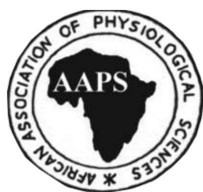

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Research Article

Ursolic acid prevents the development of metabolic syndrome in male Wistar rats fed a high-carbohydrate high-fat diet

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Keywords:

Ursolic acid, metabolic syndrome, high-carbohydrate high-fat diet, 20 weeks, obesity, insulin resistance, fasting blood glucose, dyslipidemia

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ABSTRACT

Background: Metabolic syndrome (MS); which is mostly caused by a high-carbohydrate high-fat diet (HCHFD) as well as a sedentary lifestyle; is associated with an increased risk of cardiovascular and hepatic complications. In this study, we investigated the effect of supplementation with ursolic acid (UA) on MS parameters induced by HCHFD in male Wistar rats. **Methods:** Twenty male Wistar rats, aged 8-9 weeks old, weighing 120 - 170 grams, and randomly divided into 4 groups (n =5) were used. Group I received normal diet (ND) and distilled water (DW); group II received ND and UA; group III received HCHFD and DW; group IV received HCHFD and UA. HCHFD was formulated in-house and the drinking water was augmented with 20% fructose. The animals were fed their respective diets daily for 20 weeks. A dose of 250 mg/kg body weight of ursolic acid was adopted and administered orally to UA-treated groups starting 12 weeks after initiation of the HCHFD for a further 8 weeks. Body weight, body mass index (BMI), and fasting blood glucose (FBG) were measured every four weeks and percentage increases were determined. An oral glucose tolerance test (OGTT) was performed and the area under the curve (AUC) was determined. Blood samples were obtained for serum insulin and lipid profile. Insulin resistance was determined using the homeostatic model assessment for insulin resistance (HOMA-IR). Histopathological evaluation of liver tissue was performed using the hematoxylin and eosin staining technique. **Results:** The increase in BMI and FBG of the HCHFD+UA group was significantly lower ($P<0.05$) compared to the HCHFD+DW group. The HCHFD+DW group had a higher ($P<0.05$) HOMA-IR and AUC for OGTT compared to HCHFD+UA. There was a significant decrease ($P<0.05$) in serum insulin, cholesterol, triglyceride, and LDL-C in the HCHFD+UA group compared to the HCHFD+DW group, while HDL-C significantly ($P<0.05$) increased in the HCHFD+UA group compared to HCHFD+DW group. **Conclusion:** In this study, UA supplementation prevented the development of MS in male Wistar rats fed with HCHFD for 20 weeks. This suggests that UA has the potential to be considered for the management of MS.

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Introduction

Some of the major public health challenges are cardiovascular disease (CVD) and type 2 diabetes (Zimmet *et al*., 2001). Metabolic syndrome (MS), which is a cluster of risk factors for CVD and type 2 diabetes, increases cardiovascular mortality (Hunt *et al*., 2004; Sattar *et al*., 2005). According to the Adult Treatment Panel III criteria of the National Cholesterol and Education Program (NCEP), these risk factors have been grouped as diagnostic criteria for MS which are: central obesity, elevated blood pressure, impaired glucose tolerance, insulin resistance, and dyslipidemia and the presence of any three features is considered sufficient to diagnose the syndrome (NCEP, 2001; Eckel *et al*., 2005; Cornier *et al*., 2008; Mccracken *et al*., 2018).

The global prevalence of MS is increasing and it is determined by variation in age, gender, ethnicity, race, and definition of MS (Cornier *et al*., 2008), with a sedentary lifestyle and overnutrition being major contributors. According to a recent report on the global prevalence of MS by Belete *et al*. (2021), 23.7% of patients with type 1 diabetes mellitus (T1DM) had MS. There was a high prevalence (58%) of MS in type 2 diabetics (Nsiah *et al*., 2015).

Studies conducted by Unamba (2017) showed that the prevalence of the metabolic syndrome is 28.04% in Nigeria. Patients with the risk factors of MS require about 20% more healthcare costs than those without risk factors, resulting in a huge economic burden (Marangos *et al*., 2010).

