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Weight losing, antihyperlipidemic and cardioprotective effects of the alkaloid fraction of *Hunteria umbellata* seed extract on normal and triton-induced hyperlipidemic rats

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ABSTRACT

Objective: To investigate the weight losing, antihyperlipidemic and cardioprotective effects of the alkaloid fraction of *Hunteria umbellata* (*H. umbellata*) seed.

Methods: Adult female Wistar rats (weight range: 120-150 g) were randomly divided into 4 and 5 treatment groups in the normal and triton-induced hyperlipidemic models, respectively. and were daily treated for 14 d before they were humanely sacrificed under inhaled diethyl ether anesthesia. About 5 mL of whole blood was obtained by cardiac puncture from each treated rat, from which serum for lipids assay was subsequently separated. Tissue samples of livers of treated rats were harvested and processed for histopathological analysis.

Results: Repeated daily oral treatments of normal rats with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* resulted in significant (P<0.05 and P<0.001) and dose-dependent weight loss, and decreases in the serum triglyceride, total cholesterol and low density lipoprotein cholesterol, while significantly (P<0.001) increased the serum levels of high density lipoprotein cholesterol fraction. Similarly, oral pre-treatments with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* for 14 d before induction of hyperlipidemia with triton WR-1339 significantly (P<0.01, P<0.001) and dose-dependently attenuated increases in the average body weights, serum levels of triglyceride, total cholesterol and low density lipoprotein cholesterol while also significantly (P<0.01, P<0.001) and dose-dependently attenuated significant (P<0.001) decrease in the serum high-density lipoproteincholesterol levels when compared to the untreated control values. However, the results obtained for 50 mg/kg of alkaloid fraction of *H. umbellata* in both normal and triton WR-1339-induced hyperlipidemic rats were comparable to that recorded for 20 mg/kg of simvastatin. Similarly, oral pretreatments with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* significantly improved the histological lesions of fatty hepatic degeneration induced by triton WR-1339 treatment.

Conclusions: Overall, results of this study showed that repeated oral treatments with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* elicited weight losing, antihyperlipidemic and cardioprotective effects in triton WR-1339 induced hyperlipidemic rats that were mediated via *de novo* cholesterol biosynthesis inhibition.

1. Introduction

In 2010, overweight and obesity were estimated to cause 3.4 million deaths, 3.9% of years of life lost, and 3.8% of disability-adjusted life-years worldwide[1]. Overweight and obesity result in adverse metabolic effects on blood pressure, cholesterol, triglycerides (TGs) and insulin resistance. Risks of coronary heart disease, ischemic stroke and type 2 diabetes mellitus increase steadily with increasing body mass index (BMI), a ratio of body weight (kg) relative to height squared (m^2). Raised BMI also increases the risk of cancers (of breast, colon, prostate,

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endometrium, kidney and gall bladder), osteoarthritis, hypertension, coronary heart disease, stroke, type 2 diabetes mellitus, hyperlipidemia, sleep apnea, obesity hypoventilation syndrome, cholelithiasis, infertility and menstrual disorders[2].

In 1980, the global prevalence of obesity (BMI 30 kg/m^2) was estimated to be 5% for men and 8% for women and by the 2008, these figures have doubled. Also, in 2008, 35% of the adult population (aged 20 years and above) were overweight (BMI 25 kg/m^2) (34% men and 35% women)[3]. Indeed, the prevalence of overweight and obesity were the highest in the World Health Organization (WHO) Regions of the Americas (62% for overweight in both sexes, and 26% for obesity) and the lowest in the WHO Region in South East Asia (14% overweight in both sexes and 3% for obesity). In the WHO Region for Europe and the WHO Region for the Eastern Mediterranean and the WHO Region for the Americas over 50% of women were overweight. For all three of these regions, roughly half of overweight women are obese (23% in Europe, 24% in the Eastern Mediterranean, 29% in the Americas). In all WHO regions, women were more likely to be obese than men. In the WHO regions for Africa, Eastern Mediterranean and South East Asia, women had roughly double the obesity prevalence of men[4].

Hunteria umbellata (K. Schum.) Hallier f. (*H. umbellata*) (locally known as "Abeere") (family: Apocynaceae) is tropical rainforest tree which is popularly used for the local management of diabetes mellitus and obesity[5-7]. In African folk medicine, water decoction made from the dry seeds of *H. umbellata* is employed in the local management of diabetes and obesity[5,7,8]. The crude aqueous extract of the plant have been reported to possess antihyperglycemic[7,8], anti-obesity and anti-hyperlipidemic[9], anti-inflammatory and antioxidant[10] with the crude alkaloid fraction found to be its principal antihyperglycemic, anti-inflammatory and antioxidant agent[10,11]. The aqueous seed extract of the plant has also been reported to be relatively safe when administered orally[12,13].

The present study was aimed at investigating the weight losing, antihyperlipidemic and cardioprotective potentials and mechanism(s) of 25 and 50 mg/kg/day alkaloid fraction of the *H. umbellata* seeds in normal and triton WR-1339-induced hyperlipidemic rats for 14 d.

2. Materials and methods

2.1. Plant materials

Eight fresh mature fruits of *H. umbellata* were collected from the deciduous forest of Odorasanyin District of Ijebu-Igbo in Ogun State, Nigeria, in the month of June, 2010. From these, 3 kg of fresh seeds were harvested, rinsed in tap water and air-dried at room temperature (25 ± 1) ˚C for 1 month, protected from direct heat and sunlight. Plant identification and deposit of voucher specimen were done as earlier described by Adeneye and Adeyemi[7].

About 2 kg of the dried seed was pulverized into fine powder using Laboratory Hammer Mill at the Department of Plant Sciences, College of Agriculture, University of Kentucky, USA. The powdered sample was kept in a thick water-proof and air-proof transparent white polythene bag and stored in the refrigerator at 4 ˚C.

