Antiobesity and antihyperglycaemic effects of Adiantum capillus-veneris extracts: in vitro and in vivo evaluations

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\textbf{ABSTRACT}

\textbf{Context:} Adiantum capillus-veneris L. (Adiantaceae) hypocholesterolemic activity is therapeutically praised.

\textbf{Objectives:} Pharmacological modulation of pancreatic triacylglycerol lipase (PL) and $\alpha$-amylase/$\alpha$-glucosidase by $A.\text{capillus-veneris}$ are evaluated.

\textbf{Materials and methods:} Using positive controls (acarbose, orlistat, guar gum, atorvastatin, glipizide and metformin) as appropriate, crude aqueous extracts (AEs) of $A.\text{capillus-veneris}$ aerial parts were tested via a combination of \textit{in vitro} enzymatic (0.24–100 mg/mL), \textit{acute in vivo} carbohydrate tolerance tests (125, 250 and 500 mg/kg body weight (b.wt)) and chronic \textit{in vivo} studies (500 mg/kg b.wt) in high cholesterol diet (HCD) fed Wistar rats.

\textbf{Results:} Like acarbose, $A.\text{capillus-veneris}$ as well as chlorogenic acid, with respective IC\textsubscript{50} values (mg/mL) of 0.8±0.0 and 0.2±0.0, were identified as \textit{in vitro} potent dual inhibitors of $\alpha$-amylase/$\alpha$-glucosidase. Unlike guar gum, $A.\text{capillus-veneris}$ had no glucose diffusion hindrance capacity. Equivalent to orlistat, $A.\text{capillus-veneris}$ and its phytoconstituents inhibited PL \textit{in vitro} with an ascending order of PL-IC\textsubscript{50} values (µg/mL): ferulic acid; 0.48±0.06 < ellagic acid; 13.53±1.83 < chlorogenic acid; 38.4±2.8 $< A.\text{capillus-veneris}$; 1600±100. Incomparable to acarbose or metformin and glipizide, $A.\text{capillus-veneris}$ (125, 250 and 500 mg/kg b.wt) lacked antihyperglycaemic efficacies in acute starch- or glucose-evoked postprandial hyperglycaemia increments in normoglycaemic overnight fasting rats. Superior to atorvastatin; $A.\text{capillus-veneris}$ exerted significant antiobesity ($p<0.001$) with marked triacylglycerol-reducing capacities ($p<0.001$) in comparison to rats fed with HCD for 10 weeks.

\textbf{Discussion and conclusion:} $A.\text{capillus-veneris}$, modulating pancreatic digestive enzymes, may be advocated as a combinatorial diabetes prevention/phytotherapy agent.

\textbf{Introduction}

Type 2 diabetes and obesity, referred to as diabeity, comprise global health threats (Tschoëp & DiMarchi 2012) with rising prevalence rates of cardiovascular diseases, especially in people with metabolic syndrome and diabetes (Xanthakis et al. 2015). Recently, the high prevalence of dyslipidemia, obesity and diabetes in Jordan is being alarmingly worrisome (Al-Kloub & Froelicher 2009; Al-Nsour et al. 2012). Plants have been long used for the ethnomedical integrative/complementary treatment of obesity-diabetes in various systems of medicine (Hasani-Ranjbar et al. 2008, 2009). Rosmarinus officinalis L. (Lamiaceae) (Al-Jamal & Alqadi 2011; Al-Jamal et al. 2012; Al Sheyab et al. 2012), Apium graveolens (Mill.) Pers. (Apiaceae) (Mansi et al. 2009), a combination of prickly pear (Opuntia ficus-indica L. Mill. [Cactaceae]), artichoke (Cynara scolymus L. [Asteraceae]), turmeric (Curcuma longa L. [Zingiberaceae]) and garlic extracts (Allium sativum L. [Amaryllidaceae]) (Qnna et al. 2012), Crataegus aronia L. (D.C.) (Rosaceae) (Al-Hallaq et al. 2012), and Lavandula angustifolia L. (Lamiaceae) (Issa et al. 2011) have been investigated locally for hyperlipidemic/hypocholesterolemic benefits. Keeping in mind, additional comprehensive reviews of native ethnomedicinal plants with antidiabetic therapeutic values were detailed by Afifi and Kasabri (2013). Natural inhibitors of hydrolysing enzymes in carbohydrates and lipids digestion and absorption can offer an attractive combinatorial therapeutic strategy for the management/prevention of postprandial dysglycaemia and diabesity. Hence, diverse studies were conducted to explore medicinal plants as potential therapeutic agents for dual management of diabetes and hyperlipidemia via digestive enzyme inhibition, namely pancreatic $\alpha$-amylase, intestinal $\alpha$-glucosidase and pancreatic lipase (PL) (Li et al. 2009a, 2011; Etoundi et al. 2010; Adisakwattana & Chanathong 2011; Rani et al. 2012; Sun et al. 2012). Given the comprehensive hypocholesterolemic propensities and HPLC-MS-based phytochemical characterization of $A.\text{capillus-veneris}$ L. (Adiantaceae) (Al-Hallaq et al. 2015), this study investigates the inhibitory effects of crude aqueous extracts (AEs) of $A.\text{capillus-veneris}$ on these extrapancreatic digestive enzymes \textit{in vitro}. Thus, more detailed investigations to elucidate the dual anti-diabetic antiobesity pharmacotherapeutic effects of $A.\text{capillus-veneris}$ on cell-free \textit{in vitro} systems of carbohydrate and lipid enzymatic digestion and absorption were undertaken. Additionally, acute as well chronic \textit{in vivo} effects in high fat fed rats were investigated.

