellular hormone sensitive lipase or pancreatic triacylglycerol lipase as separate pharmacological targets can be examined [30,43]. Further downstream studies into a plausible pivotal role in modulating adipogenic differentiation and accumulation maybe also conducted [44] in anticipation of *in vivo* tolerance, efficacy and safety [45-47]. All in all, pharmacological inhibition of dietary lipid digestion and absorption can induce favorable amelioration of dyslipidemia, atherosclerosis and obesity. Impressively, pancreatic triacylglycerol lipase natural inhibitors offer the utility for adjuvant or alternative treatment to statins or orlistat as likely synergies can exist between new and established lipid-lowering drugs [48]. Furthermore, the tested extracts and their isolated pure flavonoids were evaluated for their inhibitory potential of enzymatic starch digestion in comparison to acarbose therapeutic efficacy. Glucose liberation from starch was inhibited by 97.6% highly significantly with acarbose (0.1 mg/mL) as the reference drug, ($P < 0.001$, vs. drug-free control incubation, n = 3, **Figure 2**). Furthermore, **Figure 2** demonstrates that *C. aronia* aerial parts AE concentrations 1 - 10 mg/mL had highly substantial dose-related reductions in aldohexose release from culinary polymeric cornstarch ($P < 0.05$ - 0.001 vs. plant-free control determinations, n = 3). With an IC_{50} of 2.1 ± 0.3 mg/mL, the highly significant dose related ($P < 0.05$ - 0.001) % decreases in enzymatic starch hydrolysis by tested extracts of *C. aronia* are summarized in **Table 2**. Additionally, *C. aronia* fruits AEs exerted highly significant ($P < 0.001$ vs. control determinations, n = 3) concentration-dependent inhibitions of enzymatic starch digestion, with an IC_{50} of 3.5 ± 0.7 mg/mL (**Figure 3**). Percent decreases in polysaccharide hydrolysis are tabulated in **Table 2**. The exquisite anti-α-glucosidase activity of Hawthorn leaf [49] can be strongly validated by comparable activity of its phytochemicals, mainly quercetin [50], and rutin [51]. Additively, quercetin and rutin exhibited an effective competitive inhibition of α-amylase. Taken together, the overall dual α-amylase and α-glucosidase inhibitory propensities of *C. aronia* could be the result of the combination of its several constituents performing in concert, in a holistic manner, thereby contributing to the restoration of homeostasis of energy consumption and utilization [52].

Using the glucose diffusion model; *in vitro* investigation of *C. aronia* extracts and its isolated flavonoids were undertaken in comparison to standard guar gum. Mean AUC (area under 24 h glucose curve) for the viscous water-soluble gel-forming guar gum (50 mg/mL) was decreased highly significantly by 30.8% ± 2.5% ($P < 0.001$) (n = 3, **Figure 4**) compared to overnight negative control. The efficacy of guar as a classical positive control has been elsewhere detailed [33]. Incomparable to guar gum, *C. aronia* extracts (10, 20 and 40 mg/mL) lacked any marked glucose diffusional hindrances into external solution across dialysis membrane (with res-

Table 1. *In vitro* pancreatic triacylglycerol lipase IC_{50} values for increasing concentrations of different parts of *C. aronia AEs*, its bioactive phytoconstituents; rutin and quercetin, and orlistat.

Tested extract/compound	IC_{50} (μg/mL)	IC_{50} (μM - mM)
Orlistat	0.114 ± 0.0	0.2 ± 0.0 μM
C. aronia aerial parts	225.2 ± 33.4	-
C. aronia fruits	286.1 ± 37.4	-
Quercetin	30.1± 2.8	99.6 ± 0.0 μM
Rutin	77.3 ± 1.7	0.1 ± 0.0 mM

Results are mean ± SEM (n = 3 independent replicates).

