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Research Article

Evaluation of the Anti-Hyperlipidaemic and Cardioprotective Effects of Ethanol Leaf Extract of *Hyptis Verticillata* in High-Fat Diet-Induced Obese Wistar Rats

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ABSTRACT

A high-fat diet (HFD) can easily trigger obesity with a resultant distortion of metabolic homeostasis. In this study, the potential of *Hyptis verticillata* to ameliorate selected obesity associated abnormalities in rats exposed to high fat diet was evaluated. Thirty-six (36) male Wistar rats grouped in 6 were fed HFD for 8 weeks and received vehicle, orlistat or graded doses of *H verticillata* leaf extract for the last 4 weeks of the 8-week experimental duration. The untreated control rats showed increased organ and body weight, as well as distorted atherogenic coefficient and cardioprotective index relative to animals in the normal control group. These metabolic dysregulations were ameliorated by *H. verticillata* ($p<0.05$). Also, *H. verticillata* positively impacted lipid profile and attenuated HDL levels. The orlistat treated group presented a similar trend with *H. verticillata*. This study highlights the therapeutic potential of *H. verticillata* leaf extract in reversing obesity associated weight abnormalities, dyslipidaemia, improving cardioprotective index and ameliorating atherogenic and coronary risk indices triggered by HFD-induced obesity.

Keywords: *Hyptis verticillata, obesity, high fat diet, dyslipidaemia, cardioprotective indices, atherogenic index, hepatorenal.*

INTRODUCTION

Consumption of energy rich food together with a sedentary lifestyle are globally recognized factors responsible for the increasing prevalence of obesity. Obesity is a metabolic disorder characterized by accumulation of excess body fat, with deleterious effects that influence overall health of an affected individual. Although this metabolic disorder is amongst the most neglected public health challenge, despite its obvious nature, it is a major health concern for both developed and developing countries. Experimental induction of obesity in animals may be achieved by neuro-endocrine, dietary or genetic approach (Singh *et al*., 2020). However, diet-induce obesity overtime is preferred over genetic models as it gives a true reflection of obesity associated abnormalities as well as consequences associated with unhealthy dietary and lifestyle choices

(Swaraj *et al*., 2016). Feeding rats with high fat diet, HFD results in visceral fat accumulation, dyslipidaemia, hyperinsulinemia as well as hyperglycaemia.

These diet-induced metabolic alterations are strongly associated with impaired cardiac functions that may alter cardioprotective indices and atherogenic coefficient. From existing reports, increased lipoprotein levels alter arterial function and significantly contributes to atherosclerosis and cardiovascular diseases (Abu *et al*., 2015). Particularly, elevated levels of total cholesterol, low density lipoprotein and triacylglycerol accompanied by decreased high density lipoprotein levels in dyslipidaemia are well documented cardiovascular risk factors (Abu *et al*., 2015). Additionally, the atherogenic index, a ratio of triglycerides to HDL is an important marker of dyslipidaemia and related cardiovascular diseases (Cai *et al.,* 2017; Yang *et al.,* 2017).

An obese condition is strongly associated with elevated atherogenic index and can be clinically employed to access the impact of hypolipidemic drugs as a treatment regimen for obesity. Furthermore, HFD-induced obesity is strongly associated with diminished antioxidant levels and elevated oxidative stress in liver, heart and kidney tissue (Noeman *et al*., 2011; Swaraj *et al*., 2016). Although lifestyle modifications focused on improving dietary patterns and increased physical activities is recommended to manage metabolic diseases like obesity, there are insufficient and must be accompanied by drug interventions. Although a number of anti-obesity drugs exist, these available synthetic drugs are strongly associated with adverse and side effects. To circumvent adverse side effects associated with synthetic anti-obesogenic drugs, ethnobotanicals are constantly being investigated as the search for effective and reliable anti-obesity therapy with minimal side effects is ongoing.

Ethnobotanicals are essential sources of clinically effective agents. Their role in maintaining human health is gaining acceptance mostly because they are relatively safe for use with low side effects compared to conventional synthetic anti-obesity drugs. Some medicinal plants with established anti-obesity properties include Camellia sinesis, Moringa oleifera, Curcuma longa, Nigella sativa, Opuntia ficus-indica are also known as excellent sources of antioxidants and have been evaluated for their therapeutic potential to manage obesity related metabolic abnormalities (Shao *et al*., 2012; Basu *et al*., 2011; Ghasi *et al*., 2000; Datau *et al*., 2010: Godard *et al*., 2010).

In the present study, the effect of graded doses of *Hyptis verticillata* leaf extract in HFD-fed Wistar rats was assessed. Several biological effects of *H. verticillata* have been demonstrated. In our previous work, we reported its anti-hyperglycaemic effect in streptozotocin-induced diabetic rats (Ogar *et al*., 2019). In a follow up study, we showed that *H. verticillata* attenuated dyslipidaemia, anti-inflammatory, cardioprotective, atherogenic indices in diabetic Wistar rats (Ogar *et al*., 2018). The present study investigated the anti-hyperlipidaemic, anti-atherogenic and cardioprotective effects of ethanol leaf extracts of *H. verticillata* in high-fat diet-induced obese male Wistar rats by evaluating selected parameters including anthropometric indices, blood glucose, lipid profile, atherogenic index (AI) and the coronary risk index (CRI) as well as markers of hepatorenal function.

MATERIALS AND METHODS

Reagents and chemicals

Hydrochloric acid (HCL), Diethyl ether, Thiobarbituric acid (TBA), Trichloroacetic acid (TCA) n-Hexane were purchased from Sigma-Aldrich (St. Louis, Mo, USA), while Ellman's reagent (DTNB) manufactured by Thermo Scientific (Meridian Road, Rockford, IL 61105 USA). Kits used for biochemical estimations were purchased from Fortress diagnostics (Spain). All other reagents and chemicals utilised for this study were of analytical grade.