2.2. Aqueous extraction process

About 60 g of the pulverized *H. umbellata* seed was completely cold extracted in 1 L of distilled water after dissolving it in distilled water and kept in the refrigerator for 72 h. The solution was continuously stirred using magnetic stirrer for 6 h after which it was filtered using ®Celite (Analytical filter-aid)-packed filter funnel. The deep brown filtrate was completely dried in vacuo using a freeze dryer (LABCONCO ®FreeZone 18 Liter Console Freeze Dry Systems, LabConco Corporation, Kansas City, MO, USA) to give a deep brown, sweet-smelling fluffy residue. This procedure was done more than 10 times and the residues were pooled into a water-proof and air-proof container and kept in the freezer to prevent decomposition of the extract.

2.3. Alkaloid extraction from H. umbellata seeds

About 10 g of *Hunteria* seed water extract in 10 mL of distilled water (pH=4.2) was repeatedly titrated with 50 mL of 5% aqueous HCl solution (Aldrich-Sigma, St. Louis, MO, USA) to acidify the solution to a pH 2. The acidified solution was extracted with ethyl acetate (150 mL × 3) to remove the neutral compounds in the acidify *Hunteria* solution. The acidified *Hunteria* solution was then carefully basified with 5% sodium bicarbonate (Na_2CO_3) (Aldrich-Sigma, St. Louis, MO, USA) solution to a pH 10. Using a 5 L separating funnel, the mixture was repeatedly extracted with small portions of ethyl acetate (Aldrich-Sigma, St. Louis, MO, USA) (150 mL×3) until the extract turned colourless, and was negative test to the alkaloid detecting (Draggendorff's) reagents. The ethyl acetate extract obtained was evaporated to complete dryness using rotary evaporator coupled with a water bath (®BUCHI Rotavapor Model R-215, BUCHI Labortechnick AG, Flawil, Switzerland) preset at 40 ˚C until a solid residue is left behind. This procedure was repeated 10 times. Alkaloid fraction of *H. umbellata* was pooled into tight-capped container which was stored in the refrigerator at -4 ˚C until required for experimentation.

2.4. Spectra studies of alkaloid fraction of H. umbellata

Alkaloid fraction of *H. umbellata* dissolved in dry deuterated chloroform (Cambridge Isotope Labs DLM-7) was subjected to high resolution full mass spectrometry-electron impact ionization mass

spectra (EI) which were recorded at 25 eV on a JEOL JMS-700T MStation (magnetic sector instrument) at a resolution of greater than 10 000. Samples were introduced via heatable direct probe inlet and perfluorokerosene was used to produce reference masses.

2.5. Experimental animals

The animal experiment was conducted in the Animal House Facility of the Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria and this was done in two phases. A total of sixty young adult female Wistar rats (weight range: 120-140 g) were obtained from Bayo Farms, Sango-Otta, Ogun State, Nigeria in the month of August 2011 after an institutional ethical approval has been obtained. The rats were acclimatized for 14 d, fed on standard rat chow and tap water *ad libitum*. The rats were housed in a standard rat cages in the Animal House Facility of the Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria and maintained at standard laboratory conditions (12/12 h light-dark periodicity, temperature: 23-26 ℃; humidity: 55%-65%) as prescribed by the United States National Institute for Health[14].

2.6. Oral treatments of normal rats

In the first phase of the experiment which involved normal rats, two days prior to commencement of the experiment, rats were randomly divided into 4 groups of 6 rats per treatment group such that the weight differences within and between treatment groups do not exceed ± 20% of the average weight of the rat population, respectively. The rats were treated with single daily oral treatments for 14 d as follows: Group I: normal rats + 10 mL/kg of 5% Tween 20 distilled water; Group II: normal rats + 10 mg/kg of simvastatin dissolved in 5% Tween 20 distilled water; Group III: normal rats + 25 mg/kg/day of alkaloid fraction of *H. umbellata* dissolved in 5% Tween 20 distilled water; Group IV: normal rats + 50 mg/kg/day of alkaloid fraction of *H. umbellata* dissolved in 5% Tween 20 distilled water.

2.7. Induction of triton-induced hyperlipidemia and their oral treatments

In the second phase of the study involving 30 adult female rats, the rats were also treated with daily oral treatment for 14 d before treatments with intraperitoneal injection of triton WR-1339 as follows: Group I: 10 mL/kg of 5% Tween 20 distilled water (*p.o.*) + 1 mL/kg distilled water (*i.p.*); Group II: 10 mL/kg of 5% Tween 20 distilled water (*p.o.*) + 200 mg/kg triton WR-1339 (*i.p.*); Group III: 10 mg/kg simvastatin in 5% Tween 20 distilled water (*p.o.*) + 200 mg/kg triton WR-1339 (*i.p.*); IV: 25 mg/kg/day alkaloid fraction of *H. umbellata* in 5% Tween 20 distilled water (*p.o.*)+200 mg/kg triton WR-1339 (*i.p.*); Group V: 50 mg/kg/day of alkaloid fraction of *H. umbellata* in 5% Tween 20 distilled water (*p.o.*)+200 mg/kg triton WR-1339 (*i.p.*).

One hour after the last dose of simvastatin and alkaloid fraction of *H. umbellata* were given on the 14th day, and 200 mg/kg triton WR-1339 was injected intraperitoneally into Group II-V rats while 1 mL/kg of distilled water was administered to Group I rats. Twenty four hours post-triton injection, rats were sacrificed under light diethyl ether anesthesia and blood samples for serum analysis were obtained directly from the heart chamber.

Simvastatin (Teva Simvastatin®) was obtained from Teva UK Limited, Eastbourne, UK while triton WR-1339 was obtained from Sigma Chemical Company, St. Louis, USA.