Investigating the complicacy of human MS caused by unhealthy lifestyle and eating habits requires the usage of a variety of diet-induced animal models of MS. Although the pathogenesis of MS is complex and still being unraveled, various diet-induced MS animal models have been established (Aleixandre de Artinano and Miguel Castro, 2009; Lehnen *et al*., 2013) to enhance our comprehension of the cause and origin of MS as well as its pathophysiological basis and development of therapies. According to previous studies in rats, high-sucrose (Pang *et al*., 2008) and high-fructose (Thirunavukkarasu *et al*., 2004) diets induced the components of MS except for central obesity. On the contrary, rats fed with a high-fat diet developed central obesity (Buettner *et al*., 2006). Epidemiological studies of sugar consumption and diabetes/metabolic disorders' prevalence (Alam *et al*., 2013; Basu *et al*., 2013) suggest that a diet rich in fat, as well as sugar, is a greater risk factor for these disorders than a diet that is rich in either fats or sugar. To induce the pathogenesis of MS in rats, as obtainable in humans, the best diet suggested is one that contains high fats and -carbohydrates (mainly fructose) (Panchal and Brown, 2011; Wong *et al*., 2016). HCHFD in rats has been reported to induce all components of MS as well as decreased antioxidant defense systems and increased proinflammatory biomarkers (Schaalan *et al*., 2009; Panchal *et al*., 2011).

Ursolic acid (UA), is a naturally occurring pentacyclic triterpenoid. Many plants, including apples, have high concentrations of UA, and have become an integral part of the human diet (Jager *et al*., 2009). Several studies, both in vitro and in vivo, have revealed that UA has diverse biological roles, including anti-inflammatory (Kashyap *et al*., 2016), anti-oxidative (Liobikas *et al*., 2011), anti-carcinogenic (Shishodia *et al*., 2003), anti-obesity (Jayaprakasam *et al*., 2006), and anti-diabetic activities (Kwon *et al*., 2018). However, it is not known if UA could be beneficial in the prevention of MS induced by HCHFD in male Wistar rats. Therefore, this study investigated the effect of UA on MS parameters induced by HCHFD in male Wistar rats.

Materials and Methods

Ethical approval and adherence

Ethical approval for the study was obtained from the Ahmadu Bello University Committee on Animal Use and Care (Approval No: ABUCAUC/2020/37). The experiment was conducted by following the laboratory care and policy on animal research of Ahmadu Bello University, Zaria.

Experimental Animals

A total of twenty (20) male Wistar rats, 8-9 weeks old and weighing 120 - 170 grams were sourced from the animal house, Department of Human Physiology, Ahmadu Bello University, Zaria. They were housed in well-aerated plastic cages and allowed to acclimatize having free access to commercial grower mash feed and water ad libitum. After two weeks of acclimatization, the cages' beddings were changed from shaving sawdust to aluminum beddings. This allowed us to quantify spill over food and measure total daily food consumption.

Formulation of high-carbohydrate high-fat diet (HCHFD):

The high-carbohydrate, high-fat diet (HCHFD) was formulated in-house following the method of Panchal *et al*. (2011) and Wong *et al*. (2017) with little modification. The high-carbohydrate, high-fat diet consists of condensed milk (39.5%), fructose (17.5%), thermally oxidized palm oil (20%), Powdered rat food (15.5%), Hubble, Mendel & Wakeman (HMW) salt mixture (2.5%), and water (5%) together with 20% fructose in drinking water. Proximate analysis of the diets was carried out by the method of AOAC (2006) and the results are presented in table 1 below.

Table 1: Macronutrients in a normal diet and HCHF diet

Macronutrient (g/100g)	Normal diet	HCHF diet
Total carbohydrate	57.24	53.82
Total fat	4.78	17.29
Protein	25.76	14.05
Crude fibre	2.68	1.44
Ash	5.62	3.40
Moisture	4.06	10.02

Note: Drinking water in the HCHFD-fed rats was augmented with 20% fructose.

Preparation of thermally oxidized palm oil

Fresh palm oil was purchased from the Samaru market in Zaria, Nigeria. Fresh palm oil was thermally oxidized as described previously (Osim *et al*., 1992). Briefly, fresh palm oil was subjected to heat at 150°C in a stainless-steel pot. The heating was for five rounds, and each round lasted 20 minutes. After each round, the oil was allowed to cool for 5 hours. The obtained thermally oxidized palm oil was then used in the formulation of HCHFD.

Experimental Design

After two weeks of acclimatization, animals were randomly divided into 4 groups and treated as follows:

(i) Group 1: Normal diet-fed rats + Distilled water (ND+DW; $n = 5$),
(ii) Group 2: ND + Ursolic acid (ND+UA; $n = 5$),
(iii) Group 3: High-carbohydrate high-fat diet-fed rats (HCHFD) + Distilled water (DW) (HCHFD + DW, $n = 5$),
(iv) Group 4: HCHFD + Ursolic acid (HCHFD+UA, $n = 5$).