\textbf{Materials and methods}

\textbf{Chemicals, biochemicals and instruments}

Unless stated otherwise, all reference drugs (orlistat, metformin, glipizide, acarbose and atorvastatin with purities >95%) reagents,
and chemicals (chlorogenic acid, ellagic acid and ferulic acid with purities >95%) were procured 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®, Daian LabTech Co., LTD. (Hwado-eup, Korea). The Glucose GOD-PAP kit was obtained from BIOLABO Reagents (Maizy, France). In the UV determinations, the UV–VIS spectrophotometer from SpectroScan 80D (Sedico Ltd., Nicosia, UK) was used. The sonicator (Bandelin Sonorex, BANDELIN electronic GmbH & Co., Berlin, Germany) and rotary evaporator (Laborota 4000-efficient, Heidolph Instruments GmbH & Co., Schwabach, Germany) were also used.

**Plant material and preparation of the Adiantum capillus-veneris aqueous extracts (AEs)**

During the spring of 2013, dried aerial parts of *A. capillus-veneris* from northern Jordan were provided and identified by Prof. K. Abdul-Razzak using a descriptive reference (Zohary 1966) and in comparison with the herbarium specimens of the Herbarium of the Department of Biological Sciences, Faculty of Science, The University of Jordan. Voucher specimens of the plant material were deposited at the Department of Pharmaceutical Sciences, The University of Jordan (Reference No. 127). AEs were prepared as described by Kasabri et al. (2014). In brief, AEs were prepared by gentle boiling 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/mL) crude aqueous solutions. Sonication of the stock crude extract or testing concentrations was performed before implementation of investigations. For PL 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.

**Preparation of A. capillus-veneris crude AEs and its phytoconstituents for in vitro pancreatic triacylglycerol lipase activity assay**

The tested AEs were initially dissolved in Tris–HCl buffer (2.5 mM [Promega Biotechnology company, Madison, WI], pH 7.4 with 2.5 mM NaCl) to give five different stock solutions with a concentration range of 12–192 mg/mL. Subsequently, a 20 μL aliquot of each stock solution was used in the reaction mixture to give a final concentration range of 240–3840 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 (Gurbuz et al. 2003). Also ellagic acid, caffeic acid (in DMSO, separately) and chlorogenic acid (in absolute ethanol) isolated from *A. capillus-veneris* were prepared into five different stock solutions with an initial chlorogenic acid concentration range of 0.625–100 mg/mL, initial ellagic acid concentration range of 0.086–5.5 mg/mL and initial ferulic acid concentration range of 6.10–390.63 μg/mL. Thereafter, a 20-μL aliquot of each stock solution was used in the reaction mixture to give a final chlorogenic acid concentration range of 12.5–2000 μg/mL, a final ellagic acid concentration range of 1.72–110 μg/mL, and a final ferulic acid concentration range of 0.12–7.81 μg/mL. Finally, orlistat (Sigma-Aldrich, St. Louis, MO), the reference drug (1 mg/mL), was prepared into six different stock solutions with a concentration range of 0.625–20 μg/mL (Habtemariam 2012). Thereafter, a 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.