2.8. Body weight measurement

In the course of the 14 d oral treatment, body weights of rats were regularly taken from 1st to 14th day, respectively, with electronic Mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland). Absolute and percentage of weight changed on the 14th day were calculated in respect of the initial body weight on the 1st day.

2.9. Bioassays

At the termination of each experiment and at between 07:00 am and 09:00 am, overnight fasted rats had their blood samples collected directly from the heart chamber under light inhaled diethyl ether anesthesia. Blood samples were collected into plain sample bottles and the blood samples obtained were immediately frozen at -70 ℃ and centrifuged at 3 000 r/min for 20 min to separate out the serum that was then analyzed for the lipids such as TG, total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein (VLDL-c) using standard diagnostic test kits (Randox Laboratories, Crumlin, UK) on Automated Clinical System (Sychron Clinical System®, model: CX5 PRO) (Beckman Coulter Inc., Galway, Ireland). Serum LDL-c was estimated using Frieldwann's equation:

LDL-c=[TC-(HDL-c+TG/5)]

Serum VLDL-c fraction concentration was calculated by deduction of the sum of HDL-c and LDL-c concentrations from that of TC and represented by the equation:

VLDL-c=[TC-(HDL-c+LDL-c)]

2.10. Determination of atherogenic index (AI) and coronary risk index (CRI)

AI was calculated as: LDL-c (mg/dL)/HDL-c (mg/dL)[15,16],

while the CRI was calculated as: TC (mg/dL)/HDL-c (mg/dL)[9].

2.11. Statistical analysis

Results were presented as mean ± SD for body weights, weight changes (%) and feed intake while that of lipids, AI and CRI were expressed as mean ± SEM of six observations. Statistical analysis was done using Two-way ANOVA followed by *post-hoc* test, Student-Newman-Keuls test, on SYSTAT 10.6. Statistical significance were considered as $P<0.05$, $P<0.01$ and $P<0.001$.

3. Results

3.1. Alkaloid extraction

Extraction of alkaloid fraction of *H. umbellata* from the crude aqueous extract of *H. umbellata* seeds left behind a yellow-brown solid residue weighing 0.81 g with the yield of 8.10% ± 0.43%.

3.2. Spectral studies of alkaloid fraction of H. umbellata

Spectral studies of alkaloid fraction of *H. umbellata* using full mass spectrometry showed that it contains major molecules of alkaloid compounds with the molecular weights of 411, 383, 323 and 367 and of significant relative abundance of 100%, 95%, 67% and 47%, respectively (Figure 1). Although other molecules of higher molecular weights were also presented in alkaloid fraction of *H. umbellata* but were relative low abundance (Figure 1).

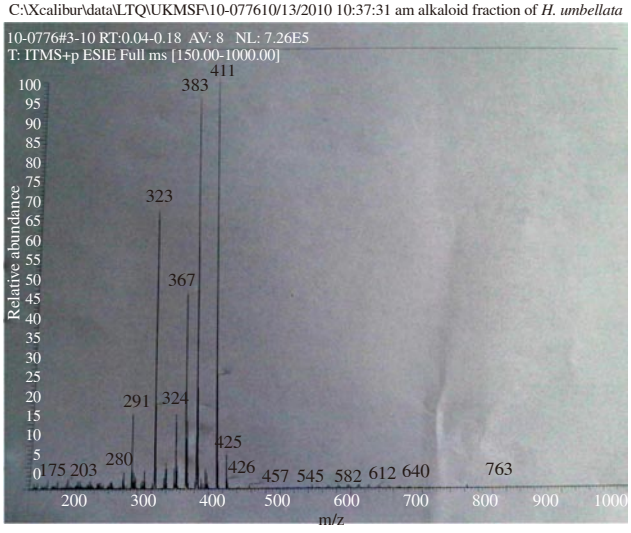

Figure 1. Full mass spectrometry of alkaloid fraction of *H. umbellata* showed major constituent alkaloid compounds of molecular weights of 411, 383, 323 and 367 and relative abundance of 100%, 95%, 67% and 47%, respectively.

3.3. Effect of alkaloid fraction of H. umbellata on the average body weight and weight changes in normal and triton-induced hyperlipidemic rats

Repeated daily oral treatments of rats with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* for 14 d, consecutively, resulted in significant ($P < 0.01$ and $P < 0.001$) and dose-dependent decreases in the average body weight and weight changes (%) when compared to the untreated normal (Group I) rats (Table 1). However, these changes in the body weights were comparable with that recorded for the group of rats treated with 20 mg/kg/day of simvastatin (Group II) (Table 1). Similar results were recorded in triton WR-1339 induced hyperlipidemic rats treated with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* (Table 2).

Table 1
Effect of repeated daily oral treatment with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* in the average body weight and weight changes (%ΔWt) of normal rats on Days 1 and 14.

Groups	Average body weight (g)		%ΔWt
	Day 1	Day 14	
I	127.30 ± 5.75	161.80 ± 6.71	27.12 ± 1.84
II	129.30 ± 7.42	146.80 ± 10.78^{c-}	13.47 ± 3.36
III	132.20 ± 6.27	154.20 ± 7.03^{b-}	16.67 ± 0.99^{b-}
IV	130.50 ± 4.72	144.00 ± 4.15^{c-}	10.45 ± 4.56^{c-}

$^{b-}$ and $^{c-}$ represent significant decreases at $P < 0.01$ and $P < 0.001$, respectively, when compared to Group I values.

Table 2
Effect of repeated daily oral treatment with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* in the average body weight and weight changes (%ΔWt) of rats on Days 1 and 14 before intraperitoneal injection of triton WR-1339.