Preparation of *H. verticillata* plant extract

Fresh leaves of *H. verticillata* were obtained from homestead garden in Calabar Municipality, Cross River State, Nigeria and transported to Botany Department, University of Calabar for botanical identification and verification by Mr Frank Apojeye. Voucher specimens were deposited in the herbarium of the same department (BOT/HV/2023/001) for future reference.

The leaves were thoroughly rinsed in clean tap water followed by distilled water, dried under shade, and afterward blended into powder and weighed. 1450 g of the powder sample was macerated in 98 % ethanol at room temperature for 48 h and thereafter, double filtrated and thereafter concentrated with a rotary evaporator to yield 149.6 g of crude extract, which represented a percentage yield of 10.3 %.

Handling of experimental animals

Thirty (30) male Wistar rats (150–210 g) were obtained from the Animal house of the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar were utilised. Male animals were used in this study to eliminate potential hormonal variations associated with the female menstrual cycle. This study followed the National Research Council's guide for the care and use of laboratory animals and the protocol was approved by the Faculty of Basic Medical Sciences Animal Ethics Committee with the following approval number: 293BCM2624. Animals to be fed high-fat diet were allowed to acclimatize to a high fat diet for one (1) week. Additionally, all experimental animals were allowed two (2) weeks to acclimatize to the environment. Water and feed were given *ad libitum*. After acclimatization, all animals were maintained in standard conditions of regular 12-h light and 12-h dark cycle all through the period of the experiment according to the guidelines of the Ethics committee, Faculty of Basic Medical Sciences, University of Calabar, Nigeria.

Formulation of high fat diet and induction of experimental obesity

In the present study, normal rat chow and high fat diet adopted by Wang *et al*. (2020) were used. In terms of feeding, the experimental animals were separated into two (2) categories that included the normal control animals in group 1 and received normal rat chow while the second category of animals received high fat diet. After 4 weeks of feeding, animals with body weight above 20 % and Lee's index above 310 were considered obese and selected for the study (Shabbir *et al*., 2016; Lee, 1929).

Experimental design

The doses of *H. verticllata* selected for the present study was based on previous reports by (Ogar et al., 2018). After induction of obesity, the animals were randomly assigned into 6 groups (n = 5) consisting of Control and Treatment groups as follows:

Group 1- normal control (distilled water, rat chow).
Group 2- fed with the high-fat diet (HFD).
Group 3- HFD + 30 mg/kg of orlistat
Group 4- HFD + 100 mg/kg of *H. verticillata* extract
Group 5- HFD + 200 mg/kg of *H. verticillata* extract
Group 6- HFD + 300 mg/kg of *H. verticillata* extract

The normal and untreated obese control animals in groups 1 and 2 received distilled water as vehicle while animals in groups 3 - 6 were treated with orlistat and graded doses of *H. verticillata* leaf extract for four (4) weeks to ascertain its impact on obesity-induced with high fat diet. Treatment with orlistat and *H. verticillata* leaf extract was administered orally for 12 h at night 7.00 pm to 7.00 am. This exposure cycle was maintained throughout the study period. At the end of the 8 weeks experimental duration, the animals were euthanized using ketamine and thereafter sacrificed. Serum and selected organs were harvested and used for biochemical assays.

Measurement of anthropometric parameters

This was done according to the previously reported method by Novelli *et al*. (2007). The length of the body (nose-to-anus length) was also determined weekly using a calibrated measuring tape and measured in centimetres (cm) (Novelli *et al*., 2007). The length and body weight were utilized in the calculation of body mass index (BMI).

Estimation of serum lipid parameters

Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and triglyceride (TG) in serum was estimated using spectrophotometric assay kits (Sigma-Aldrich, USA) (Allain et al., 1974; Assmann et al., 1983; Sullivan et al., 1985). Serum low density lipoprotein cholesterol (LDL-C) was estimated based on calculations as previously described (Friedewald & Levy, 1972). Additionally, cardioprotective index (CPI) was assessed based on HDL-C/LDL-C ratio, while atherogenic coefficient (AC), atherogenic (AI) and coronary risk (CRI) indices were determined using the formula: AC = [TC – HDL-C / HDL-C], AI = Log [TG/HDL-c] and CRI = [TC/HDL-C], respectively, as described earlier (Barter *et al*., 2007).

Assessment of serum glucose concentration

Blood was collected via cardiac puncture, serum was obtained through centrifugation, and aliquots were taken to assay for glucose concentration using commercial kits from Randox laboratories ltd, (55 Diamond Road, Crumlin, Country Antrim, BT29 4QY, United Kingdom). The serum glucose was measured according to a previously reported method (Kumar & Gill, 2018).

Assessment of markers of hepatic and renal function

Selected biomarkers of hepatic (AST, ALT, ALP, total protein, albumin and globulin) and renal (creatinine and urea) function were assessed in serum spectrophotometrically using assay kits from Sigma-Aldrich, USA according to previously reported method (Sedlak & Lindsay, 1968; Murray *et al*., 2004).

Statistical analysis

GraphPad prism software, version 7.0 (GraphPad Software, San Diego, CA, USA) was used to analyse data for statistical significance using One-way analysis of variance. This was followed by a Tukey post hoc test and results were considered significant at $p<0.05$. All data were expressed as mean ± standard error of mean (SEM).

RESULTS

Effect of *H. verticillata* leaf on anthropometric in HFD-induced obese rats

Anthropometric indices evaluated in this study included body and organ weights, Naso-anal lengths, Waist circumference, and Lee's index (*Tables 1-4*). From the result, a significant effect ($p<0.05$) on the body weights was observed following treatment with *H. verticillata* leaf extracts compared to obese controls, and these were similar to standard treatments (orlistat). Also, the obese control group showed significantly higher weights of liver, kidneys, heart and adipose tissue compared to the normal controls ($p<0.05$) (*table 1*).