**Spectrophotometric quantification of pancreatic lipase (PL) activity and assaying PL inhibition by crude AEs and phytochemicals**

According to Bustanji et al. (2011), *in vitro* enzymatic PL activity was assayed. Subsequent determinations were undertaken for the tested extracts/phytoconstituents in comparison to the control evaluations to calculate the concentration value required for PL 50% inhibition (IC_{50}).

**In vitro α-amylase/α-glucosidase assay**

*In vitro* enzymatic starch digestion was assayed with acarbose as the reference drug (Kasabri et al. 2014, 2015). The extent of polysaccharide breakdown into glucose was evaluated in a concentration range of different parts of plant AE 1, 5, 10, 12.5, 25, 50 and 100 mg/mL. The chlorogenic acid tested concentration gradient was 0.0625, 0.125, 0.25, 0.5, 1 and 2 mg/mL. The effects of acarbose at 1000 μg/mL concentration were evaluated as well. Control (tap water only) samples did not contain acarbose, plant extract or chlorogenic acid.

**Glucose movement in vitro assay**

*In vitro* glucose movement was assayed according to Kasabri et al. (2015). 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, 25 and 50 mg/mL of *A. capillus-veneris* 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 (Gallagher et al. 2003; Butt et al. 2007).

**In vivo confirmatory studies**

**Oral starch tolerance test (OSTT) and oral glucose tolerance test (OGTT)**

With the treatment plant administered in doses 125, 250 and 500 mg/kg b.wt; OSTT and OGTT were conducted according to Kasabri et al. (2015) in the Experimental Animal Laboratory of the School of Medicine, 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 (registration number 218/2007–2008) obtained from the Scientific Research Council at the Deanship of Academic Research and the School of Pharmacy.

**Body weight and triglycerides determinations**

**Experimental animal groups**

At the Experimental Animal Laboratory of the Department of Biological Science, Faculty of Science, The University of Jordan,
the study was conducted as briefed in Al-Hallaq et al. (2015). All animals were housed, fed and treated in accordance with the University of Jordan ethical guidelines for animal protection and experimental approval. It also fulfills the accepted international requirements in this field. 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 h fasting period preceding cholesterol administration and blood collection. Rats were divided into the following groups (n = 6). Control: The control group was given only the control diet ad libitum for 10 weeks. HCD: The hypercholesterolemic control group was given the control diet ad libitum for 10 weeks. HCD + A. capillus-veneris (500 mg/kg b.wt): The treated group was given the control diet ad libitum. HCD was administered once daily for 10 weeks, while A. capillus-veneris 500 mg/kg b.wt was fed in the last four weeks once daily (week 7–10). HCD + Atorvastatin (10 mg/kg b.wt): This group was given the control diet ad libitum. HCD was fed once daily for 10 weeks. Atorvastatin (positive control) 10 mg/kg b.wt was administered once daily for the last four weeks (week 7–10). LD50 determinations are reported in Al-Hallaq et al. (2015). Thereafter, A. capillus-veneris AEs were selected based on the obtained LD50 values and administered to animals of groups 3 and 4 by gavage at 6:30 am daily. Body weight was measured daily; doses of different treatment and plant extract were calculated and given according to b.wt.

**Statistical analysis**

The values are presented as mean ± S.E.M. (standard error of the mean) for the 3–6 independent experiments. Statistical differences between the control and different treatment groups and A.U.Cs (incremental area under the glucose curve) were determined using GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego, CA) one-way analysis of variance (ANOVA) followed by Dunnett’s post-test whenever appropriate. Values were considered significantly different if p < 0.05, p < 0.01 and p < 0.001.