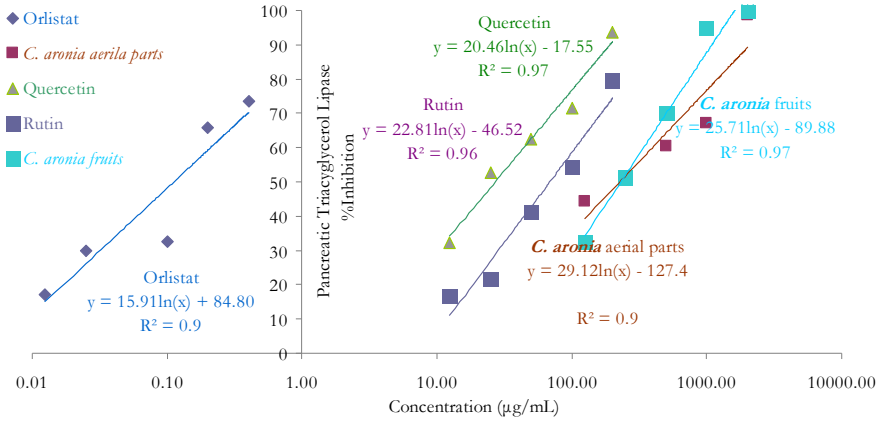

Figure 1. *In vitro* inhibitory effects of *C. aronia* (AE) different parts and its bioactive phytoconstituents concentrations in µg/mL on pancreatic triacylglycerol lipase activity.

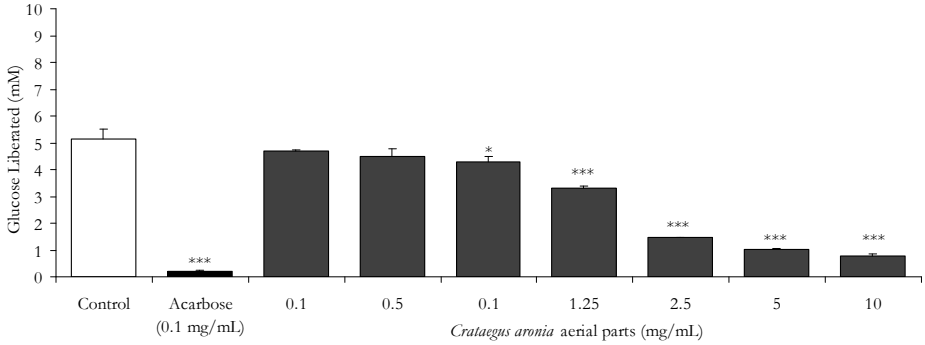

Figure 2. *In vitro* inhibitory effects of *C. aronia* (AE) aerial parts concentrations in mg/mL on enzymatic starch digestion. Results are mean ± SEM (n = 3 independent replicates). $^{*}P < 0.05$ and $^{***}P < 0.001$ compared to control (drug-free or plant-free) incubations, as determined by ANOVA followed by Dunnett post test.

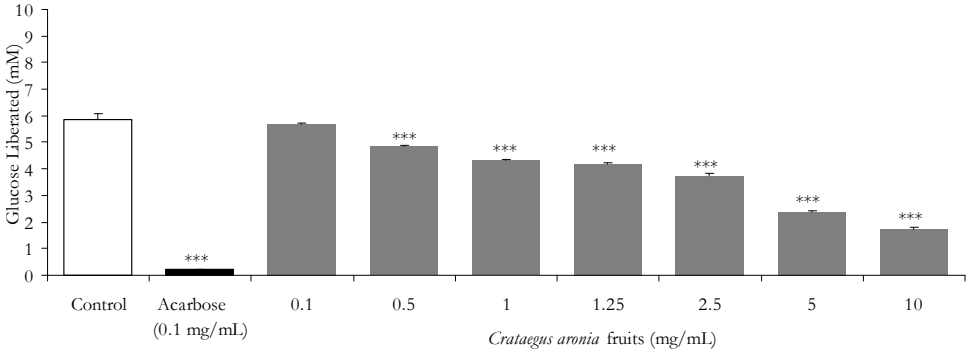

Figure 3. *In vitro* inhibitory effects of *C. aronia* (AE) fruits concentrations in mg/mL on enzymatic starch digestion. Results are mean ± SEM (n = 3 independent replicates). $^{*}P < 0.05$ and $^{***}P < 0.001$ compared to control (drug-free or plant-free) incubations, as determined by ANOVA followed by Dunnett post test.

