



Research Article

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Anti-obesity activity of rida herbal bitters in high fat diet-induced obese rats

Ajao Folasade Omobolanle¹, Marcus Olaoye Iyedupe¹, Akanmu Oluwatosin¹, Noheem Olaolu Kalejaiye¹

¹Physiology Department, Faculty of Basic Medical Science, Ladake Akintola University of Technology, P.M.B. 4000, Ogbomoso, Oyo State, Nigeria

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 estimations. **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 malondialdehyde (MDA) level 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 level 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.

Keywords:Rida Herbal Bitters (RHB), High-Fat Diet, Dyslipidemia, Antioxidant, Metabolic Hormones.

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 1.9 billion 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) $\geq 30\text{kg/m}^2$. 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 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 *Curculigopilosa*, *Citrullus colocynthis*, *Hunteriaumbellata*, *Uvariachamae*, and *Senna alata*. Traditionally and like others therapeutic herbal bitters^[10]. Rida herbal bitters is usedfor the management of many ailments and claimed to possess anti-diabetic,antioxidant, anti-hyperlipidemic,anti-inflammatory,

*Corresponding author:

Dr. Ajao Folasade O.

Department of Physiology,
Ladake Akintola University of
Technology, P.M.B. 4000
Ogbomoso, 210214, Nigeria
Email:foajao@lautech.edu.ng

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, Ladoko 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 ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$), relative humidity ($45\% \pm 5\%$) and natural photoperiodicity (12:12h light/dark cycle) prior the initiation of the experiment. All experimental procedures were approved by the Research Ethical Committee of Ladoko 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 cannula. 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 (40mg/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 -4°C 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. Levels of low-density lipoprotein cholesterol (LDL-C), and very-low density lipoprotein cholesterol (VLDL-C) were calculated based on Friedewald *et al.* equation $\text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{TG}/5)$, and $\text{VLDL-C} = \text{Triglyceride (TG)}/5^{[11]}$.

Also, atherogenic index (AI), and cardiac risk index (CRI), were calculated using the following equations

Atherogenic Index (AI) = $(\text{LDL-C}/\text{HDL-C})$

Cardiac Risk Index (CRI) = $(\text{TC}/\text{HDL-C})$

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 \pm 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 ($p < 0.05$), 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).

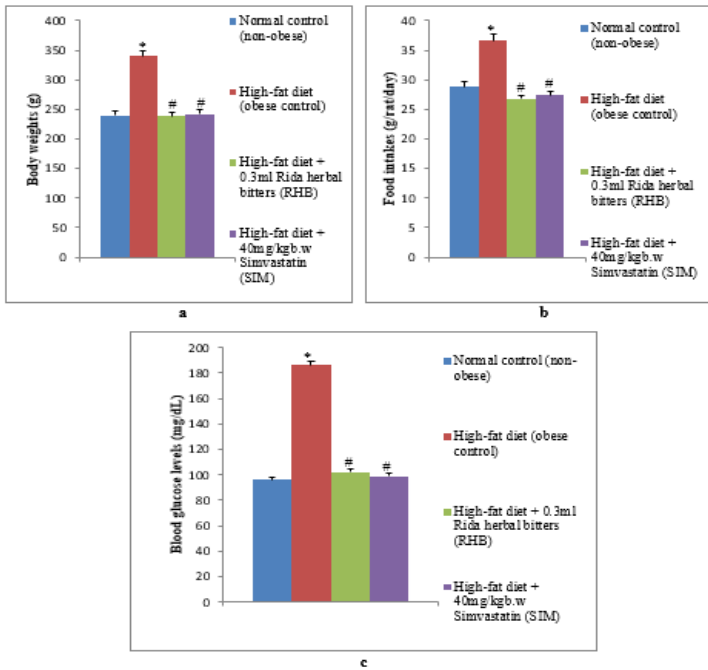


Fig. 1: Effects of Rida herbal bitters (RHB) on (a) body weights (b) food intakes (c) blood glucose levels in high-fat diet induced obese rats. Values are expressed as mean \pm SEM (n=8), *significant at ($p < 0.05$) compared with control; #significant at ($p < 0.05$) compared with high-fat diet untreated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

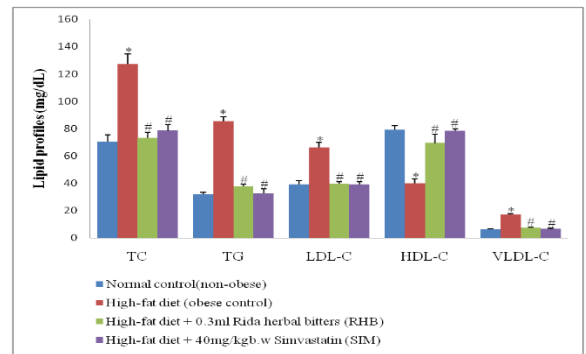


Fig. 2: Effects of Rida herbal bitters (RHB) on (a) lipid profiles (b) atherogenic index (AI), (c) cardiac risk index (CRI) in high-fat diet induced obese rats. Values are expressed as mean \pm SEM (n=8), *significant at ($p < 0.05$) compared with control; #significant at ($p < 0.05$) compared with high-fat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