Groups	Average body weight (g)		%ΔWt
	Day 1	Day 14	
I	131.80 ± 7.33	161.20 ± 4.75	22.48 ± 6.12
II	132.50 ± 5.43	159.80 ± 6.68	21.42 ± 4.02
III	133.50 ± 7.12	146.00 ± 12.46^{c-}	13.42 ± 2.57^{c-}
IV	134.80 ± 4.75	154.80 ± 5.91^{b-}	15.85 ± 2.66^{b-}
V	133.80 ± 5.78	139.50 ± 10.43^{c-}	12.08 ± 3.57^{c-}

$^{b-}$ and $^{c-}$ represent significant decreases at $P < 0.01$ and $P < 0.001$, respectively, when compared to Group I values.

3.4. Effect of alkaloid fraction of H. umbellata on the lipid profile of normal and triton-induced hyperlipidemic rats

Repeated daily oral treatments of rats with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* for 14 d, consecutively, resulted in significant ($P < 0.05$, $P < 0.01$ and $P < 0.001$) and dose-dependent reductions in the serum TG, TC, LDL-c and VLDL-c levels while 50 mg/kg/day of alkaloid fraction of *H. umbellata* alone caused a significant ($P < 0.05$) elevation in the serum HDL-c levels when compared to that recorded for the untreated normal rats (Table 3). However, in the triton WR-1339

induced hyperlipidemic rats, intraperitoneal injection of 200 mg/kg of triton WR-1339 to rats after 24 h resulted in significant ($P < 0.001$) elevations in the serum TG, TC, LDL-c and VLDL-c levels as well as significant ($P < 0.001$) decrease in the serum HDL-c levels (Group II rats) when compared to untreated normal values (Group I rats) (Table 4). Oral pre-treatments with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* significantly ($P < 0.01$ and $P<0.001$) and dose-dependently attenuated increases in the serum TG, TC, LDL-c and VLDL-c levels as well as decreases in the serum HDL-c when compared to Group II values (Table 4). These significant attenuations were comparable to triton-induced hyperlipidemic rats pre-treated with 20 mg/kg/day of simvastatin (Group II) (Table 4).

Table 3

Effect of repeated daily oral treatment with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* on the lipid profile of normal rats.

Groups	TG (mg/dL)	TC (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)	VLDL-c (mg/dL)
I	94.83 ± 2.46	85.50 ± 2.28	46.17 ± 1.22	22.17 ± 1.47	17.17 ± 0.79
II	74.17 ± 2.12^{c-}	63.17 ± 2.80^{c-}	43.33 ± 2.62	13.50 ± 1.36^{b-}	6.33 ± 0.92^{c-}
III	80.50 ± 2.83^{a-}	70.17 ± 2.65^{a-}	48.50 ± 1.12	22.33 ± 1.17	10.33 ± 1.17^{a-}
IV	69.17 ± 2.71^{c-}	65.17 ± 2.65^{c-}	54.00 ± 2.34^{a}	9.17 ± 1.08^{c-}	2.00 ± 0.26^{c-}

$^{a-}$, $^{b-}$ and $^{c-}$ represent significant decreases at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, when compared to Group I values, while ^{a} represents a significant increase at $P < 0.05$ when compared to Group I values.

Table 4

Effect of repeated daily oral treatment with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* on the lipid profile in triton WR-1339 induced hyperlipidemic rats.

Groups	TG (mg/dL)	TC (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)	VLDL-c (mg/dL)
I	95.17 ± 1.94	85.83 ± 1.97	47.17 ± 1.17	21.50 ± 0.89	17.17 ± 0.75
II	161.50 ± 1.92^{c}	129.70 ± 1.43^{c}	25.00 ± 1.69^{f-}	70.83 ± 4.18^{c}	33.83 ± 1.76^{c}
III	116.00 ± 5.03^{f}	100.80 ± 4.05^{f}	59.17 ± 3.89^{c+}	18.83 ± 1.60^{f}	18.83 ± 1.60^{f}
IV	134.30 ± 6.24^{e}	115.00 ± 4.03^{e}	60.33 ± 1.23^{c+}	35.50 ± 2.19^{f}	19.17 ± 1.68^{f}
V	125.80 ± 2.14^{f}	105.80 ± 3.79^{f}	69.17 ± 3.05^{c+}	23.67 ± 3.63^{f}	13.33 ± 2.46^{f}

^{c} and $^{f-}$ represent significant increase and decrease at $P < 0.001$, respectively, when compared to Group I values while ^{e} and ^{f} represent significant decreases at $P < 0.01$ and $P < 0.001$, respectively, when compared to Group II values; $^{c+}$ represents a significant increase at $P < 0.001$ when compared to Group II values.

3.5. Effect of alkaloid fraction of H. umbellata on cardiovascular risk indices (AI and CRI) of normal and triton-induced hyperlipidemic rats

Repeated oral treatments of normal rats with 50 mg/kg/day of alkaloid fraction of *H. umbellata* resulted in significant ($P < 0.001$) reductions in the AI and CRI values, effects that were comparable to that caused by 20 mg/kg/day of simvastatin (Figure 2). However, in the triton WR-1339-treated rats, oral pre-treatment with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* significantly ($P < 0.001$) reduced both AI and CRI values when compared to the untreated triton-induced hyperlipidemic rats (Figure 3).

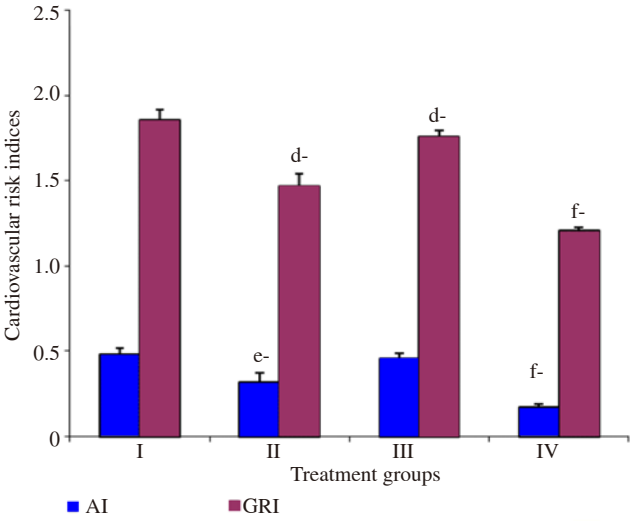

Figure 2. Effects of 14 d of repeated daily oral treatments with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* on the AI and CRI in normal rats.