Feeding of animals and administration of ursolic acid

The animals were fed their respective diets daily for 20 weeks. The ND-fed rats were provided with normal tap water while drinking water in the HCHFD-fed rats was augmented with 20% fructose. A dose of 250 mg/kg body weight of ursolic acid (Zhang *et al*., 2016) was adopted. Ursolic acid was dissolved in an equal volume of distilled water and 50% DMSO, and administered orally through oral gavage to both ND+UA and HCHFD+UA groups starting 12 weeks after initiation of the HCHFD for a further 8 weeks period.

Determination of percentage change in body mass index

All rats were monitored daily for food and water intake. The body weight of rats was measured every 4 weeks from week 0 until week 12 and measured weekly from week 12 until week 20 using a standard weighing scale, while body length (nose to anus) and abdominal circumference were measured using a standard measuring tape every 4 weeks. The body mass index (BMI) of each rat was calculated as body weight (in grams) / [body length (in centimeters)]2 (Panchal *et al*., 2011). The difference between BMI values at weeks 0 and 20 were determined and used to calculate the percentage change (increase).

Determination of percentage change in fasting blood glucose level and area under curve for oral glucose tolerance test

Fasting blood glucose (FBG) was measured at weeks 0, 4, 8, 12, 16, and 20 after overnight fasting using a blood sample taken from the tail vein with ACCU Check Performa glucometer (Roche Diagnostic, USA). For overnight fasting, rats were deprived of all types of diets for 12 hours. The difference between FBG values at weeks 0 and 20 were determined and used to calculate the percentage change (increase). After measuring the fasting blood glucose in the 20th week, an oral glucose

tolerance test (OGTT) was performed. Rats were given a glucose load of 2 g/kg body weight as 40% glucose solution via oral gavage (Tang *et al.*, 2018). Measurement of blood glucose concentrations was done at 30, 60, 90, and 120 min after oral glucose administration. The area under the curve (AUC) of the concentration-time curve was calculated via the trapezoidal rule (Tai, 1994).
The total area under the fasting blood glucose curve was determined using the formula described by Tai, (1994).

$$\text{Area} = \tfrac{1}{2} \sum_{i=1}^{n} \mathrm{Xi} - 1\ (\mathrm{Yi} - 1 + \mathrm{Yi})$$

$$= \frac{1}{2}[X_0 \times (Y_0 + Y_1) + X_1 \times (Y_1 + Y_2) + ... + X_{n-1} \times (Y_{n-1}$$

Where, X is time and Y is glucose concentration.

Calculation of percentage change or increase

Change = values at week 20 – values at week 0.

If the change is positive, it means there is an increase and if negative, it means there is a decrease. In this study, all changes were positive, meaning there were increases. Therefore, in this study, the percentage increase was calculated as follows:

Percentage increase (%) = increase/values at week 0 x 100.

Animal termination and collection of blood samples

At the end of 20 weeks, animals were fasted overnight for 12 hours and anesthetized using a combination of 75 mg/kg body weight of ketamine and 5 mg/kg body weight of diazepam injection, blood sample was collected via cardiac puncture (Flecknell, 2009). The blood sample, collected in a plain bottle, was centrifuged for 10 minutes at 6000RPM to separate the blood into serum and clotted blood cells. The serum was collected and stored at -70°C for further biochemical analyses.

Biochemical analyses

The lipid profile was analyzed by measuring serum levels of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and triglycerides (TG) using commercial colorimetric assay kits (Agape, Switzerland).

Serum insulin concentration was measured using a Fine Test Rat Insulin ELISA kit (Coon Koon Biotech, Shanghai, China) according to the manufacturer's instructions.

Histopathological evaluation of liver tissue

Liver tissues were harvested and prepared using routing tissue processing techniques outlined by Bancroft (2018). The prepared tissues were placed immediately into the fixative (10 % formal saline). After proper fixing for about 48 hours, the tissues were dehydrated through ascending grades of alcohol from 70% alcohol to 90% alcohol and absolute (100%) alcohol for 16 hours. The tissues were then cleared in toluene for 2 hours after which they were impregnated in molten paraffin for 4 hours. Thereafter, the tissues were then embedded in paraffin wax and sectioned using a rotary microtome at 5 µ thickness. The sections were then stained using the hematoxylin and eosin (H&E) staining technique. The stained sections were examined using a light microscope and relevant photomicrographs were taken at the Histology unit of the Department of Human Anatomy, Ahmadu Bello University, Zaria, using an Amscope digital camera for microscope (DCM500), 5M pixels, made in Japan.

STATISTICAL ANALYSIS

Data were expressed as mean ± standard error of the mean (SEM). Data were analyzed using one-way analysis of variance (ANOVA), followed by the Tukey post-hoc test. Values at P<0.05 was considered statistically significant. The SPSS version 23 (IBM, Armonk, NY, USA) was used for statistical analysis.