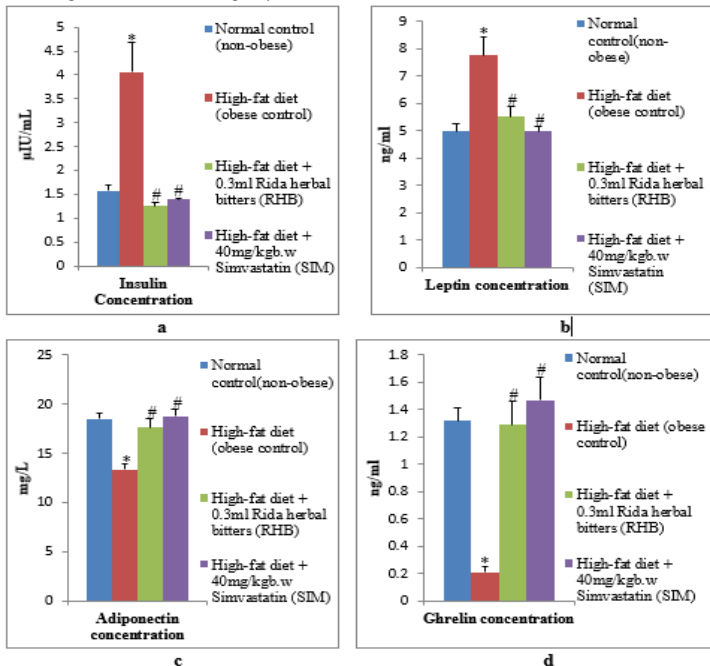


Fig. 3: Effects of Rida herbal bitters (RHB) on (a) insulin concentration (b) leptin concentration (c) adiponectin concentration (d) ghrelin concentration in high-fat diet induced obese rats. Values are expressed as mean \pm SEM (n=8), *significant at ($p < 0.05$) compared with control; #significant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

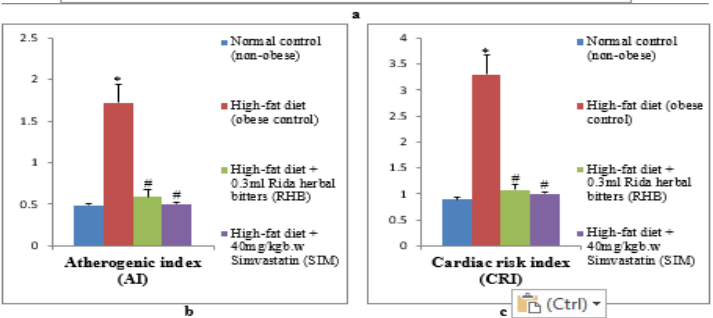


Fig. 4: Effects of Rida herbal bitters (RHB) on (a) uric acid level (b) urea level (c) creatinine level in high-fat diet induced obese rats. Values are expressed as mean \pm SEM (n=8), *significant at ($p < 0.05$) compared with control; #significant at $p < 0.05$ compared with high-fat diet untreated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

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+ 0.99	338.95+5.95*	566.06+11.28#	581.12+11.08#
SOD (u/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 \pm 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 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 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 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.

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.

Conflicts of interest

None declared.

Financial support

None declared.