$^{d-}$, $^{e-}$ and $^{f-}$ represent significant decreases at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, when compared to Group I values.

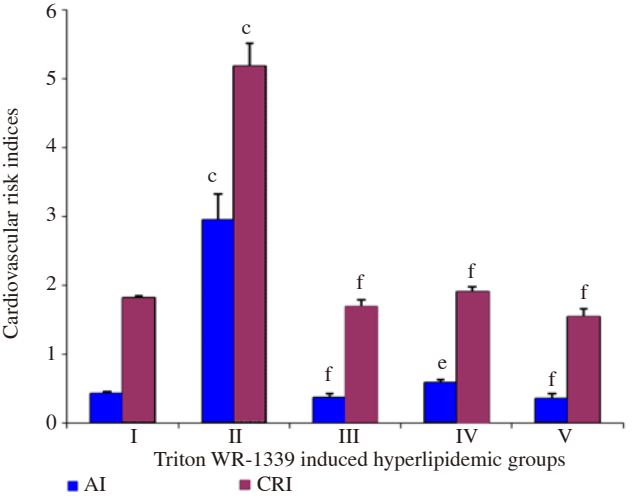

Figure 3. Effects of 14 d of repeated daily oral pretreatments with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* on the AI and CRI in triton WR-1339 induced hyperlipidemic rats.

^{c} represents a significant increase at $P < 0.001$ when compared to Group I values, while ^{e} and ^{f} represent significant decreases at $P < 0.01$ and $P < 0.001$, respectively, when compared to Group II values.

3.6. Histopathological effect of alkaloid fraction of H. umbellata pre-treatment on the hepatic tissues of normal and triton-induced hyperlipidemic rats

Treatment with 200 mg/kg of triton WR-1339 caused global fatty degeneration of hepatocytes with central hepatic vein and sinusoidal congestion (Figure 4) when compared to normal hepatic architecture (Figure 5). These histological changes were significantly attenuated by alkaloid fraction of *H. umbellata* pre-treatment with the most significant improvement induced by 50 mg/kg of alkaloid fraction of *H. umbellata* (Figure 6), in a similar pattern to that observed in simvastatin pretreated rats (Figure 7).

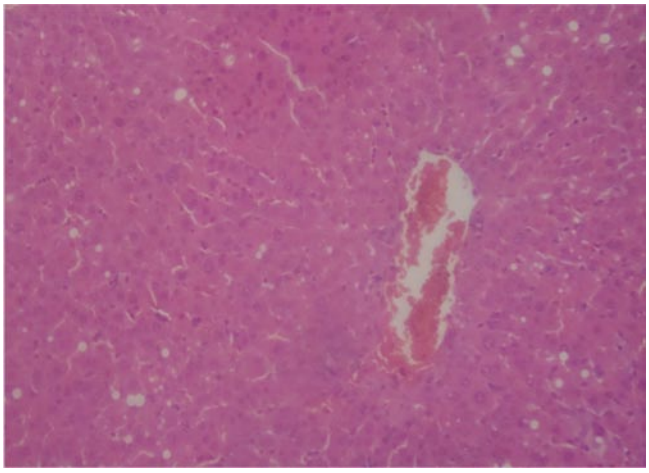

Figure 4. A representative section of triton WR-1339-treated rats liver showed congested central hepatic vein and sinusoids as well as global hepatic fatty degeneration (hematoxylin and eosin stain, ×100 magnification).

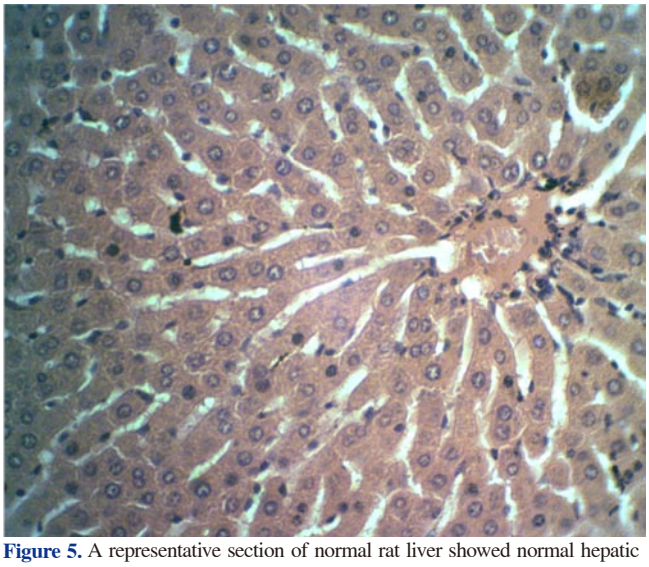

Figure 5. A representative section of normal rat liver showed normal hepatic architecture (hematoxylin and eosin stain, ×400 magnification).

