Int J Biol Med Res.2022 ;13(2):7450-7456

Contents lists available at BioMedSciDirect Publications

International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com

Original article

ANTI-OBESITY ACTIVITY OF RIDA HERBAL BITTERS IN HIGH FAT DIET-INDUCED OBESE RATS

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ARTICLE INFO

Keywords:
Rida herbal bitters (RHB)
High-fat diet
Dyslipidemia
Antioxidant
Metabolic hormones

ABSTRACT

Background: Obesity therapies still a serious issue worldwide and herbs from medicinal plants have play crucial roles in management of metabolic diseases. This study investigated the anti-obesity effects of Rida herbal bitters in high-fat diet-induced obese rats. Materials and Methods: Thirty two (32) male Wistar rats were used and randomly divided into four (4) groups, 8rats/group. Group I: normal control; Group II: high-fat diet (obese control); Group III & IV: high-fat diet (obese rats) administered with 0.3ml Rida herbal bitters and 40mg/kgb.w Simvastatin respectively for 6 weeks. Food intake was assessed daily and body weight weekly. The animals were sacrificed at the end of the experiment and blood samples were collected for biochemical parameters estimation. Results: High-fat diet significantly ($p<0.05$) increased the body weight, food intake, blood glucose, insulin, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), very-low density lipoprotein cholesterol (VLDL-C),atherogenic index, cardiac risk index, leptin, creatinine, urea, uric acid, and malodialdehyde (MDA) levels and significantly ($p<0.05$) reduced high-density lipoprotein cholesterol (HDL-C), ghrelin, adiponectin, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and reduced glutathione (GSH) levels. Treatments with Rida herbal bitters significantly ($p<0.05$) lowered the body weight, food intake, blood glucose, insulin, TC, TG, LDL-C, VLDL-C, atherogenic index, cardiac risk index, creatinine, uric acid, urea, leptin and MDA levels and significantly ($p<0.05$) elevates HDL-C, ghrelin, adiponectin, SOD, GPx, CAT and GSH levels.Conclusion: Rida herbal bitters ameliorate the metabolic alterations induced by high-fat diet and may be a promising therapy for obesity and its complications.

Introduction

Obesity is a global public health concern and the prevalence of this common epidemic disease has tripled in 2016 than 1976. In 2016, World Health Organization (WHO) estimated that 1.9billion adults were overweight with over 650 million obese cases, representing about 13% of the world's adult population and nearly half population of the adult forecasted to suffer from overweight and obese by 2030 [1, 2].

The imbalance between energy intake and expenditure is the fundamental etiology of obesity. Dietary choice for high-fat diet (HFD) and sedentary lifestyle contribute to the energy imbalance [3, 4]. Obesity is defined as metabolic disorder resulting from excessive fat accumulation in the body and clinically diagnosed at body mass index (BMI) ≥ 30kg/m2. Obesity enormously contribute to the pathogenesis of several chronic metabolic diseases such as diabetes mellitus, hyperglycemia, dyslipidemia, cardiovascular disease, hypertension, fatty liver disease and others, all of which reduce the life quality and expectancy [5].

Obesity has been linked with induction of oxidative stress with releasing of excessive free radicals and declining in endogenous antioxidant defense system to eliminate free radicals also contribute to the development of obesity-related metabolic comorbidities [6]. More so, in obesity condition, extreme fat accumulation in the body affects vital organs, including the kidneys [7].

Currently, treatments and preventions options for obesity and overweight are lifestyle modification (physical exercise and calorie diet restriction), drugs and at times, surgery [8]. However, due to the high cost and unexpected adverse side effects such as, elevated blood pressure, constipation, cardiac arrest and insomnia posed by anti-obesity medications, the use of herbal medicines for

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management and prevention of obesity and overweight globally increasing owing to their natural origin, availability, cheap and minimal/no side effects [9].