**Results, discussion and future directives**

**In vitro extrapancreatic inhibitory effects of A. capillus-veneris crude AEs and its bioactive phytoconstituents on PL activity**

PL inhibition is one of the most widely studied mechanisms to determine the potential efficacy of natural products and ethnomedicinal botanicals as obesity modulating agents (Mansi et al. 2009; Habtemariam 2012; Marrelli et al. 2013, 2014). In this study, the pancreatic triacylglycerol anti-lipase activity profiles of the crude AEs of A. capillus-veneris and its bioactive phytoconstituents (chlorogenic acid, ellagic acid and ferulic acid) are shown in Figure 1. Orlistat PL IC50 value of 114.0 ± 4.0 ng/mL, equivalent to 0.2 ± 0.0 μM, is comparable to reported PL IC50 values elsewhere (Habtemariam 2012) (Table 1). Comparable to orlistat performance, a marked concentration dependent PL inhibition trend was obtained per tested extracts (Figure 1) and their pure phytoconstituents PL-IC50 values obtained for a minimum of triple separate determinations are also illustrated (Table 1).

These significant anti-lipase effects may be solidly related to the effect of the major compounds identified in the crude extract (Habtemariam 2012; Al-Hallaq et al. 2013). Caffeic acid and chlorogenic acid contributed to the PL and α-amylase inhibitory potential of Clematis vitalba L. (Ranunculaceae) and Echium vulgare L. (Boraginaceae) extracts (Marrelli et al. 2013, 2014). Evidently, chlorogenic acid could modulate glucose and lipid metabolism in vivo (Li et al. 2009b; Karthikesan et al. 2010) and in vitro (Sakulnarmrat & Konczak 2012). Ellagic acid could attenuate the metabolism characteristic changes in association with

| Tested extract/compound | IC50 (ng/mL-mg/mL) | IC50 (μM–mM) |
|-------------------------|-------------------|---------------|
| Orlistat                | 114.0 ± 4.0 ng/mL | 0.23 ± 0.0 μM |
| A. capillus-veneris AEs | 1.6 ± 0.1 mg/mL  | –             |
| Chlorogenic acid        | 38.4 ± 2.8 μg/mL  | 0.11 ± 0.0 mM |
| Ellagic acid            | 13.53 ± 1.83 μg/mL | 44.78 ± 6.07 μM |
| Ferulic acid            | 0.48 ± 0.06 μg/mL | 2.49 ± 0.29 μM |

![Figure 1](image_url) 

*Figure 1. In vitro inhibitory effects of Adiantum capillus-veneris AE, its bioactive phytoconstituents; chlorogenic acid, ellagic acid and ferulic acid, and orlistat concentrations on pancreatic triacylglycerol lipase activity. Results are mean ± SEM (n = 3 independent replicates).*
with high carbohydrate and high fat diet induced metabolic syndrome in rats (Panchal et al. 2013). Furthermore, ellagic acid could inhibit the fatty acid synthase and adipogenesis of 3T3L1 adipocytes (Dan et al. 2013), and it was linked to porcine lipase inhibitory potential of pomegranate peel extracts (Hadrich et al. 2014). Besides that, *A. capillus-veneris* content of rutin and quercetin were recognized for their in vitro antilipolytic properties (Al-Hallaq et al. 2014). In effect, the results indicate that the pancreatic triacylglycerol lipase (PL) inhibitory efficacy of *A. capillus-veneris* may be attributable to its multiple components acting additively or synergistically in optimal ratio (Bansal et al. 2011). Overall, pharmacological inhibition of dietary lipid digestion and absorption can induce favourable amelioration of dyslipidemia, atherosclerosis and obesity. Impressively, PL 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 (Wierzbicki et al. 2012).

**In vitro extrapancreatic inhibitory effects of *A. capillus-veneris* crude extracts and its bioactive phytoconstituent on extrapancreatic enzymatic starch digestion**