Table 2. Effects of ascending concentrations of different parts of *C. aronia* (AE) (mg/mL) on %reduction of enzymatic starch digestion *in vitro*.

Plant AE (mg/mL)	0.1	0.5	1	1.25	2.5	5	10
C. aronia aerial parts	8.9 ± 1.4	13.0 ± 0.6	17.0 ± 3.4*	36.0 ± 1.5***	71.7 ± 0.4***	79.8 ± 0.3***	85.1 ± 1.5***
C. aronia fruits	3.4 ± 0.7	22 ± 2.3***	27.7 ± 4.4***	30.2 ± 1.3***	40.7 ± 5.8***	66.6 ± 2.5***	70.7 ± 3.9***

Results expressed as % decrease in control values are mean ± SEM (n = 3 independent replicates). $^{*}P < 0.05$ and $^{***}P < 0.001$ compared to control (drug-free or plant-free) incubations as determined by ANOVA followed by Dunnett post test.

pective 1.4% ± 0.8%, 1.0% ± 0.0% and 1.0% ± 0.1% AUC % reductions, P > 0.05, **Figure 4**).

Based on the optimal *in vitro* findings of the aqueous extract of *C. aronia* aerial parts, confirmatory acute *in vivo* studies (OSTT and OGTT) were conducted. At −30 min time point, the administration of acarbose 3 mg/Kg b.wt reduced highly significantly the starch induced postprandial hyperglycaemia at 45, 90 and 135 min post corn starch load at 0 min, thus evoking highly substantial reduction (P < 0.001 vs. untreated animals, n = 5 - 8) of the overall glycemic excursion AUC compared to controls (**Figure 5**).

Exceedingly superior to acarbose, *C. aronia* AE at concentrations 100, 200 and 400 mg/Kg b.wt diminished highly markedly (p < 0.001 vs. untreated rats, n = 5 - 8) AUC of overall glycemic excursions (**Figure 5**). Compared to control rats, **Figure 5** mirrors the highly significant minimized increments in acute postprandial hyperglycemia evoked by *C. aronia* AE (100, 200 and 400 mg/Kg b.wt) at the determination time points 45 min (P < 0.001), 90 min (P < 0.001) and 135 min (P < 0.001) postculinary cornstarch oral intake. Most importantly and impressively, such pronounced *C. aronia* bioeffects on rats' gastrointestinal starch digestion were comparable to those effectively obtained *in vitro*; a finding that can complement its claimed antihyperglycemic effects in STZ-diabetic rats [20]. 30 min pre-glucose-load in OGTTs; treatments with metformin (300 mg/Kg b.wt) or glipizide (0.6 mg/Kg b.wt) minimized highly significantly (P < 0.001 compared to control rats, n = 5 - 8) the overall glycemic excursions (**Figure 6**). The figure demonstrates the highly substantial antihyperglycemic efficacies of both oral antidiabetic therapeutics 45 (P < 0.001), 90 (P < 0.001) and 135 min (P < 0.001) following sugar load. Oral administration of *C. aronia* AEs did not evoke any marked improvement of glucose tolerance AUCs in comparison to control determinations respective AUCs, contrary to metformin and glipizide therapeutic propensities (**Figure 6**).