However, treatment with *H. verticillata* prevented the increase in organ weights. Furthermore, a significant ($p<0.05$) elevation of serum glucose concentration was observed in the HFD-Obese control group 2 relative to the normal control group 1 and upon treatment with treatment with orlistat and *H. verticillata* extract doses, the serum glucose level declined significantly ($p<0.05$) compared to the untreated control (*table 1)*. Interestingly, serum glucose concentration following treatment with 200 mg/kg b. w extract compared well with the normal control animals. Results for naso-anal lengths, waist circumference and Lee obesity index (*tables 2-4*) was similar to that observed for organ weights in this study and showed a significant increase in the corresponding values of the measured indices compared to the normal control

and these effects were significantly decreased (p<0.05) on administration of *H. verticillata* leaf extract.

Table 1. Effect of *H. verticillata* on body weight, Organ weight and Serum Glucose Concentration in HFD-induced Obese Rats

	Treatment and groups					
	Normal control	HFD control	HFD/orlistat control	100 mg/kg b.w.	200 mg/kg b.w.	300 mg/kg b.w.
Body weight						
Initial body weight (g)	148.60 ± 2.23^{a}	265.44 ± 13.34^{b}	235.66 ± 14.66^{b}	229.33 ± 8.28^{b}	234.41 ± 6.51^{b}	227.58 ± 26.12^{b}
Final body weight (g)	167.68 ± 3.62^{a}	285.63 ± 13.26^{c}	237.51 ± 14.92^{b}	218.27 ± 7.04^{b}	234.95 ± 4.72^{b}	209.72 ± 20.36^{b}
Organ weight (g)						
Liver	5.54 ± 0.33^{a}	8.73 ± 0.84^{c}	7.61 ± 0.88^{bc}	5.79 ± 0.12^{ab}	6.59 ± 0.08^{ab}	5.94 ± 0.27^{ab}
kidneys	1.04 ± 0.07^{a}	1.66 ± 0.20^{b}	1.43 ± 0.12^{ab}	1.28 ± 0.08^{ab}	1.30 ± 0.05^{ab}	1.24 ± 0.14^{a}
Heart	0.46 ± 0.07^{a}	0.98 ± 0.02^{c}	0.81 ± 0.07^{b}	0.66 ± 0.03^{b}	0.80 ± 0.04^{b}	0.66 ± 0.06^{b}
Adipose tissue	0.67 ± 0.27^{a}	2.81 ± 0.35^{b}	2.90 ± 0.46^{b}	1.85 ± 0.37^{b}	2.41 ± 0.48^{b}	1.94 ± 0.44^{b}
Serum glucose (g/dl)	79.10 ± 3.69^{ab}	82.89 ± 13.19^{b}	57.20 ± 6.39^{ab}	54.11 ± 7.56^{a}	72.61 ± 10.25^{ab}	58.92 ± 6.36^{ab}

Results are presented as Mean ± SEM. Means with the same alphabets are not significantly different, however, means with different alphabets are significantly different P<0.05.

Table 2. Effect of *H. verticillate* on Naso-anal length of HFD-induced obese rats

Treatment	Naso- anal length (cm) of the treatment period			
	Week 1	Week 2	Week 3	Week 4
Normal control	17.70 ± 0.09^{a}	18.16 ± 0.20^{a}	17.78 ± 0.01^{a}	19.77 ± 0.34^{a}
HFD control	21.18 ± 0.48^{b}	21.59 ± 0.40^{c}	21.08 ± 0.51^{c}	21.49 ± 0.41^{b}
HFD/Orlistat control	20.53 ± 0.51^{b}	20.74 ± 0.44^{ab}	20.74 ± 0.42^{bc}	20.74 ± 0.42^{ab}
100 mg/kg bw of extract	20.68 ± 0.47^{b}	20.47 ± 0.30^{b}	19.81 ± 0.51^{abc}	20.83 ± 0.51^{ab}
200 mg/kg bw of extract	20.32 ± 0.01^{b}	20.32 ± 0.01^{b}	18.80 ± 1.53^{ab}	20.74 ± 0.42^{ab}
300 mg/kg bw of extract	20.07 ± 0.84^{b}	20.07 ± 0.48^{b}	20.32 ± 0.80^{bc}	20.07 ± 0.62^{ab}

Results are presented as Mean ± SEM. Means with the same alphabets are not significantly different, however, means with different alphabets are significantly different P<0.05.

Table 3. Effect of *H. verticillate* on Waist circumference of HFD-induced obese rats

Treatment	Waist circumference(cm) of the treatment period			
	Week 1	Week 2	Week 3	Week 4
Normal control	5.12 ± 0.12^{a}	4.82 ± 0.09^{a}	4.80 ± 0.09^{a}	5.48 ± 0.13^{a}
HFD control	6.40 ± 0.19^{b}	6.46 ± 0.20^{c}	6.50 ± 0.22^{b}	7.00 ± 0.16^{b}
HFD/Orlistat control	6.27 ± 0.30^{b}	6.05 ± 0.26^{bc}	6.37 ± 0.36^{b}	6.17 ± 0.31^{a}
100mg/kg bw of extract	6.00 ± 0.16^{b}	5.68 ± 0.13^{b}	5.80 ± 0.12^{b}	5.60 ± 0.19^{a}
200mg/kg bw of extract	6.13 ± 0.13^{b}	6.00 ± 0.01^{bc}	5.93 ± 0.08^{b}	6.00 ± 0.01^{a}
300mg/kg bw of extract	5.90 ± 0.33^{b}	5.68 ± 0.34^{b}	6.00 ± 0.32^{b}	5.62 ± 0.19^{a}

Results are presented as Mean ± SEM. Means with the same alphabets are not significantly different, however, means with different alphabets are significantly different P<0.05.