With acarbose (0.1 mg/mL) as the reference drug, glucose liberation from starch was inhibited by 97.6% (*p < 0.001 vs. drug-free control incubation, n = 3, Figures 2 and 3). Furthermore, Figure 2 demonstrates that *A. capillus-veneris* AE concentrations 0.1–10 mg/mL had pronounced dose-related reductions in aldohexose release from culinary polymeric corn starch (*p < 0.05–0.001 vs. plant-free control determinations, n = 3). With an IC50 value of 0.8 ± 0.0 mg/mL, the highly significant dose related (*p < 0.05–0.001) percentage decreases in enzymatic starch hydrolysis by *A. capillus-veneris* dosage gradient (1–10 mg/mL) are summarized in Table 2. Additionally, chlorogenic acid, *A. capillus-veneris* bioactive phyto-principle, exerted highly significant (*p < 0.001 vs. basal control determinations, n = 3) concentration dependent inhibitions of polymeric starch enzymatic digestion, with an IC50 value of 0.2 ± 0.0 mg/mL (0.5 ± 0.1 mM) over a dose range of 6.25–200 μg/mL (Figure 3). Further details of percentage decreases in polysaccharide hydrolysis are tabulated (Table 2). Importantly, the exquisite dual anti-α-amylase/anti-α-glucosidase activity of *A. capillus-veneris* can be strongly attributed to its phytochemicals, mainly chlorogenic acid (Sakulnarmrat & Konczak 2012). Suggestively, co-incubations *A. capillus-veneris* with acarbose can reduce the therapeutic concentration required for its effective dual amylase and glucosidase inhibitions. Such synergic interactions can impact/substitute the clinical prescriptions of acarbose for type-2 diabetics’ postprandial glycaemia management (Adisakwattana & Chanathong 2012).
Taken together, as pancreatic enzymes elevation in type 2 diabetes has recently been marked (Maolly et al. 2012), gastrointestinal enzyme inhibition serves multiple pharmacotherapeutic targets in the treatment of diabetes, obesity and hyperlipoproteinemia (Raju et al. 2010).

Acute in vivo studies

OSTT

At the 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 a highly substantial reduction \( p < 0.001 \) vs. untreated animals, \( n = 6 \) of the overall glycaemic excursion AUC compared to controls (Figure 4). Unlike acarbose, *Adiantum capillus-veneris* at concentrations 125, 250 and 500 mg/kg b.wt, AE could not diminish overall glycaemic excursions AUCs (Figure 4). Except for *Adiantum capillus-veneris* 250 mg/kg b.wt eliciting a significant decrease in culinary starch-related postprandial hyperglycaemia at 45 min \( p < 0.05 \) vs. control rats, Figure 4). *Adiantum capillus-veneris* tested doses at the determination time points lacked acute anti-hyperglycaemic efficacies. These outcomes did not correspond with those obtained *in vitro*.

OGTT

Thirty-min pre-glucose-load treatments with metformin (300 mg/kg b.wt) or glipizide (0.6 mg/kg b.wt) significantly minimized \( p < 0.001 \) compared to control rats, \( n = 6 \) the overall glycaemic excursions in OGTTs (Figure 5(A)). The same figure demonstrates the highly substantial anti-hyperglycaemic efficacies of both oral anti-diabetic therapeutics 45 min \( p < 0.001 \), 90 min \( p < 0.001 \) and 135 min \( p < 0.001 \) following sugar load. Oral administration of *A. capillus-veneris* 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 5(A)). In parallel terms, none of *Adiantum capillus-veneris* AEs exhibited any postprandial acute antihyperglycaemic activity in glucose fed rats at any determination point (Figure 5(A)). This was further ascertained by the lack of *in vitro* efficacies of *A. capillus-veneris* AEs on the impedance of glucose movement *in vitro*. Using the diffusion model described in Kasabri et al. (2015), viscous water-

| Plant AE (mg/mL) | 0.1  | 0.5  | 1    | 1.25 | 2.5  | 5    | 10   |
|------------------|------|------|------|------|------|------|------|
| *Adiantum capillus-veneris* | 31.3 ± 2.0 | 49.0 ± 4.3* | 51.3 ± 1.2* | 51.0 ± 0.8* | 60.2 ± 1.3* | 63.3 ± 0.6* | 64.0 ± 0.3* |
| Chlorogenic acid (µg/mL) | 6.25 | 12.5 | 25   | 50   | 100  | 200  |      |
| Chlorogenic acid | 30.6 ± 0.9* | 31.4 ± 1.9* | 32.6 ± 1.3* | 36.8 ± 1.4* | 43.7 ± 1.4* | 57.2 ± 0.9* |

* \( p < 0.001 \) compared to control (drug-free or plant-free) incubations as determined by ANOVA followed by Dunnett’s post-test.

Figure 4. *In vivo* modulatory postprandial antihyperglycaemic effects of *Adiantum capillus-veneris* AE (mg/kg b.wt) on oral starch tolerance over 165 min and AUC in normoglycaemic overnight fasting rats. * \( p < 0.05 \) and *** \( p < 0.001 \) compared to control untreated animals, as determined by ANOVA followed by Dunnett’s post-test.
soluble gel-forming guar gum (50 mg/mL) significantly decreased the mean AUC (area under 24 h glucose curve) by 30.8 ± 2.5% \((p < 0.001)\) \((n = 3, \text{vs. overnight negative control, Figure 5(B))}\).