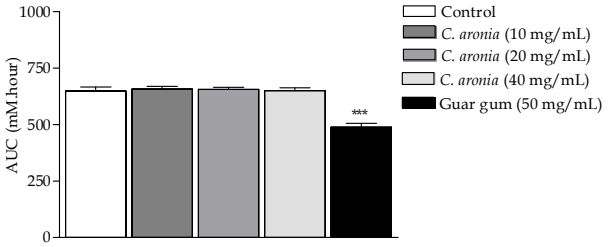

Figure 4. *In vitro* effects of *C. aronia* AE concentrations (mg/mL) in on the incremental AUC of 24 h glucose movement. ***P < 0.001 compared to control (drug-free or plant-free) incubations, as determined by ANOVA followed by Dunnett post test.

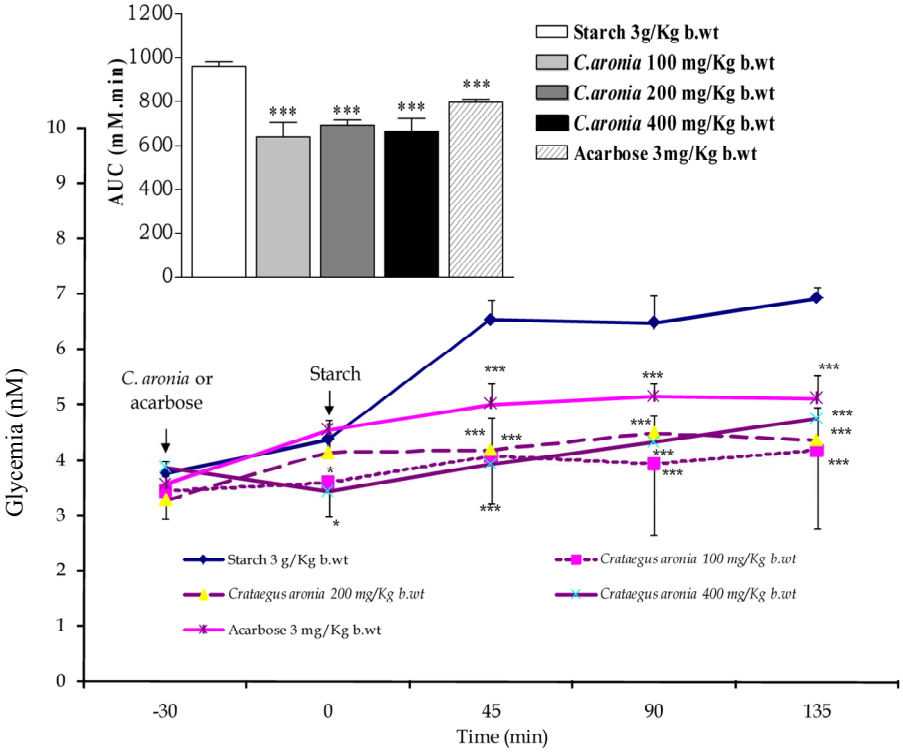

Figure 5. Modulatory postprandial antihyperglycemic effects of *C. aronia* (AE) concentrations in mg/Kg b.wt on oral starch tolerance over 165 min and AUC in normoglycemic overnight fasting rats. *P < 0.05 and ***P < 0.001 compared to control untreated animals, as determined by ANOVA followed by Dunnett post test.