Rida herbal bitter (RHB) is a polyherbal formulation prepared in Nigeria aqueously from mixture of Curculigo pilosa, Citrullus colocynthis, Hunteria umbellata, Uvaria chamae, and Senna alata. Traditionally and like others therapeutic herbal bitters [10], Rida herbal bitters is used for the management of many ailments and claimed to possess anti-diabetic, antioxidant, anti-hyperlipidemic, anti-inflammatory, analgesic and immunomodulatory properties with uncertain and unverified anti-obesity property. Due to these medicinal claims of Rida herbal bitter without much scientific experimental evidences and its unconfirmed anti-obesity efficacy, this study therefore experimentally investigated the anti-obesity effect of Rida herbal bitters (RHB) in high-fat diet induced obese.

MATERIALS AND METHODS

Drugs and Chemicals: Simvastatin, Xylazine and ketamine,

Experimental Animals

Thirty two (32) male Wistar Albino rats weighing (180-200g) were used and procured from Animal House of Physiology Department, Ladoke Akintola University of Technology, Ogbomoso, Oyo-state, Nigeria. The rats were kept in a clean ventilated plastic cage of 8rats each and acclimatized for two weeks with pelletized fed and water ad libitum under pathogen-free standard ambient condition at temperature (25 ± 2oC), relatively humidity (45% ± 5%) and natural photoperiodicity of 12:12h light/dark cycle) prior the initiation of the experiment. All experimental procedures were approved by the Research Ethical Committee of Ladoke Akintola University of Technology and conducted according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Obesity Induction

After the acclimatization period, the rats were fed with high-fat diet (HFD) for 6 weeks to induce obesity, excluding the normal control rats. Animals fed on HFD with body weight (>300g) were selected as obese rats and used for the experiment.

Experimental Design and Treatment

The Thirty two (32) rats were randomly allotted into 4groups of 8rats/group as follow:

Group I: Normal control (non-obese rats)

Group II: HFD (obese control rats)

Group III: HFD (obese rats) + 0.3ml Rida herbal bitters (RHB)

Group IV: HFD (obese rats) + 40mg/kgb.wt Simvastatin (SIM)

Treatment period lasted for 6 weeks and administrations of Rida herbal bitters and Simvastatin was done orally with oral cannular. At day 1, body weight was recorded followed by weekly intervals throughout the experimental treatment phase. Also, daily food intake was recorded at the treatment phase and the experimental duration was 12 weeks.

Determination of Fasting Plasma Glucose Levels

The fasting plasma glucose level of the rats was measured at day 1 and weekly intervals during the experimental treatment period. Glucose oxidase/peroxidase (GOD-POD) method was used to determine fasting plasma glucose levels using a digital glucometer with test stripes (Accu-Chek Advantage,Roche Diagnostic, Germany).

Blood Collection and Biochemical Estimations

At the end of the experiment, all the rats were subjected to overnight fasting, rapidly anaesthetized with single intraperitoneal injection of ketamine (40 mg/kgb.w) and xylazine (20mg/kgb.w) dose and sacrificed by cervical dislocation. Fasting blood samples were withdrawn from the rats' hearts via cardiac punctured into heparinized tube and centrifuge at 3000rpm for 5mins. The supernatant plasma collected after the centrifugation was stored at -4oC and used for biochemical parameters estimations. The plasma levels of total cholesterol (TC), triglyceride (TG), and high-density lipoprotein-cholesterol (HDL-C) were estimated using enzymatic colorimetric method with commercial assay kits according to the instructions of the manufacturers. Level of low-density lipoprotein cholesterol (LDL-C) and very-low density lipoprotein cholesterol (VLDL-C) were calculated based on Friedewald et al. equation; LDL-C = TC-(HDL-C + TG/5) and VLDL-C = Triglyceride (TG)/5 [11].

Also, atherogenic index (AI) and cardiac risk index (CRI) were calculated using the following equations; Atherogenic Index (AI) = LDL-C/HDL-C

Cardiac Risk Index (CRI) = TC/HDL-C.

Plasma levels of insulin, ghrelin, leptin and adiponectin were estimated using their specific rat enzymes link immunosorbent assay (ELISA) kits following manufacturers' protocols. The plasma activities levels of antioxidants superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) and oxidative stress biomarker malondialdehyde (MDA) level were determined using specific rat SOD, GPx, CAT, and MDA ELISA kits following the procedures described by the manufacturers (Elabscience, China).Glutathione reductase (GSH) was measured based on the Gupta and Gupta method [12].