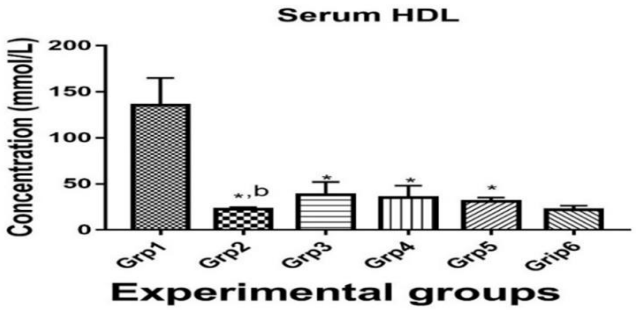
Table 4. Effect of *H. verticillate* on Lee obesity index of HFD-induced obese Rats

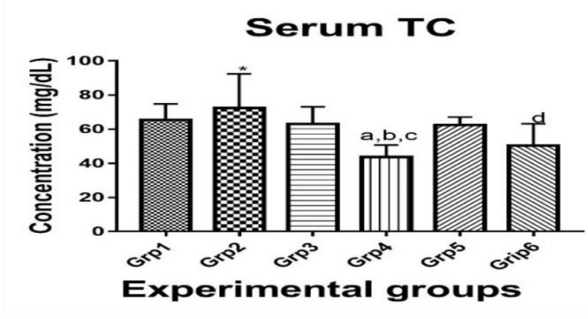
Treatment	Lees index (g/cm) of the treatment period			
	Week 1	Week 2	Week 3	Week 4
Normal control	299.61 ± 1.71^{a}	291.39 ±0.78^{a}	299.39 ± 1.88^{a}	281.06 ± 1.73^{a}
HFD control	303.25 ± 3.67^{a}	294.06 ± 9.00^{a}	311.93 ± 4.94^{ab}	307.41 ± 6.05^{b}
HFD/Orlistat control	306.59 ± 4.67^{a}	303.53 ± 3.23^{a}	306.72 ± 5.00^{ab}	306.85 ± 5.63^{b}
100mg/kg bw of extract	296.19 ± 3.77^{a}	291.81 ± 3.93^{a}	302.11 ± 6.17^{ab}	289.72 ± 8.45^{ab}
200mg/kg bw of extract	303.36 ± 2.83^{a}	303.63 ± 1.86^{a}	336.17 ± 3.54^{b}	296.42 ± 7.62^{ab}
300mg/kg bw of extract	302.90 ± 2.70^{a}	298.78 ± 4.44^{a}	291.36 ± 3.58^{a}	288.70 ± 8.09^{ab}

Results are presented as Mean ± SEM. Means with the same alphabets are not significantly different, however, means with different alphabets are significantly different P<0.05

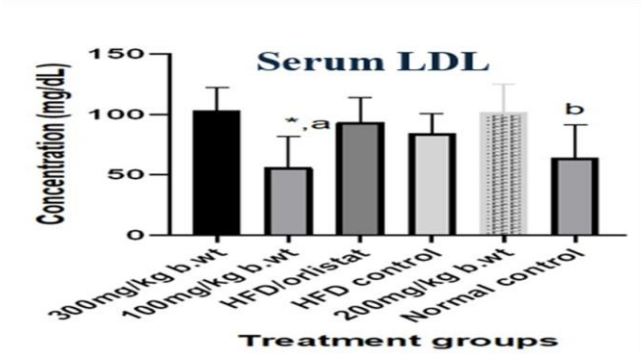
Effect of *H. verticillata* on serum and tissue lipid profile in HFD-induced obese Wistar ratsFigures 1a – f indicates the effect of administration of *H. verticillata* leaf extract on serum lipid profile of high-fat diet induced Wistar rats following treatment with extract doses of *H. verticillata* leaf. From our observation (figures 1a-f), lipid profile was significantly (p<0.05) distorted in the untreated obese group, a possible indication of obesity-induced dyslipidaemia. Interestingly, treatment with the doses of *H. verticillata* extract resulted in improved serum and

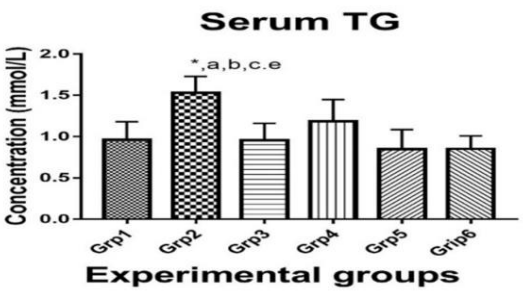
tissue lipid profile by significantly improving serum HDL concentration and decreasing the concentration of TC, LDL and TG both in the serum and liver tissue (figures 1b-f). This observation compared well with normal control and the group treated with the reference drug, orlistat and was significant at $p<0.05$. It is worth noting that the impact of extract doses of *Hyptis verticillata* on HDL was not dose dependent as no significant difference was observed between Group 2 obese control, and 300 mg/kg bw.



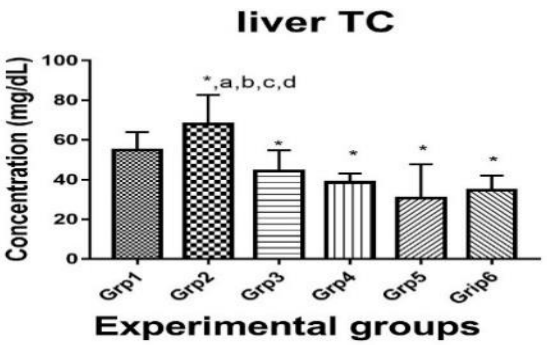


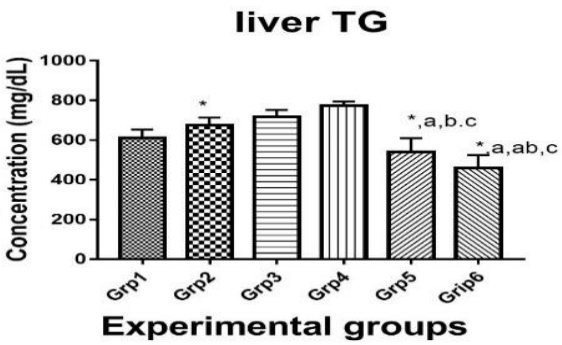
Figures 1a-b: Effect of extract doses of *H. verticillata* leaf on serum HDL & TC. Grp1: normal control, Grp2: HFD control, Grp3: HFD/Orlistat control, Grp4: 100 mg/kg bw extract, Grp5: 200 mg/kg bw extract; Grp6: 300 mg/kg bw extract.
Data is expressed as mean ± SEM. Bars with different superscripts are significantly different. n = 5, $p<0.05$





Figures 1c-d: Effect of extract doses of *H. verticillata* leaf on serum LDL &TG. Grp1: normal control, Grp2: HFD control, Grp3: HFD/Orlistat control, Grp4: 100 mg/kg bw extract, Grp5: 200 mg/kg bw extract; Grp6: 300 mg/kg bw extract.
Data is expressed as mean ± SEM. Bars with different superscripts are significantly different.