Results

Effect of ursolic acid on body mass index in rats fed a high-carbohydrate high-fat diet

Figure 1 shows the effects of HCHFD and UA treatment on the percentage increase in BMI of all the groups. The ND+UA-fed animals had a significantly (P<0.05) lower BMI increase (12.81±1.07) compared to the ND+DW group (23.84±0.57). The HCHFD+DW group had a significantly (P<0.05) higher BMI increase (50.0±0.69) compared to the ND+DW group (23.84±0.57). The HCHFD+UA-fed animals had a significantly (P<0.05) lower BMI increase (34.78±0.30) compared to the HCHFD+DW group (50.0±0.69).

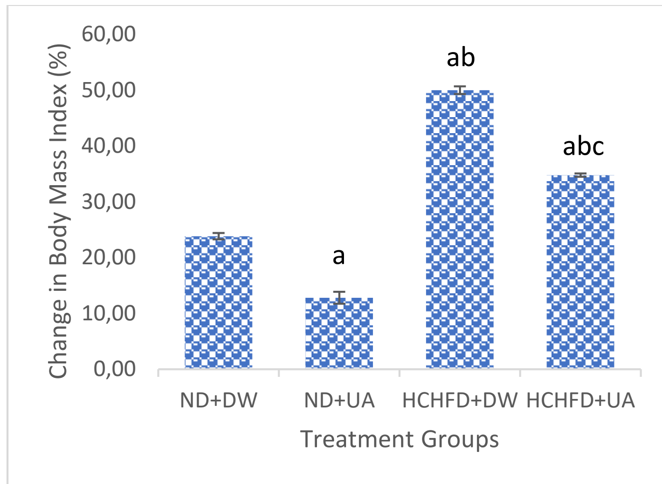

Figure 1: Percentage change in body mass index (BMI) of male Wistar rats fed HCHFD and UA. Data were expressed as Mean±SEM (n=5). a = significant vs ND+DW at P<0.05; b = significant vs ND+UA at P<0.05; c = significant vs HCHFD+DW at P<0.05. ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.

Fasting blood glucose changes in male Wistar rats fed with HCHFD and UA at weeks 0, 4, 8, 12, 16 and 20

Figure 2 shows the four-weekly trend in fasting blood glucose (FBG) changes in all the groups from week 0 to 20.

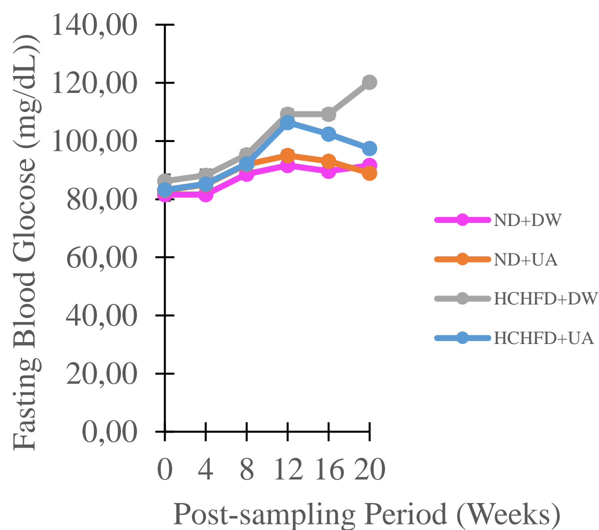

Figure 2: Fasting blood glucose changes in male Wistar rats fed with HCHFD and UA at weeks 0, 4, 8, 12, 16, and 20. Data were expressed as Mean±SEM (n=5). ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.

Effect of ursolic acid on fasting blood glucose in rats fed a high-carbohydrate high-fat diet

Figure 3 shows the effects of HCHFD and UA treatment on the percentage increase in FBG of all the groups. The ND+UA-fed animals had a significantly (P<0.05) lower FBG increase (7.39±1.57) compared to the ND+DW group (12.59±3.47). The HCHFD+DW group had a significantly (P<0.05) higher FBG increase (39.68±2.32) compared to the ND+DW group (12.59±3.47). The HCHFD+UA-fed animals had a significantly (P<0.05) lower FBG increase (17.26±1.97) compared to the HCHFD+DW group (39.68±2.32).