Kidney functions biomarkers; urea, uric acid and creatinine levels were estimated using commercial assay kits (Siemens Health Care Diagnostics).

Statistical Analysis

All the data collected were analyzed using statistical package for social science (SPSS version 21.0 software). Data were presented as standard error of means (Mean ± SEM) and statistical significance difference between groups was determined using one-way analysis of variance (ANOVA) followed by Bonferoni post-hoc test. Data at P-value (<0.05) was considered statistically significant.

RESULTS

Effects of Rida herbal bitters on body weight and food intake in high-fat diet induced obese rats.

The rats fed with high-fat diet exhibited significant ($p<0.05$) increase in body weight and food intake in comparison with normal control (non-obese) rats. Administrations of Rida herbal bitters drastically lowered the increase in body weight and food intake, even more than the simvastatin compared to the high- fat diet induced obese non-treated rats (fig.1a & b)

Effects of Rida herbal bitters on plasma blood glucose levels, lipid profiles, atherogenic index and cardiac risk index in high-fat diet induced obese rats.

High-fat diet induced obese rats showed significant ($p<0.05$) elevated blood glucose levels, total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C), very-low density lipoprotein-cholesterol (VLDL-C), atherogenic index (AI) and cardiac risk index (CRI) levels, whereas, the level of high-density lipoprotein-cholesterol (HDL-C) significantly ($p<0.05$) reduced

compared to the normal control rats. Rida herbal bitter administration significantly lowered the blood glucose levels, TC, TG, LDL-C, VLDL-C, atherogenic index, and cardiac risk index levels while HDL-C level was increased in comparison with the high-fat diet induced obese non-treated group (fig.1c; fig. 2a, b, & c)

Effects of Rida herbal bitters on insulin, leptin, adiponectin and ghrelin concentrations in high-fat diet induced obese rats.

The plasma insulin and leptin concentrations were significantly ($p<0.05$) higher in high-fat diet induced obese rats while adiponectin and ghrelin concentrations were significantly lowered in comparison with normal control rats. Rida herbal bitters administrations significantly diminished the plasma insulin and leptin concentrations and significantly elevated the adiponectin and ghrelin concentrations in comparison with the high-fat diet induced obese non-treated group (fig. 3a, b, c & d).

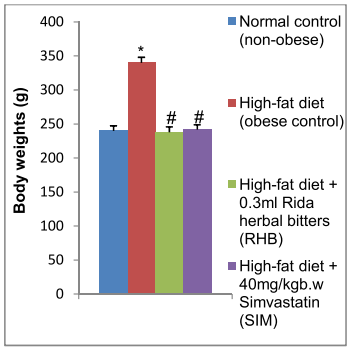
Effect of Rida herbal bitters on renal functions markers in high-fat diet induced obese rats.

There was significant ($p<0.05$) increase in renal functions markers uric acid, urea, and creatinine levels in high-fat diet induced obese rats compared to the normal control rats. The levels of urea, uric acid and creatinine were significantly decreased after treatments with Rida herbal bitter and simvastatin in comparison with the high-fat diet induced obese non-treated group (fig.4a, b & c).

Effects of Rida herbal bitters on oxidative stress marker and antioxidant activity in high-fat diet induced obese rats.

The level of oxidative stress marker malondialdehyde (MDA) was significantly ($p<0.05$) higher in high-fat diet induced obese rats, while the levels of antioxidants superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and reduced glutathione (GSH) were significantly lowered when compared with the normal control rats. However, in the high-fat diet induced obese rats treated with Rida herbal bitter, there was significant reduction in the MDA level and increased activities of antioxidants SOD, GPx, CAT and GSH when compared to the high-fat diet induced obese non-treated group (table 1).

Fig. 1: Effects of Rida herbal bitters (RHB) on (a) body weights (b) food intakes (c) blood glucose levels in high-fat diet induced obese rats. Values are expressed as mean ± SEM (n=8). *significant at $p<0.05$ compared with control; #significant at $p<0.05$ compared with high-fat diet untreated group.