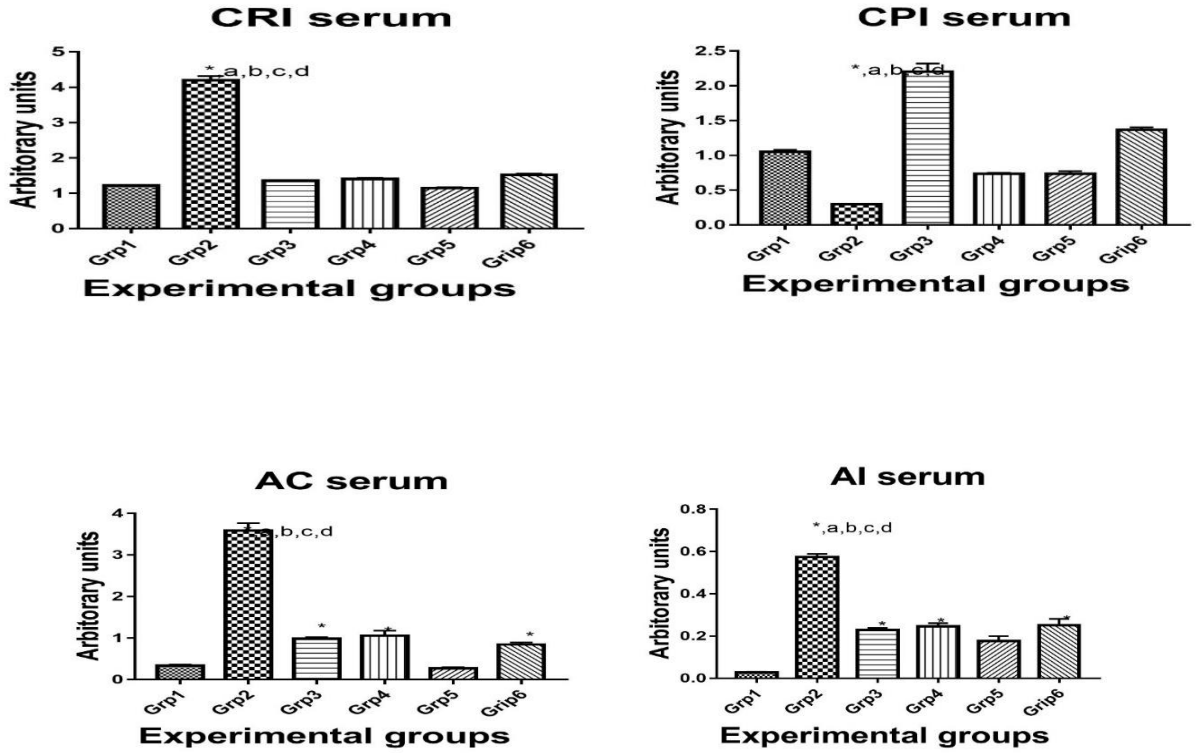




Figures 1e-f: Effect of extract doses of *H. verticillata* leaf on tissue TC and TG. Grp1: normal control, Grp2: HFD control, Grp3: HFD/Orlistat control, Grp4: 100 mg/kg bw extract, Grp5: 200 mg/kg bw extract; Grp6: 300 mg/kg bw extract. Data is expressed as mean ± SEM. Bars with different superscripts are significantly different. n = 5, $p<0.05$.

Effect of *H. verticillata* on cardiovascular indices in high-fat diet induced male Wistar rats

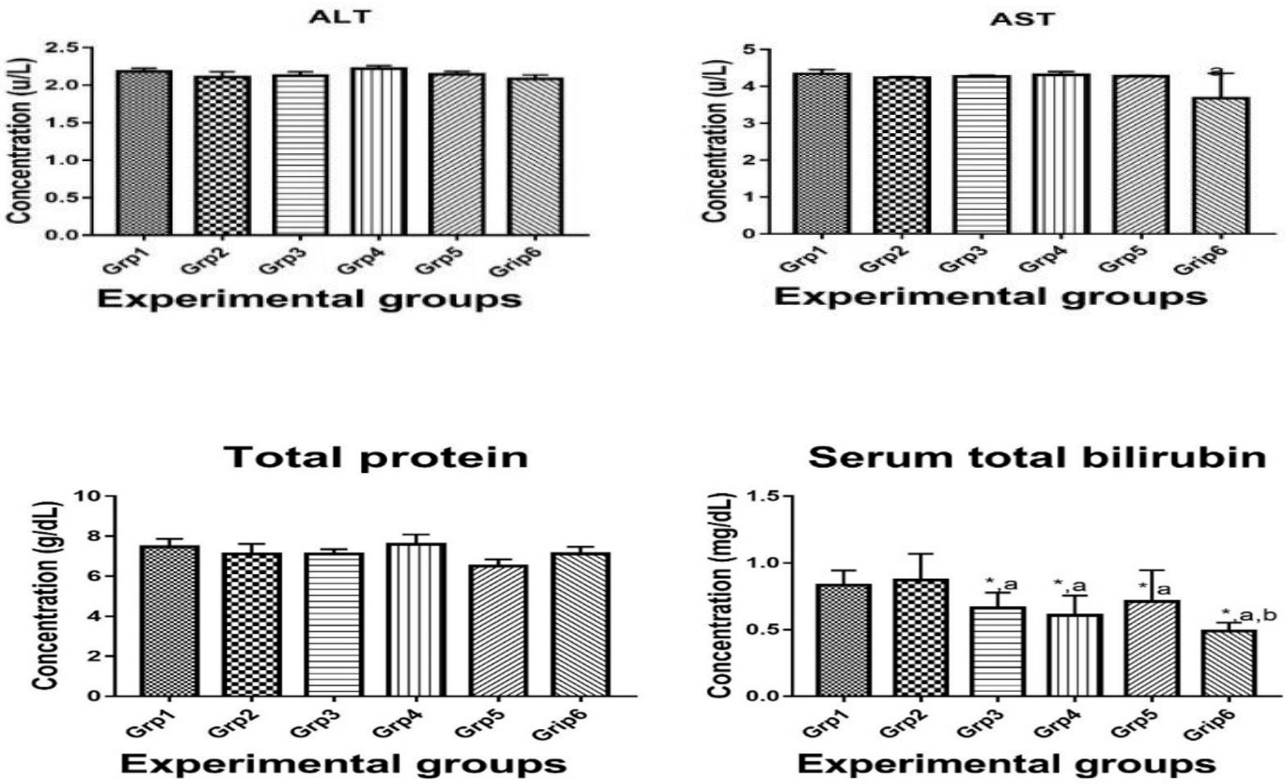
The impact of the treatment on indices of cardiovascular disease risk panel is presented (*figures 2a-d*). From *figure 2a*, the untreated obese control group 2 animals showed a significantly higher coronary risk index compared to the normal control ($p<0.05$). Upon treatment with *H. verticillata,* this observed elevation of coronary risk index was decreased significantly ($p<0.05$), across all treatment groups, and the effects compared favourably with the orlistat treated control (group 3). Furthermore, cardioprotective risk index decreased significantly ($p<0.05$) in obese condition compared to normal control was improved upon on treatment (*figure 2b*), while the Atherogenic index (AI) and atherogenic coefficient (AC) (*figures 2c-d*), indicated a possible coronary risk in obese condition with increased values of AI and AC compared to normal control (Grp1) ($p<0.05$). Similarly, treatment with *H. verticillata* decreased AI and AC values significantly ($p<0.05$), and these were similar to standard treatment (Group 3).