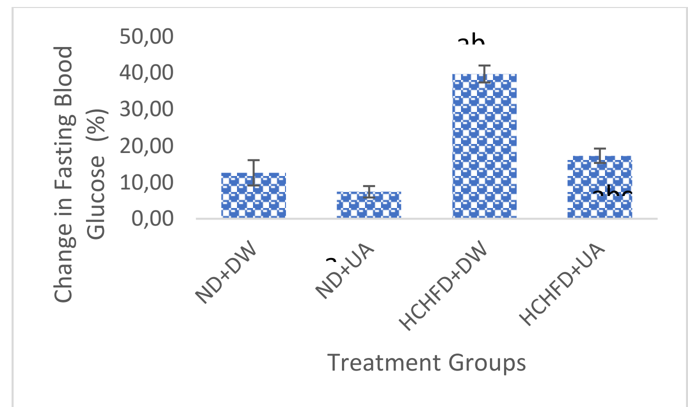

Figure 3: Percentage change in fasting blood glucose (FBG) of male Wistar rats fed HCHFD and UA. Data were expressed as Mean±SEM (n=5). a = significant vs ND+DW at P<0.05; b = significant vs ND+UA at P<0.05; c = significant vs HCHFD+DW at P<0.05. ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.

Effect of ursolic acid on oral glucose tolerance test in rats fed a high-carbohydrate high-fat diet

Figure 4 shows the effects of HCHFD and UA supplementation on the area under the curve (AUC) of blood glucose measurement for OGTT in all the groups. The ND-fed groups had similar AUC of blood glucose ($AUC_{glucose}$). The HCHFD+DW group had a significantly higher (P<0.05) $AUC_{glucose}$ (5008.90±22.91) compared to the ND+DW group (3788.80±17.23). The HCHFD+UA-fed animals had a significantly lower (P<0.05) $AUC_{glucose}$ (4066.70±19.87) compared to the HCHFD+DW group (5008.90±22.91).

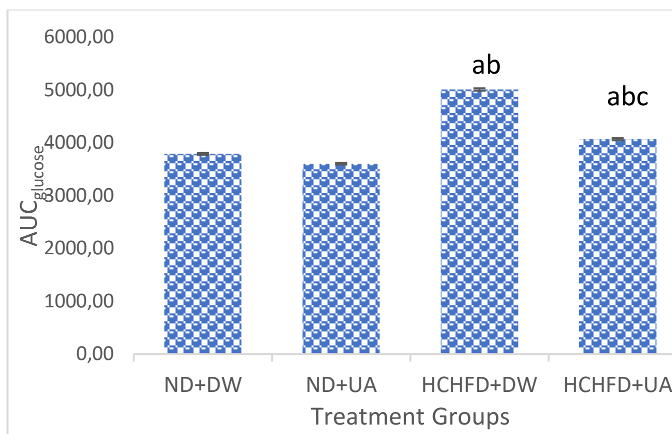

Figure 4: Area under the curve for glucose tolerance test of male Wistar rats fed HCHFD and UA. Data were expressed as Mean±SEM (n=5). a = significant vs ND+DW at P<0.05; b = significant vs ND+UA at P<0.05; c = significant vs HCHFD+DW at P<0.05. ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.

Effect of ursolic acid on fasting insulin and insulin resistance in rats fed a high-carbohydrate high-fat diet

Figure 5 shows levels of serum insulin in all the groups. The level of insulin was similar among ND-fed groups. There was a significant increase (P<0.05) in fasting insulin level in the HCHFD+DW group (123.76±0.95) compared to the ND+DW group (73.84±0.72). The HCHFD+UA-fed animals had a significantly lower (P<0.05) insulin level (95.64±0.48) compared to the HCHFD+DW group (123.76±0.95).

Figure 6 shows levels of homeostasis model assessment for insulin resistance (HOMA-IR) in all the groups. HOMA-IR level was similar among ND-fed groups. There was a significant increase (P<0.05) in HOMA-IR level in the HCHFD+DW group (36.72±0.53) compared to the ND+DW group (16.70±0.34). The HCHFD+UA-fed animals had a significantly lower (P<0.05) HOMA-IR level (23.0±0.34) compared to the HCHFD+DW group (36.72±0.53).

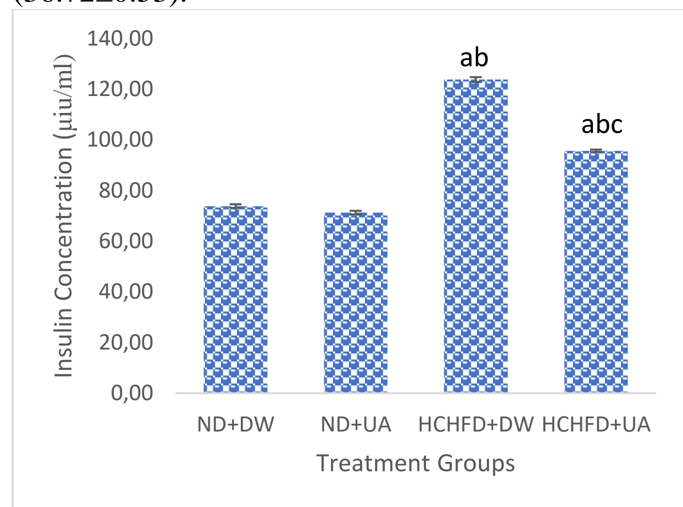

Figure 5: Serum insulin concentration of male Wistar rats fed HCHFD and UA. Data were expressed as Mean±SEM (n=5). a = significant vs ND+DW at P<0.05; b = significant vs ND+UA at P<0.05; c = significant vs HCHFD+DW at P<0.05. ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.