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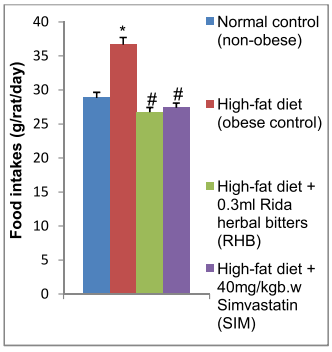

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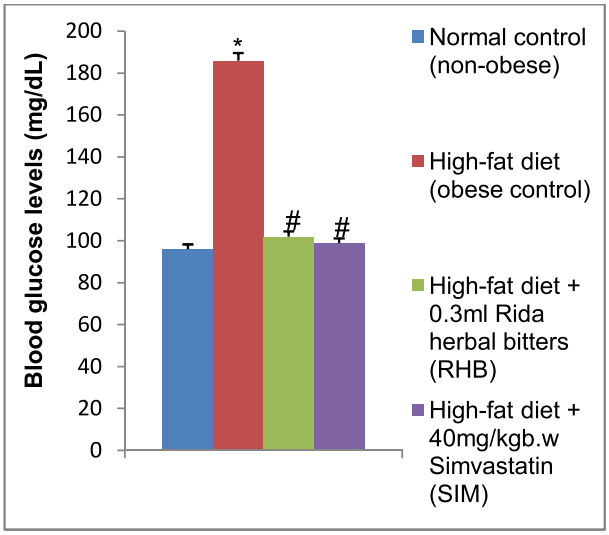

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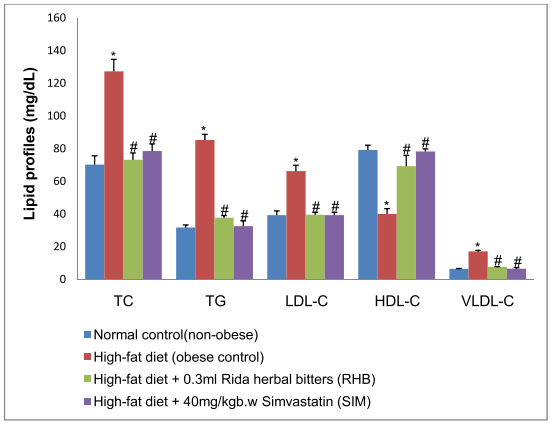
Fig. 2: Effects of Rida herbal bitters (RHB) on (a) lipid profiles (b) atherogenic index (AI) (c) cardiac risk index (CRI) in high-fat diet induced obese rats. Values are expressed as mean ± SEM (n=8). *significant at $p<0.05$ compared with control; #significant at $p<0.05$ compared with high-fat diet untreated group.



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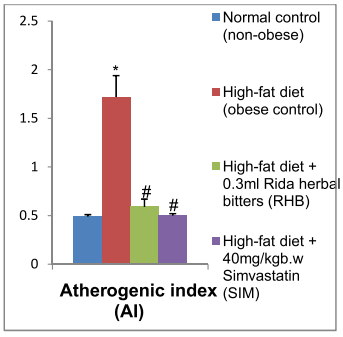

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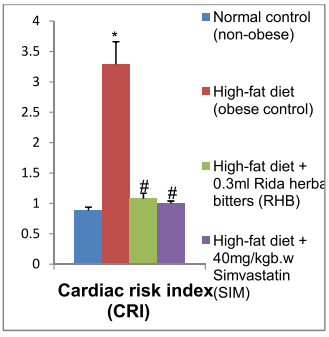

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Fig. 3: Effects of Rida herbal bitters (RHB) on (a) insulin concentration (b) leptin concentration (c) adiponectin concentration (d) ghrelin concentration in high-fat diet induced obese rats. Values are expressed as mean ± SEM (n=8). *significant at p<0.05 compared with control; #significant at p<0.05 compared with high-fat diet untreated group.