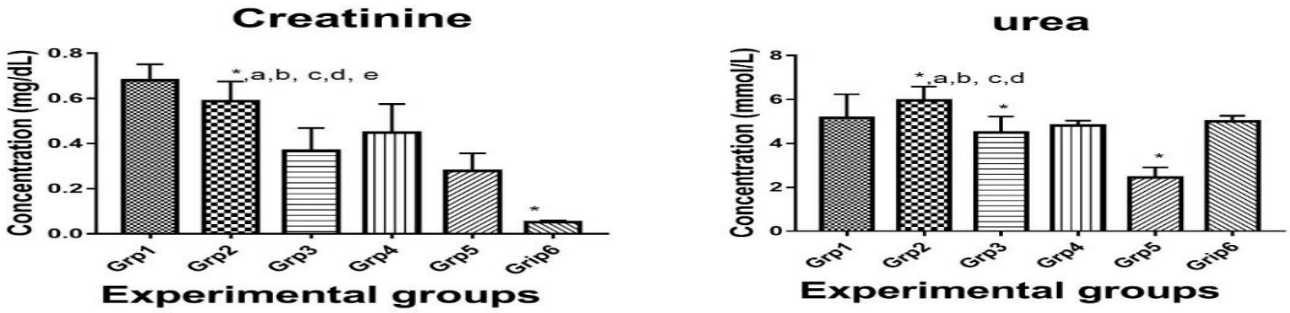
Figures 2a-d: Effect of extract doses of *H. verticillata* leaf on CRI, CPI, AI and AC. Grp1: normal control, Grp2: HFD control, Grp3: HFD/Orlistat control, Grp4: 100 mg/kg bw extract, Grp5: 200 mg/kg bw extract; Grp6: 300 mg/kg bw extract. Data is expressed as mean ± SEM. Bars with different superscripts are significantly different. n = 5, $p<0.05$

Effect of *Hyptis verticillata* on markers of hepatic and renal function in high fat diet-induced obese Wistar rats.

Indices of hepatic function studied included alanine aminotransferase activity, aspartate aminotransferase activity, total protein, albumin, and serum total bilirubin (*figures 3a-d*). Results indicated that there was no significant impact of HFD on the liver as reflected in serum concentrations of liver enzymes and total protein (figures 3a-c). However, it was observed that *H. verticillata* improved hepatic bilirubin clearance relative to the untreated obese group and comparable to animals in the normal control group and this was significant at $p<0.05$ (figure3d). Additionally, serum levels of creatinine and urea were observed to decline significantly ($p<0.05$) following treatment with both doses of *H. verticillata* compared to the untreated obese group as presented on figures 4a-b.



Figures 3a-d: Effect of *H. verticillata* leaf serum markers of liver function. Grp1: normal control, Grp2: HFD control, Grp3: HFD/Orlistat control, Grp4: 100 mg/kg bw extract, Grp5: 200 mg/kg bw extract; Grp6: 300 mg/kg bw extract. Data is expressed as mean ± SEM. Bars with different superscripts are significantly different. n = 5, p<0.05



Figures 4a-b: Effect of extract doses of *H. verticillata* leaf on serum markers of renal function Grp1: normal control, Grp2: HFD control, Grp3: HFD/Orlistat control, Grp4: 100 mg/kg bw extract, Grp5: 200 mg/kg bw extract; Grp6: 300 mg/kg bw extract. Data is expressed as mean ± SEM. Bars with different superscripts are significantly different. n = 5, p<0.05