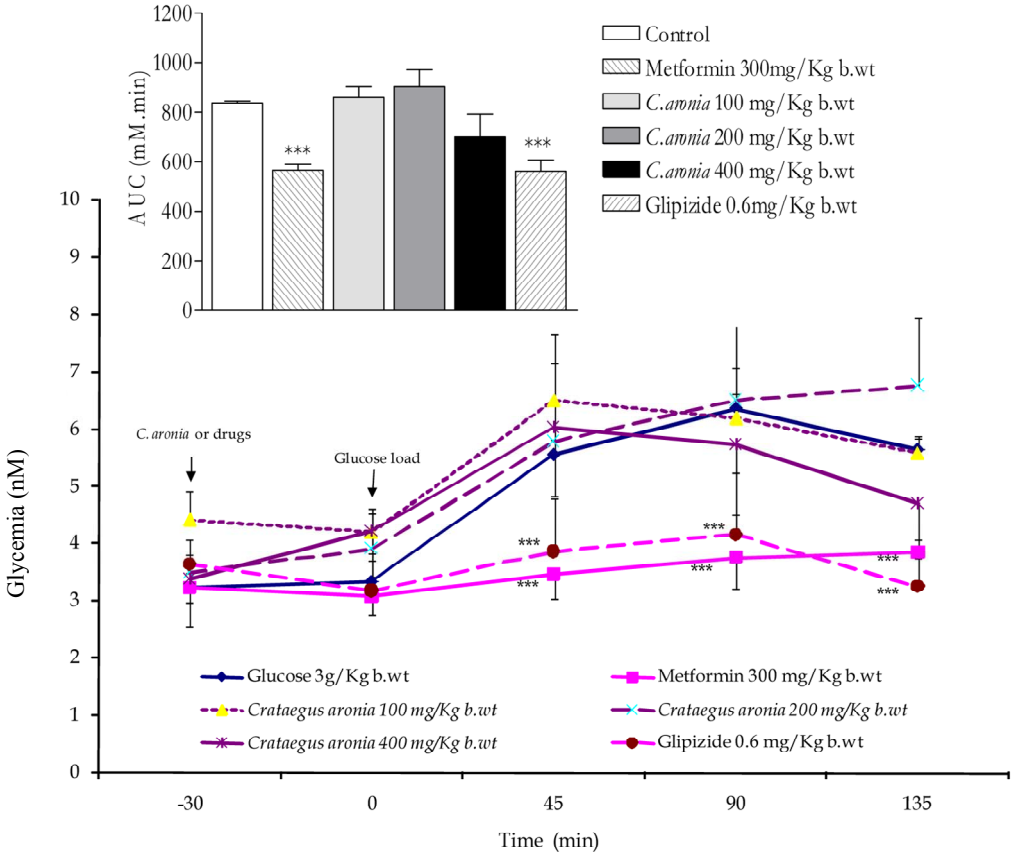

Figure 6. Effects of *C. aronia* (AE) concentrations in mg/Kg b.wt on oral glucose tolerance over 165 min and AUC in normoglycemic overnight fasting rats. $^{*}P < 0.05$ and $^{***}P < 0.001$ compared to control untreated animals, as determined by ANOVA followed by Dunnett post test.

In parallel terms, none of *C. aronia* AEs exhibited any postprandial acute antihyperglycemic activity in glucose fed rats at any determination point (**Figure 6**), thus further ascertaining the lack of *in vitro* efficacies on gastrointestinal glucose movement. Suggestively, co-administration *C. aronia* with acarbose may reduce the therapeutic concentration required for its effective dual α-amylase and α-glucosidase inhibitions. Such anticipated synergistic interactions, at best, may impact/ substitute the clinical prescriptions of acarbose for type-2 diabetics' postprandial glycaemia management [53,54]. As pancreatic enzymes elevation in type 2 diabetes has been marked; hence inhibition of these enzymes serves multiple pharmacotherapeutic targets in treatment of diabetes, obesity and hyperlipoproteinemia [55-57]. Starch blockers with dietary lipid blockers are successful antiobesity therapeutic perspectives [58-61].