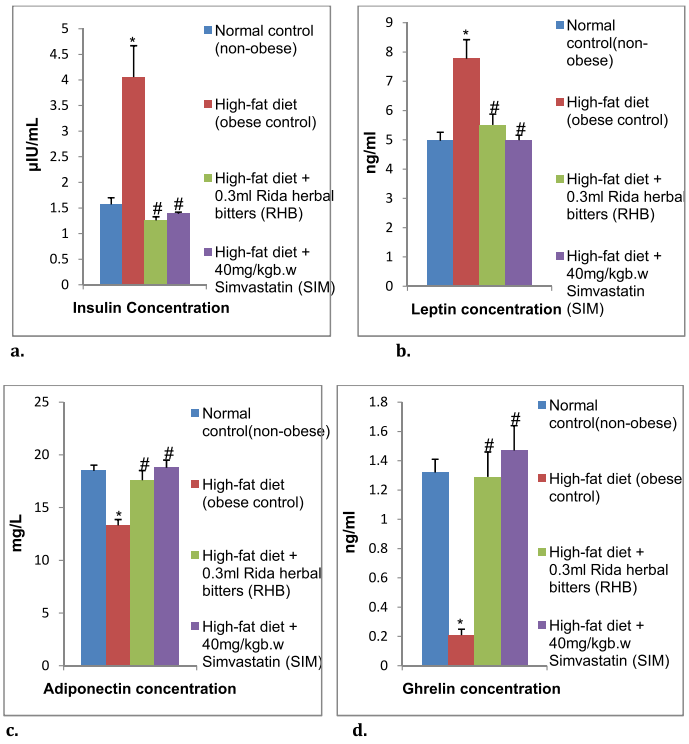
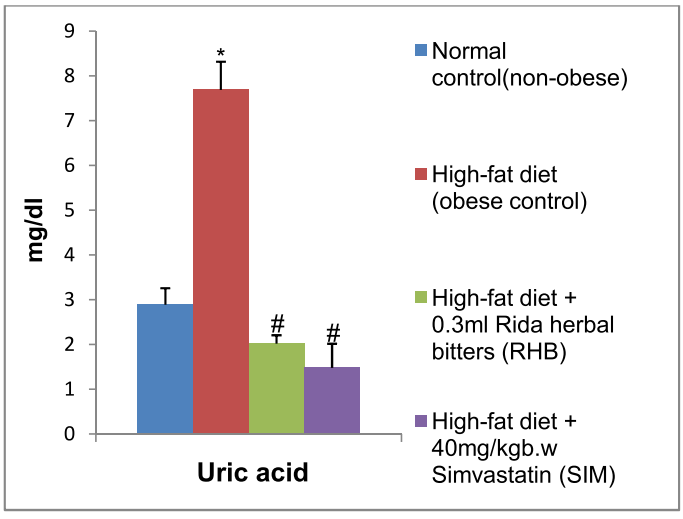


Fig. 4: Effects of Rida herbal bitters (RHB) on (a) uric acid level (b) urea level (c) creatinine level in high-fat diet induced obese rats. Values are expressed as mean ± SEM (n=8). *significant at p<0.05 compared with control; #significant at p<0.05 compared with high-fat diet untreated group.



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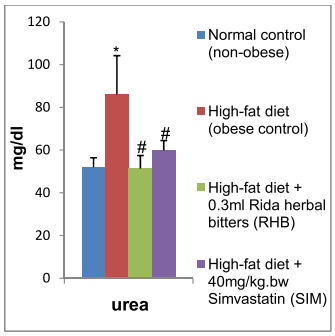

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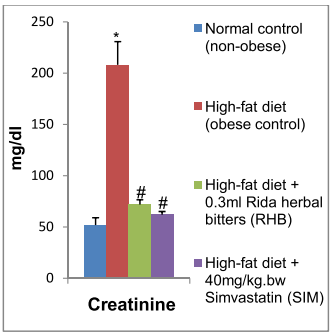

c.

Table 1: Effects of Rida herbal bitters on oxidative stress marker and antioxidant activity in high-fat diet induced obese rats.

Parameters	Normal control (non-obese)	High-fat diet (obese control)	High-fat diet + 0.3ml Rida herbal bitters (RHB)	High-fat diet + 40mg/kgb.w Simvastatin (SIM)
MDA (μM)	7.79 ± 0.63	11.92 ± 0.64*	8.24 ± 0.58#	8.49 ± 0.25#
GPx (U/L)	603.43 ± 10.99	338.95 ± 5.95*	566.06 ± 11.28#	581.12 ± 11.08#
SOD (μ/ml)	1.89 ± 0.09	0.91 ± 0.05*	1.66 ± 0.18#	1.71 ± 0.15#
CAT (mol/ml/mins)	16.39 ± 0.98	10.17 ± 0.41*	17.39 ± 0.89#	16.69 ± 0.99#
GSH (mM)	1.93 ± 0.07	0.88 ± 0.03*	1.86 ± 0.05#	1.61 ± 0.14#