DISCUSSION

In this study, the anti-hyperlipidaemic, anti-atherogenic and cardioprotective effects of ethanol leaf extracts of *H. verticillata* in high-fat diet induced obese Wistar rats was evaluated. Orlistat, a saturated derivative of lipstatin was used as a reference drug to compare effectiveness of *H. verticillata* ethanol leaf extracts in obese rats. From our result, it was evident that chronic intake of high fat diet triggered obesity in Wistar rats and was characterized by dyslipidaemia and anthropometric distortions. It is well established that obesity ensues from a combination of physical inactivity and excessive calorie intake (Prasatthong *et al*., 2021). High fat diet-induced obese rodents are reliable experimental models for human obesity and have been shown to present symptoms such as hyperglycaemia,

insulin resistance, dyslipidaemia, hepatic steatosis, which characterise human obesity (Jin *et al*., 2013). From our investigation, we observed that oral administration of *H. verticillata* leaf extract impacted body weight as observed by a significant reduction in body weight of the extract treated animals compared to the untreated group. This observation agrees with a study by Wahabi *et al*. (2023) where they reported the weight modulation effect of aqueous extract from Arbutus unedo and Crataegus monogyna fruits in high-fat diet obese rats. Additionally, research has shown that the weight modulation effect of plant extract may be associated with the activities of phytochemicals like carotenoids, polyphenols, flavonoids, terpenoids and squalene that directly or indirectly influence overall body weight in experimental animals by either inhibiting the activity of digestive enzymes like pancreatic lipase and amylase (Athesh et al., 2020), appetite regulation as well as reducing the formation of white adipose tissue (Fu *et al*., 2016). Also, the naso-anal length, waist circumference and Lee obesity index were all positively ameliorated following treatment with the three doses of *H. verticillata* leaf extract compared to the untreated control group. From published data, it is evident that cessation of growth accompanied by stunting of body length and increased body weight are consistent with obesity in Wistar rat models (Prasatthong et al., 2021). This observation compared favourably with the group that received orlistat, the reference drug. Lee obesity index, naso-anal length and waist circumference are excellent indicators of intra-abdominal fat thickness in obese rats as well as central obesity (Gerbaix *et al*., 2010). In human subjects, waist and abdominal circumference, as well as waist to hip and waist to height ratios are used as reliable estimations of body fat content and are acceptable diagnostic markers for metabolic syndromes (Arika *et al*., 2019; Gerbaix *et al*., 2010). The observed amelioration in these indices following treatment with extract doses of *H. verticillata* leaf suggests a possible decline in levels of circulating lipids which may have resulted from the action its rich composition of flavonoids such as sideritoflavone and terpenoids such as squalene (Ogar *et al*., 2018) which have been previously reported to play a vital role in maintenance of homeostatic balance via regulating hunger, supressing appetite signals (Huang *et al*., 2016) and reducing body fat (Arika *et al*., 2019) by acting on adenylate cyclase to convert adenosine triphosphate, ATP to cyclic adenosine monophosphate, cAMP which in turn upregulates lipolysis and basal metabolic rate while promoting protein degradation and supressing protein synthesis (Singh, 2015). Again, excessive weight gain, characteristic of an obese state is usually as a result of increased acylation of saturated fatty acids into triglycerides which are eventually deposited in the adipose tissue and organs such as liver, kidneys and heart (Boniface *et al*., 2016; Arika *et al*., 2019). We assessed the weights of the liver, kidneys and heart across the experimental groups and found an increase in the weight of these organs in the untreated HFD-induced obese animals. Interestingly, this increase in organ weight was reversed following treatment with *H. verticillata (Table 1)*. It is plausible to suggest that the decline in organ weight following treatment with *H. verticillata* extract may have contributed to the overall decrease in body weight as observed.

Evaluation of serum glucose concentration in this study revealed an elevation in the untreated HFD-induced obese rats. Induction of obesity is usually accompanied by an elevation in blood glucose Kadir *et al*. (2015), likewise type 2 diabetes mellitus may occur secondary to obesity as a result of insulin resistance triggered by impaired sensitivity of insulin-to-insulin receptors. Our findings that animals in the untreated HFD-induced obese group presented higher concentration of fasting serum glucose compared to the normal control animals is consistent with earlier explanations that exposing animals to high fat diet triggered significant increase in blood glucose concentration, especially because sustained consumption of fat packed diet decreases glucose oxidation and while increasing beta oxidation of fatty acids, thus, favouring hyperglycaemia (Kadir *et al*., 2015; Chen & Reaven, 2019). *H. verticillata* ameliorated the elevated serum glucose concentration in groups that received extract doses of the leaf. This observation is consistent with findings from Ogar *et al*. (2018) that reported the antihyperglycemic activity of crude ethanol extract of *H. verticillata* in streptozotocin induced diabetic rat model. *H. verticillata* may have possibly attenuated insulin sensitivity and reduced the concentration of glucose channelled into the hepatic portal circulation. Additionally, the high flavonoids and polyphenols contained in the study plant Egbung *et al*. (2017) may have contributed to the observed decrease in serum glucose as these phytochemicals have demonstrable hypoglycaemic effect and act by decreasing glucose concentration in the blood, enhancing cellular glucose uptake and modulating the activities of carbohydrate metabolising enzymes such as alpha amylase and alpha glucosidase to downregulate glucose absorption and improving glucose tolerance (Gupta *et al*., 2012).

Furthermore, the impact of administration of extract doses of *H. verticillata* leaf on the lipid profile of high-fat diet induced obesity was assayed (*Figures 1-6*). Obesity induced dyslipidaemia has been extensively investigated (Atangwho *et al*., 2013; Arika *et al*., 2019) and was confirmed in the present study by elevated levels of total cholesterol (TC), triglycerides (TGs), low density lipoprotein (LDL) with an accompanying suppression of high-density lipoprotein (HDL) concentration. Interestingly, intervention with *H. verticilllata* leaf extract reversed the observed dyslipidaemia. This agrees with earlier reports by Ogar *et al*. (2019) and suggests that *H. verticillata* leaf extract could improve lipid metabolism. Additionally, the raised serum concentration of TGs in the untreated obese control group may have resulted from increased de novo synthesis of lipid. TGs are well implicated in development of metabolic syndrome because of their roles in facilitating deposition of lipid stores around the hepatic and adipose tissues (Welty, 2013) leading to hypertrophied and hyperplastic adipocytes which eventually promote free fatty acids synthesis and