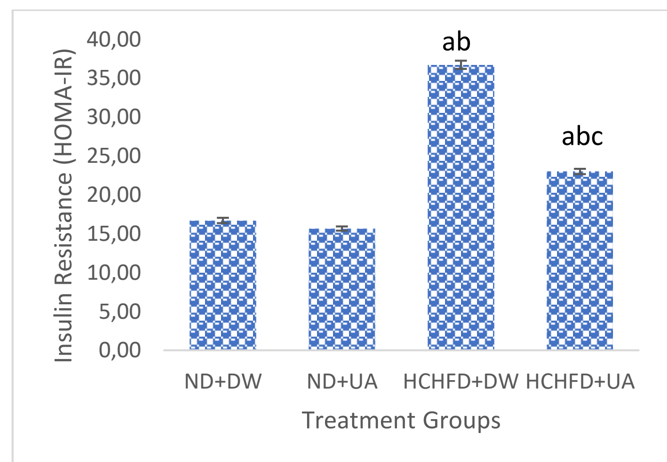

Figure 6: Homeostatic model assessment for insulin resistance of male Wistar rats fed HCHFD and UA. Data were expressed as Mean±SEM (n=5). a = significant vs ND+DW at $P<0.05$; b = significant vs ND+UA at $P<0.05$; c = significant vs HCHFD+DW at $P<0.05$. ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.

Effect of ursolic acid on lipid profile in rats fed a high-carbohydrate high-fat diet

Table 2 shows the effects of HCHFD and UA on serum levels of lipid profile in all the groups. Levels of TC, TG, and LDL-C were similar in ND-fed groups, whereas the level of HDL-C was significantly higher ($P<0.05$) in ND+UA-fed animals (38.40±0.99) compared to the ND+DW (28.30±0.91) group. In the HCHFD+DW group, levels of TC (126.18±1.70), TG (118.18±1.25), and LDL-C (72.86±1.05) were all significantly higher ($P<0.05$) compared respectively to the ND+DW group - TC (65.10±1.75), TG (57.94±1.22) and LDL-C (43.52±1.30), while HDL-C was similar in both groups. In HCHFD+UA-fed animals, levels of TC (105.62±1.84), TG (83.38±1.19), and LDL-C (54.74±1.03) were significantly lower ($P<0.05$) compared respectively to the HCHFD+DW group - TC (126.18±1.70), TG (118.18±1.25) and LDL-C (72.86±1.05), whereas in HCHFD+UA fed animals, level of HDL-C (78.86±0.97) was significantly higher ($P<0.05$) compared to the HCHFD+DW group (36.12±1.91).

Table 2: Levels of serum lipids following high-carbohydrate high-fat diet feeding and treatment with ursolic acid in male Wistar rats.

Lipid Profile (mg/dl)	ND+DW	ND+UA	HCHFD+ DW	HCHFD+ UA
TChol	65.10 ± 1.75^{a}	65.20 ± 1.85^{a}	126.18 ± 1.70^{c}	105.62 ± 1.84^{b}
Trigly	57.94 ± 1.22^{a}	58.04 ± 0.96^{a}	118.18 ± 1.25^{c}	83.38 ± 1.19^{b}
HDL-C	28.30 ± 0.91^{a}	38.40 ± 0.99^{a}	36.12 ± 1.91^{a}	78.86 ± 0.97^{b}
LDL-C	43.52 ± 1.30^{a}	43.64 ± 1.37^{a}	72.86 ± 1.05^{c}	54.74 ± 1.03^{b}

Data were expressed as Mean±SEM (n = 5). Values with different superscripts within rows are significantly different ($P<0.05$). ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.
HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; T Cholesterol: Total cholesterol.