In vivo studies were conducted to evaluate the effects of 10 weeks administration of *C. aronia* AE on body weight, triacylglycerol levels and atherogenic indices in HCD fed rats. Weights were standardized by considering the starting weight for each animal as 100%. Body weights of all groups increased with time. As shown in **Figure 7**, *C. aronia* 400 mg/Kg-group exhibited significant decrease in body weight compared to control ($P < 0.05$) group. Additionally, in the same figure, significant body weight decrease was obtained for the HCD + *C. aronia* 200 mg/Kg b.wt when compared to HCD alone ($p < 0.01$ - 0.001). Body weight $AUC_{10weeks}$ in HCD-rats was significantly increased compared to control animals (1192.5 ± 81.5 vs. 1032.4 ± 60.8, n = 6 - 8 rats/group, $P < 0.05$, **Figure 7**). Most impressively, body weight-$AUC_{10weeks}$ in rat groups of HCD + *C. aronia* 200 mg/Kg b.wt and *C. aronia* 400 mg/Kg b.wt were normalized to control's AUC (953.3 ± 29.7 and 962.7 ± 13.1 vs. 1032.4 ± 60.8, n = 6 - 8 rats/group, $P > 0.05$, respectively, **Figure 7**). Interestingly, body weight AUC10weeks in HCD + *C. aronia* 200 mg/Kg b.wt was highly significantly less than those of HCD rats (respective 953.3 ± 29.7 and 962.7 ± 13.1 vs. 1192.5 ± 81.5, n = 6 - 8 rats/group, $P < 0.01$, **Figure 7**). Unlike *C. aronia* effects, chronic atorvastatin oral treatment did not change markedly body weight $AUC_{10weeks}$ (1054.8 ± 26.9 vs. either 1032.4 ± 60.8 or 1192.5 ± 81.5, n = 6 - 8 rats/group, $P > 0.05$).

Figure 8 demonstrates the effect of *C. aronia* AE on triacylglycerol levels (TAG). TAG-$AUC_{10\ weeks}$ in HCD fed-animals is highly significantly greater than control's

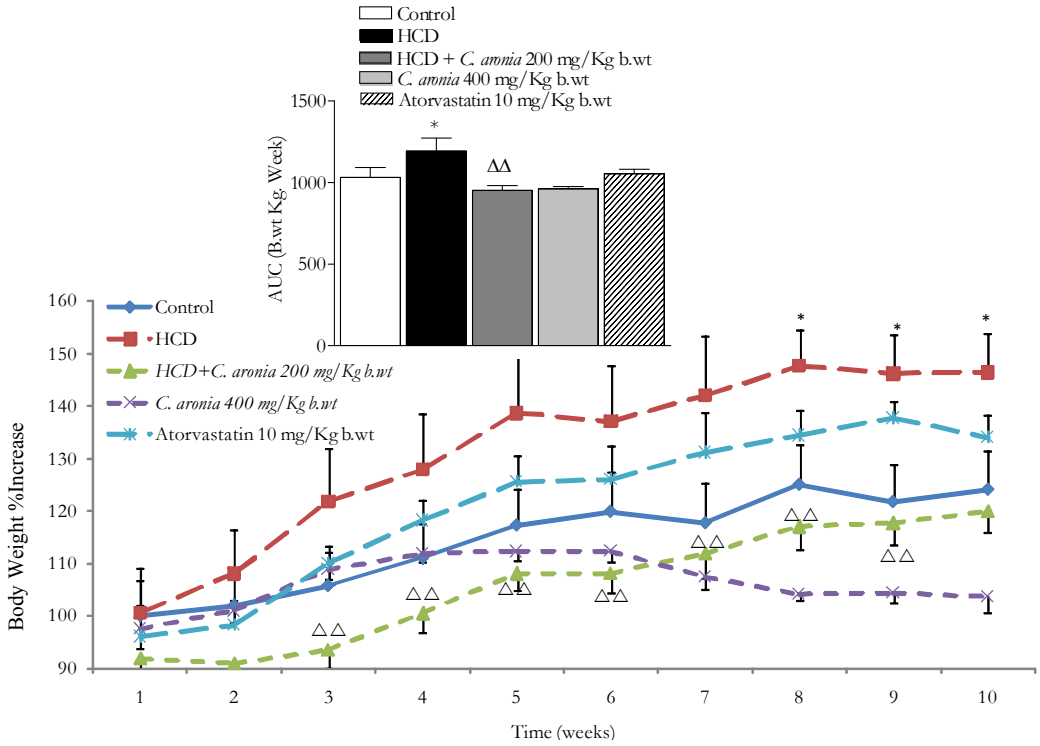

Figure 7. ***In vivo*** **chronic study effects of** ***C. aronia*** **AE on the body weight and the incremental AUC of 10 weeks treatment.** ***P < 0.05 compared to control group and** $^{\Delta\Delta}$**P < 0.01 compared to HCD as determined by ANOVA followed by Dunnett post test.**