REFERENCES

- World Health Organization. Obesity and overweight 2020. Available from: <https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight> [cited 17 February 2021].
- Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. *International journal of obesity*. 2008 Sep;32(9):1431-7.
- González-Muniesa P, Martínez JA. Precision nutrition and metabolic syndrome management. *Nutrients*. 2019;11(10):2411.
- Oussaada SM, van Galen KA, Cooman MI, Kleinendorst L, Hazebroek EJ, van Haelst MM, *et al*. The pathogenesis of obesity. *Metab Clin Exp*. 2019;92:26–36.
- Blüher M. Obesity: global epidemiology and pathogenesis. *Nature Reviews Endocrinology*. 2019;15(5):288-98.
- Ulla A, Alam MA, Sikder B, Sumi FA, Rahman MM, Habib ZF, Mohammed MK, *et al*. Supplementation of *Syzygiumcumini* seed powder prevented obesity, glucose intolerance, hyperlipidemia and oxidative stress in high carbohydrate high fat diet induced obese rats. *BMC complementary and alternative medicine*. 2017;17(1):1-3.
- Szeto HH, Liu S, Soong Y, Alam N, Prusky GT, Seshan SV, *et al*. Protection of mitochondria prevents high-fat diet-induced glomerulopathy and proximal tubular injury. *Kidney international*. 2016;90(5):997-11.
- Ryder JR, Fox CK, Kelly AS. Treatment options for severe obesity in the pediatric population: current limitations and future opportunities. *Obesity*. 2018;26(6):951-60.
- de Freitas Junior LM, de Almeida Jr EB. Medicinal plants for the treatment of obesity: ethnopharmacological approach and chemical and biological studies. *American journal of translational research*. 2017;9(5):2050.
- Obasi DC, Ogugua VN, Obasi JN, Okagu IU. Phytochemical, nutritional and anti-nutritional analyses of Ruzu herbal bitters. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*. 2020;15(1):04-17.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*. 1972;18(6):499-502.
- Gupta R, Gupta RS. Protective role of *Pterocarpus marsupium* in diabetes-induced hyperlipidemic condition. *Journal of complementary and integrative medicine*. 2009;21:6(1).
- Hruby A, Manson JE, Qi L, Malik VS, Rimm EB, Sun Q, *et al*. Determinants and consequences of obesity. *Am J Public Health*. 2016;106:1656–62.
- Dietrich MO, Horvath TL. Limitations in anti-obesity drug development: the critical role of hunger-promoting neurons. *Nature reviews Drug discovery*. 2012;11(9):675-91.
- Liu Y, Sun M, Yao H, Liu Y, Gao R. Herbal medicine for the treatment of obesity: an overview of scientific evidence from 2007 to 2017. *Evidence-Based Complementary and Alternative Medicine*. 2017; 8943059.
- Arika WM, Kibiti CM, Njagi JM, Ngugi MP. Anti-obesity effects of dichloromethane leaf extract of *Gnidia glauca* in high fat diet-induced obese rats. *Heliyon*. 2019;5(11):e02800.
- Sakuludomkan W, Yeewa R, Subhawa S, Khanaree C, Bonness AI, Chewonarin T. Effects of fermented *houltuyunia cordata* thunb. On diabetic rats induced by a high-fat diet with streptozotocin and on insulin resistance in 3T3-L1 adipocytes. *Journal of Nutrition and Metabolism*. 2021;15.
- Lennox R, Moffett RC and Porter DW. Effects of glucose-dependent Insulinotropic Polypeptide receptor Knockout and a high-fat diet on cognitive function and hippocampal Gene expression in mice. *Mol. Med. Rep*. 2015;12:1544–48.
- Storlien LH, Huang XF, Lin S, Xin X, Wang HQ, Else PL, *et al*. Dietary fat subtypes and obesity. *Fatty acids and lipids: new findings*. 2001:148-54.
- Arika WM, Nyamai DW, Agyrifio DS, Ngugi MP, Njagi EN. In vivo antidiabetic effect of aqueous leaf extract of *Azadirachta indica*, A. juss in alloxan induced diabetic mice. *Journal of Diabetic Complications and Medicine*. 2016;1(2):1-6.
- Ikemoto S, Takahashi M, Tsunoda N, Maruyama K, Itakura H, Ezaki O, *et al*. High-fat diet-induced hyperglycemia and obesity in mice: differential effects of dietary oils. *Metabolism*. 1996;45(12):1539-46.
- Hariri N, Thibault L. High-fat diet-induced obesity in animal models. *Nutrition research reviews*. 2010;23(2):270-99.
- Seo SH, Fang F, Kang I. Ginger (*Zingiber officinale*) attenuates obesity and adipose tissue remodeling in high-fat diet-fed c57bl/6 mice. *International Journal of Environmental Research and Public Health*. 2021;18(2):631.
- Pandeya PR, Lamichhane R, Lee KH, Lamichhane G, Kim SG, Jung HJ, *et al*. Efficacy of a novel herbal formulation (F2) on the management of obesity: In vitro and in vivo study. *Evidence-Based Complementary and Alternative Medicine*. 2021;8854915:14.
- Piero NM, Eliud NN, Susan KN, George OO, David NJ. In vivo antidiabetic activity and safety in rats of *Cissampelos pareira* traditionally used in the management of diabetes mellitus in Embu County, Kenya. 2015;(6):184.
- Sifat N, Zihad SN, Lovely F, Rouf R, Shajib GM, Alam MA, Shilpi JA, *et al*. Supplementation of *Heliotropium indicum* Linn attenuates obesity and associated metabolic disorders in high-carbohydrate-high-fat diet-induced obese rats. *Journal of Food Biochemistry*. 2020;44(11):e13444.
- Rahman MM, Alam MN, Ulla A, Sumi FA, Subhan N, Khan T, Sikder B, *et al*. Cardamom powder supplementation prevents obesity, improves glucose intolerance, inflammation and oxidative stress in liver of high carbohydrate high fat diet induced obese rats. *Lipids in health and disease*. 2017;16(1):1-2.
- Mamun M, Al A, Faruk M, Rahman M, Nahar K, Kabir F, Alam MA, *et al*. High carbohydrate high fat diet induced hepatic steatosis and dyslipidemia were ameliorated by *Psidium guajava* leaf powder