Effect of ursolic acid on the histology of liver in rats fed a high-carbohydrate high-fat diet

Plate 1A and 1B show the normal histological structure of the liver in rats fed with a normal diet indicated by a clear central vein as shown with green arrows and sinusoids as shown with black arrows. Plate 1C shows the damaged histological structure of the liver in rats fed only HCHFD, showing steatosis as indicated by blue arrows; vesicular fatty change as indicated by yellow arrowheads; cellular infiltration as indicated by green arrowheads; and congested central vein with thickening of its wall as indicated by green arrows. Plate 1D shows a near-normal architecture of the liver parenchyma in rats fed an HCHFD+UA, showing a clear central vein as indicated by the green arrow.

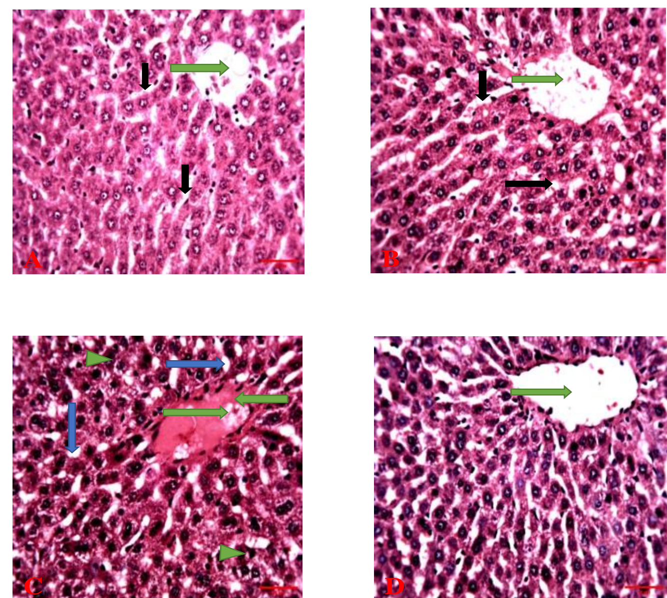

Plate I: Summary of a photomicrograph of liver tissues of HCHFD-fed and UA-treated male Wistar rats (H&E; X 250). A: ND+DW group; **B:** ND+UA group; **C:** HCHFD+DW group; and **D:** HCHFD+UA group. ND+DW: normal diet + distilled water group; ND+UA: normal diet + ursolic acid group; HCHFD+DW: high-carbohydrate high-fat diet + distilled water group; HCHFD+UA: high-carbohydrate high-fat diet + ursolic acid group.

Discussion

We reported that HCHFD caused a higher BMI increase which is a marker of obesity (Novelli *et al*., 2007). This HCHFD-induced higher BMI increase is attributed to increased abdominal circumference change and body length retardation (Panchal *et al*., 2011; Chu *et al*., 2015; Senaphan *et al*., 2015; Wong *et al*., 2017). However, in this study, 8 weeks of supplementation with UA was able to mitigate obesity by preventing increased BMI change. Anti-obesity effect of UA in rats is mediated through increased Akt phosphorylation and improved skeletal muscle glucose uptake (Chu *et al*., 2015; Zhang *et al*., 2016; Ramirez-Rodriquez *et al*., 2017). This is believed to occur by inhibition of adipocyte and hepatic lipogenesis and enhanced adipocyte lipolysis (Chu *et al*., 2015). Inhibition of adipocyte and hepatic lipogenesis and enhanced adipocyte lipolysis releases free fatty acid (FFA) into circulation (Rao *et al*., 2011; He *et al*., 2013). However, according to Jang *et al*. (2010) and Sundaresan *et al*. (2012), UA supplementation does not induce an elevation of circulating FFA level, and conversely, significantly decreases its concentration in diet-induced obese mice. This suggests that UA prevents high-energy diet-induced obesity, as shown by our findings, through enhanced adipocyte lipolysis, and at the same time promotes FFA utilization probably by the skeletal muscle.

Results from this study showed that HCHFD significantly caused a higher fasting blood glucose (FBG) and AUC increase. These indicate hyperglycemia and impaired glucose tolerance respectively. This is similar to findings that were reported by Panchal *et al*. (2011); Poudyal *et al*., (2012); Senaphan *et al*. (2015), and Wong *et al*. (2017). Our findings showed that UA has both hypoglycemic and anti-hyperglycemic effects, this is because UA decreased FBG change in ND-fed and HCHFD-fed animals respectively compared to ND-fed and HCHFD-fed animals that were not treated with UA. Also, UA significantly decreased the AUC for blood glucose in OGTT of both ND-fed and HCHFD-fed animals, indicating improved glucose tolerance effects of UA. Jung *et al*. (2007) demonstrated that UA supplementation in diabetic models prevented the development of insulin resistance and the cells exhibited normal glucose transporter type 4 translocation and insulin receptors via Akt activation. Our findings showed that UA has an anti-diabetic effect by regulating glucose levels in HCHFD-induced MS.