*Values are expressed as mean ± SEM (n=8). *significant at p<0.05 compared with control; #significant at p<0.05 compared with high-fat diet untreated group.*

DISCUSSION

Obesity is well known as a serious health issue up to date. The consumption of high-fat and carbohydrate-rich diet plays a fundamental role in etiology of obesity [13].Current conventional anti-obesity drugs have many adverse side effects and research has focused on the use of nutraceutical and herbal medicines from natural compound as alternative therapy for obesity [14, 15]. Here in this study, anti-obesity effect of Rida herbal bitters in high-fat diet induced obese was investigated.

High-fat diet induced obesity models in animal has been reported to develop similar metabolic complications identical as humans' obesity [16].In this present study, intake of calorically high-fat diet (HFD) induced higher body weight and food intake which consistent with findings of Sakuludomkan et al. [17]. These results confirmed that chronic consumption of high-fat diet results in obesogenic states which is coupled with positive energy imbalance [18].This body weight gain could be attributed to the high rate of acylation of saturated fatty acids into triglycerides which resulted in excessive fat deposition in adipose tissues[19]. The body weight gain and food intakes were reduced on administration of Rida herbal bitters (RHB) which revealed body weight gain inhibition efficacy of the herb and may be attributed to the thermogenic effects of many phytochemicals in Rida herbal bitters and their synergistic action on fat metabolism.

Obesity-related hyperglycemia is a core factor for etiology of diabetes mellitus, primarily as a result of insufficient insulin secretion and/ or ineffective of insulin at target tissues (insulin resistance) [20, 21].It has been reported that high-fat diet cause insulin resistance, reduction in insulin receptors number, and decrease in hepatic and muscle glycogen synthesis, which resulted in elevated blood glucose [22]. The results of this finding also revealed significant elevated blood glucose levels in high-fat diet induced obese which are in line with the report of Seo et al [23]. Rida herbal

bitter supplementation attenuates the elevated blood glucose levels, indicating its anti-hyperglycemic activity and this support the finding of Prakash Raj Pandeya et al on the blood glucose lowering effect of a novel herbal formulation (F2)[24]. The hypoglycemic activity of Rida herbal bitters might stem from the insulin sensitivity restoration, facilitation of glucose uptake at peripheral tissues, increase hepatic and muscle glycogenesis and potentiation of insulin secretion from beta-cell of pancreas which harmonizes the findings of Piero et al [25].

High-fat diet intake have been previously reported to induce dyslipidemia, characterized by elevated total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and very-low density lipoprotein cholesterol (VLDL-C) and decreased high-density lipoprotein cholesterol (HDL-C) [26-28]. Chronic dyslipidemia increases the risk and progression of several obesity-related metabolic complications such as type-2-diabetes mellitus and cardiovascular disease [29, 30].In this study, consumption of high-fat diet cause an aberration in lipid profiles markedly by increased TC, TG, LDL-C and VLDL-C levels and reduced HDL-C level, indicating development of dyslipidemia and corroborate with the findings of Prince et al [31]. Rida herbal bitters (RHB)supplementation lessen the TC, TG, LDL-C and VLDL-C levels and upsurge HDL-C level, parallel with the findings of Marcéline Joëlle Mbouche Fanmoe et al. [32].

Atherogenic index (AI) is a good predictor of cardiovascular disease and cardiac risk index (CRI) is known as a hyperlipidemia risk indicator [33].High atherogenic and cardiac risk index is an evidence of occurrence of obesity, dyslipidemia and vulnerable of cardiovascular disease in untreated obese in this study. These levels were ameliorated on administration of Rida herbal bitters; connoting its anti-dyslipidemic effect and prevention of cardiovascular diseases complications induced by high-fat diet and are in accord with the findings of Niroumand et al [34].