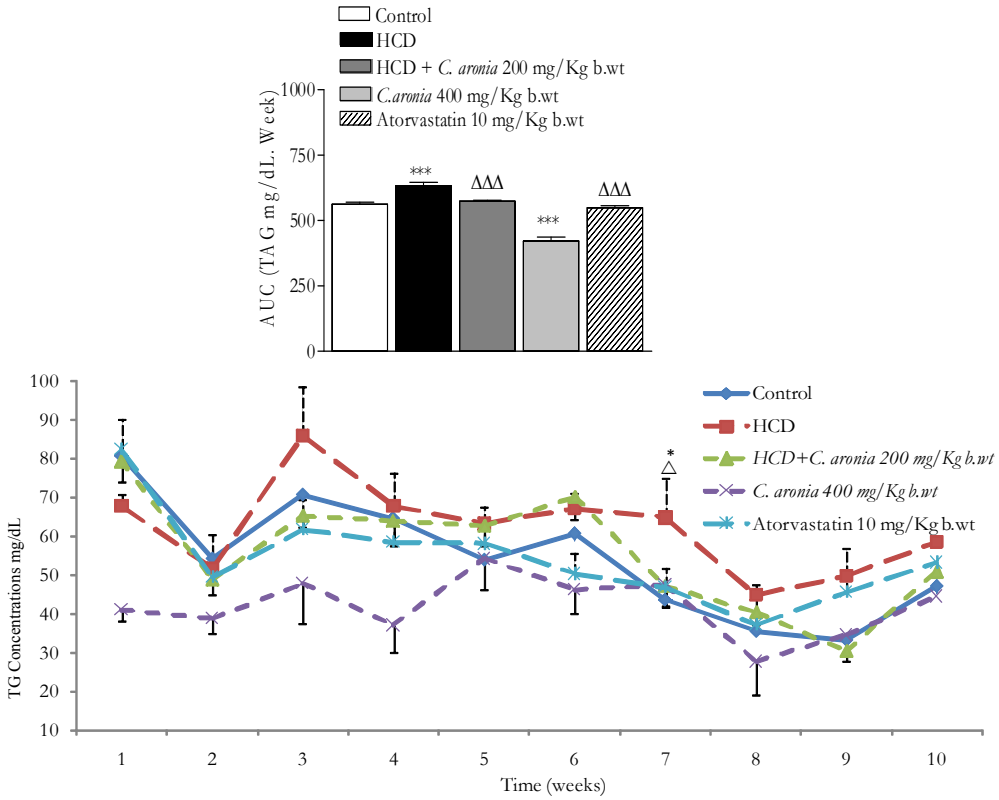

Figure 8. ***In vivo*** **chronic study effects of** ***C. aronia*** **AE on the incremental AUC of 10 weeks treatment on triglycerides concentrations.** *****P < 0.001 compared to control group,** $^{\Delta\Delta}$**P < 0.01 compared to HCD as determined by ANOVA followed by Dunnett post test.**

AUC (633 ± 30.0 vs. 562 ± 20.4, n = 6 - 8 rats/group, $P < 0.01$). In the same figure, orally administrated atorvastatin 10 mg/Kg b.wt normalised TAG-AUC $_{10weeks}$ in HCD fed-animals in comparison to HCD fed rats (548.6 ± 17.8 vs. 633 ± 30.0 and 548.6 ± 17.8 vs. 562 ± 20.4, n = 6 - 8 rats/group, $P < 0.001$ and $P > 0.05$ respectively). Likewise, TAG-AUC$_{10weeks}$ in HCD + *C. aronia* 200 mg/Kg b.wt-animals has been normalized as in that of control's (573.6 ± 14.2 vs. 562 ± 20.4, n = 6 - 8 rats/group, $P > 0.05$, **Figure 8**) and is highly significantly less than HCD-fed animals' AUC (573.6 ± 14.2 vs. 633 ± 30.1, n = 6 - 8 rats/group, $P < 0.01$, **Figure 8**). Interestingly, TAG-AUC$_{10weeks}$ in *C. aronia* 400 mg/Kg b.wt-animals is highly significantly less than control's (421.3 ± 45.0 vs. 562 ± 20.4, n = 6 - 8 rats/group, $P < 0.001$) This finding confirms the *in vitro* PL inhibitory potential of *C. aronia* extracts.