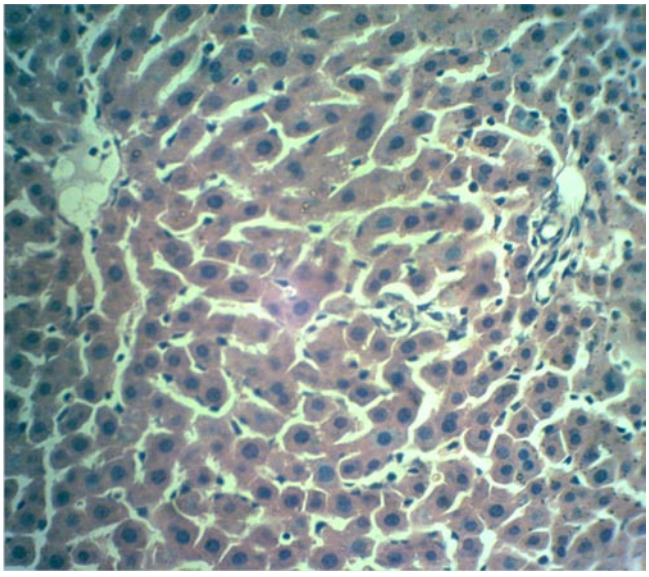

Figure 6. A representative section of 50 mg/kg of alkaloid fraction of *H. umbellata*-pretreated rat liver showed mildly congested central hepatic vein, portal triad, sinusoidal congestion and normal hepatocytes (hematoxylin and eosin stain, ×400 magnification).

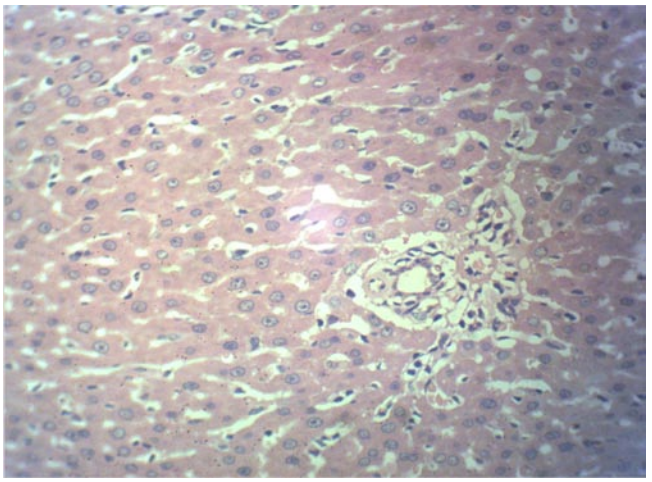

Figure 7. A representative section of 20 mg/kg of simvastatin-pretreated rat liver showed congested hepatic portal triad, mild sinusoidal congestion and normal hepatocytes (hematoxylin and eosin stain, ×400 magnification).

4. Discussion

The non-ionic detergent, triton WR-1339 (tyloxapol or an oxyethylated tertiary octyl phenol formaldehyde polymer), is a widely used acute experimental hyperlipidemia-inducing agent in animals[17,18]. It acts by inhibiting lipoprotein lipase activity, thus, blocking the uptake of triacylglycerol-rich lipoproteins from plasma by peripheral tissues and resulting in enhanced hepatic cholesterol biosynthesis through stimulation of HMG-CoA reductase activity, in addition to causing plasma/serum accumulation of TGs, LDL-c and VLDL-c[19,20]. Previous experimental data strengthens the fact that triton WR-1339 physically alters VLDL-c, rendering them refractive to the action of lipolytic enzymes of blood and tissue[21]. This prevents or delays their removal from blood and secondarily stimulates the hepatic cholesterol biosynthesis, thus, enhancing hyperlipidemia[22-24]. Thus, triton-induced hyperlipidemia is marked by significant increases in the serum TC, TGs, phospholipids, LDL, VLDL levels and a decrease in the serum HDL-c level in the triton-treated rats[24,25]. The fact that single intraperitoneal injection of 200 mg/kg of triton WR-1339 to the treated rats resulted in profound hypertriglyceridemia and hypercholesterolemia (especially with TC, LDL-c and VLDL-c) within 24 h post-triton administration which is consistent with reports of other studies is a strong evidence that acute hyperlipidemia was successfully established[17,26-28]. However, oral pretreatments with 25 and 50 mg/kg/day of alkaloid fraction of *H. umbellata* for 14 d effectively attenuated significant increases in the serum TG, TC, LDL-c, and VLDL-c levels as well as significantly attenuated the decreases in the serum HDL-c levels in the treated rats, thereby, demonstrating the antihyperlipidemic activity of alkaloid fraction of *H. umbellata*. Literature has shown that a sudden increase in the serum lipid levels reaching a peak of 2-3 folds within 24 h post-triton WR-1339 injection represents Phase I (synthesis phase) while a significant decrease in hyperlipidemia afterwards indicates Phase II (the elimination phase) of cholesterol metabolism[24]. The fact that there was a significant decrement in the lipid profile of the alkaloid

fraction of *H. umbellata*-pretreated hyperlipidemic rats within 24 h following hyperlipidemia induction with triton WR-1339 suggests that alkaloid fraction of *H. umbellata* mediates its antihyperlipidemic effect via cholesterol biosynthesis inhibitory mechanism which is related to HMG-CoA reductase and/or lipoprotein lipase inhibitory activities (established antihyperlipidemic mechanisms for the standard drug, simvastatin and other statins). Moreover, results of the present study corroborate that of earlier studies in which the crude aqueous extract of *H. umbellata* seeds was reported to control hyperlipidemia via *in vivo* inhibition of cholesterol biosynthesis[9].

Another notable finding of this study is the effect of alkaloid fraction of *H. umbellata* on both the coronary artery and atherogenic indices. Hyperlipidemia has been reported to be associated with the increased ~~the~~ risk of coronary artery disease and atherogenesis[29-31]. Thus, the profound attenuations of significant increases in both the coronary artery and atherogenic indices by alkaloid fraction of *H. umbellata* pretreatment also demonstrated the cardioprotective effect of alkaloid fraction of *H. umbellata*. In addition, the results presented here were significant since it is the first study designed at investigating the antihyperlipidemic effect of alkaloid fraction of *H. umbellata* in female Wistar rats. Literature has shown that the female gender increases the preponderance for cardiovascular and coronary artery disease including hyperlipidemia[32,33].