Furthermore, excessive fat accumulation in obese state has been linked with induction of oxidative stress with tremendous releasing of free radicals and diminution in free radicals scavenge activity of antioxidant defense system also contribute to the etiology of several obesity-related metabolic complications [35]. In this study, high-fat diet intake elevated the oxidative stress marker malondialdehyde (MDA) level and altered the activity of antioxidant defense system superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) levels, which agree with the findings of Orabi et al [36]. However, Rida herbal bitters administration enhanced the activity of endogenous antioxidant defense system SOD, GPx, CAT and GSH, and suppressed oxidative stress marker MDA level. This ascertain the antioxidant properties and free radicals neutralizing efficacy of Rida herbal bitters, agree with the findings of Nayan et al [37] who worked on ameliorative effect of Senna alexandrina leaf powder on oxidative stress.

Obesity can lead to renal dysfunction, primarily manifested by changes in the renal function biomarkers [38]. Renal damage results in increase levels of urea, uric acid and creatinine [39].These renal function biomarkers uric acid, urea, and creatinine were elevated in this study, which justify renal dysfunction in the high-fat diet induced obese and corresponded with the results of Vangoori et al [40]. Supplementation of Rida herbal bitters restored and normalized the biomarkers of renal function. This indicates nephro-protective effect of Rida herbal bitters and could be ascribed from its strong antioxidant properties, consistent with the report of Anyanwu et al [41].

In addition, high-fat diet is known to induce hyperinsulinemia and an elevated insulin level is indicative of insulin resistance [42]. In line with the findings of Jae Hyun Jung et al [43], this study observed increase in circulating insulin level in high-fat diet induced obese, suggesting a state of insulin resistance. The plasma insulin level extensively reduced on administration of Rida herbal bitter. This implies that Rida herbal bitters prevent progression of insulin resistance and improve insulin sensitivity at target tissues, which was in consonance with the findings of Kim et al [44].

Ghrelin is an orexigenic hormone secreted by stomach, gastrointestinal tract and other organs, increases its expression during fasting and is suppressed during the postprandial period. Obesity is associated with a low release of circulating ghrelin [45]. Leptin is an anorexigenic hormone produced in adipocytes, is released to circulate in proportion with body fat mass and having antagonist action to ghrelin. Hunger, appetite and satiety are regulated by leptin through central nervous system [46]. Obesity increases the plasma leptin concentration causing leptin resistance. Also, adiponectin, one of the adipokines, inhibits hepatic glucose synthesis, lessening insulin resistance by increasing glucose uptake and fatty acid oxidation in muscle muscles [47]. There is negative correlation between adiponectin level and body fat mass. In obese condition, plasma adiponectin concentration reduced leading to metabolic syndrome, insulin resistance, and cardiovascular disease [48]. This study also observed higher leptin concentration and lower ghrelin and adiponectin concentrations in obese rats, consistent with the findings of Stoica et al [49]. The circulating ghrelin and adiponectin concentrations were improved and leptin concentration diminished on administration of Rida herbal bitters. Studies have shown that body weight loss increase the ghrelin concentration and reduced the leptin concentration [50]. The observed enhancement in the concentrations of ghrelin and adiponectin concentrations, and reduced leptin concentration may be as a result of body weight reduction exhibited by Rida herbal bitters and this is in agreement with report of Juan José Hernández Morante et al [51].

CONCLUSION

In view of this study, Rida herbal bitters exhibited anti-obesity effect by inhibiting body weight gain and its associated dyslipidemia, oxidative stress and altered metabolic hormones. It could therefore be used as a potential alternative medication for management of obesity and its related complications without any deleterious effects. Further investigation is however necessary to isolate bioactive compounds in Rida herbal bitters responsible for these actions.

DECLARATIONS

Authors' Contributions

FO conceived the original idea, designed and supervised the research. MO, AO and NO performed the experiments and data collection with the support of FO. MO analyzed the data and wrote the manuscript. FO reviewed the manuscript. All authors' have read and approved the final manuscript.

Ethics Approval

All procedures were approved by the Animal Care committee of the Ladoke Akintola University of Technology and conducted according to the “Principles of Laboratory Animal Care” and specific national laws where applicable.

Consent for Publication

All authors agreed to publish the article.

Availability of Data and Materials

All data generated and analyzed during this study are included in this article.

Competing Interests

No competing interests.

Funding

This research work did not receive any specific funding/financial support.

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