The American Heart Association has classified obesity as one of the major risk factors of coronary heart diseases (CHD) [62]. Therefore, prevention of obesity can minimize the occurrence of CHD. Additionally, amelioration of the different ratios such as HDL/LDL is considered as target for preventing/reduction risk of CHD [63]. **Table 3** shows the ratios of TC/HDL and HDL/LDL. HCD group has high TC/HDL ratio after 10 weeks of treatment and low HDL/LDL ratio when compared to preventive group that was treated similarly except that *C. aronia* AE (200 mg/Kg/day) was given along with HCD (4.1 ± 0.5 vs. 2.3 ± 0.2, and 0.9 ± 0.5 vs. 1.4 ± 0.3, respectively). On the other hand, both TC/HDL and HDL/LDL ratios for group (*C. aronia* 400 mg/dL/Kg) are close to those of control group, both groups took the same diet except that *C. aronia* AE was administered besides in treated group (*C. aronia* 400 mg/Kg) (1.9 ± 0.1 vs.1.7± 0.1 and 1.8 ± 0.3 vs. 3.3 ± 0.8, respectively). The above results are further indications of beneficial effects of *C. aronia* AE on atherogenic indices (predictors of CHD). As the occurrence of obesity is on the rise, various recent studies were accomplished on obesity treatment through suppression of triglycerides accumulation by inhibiting the digestion of dietary lipids and minimizing intestinal fat absorption [30].

In this study it was shown that *C. aronia* AE results *in vivo* regarding triglyceride and those of body weight were significantly lower when compared to normal diet. However, compared to the HCD group, *C. aronia* AE in preventive group was able to preserve lower percent of body weight and TG concentration throughout the whole period (10 weeks). These data pursue the same line of those *in vitro* results for pancreatic lipase when *C. aronia* and/or its pure phytocomponents compared to orlistat, potent drug known for PL inhibition. These results may suggest that *C. aronia* AE has the same mode of action of orlistat through inhibition of PL to digest dietary fats

Table 3. *In vivo* effects of *C. aronia* extracts on TC/HDL and HDL/LDL ratios.

Group	TC/HDL	HDL/LDL
Control	1.7 ± 0.1	3.3 ± 0.8
HCD	4.1 ± 0.5***	1.0 ± 0.5**
HCD + *C. aronia* 200 mg/Kg b.wt	2.3 ± 0.2$^{\Delta\Delta\Delta}$	1.4 ± 0.3*
C. aronia 400 mg/Kg b.wt	1.9 ± 0.1	1.8 ± 0.3
Atorvastatin 10 mg/Kg b.wt	3.2 ± 0.3**	1.1 ± 0.5*

Results are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to control group and $^{\Delta\Delta\Delta}P < 0.001$ compared to HCD group as determined by ANOVA followed by Dunnett post test.

[30]. Moreover, our findings are in agreement with those recently reported by Mnafgui *et al.*, indicating that PL inhibition leads to decreases in lipid profiles [64]. Additionally, previous studies found that flavonoids have an effective role in PL inhibition, so did our *in vitro* study when using *C. aronia* pure compounds [64].

Succinctly *C. aronia* phytochemicals in optimal ratio can inhibit crucial gastrointestinal enzymes involved in carbohydrate and lipid digestion and absorption thus advocating a dual-target phytotherapeutic/preventive strategy in glycaemia control of obesity-diabetes (diabesity) [65].

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