The weight losses recorded in this study are also significant findings. Repeated oral pretreatments with alkaloid fraction of *H. umbellata* for 14 d showed that alkaloid fraction of *H. umbellata*, apart from having antihyperlipidemic and cardioprotective effects also possesses weight losing effect. Literature has shown that excessive weight gain (obesity) increases the risk of coronary and cardiovascular diseases while weight loss decreases the preponderance for cardiovascular desease[34-41]. The fact that alkaloid fraction of *H. umbellata* caused significant weight loss in the treated rats is a strong indication that alkaloid fraction of *H. umbellata* has cardioprotective effect, thus, corroborating our earlier assertion. However, previous studies have reported *H. umbellata* possesses weight losing, antihyperlipidemic and cardioprotective activities[9]. Therefore, results of the present study suggest that one or more of the compounds in alkaloid fraction of *H. umbellata* highlighted in the spectral analysis of alkaloid fraction of *H. umbellata* could be responsible for these observed pharmacological effects.

Overall, the present study has demonstrated that alkaloid fraction of *H. umbellata*, at oral doses of 25 and 50 mg/kg/day, elicited weight losing, antihyperlipidemic and cardioprotective effects in triton WR-1339-induced hyperlipidemic rats since it was able to profoundly attenuate hypertriglyceridemia and hypercholesterolemia as well as attenuated increases in both the coronary artery and atherogenic indices in the treated female Wistar rats which was probably mediated via *de novo* cholesterol biosynthesis.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014; **384**(9945): 766-81.

[2] National Heart, Lungs and Blood Institute. What are the health risks of overweight and obesity? Bethesda: National Heart, Lungs and Blood Institute. [Online] Available from: http://www.nhlbi.nih.gov/health/health-topics/topics/obe/risks [Accessed on 15th October, 2014]

[3] Stevens GA, Singh GM, Lu Y, Danaei G, Lin JK, Finucane MM, et al. National, regional, and global trends in adult overweight and obesity prevalences. *Popul Health Metr* 2012; **10**: 22.

[4] Boutayeb A, Boutayeb S, Boutayeb W. Multi-morbidity of non communicable diseases and equity in WHO Eastern Mediterranean countries. *Int J Equity Health* 2013; **12**: 60.

[5] Adegoke EA, Alo B. Abere-amines: water soluble seed alkaloids from *Hunteria umbellata*. *Phytochemistry* 1986; **25**(6): 1461-8.

[6] Falodun A, Nworgu ZA, Ikponmwonsa MO. Phytochemical components of *Hunteria umbellata* (K. Schum) and its effect on isolated non-pregnant rat uterus in oestrus. *Pak J Pharm Sci* 2006; **19**(3): 256-8.

[7] Adeneye AA, Adeyemi OO. Hypoglycaemic effects of the aqueous seed extract of *Hunteria umbellata* in normoglycaemic and glucose- and nicotine-induced hyperglycaemic rats. *Int J Appl Res Nat Prod* 2009; **2**(1): 9-18.

[8] Igbe I, Omogbai EKI, Ozolua RI. Hypoglycemic activity of aqueous seed extract of *Hunteria umbellata* in normal and streptozotocin-induced diabetic rats. *Pharm Biol* 2009; **47**(10): 1011-6.

[9] Adeneye AA, Adeyemi OO, Agbaje EO. Anti-obesity and antihyperlipidaemic effect of *Hunteria umbellata* seed extract in experimental hyperlipidaemia. *J Ethnopharmacol* 2010; **130**(2): 307-14.

[10] Adeneye AA, Sofidiya MO, Adenekan SO. Anti-inflammatory and antioxidant activities of *Hunteria umbellata* seed fractions. *Pharmacologia* 2011; **2**(6): 165-71.

[11] Adeneye AA, Crooks PA, Miller AF, Goodman J, Adeyemi OO, Agbaje EO. Isolation and structure elucidation of a new indole alkaloid, erinidine, from *Hunteria umbellata* seeds. *Pharmacologia* 2012; **3**(7): 204-14.

[12] Ibeh IN, Idu M, Ejimadu M. Toxicological assessment of 'Abeere' seed

Hunteria umbellata K. Schum. (Apocynaceae). *Biociência* 2007; **15**(1): 4-7.

[13] Adeneye AA, Adeyemi OO, Agbaje EO, Banjo AA. Evaluation of the toxicity and reversibility profile of the aqueous seed extract of *Hunteria umbellata* (K. Schum.) Hallier f. in rodents. *Afr J Tradit Complement Altern Med* 2010; **7**(4): 350-69.

[14] Council for International Organizations of Medical Science and the International Council for Laboratory Animal Science. International guiding principles for biomedical research involving animals 2012. Geneva: Council for International Organizations of Medical Science; 2012. [Online] Accessible from: http://grants.nih.gov/grants/olaw/Guiding_Principles_2012.pdf

[15] Kanthe PS, Patil BS, Bagali S, Deshpande A, Banu Shaikh G, Aithala M. Atherogenic index as a predictor of cardiovascular risk among women with different grades of obesity. *Int J Collab Res Int Med Public Health* 2012; **4**(10): 1767-74.

[16] Gil-Guillén VF, Orozco-Beltrán D, Pita-Fernández S, Carratalá-Munuera C, Redón J, Navarro J, et al. In the identification of cardiovascular risk with the SCORE model, could we recommend its calculation interchangeably with total cholesterol or atherogenic index? Concordance between total cholesterol and atherogenic index in the SCORE table. *Rev Esp Cardiol* 2011; **64**(5): 421-3.

[17] Harnafi H, Caid HS, Bouanani NEH, Aziz M, Amrani S. Hypolipemic activity of polyphenol-rich extracts from *Ocimum basilicum* in triton WR-1339-induced hyperlipidemic mice. *Food Chem* 2008; **108**(1): 205-12.