Results from this study showed that HCHFD significantly increased fasting serum insulin (FSI) and HOMA-IR. This is in concordance with previous findings (Senaphan *et al*., 2015; Zhang *et al*., 2016; Wong *et al*., 2017). The increased HOMA-IR, which is a result of increased FBG earlier reported and increased serum insulin, suggests insulin resistance. In our present study, the fasting hyperinsulinemia and increased insulin resistance induced by HCHFD were significantly alleviated by UA, suggestive of the insulin-sensitizing effect of UA, thereby supporting our earlier claim of its anti-diabetic effect. UA supplementation in diabetic models prevented the development of insulin resistance and the cells exhibited normal glucose transporter type 4 (GLUT-4) translocation and insulin receptors via Akt activation (Jung *et al*., 2007), indicating that UA effectively controls glucose levels in diabetes. Also, UA improves impaired glucose tolerance and insulin resistance by protecting pancreatic β-cells in diabetic mice from damage (Jang *et al*., 2009). It's likely UA suppressed inflammatory cytokine-induced insulin resistance to exert its insulin-sensitizing effect. Boonloh *et al*. (2015) reported that in the human liver cancer cell line (HepG2), insulin resistance was induced by inflammatory cytokines, whilst on the contrary, its signaling pathway was suppressed to restore insulin sensitivity.

Results from this study showed that HCHFD induced dyslipidemia by significantly increasing TG, TC, and LDL-C. This is in agreement with the findings of Panchal *et al*. (2011); Poudyal *et al*., (2012); Senaphan *et al*., (2015), and Wong *et al*., (2017). Apart from HCHFD, an excess of dietary fructose (Mamikutty *et al*., 2014), fat (Suman *et al*., 2016), or a combination of fructose and fat (Gancheva *et al*., 2015) has been widely reported to contribute to insulin resistance and dyslipidemia. Decreased rate of glucose clearance, as earlier reported in this study, may be responsible for dyslipidemia observed in HCHFD+DW animals, as a result of increased hepatic and adipocyte lipogenesis. An important transcription factor known as sterol regulatory element-binding protein 1c (SREBP-1c) has been reported to increase lipogenesis by stimulating the expression of lipogenic genes such as Acetyl-Coenzyme A carboxylase, fatty acid synthase, and saturated fatty acid dehydrogenase (Kim *et al*., 1998). High fructose feeding increases lipogenesis through the upregulation of SREBP-1c (Haas *et al*., 2012). Endogenous Peroxisome proliferator-activated receptor γ (PPARγ)

ligands are associated with activated SREBP-1c and this, consequently, increases adipogenesis/lipogenesis (Kim and Spiegelman, 1996; Kim *et al*., 1998). However, our present study showed that UA significantly decreased TG, TC, and LDL-C while HDL-C was increased. Our findings suggest that UA has hypolipidemic and anti-hyperlipidemic effects. Prevention of insulin resistance and improved glucose tolerance by UA (due both to its lipolytic and FFA utilization effects) as earlier observed, may be responsible for the amelioration of dyslipidemia.

As shown by the histological analysis of liver tissue (plate 1 A-D), HCHFD altered the architecture of liver parenchyma indicated by: hepatic steatosis as shown by fat droplets; cellular infiltration as shown by accumulated nuclei; and congested central vein as shown by accumulated blood cells (plate 1C). The structure of liver tissue may be affected by a high-fructose diet (Coate *et al*., 2010). It was reported that a high-fructose high-fat diet induced fatty liver disease and its progression into non-alcoholic steatohepatitis (NASH) (Lozano *et al*., 2016). This has been explained by several mechanisms. Many important factors involved in the mechanisms include alteration in the metabolism of lipids; increased steatotic hepatocytes vulnerability; insulin resistance; mitochondrial dysfunction and oxidative stress. The insulin resistance factor is not limited to its metabolic effects and hyperinsulinemia, it also involves the pro-inflammatory effects of adipokines secreted by the adipose tissues (Sutti *et al*., 2014). Findings from our study showed that UA restored the architecture of liver parenchyma to near normal as indicated by a clear central vein (plate 1 D). UA reduced the markers of fatty liver disease such as hepatocellular steatosis and triglycerides in the hepatocytes and also reduced the expression of SREBP-1c, which is a promoter of lipogenesis and fatty liver disease (Horton *et al*., 2002).

Conclusion

Based on the findings from this study, it was concluded that UA supplementation prevents obesity, hyperglycemia, glucose intolerance, dyslipidemia, and insulin resistance in male Wistar rats fed with HCHFD for 20 weeks. The molecular mechanism involved in these actions of UA is still being elucidated.

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