\(A. \text{capillus-veneris}\) extracts (10, 25 and 50 mg/mL), nevertheless, lacked any marked glucose diffusion hindrances in external solution across dialysis membrane (with respective 1.4 ± 0.8, 1.0 ± 0.0 and 1.0 ± 0.1% AUC reductions, \(p > 0.05\) vs. plant free basal control, Figure 5(B)).

**Chronic effects of \(A. \text{capillus-veneris}\) on body weight in HCD rats**

Weights were standardized by considering the starting weight for each animal as 100%. Body weights of all groups increased with time. Figure 6 illustrates the effect of \(A. \text{capillus-veneris}\) on b.wt over 7–10 weeks in 10-week-HCD fed rats. The b.wt AUC\(_{10}\) weeks in the HCD group and in atorvastatin of the HCD group were significantly increased compared to the normal diet control animals (with respective 1200.3 ± 77.3 and 1122.5 ± 51 vs. 941.9 ± 28.6, \(n = 6\) rats/group, \(p < 0.05\) – 0.01, Figure 6). Most interestingly, the b.wt AUC\(_{10}\) weeks in \(A. \text{capillus-veneris}\) (500 mg/kg b.wt) of the HCD rats was highly substantially reduced in comparison to the HCD fatty rats (870.7 ± 38.8 vs. 1200.3 ± 77.3, \(p < 0.001\), Figure 6). Impressively, the b.wt in the HCD group \(A. \text{capillus-veneris}\) 500 mg/kg b.wt was normalized to negative controls (870.7 ± 38.8 vs. 941.9 ± 28.6, \(n = 6\) rats/group, \(p > 0.05\), Figure 6). Strikingly, over a four-week intervention period, daily plant treatment proved markedly more effective in weight reduction \((p < 0.01, n = 6)\) than atorvastatin, the reference hypocholesterolemic drug (Figure 6).

**Chronic effects of \(A. \text{capillus-veneris}\) on plasma triglycerides (TAG) in HCD rats**

Figure 7 demonstrates the effect of \(A. \text{capillus-veneris}\) on plasma triacylglycerols (TAG) over 7–10 weeks of treatment in the 10-week-HCD fed rats. TAG AUC\(_{10}\) weeks in HCD fed-animals is significantly greater than the control rats’ (648.5 ± 33.3 vs. 552.3 ± 22, \(n = 6\) rats/group, \(p < 0.05\), Figure 7). TAG AUC\(_{10}\) weeks in atorvastatin-HCD fed rats is substantially less than the HCD-fed rats (548.6 ± 17.8 vs. 648.5 ± 33.3, \(n = 6\) rats/group, 0.05). Also, it has impressively been normalized as in
control’s (548.6 ± 17.8 vs. 552.3 ± 22, n = 6 rats/group, p > 0.05, Figure 7). In A. capillus-veneris 500 mg/kg b.wt HCD fed rats, TAG AUC10 weeks is significantly less than HCD-fed animals’ (443.1 ± 18 vs. 648.5 ± 33.3, n = 6 rats/group, p < 0.001, Figure 7) and is proven substantially less than controls’ (443.1 ± 18.0 vs. 552.3 ± 22, n = 6 rats/group, p < 0.01); thus, it is brought to levels pronouncedly less than the reference hypocholesterolemic drug atorvastatin (443.1 ± 18.0 vs. 548.6 ± 17.8, p < 0.01, n = 6 rats/group, Figure 7). These outcomes are perfectly aligned with the in vitro PL inhibitory effects of A. capillus-veneris. In a study performed by Mnafgui et al. (2012), it was found that the inhibitory action of PL leads to a decrease in lipid profiles which agrees with our in vitro and in vivo findings combined. As the occurrence of obesity is on the rise, various studies were accomplished on obesity treatment through the suppression of triglycerides accumulation by inhibiting the digestion of dietary lipids and
minimizing intestinal fat absorption (Bustanji et al. 2011; Jeong et al. 2012).

Conclusions
Succinctly, A. capillus-veneris phytochemicals in an 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).

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