release from the adipose tissue. Upon release from the adipose tissue, free fatty acids are transported to the hepatocytes for re-esterification to TGs and VLDL-c and are eventually secreted into the systemic circulation, thus contributing to lipotoxicity (Welty, 2013). Also, decreased HDL levels in obesity predisposes to developing cardiovascular diseases (Koliaki *et al*., 2019). *H. Verticillata* leaf extract at various doses increased serum HDL concentration in a manner similar to the group that received orlistat. The study plant reportedly possesses a rich profile of bioactive compounds like flavonoids and polyphenols with potent antioxidant activity, (Momoh *et al*., 2022) and squalene that inhibits hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Ibrahim *et al*., 2020), which have all shown demonstrable cholesterol decreasing effects. These bioactive components, together with squalene and phytochemicals like tannins and alkaloids present in *H. verticillata* (Ogar *et al*., 2019; Ogar *et al*., 2018) may have contributed to improving the levels of the 'good cholesterol' HDL and invariably alleviating the dyslipidaemia as observed in this study. The effects of the treatment on indices of cardiovascular disease risk panel were also investigated in this study. Again, we report that HFD-induced obesity significantly contributed to elevating atherogenic index, coronary risk index and decreased cardioprotective index in the untreated obese control rats compared to the normal control. Lipid profile, atherogenic and coronary risk indices are important in predicting metabolic compromise such as cardiovascular diseases and atherosclerosis (Barrett *et al*., 2016). Individuals with elevated levels of LDL and decreased HDL are particularly susceptible to developing cardiovascular related abnormalities like endothelial dysfunction and formation of atherosclerotic plaques (Barrett *et al*., 2016).

Additionally, it has long been established that the oxygen free radicals such as superoxide anion radical(O2·–), singlet oxygen (O2), hydroxyl radical (·OH) and perhydroxyl radical (HO2·) can oxidize LDL-c in the walls of the arteries to yield oxidized LDL-c, which is a potent chemoattractant for monocytes and macrophages (Ntchapda *et al*., 2015). The consequent accumulation of these immune scavengers at the arterial wall accompanied by elevated levels of TC and TG favours the formation of atherosclerosis and related complications such as coronary heart disease as well as ischemic stroke (Aladaileh *et al.,* 2019). In the present study, treatment with *H. verticillata* leaf extract, reversed the negative impact of HFD-obesity on cardiovascular disease risk factors by decreasing atherogenic index and coronary risk index with a concurrent increase in cardioprotective index. According to Kahn *et al*. (2020), the cardioprotective index reflects the overall protective effect of a diet or specific compounds on the cardiovascular system, with a high CPI suggesting a reduced risk of developing heart diseases. Also, atherogenic index is a measure of the balance between atherogenic (risk-increasing) and anti-atherogenic (protective) lipids in the blood. A lower AI indicates a healthier lipid profile and a reduced risk of atherosclerosis and cardiovascular events.

Chronic inflammation is a significant contributor to the development of cardiovascular diseases. Plant-derived compounds have been extensively studied for their role in the modulating cardiovascular disease risk factors (Bachheti *et al*., 2022). Polyphenols, flavonoids, carotenoids and fibre have especially demonstrated cardioprotective effects and may help improve cardiovascular health. The observed modulation of these cardiovascular disease risk factor following intervention with *H. verticillata* leaf extract may be attributable to the action of a single or synergistic effect of phytochemicals present in the study plant, making it an excellent source of cardio-protective agent and this is consistent with earlier reports from Meril-Mamert *et al.* (2022).

Also, indices of hepatic (ALP, ALT, AST, total protein, albumin, and serum total bilirubin) and renal (creatinine and urea) function were also assessed in this study. From our findings, the impact of HFD-induced obesity of the liver was minimal as we observed insignificant changes in these indices, except for the concentration total bilirubin in the serum which was significantly raised compared to the normal control group (*figure 16*). It was evident that extract doses of *H. verticillata* improved bilirubin clearance in the treated animals, an indication that may have been contributed by the potent antioxidant activity of flavonoids and polyphenol content of the study plant (Egbung *et al*., 2017) Contrary to our observation for markers of hepatic function, significantly elevated serum creatinine and urea levels were in the untreated obese animals were decreased following intervention with *H. verticillata* leaf extract doses. Plant-derived compounds can have various effects on renal function, and their impact can be both beneficial and detrimental depending on the specific compound, its concentration, duration of exposure, and individual factors such as overall health and any pre-existing kidney conditions (Khan *et al*., 2020). Most of these compounds have been confirmed in *H. verticillata*, presenting it a good candidate in the management of renal complications in obesity.

CONCLUSION

The present study investigated the potential effects of ethanol leaf extract from *H. verticillata* on high fat diet-induced obese Wistar rats. Our results revealed that *H. verticallata* ameliorated obesity related physiological alterations such as increased body and organ weight as well as metabolic dysregulations including dyslipidaemia, atherogenic and cardioprotective indices. Although the exact mechanism of antihyperlipidemic action is not known at the moment, our results suggest that *H. verticillata* may mediates its beneficial effect partly by various mechanisms that may include lowering LDL-c levels, limiting endogenous hepatic synthesis of TG and TC and appetite suppression. We conclude that *H. verticillata* showed promising effects in mitigating the negative physiological and metabolic changes associated with obesity in Wistar rats and maybe therapeutically beneficial in the management of obesity related complications.

Nevertheless, further research is necessary to elucidate the exact underlying mechanisms of action and to explore its potential as a therapeutic agent for obesity and related metabolic disorders in humans, some of which is already ongoing in our laboratory.

AUTHORS' CONTRIBUTIONS

Conceptualization, CIOU and GEE.; methodology, FCE and GEE.; validation, CIOU, GEE, FCO and MUA.; formal analysis, FCO.; investigation, CIOU, FCE, MUA and IAI.; resources, IAI, MOO and JEE.; data curation, CIOU, DEU and FCE; writing—original draft preparation, CIOU; writing—review and editing, CIOU and GEE.; supervision, GEE.; project administration, CIOU and GEE. All authors have read and agreed to the published version of the manuscript

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CONFLICT OF INTEREST

None declared

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