[18] Bertges LC, Mourão CAJ, Souza JB, Cardoso VAC. Hyperlipidemia induced by triton WR1339 (tyloxapol) in Wistar rats. *Rev Bras Cien Med Saúde* 2011; **1**(1): 32-4.

[19] Janicki BW, Aron SA. Effect of triton WR-1339 on lipoprotein lipase of guinea pig plasma. *Proc Soc Exp Biol Med* 1962; **109**: 507-9.

[20] Lomnicky Y, Friedman M, Luria MH, Raz I, Hoffman A. The effect of the mode of administration on the hypolipidaemic activity of niacin: continuous gastrointestinal administration of low-dose niacin improves lipid-lowering efficacy in experimentally-induced hyperlipidaemic rats. *J Pharm Pharmacol* 1998; **50**: 1233-9.

[21] Friedman M, Byers SO. The mechanism responsible for the hypercholesteremia induced by triton WR-1339. *J Exp Med* 1953; **97**: 117-30.

[22] Goldfarb S. Rapid increase in hepatic HMG CoA reductase activity and *in vivo* cholesterol synthesis after triton WR 1339 injection. *J Lipid Res* 1978; **19**: 489-94.

[23] Rajasekaran A, Sivakumar V, Darlinquine S. Effect of *Blepharis maderaspatensis* L. Roth. extracts on serum lipids in triton WR-1339 and high cholesterol diet induced hyperlipidemia in rats. *Afr J Pharm Pharmacol* 2013; **7**(37): 2577-83.

[24] Sikarwar MS, Patil MB. Antihyperlipidemic activity of *Salacia chinensis* root extracts in triton-induced and atherogenic diet-induced hyperlipidemic rats. *Indian J Pharmacol* 2012; **44**: 88-92.

[25] Pandit K, Mishra R, Brijesh S, Bhagwat A, Bhatt P. Lipid lowering activity of *Feronia limonia* leaf in triton WR-1339 (tyloxapol) induced hyperlipidemic rats. *Int J Pharm Pharm Sci* 2014; **6**(8): 156-8.

[26] Otway S, Robinson DS. The effect of a non-ionic detergent (triton WR 1339) on the removal of triglyceride fatty acids from the blood of the rat. *J Physiol* 1967; **190**: 309-19.

[27] Mishra R, Karmarkar SM, Bhagwat AM. Preliminary dose dependent study on anti-hyperlipidemic activity of *Hibiscus rosa sinensis* Linn. leaves on triton WR 1339 induced hyperlipidemic mice model. *Asian J Pharm Clin Res* 2011; **4**(Suppl 2): 100-2.

[28] da Rocha JT, Sperança A, Nogueira CW, Zeni G. Hypolipidaemic activity of orally administered diphenyl diselenide in Triton WR-1339-induced hyperlipidaemia in mice. *J Pharm Pharmacol* 2009; **61**: 1673-9.

[29] Mente A, de Koning L, Shannon HS, Anand SS. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. *Arch Intern Med* 2009; **169**(7): 659-69.

[30] Kanter MM, Kris-Etherton PM, Luz Fernandez M, Vickers KC, Katz DL. Exploring the factors that affect blood cholesterol and heart disease risk: is dietary cholesterol as bad for you as history leads us to believe? *Adv Nutr* 2012; **3**: 711-7.

[31] Djoussé L, Gaziano JM. Dietary cholesterol and coronary artery disease: a systematic review. *Curr Atheroscler Rep* 2009; **11**(6): 418-22.

[32] Schwab KO, Doerfer J, Naeke A, Rohrer T, Wiemann D, Marg W, et al. Influence of food intake, age, gender, HbA1c, and BMI levels on plasma cholesterol in 29,979 children and adolescents with type 1 diabetes–reference data from the German diabetes documentation and quality management system (DPV). *Pediatr Diabetes* 2009; **10**: 184-92.

[33] Kautzky-Willer A, Stich K, Hintersteiner J, Kautzky A, Kamyar MR, Saukel J, et al. Sex-specific-differences in cardiometabolic risk in type 1 diabetes: a cross-sectional study. *Cardiovasc Diabetol* 2013; **12**: 78.

[34] Haque AK, Gadre S, Taylor J, Haque SA, Freeman D, Duarte A. Pulmonary and cardiovascular complications of obesity: an autopsy study of 76 obese subjects. *Arch Pathol Lab Med* 2008; **132**(9): 1397-404.

[35] Held M, Mittnacht M, Kolb M, Karl S, Jany B. Pulmonary and cardiac function in asymptomatic obese subjects and changes following a structured weight reduction program: a prospective observational study. *PLoS One* 2014; doi: 10.1371/journal.pone.0107480.

[36] Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association scientific statement on obesity and heart disease from the obesity committee of the council on nutrition, physical activity, and metabolism. *Circulation* 2006; **113**: 898-918.

[37] Litwin SE. Which measures of obesity best predict cardiovascular risk? *J Am Coll Cardiol* 2008; **52**: 616-9.

[38] Gelber RP, Gaziano JM, Orav EJ, Manson JE, Buring JE, Kurth T. Measures of obesity and cardiovascular risk among men and women. *J Am Coll Cardiol* 2008; **52**(8): 605-15.

[39] Lavie CJ, Milani RV, Ventura HO. Obesity and cardiovascular disease: risk factor, paradox, and impact of weight loss. *J Am Coll Cardiol* 2009; **53**(21): 1925-32.

[40] Boden G, Salehi S. Why does obesity increase the risk of cardiovascular disease? *Curr Pharm Des* 2013; **19**(32): 5678-83.

[41] Wing RR, Lang W, Wadden TA, Safford M, Knowler WC, Bertoni AG, et al. Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 diabetes. *Diabetes Care* 2011; **34**(7): 1481-86.