- supplementation in rats. Evidence-Based Complementary and Alternative Medicine. 2019;1897237.
29. Musunuru K. Atherogenic dyslipidemia: cardiovascular risk and dietary intervention. *Lipids*. 2010;45(10):907-14.
 30. Stahel P, Xiao C, Hegele RA, Lewis GF. The atherogenic dyslipidemia complex and novel approaches to cardiovascular disease prevention in diabetes. *Canadian Journal of Cardiology*. 2018;34(5):595-04.
 31. Prince MR, Zihad SN, Ghosh P, Sifat N, Rouf R, Al Shajib GM, Alam MA, Shilpi JA, et al. *Amaranthus spinosus* Attenuated Obesity-Induced Metabolic Disorders in High-Carbohydrate-High-Fat Diet-Fed Obese Rats. *Frontiers in nutrition*. 2021;(8):653918.
 32. MboucheFanmoe MJ, TatsadjieuNgonne L, Ndjouenkeu R. Ipomea batatas Leaf Powder from Cameroon: Antioxidant Activity and Antihyperlipidemic Effect in Rats Fed with a High-Fat Diet. *Journal of Lipids*. 2021; <https://doi.org/10.1155/2021/5539878>
 33. Kang HJ, Jeon IH, Kwon TO, Choi J, Kim SZ, Jang SI, et al. Effect of Mori Folium extract on improvement of blood flow in ferric chloride-induced carotid artery damage rat model. *Journal of Physiology & Pathology in Korean Medicine*. 2014;28(6):607-13.
 34. Niroumand S, Khajedaluae M, Khadem-Rezaiyan M, Abrishami M, Juya M, Khodae G, Dadgarmoghaddam M, et al. Atherogenic Index of Plasma (AIP): A marker of cardiovascular disease. *Medical journal of the Islamic Republic of Iran*. 2015;29:240.
 35. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. *The Journal of clinical investigation*. 2017;127(12):1752-61.
 36. Orabi SH, Al-Sabbagh ES, Khalifa HK, Mohamed MA, Elhamouly M, Gad-Allah SM, Abdel-Daim MM, et al. Commiphora myrrha resin alcoholic extract ameliorates high fat diet induced obesity via regulation of UCP1 and adiponectin proteins expression in rats. *Nutrients*. 2020;12(3):803.
 37. Nayan SI, Chowdhury FI, Akter N, Rahman MM, Selim S, Saffoon N, Khan F, et al. Leaf powder supplementation of Senna alexandrina ameliorates oxidative stress, inflammation, and hepatic steatosis in high-fat diet-fed obese rats. *Plos one*. 2021;16(4):e0250261.
 38. Maksoud HA, Elharrif MG, Mohammed RR, Omnia MA, El Sayed NE. Biochemical changes associated with low and very low calorie diets on lipid metabolism, iron profile and kidney function in obese rats. *Clinical Nutrition Experimental*. 2020;33:32-8.
 39. Alsufyani HA, Zawawi BM. Protective effect of garlic juice on renal function and lipid profile in rats fed with high-fat diet. *Saudi J Health Sci*. 2021;10:138-142.
 40. YakaiahVangoori, Anusha Dakshinamoorthi and Kavimani S. Effect of *Myristica Fragrans* Extract on Lipid Profile, Glucose, Body Weight, Food Intake, Liver and Renal Functions in Experimental Obese Rats. *Biomedical & Pharmacology Journal*. 2019;12(2):677-82.
 41. Anyanwu AA, Jimam NS, Dangiwa DA, Wannang NN, Falang KD. Protective effects of alkaloids of *Cucumis metuliferus* isolated from the fruit pulp on some vital organs. 2014;3:259-63.
 42. Wu T, Qi X, Liu Y, Guo J, Zhu R, Chen W, Zheng X, et al. Dietary supplementation with purified mulberry (*Morus australis* Poir) anthocyanins suppresses body weight gain in high-fat diet fed C57BL/6 mice. *Food chemistry*. 2013;141(1):482-7.
 43. Jung JH, Hwang SB, Park HJ, Jin GR, Lee BH. Antiobesity and Antidiabetic Effects of *Portulaca oleracea* Powder Intake in High-Fat Diet-Induced Obese C57BL/6 Mice. *Evidence-Based Complementary and Alternative Medicine*. 2021;28: <https://doi.org/10.1155/2021/5587848>
 44. Kim NY, Thomas SS, Hwang DI, Lee JH, Kim KA, Cha YS, et al. Anti-Obesity Effects of *Morus alba* L. and *Aronia melanocarpa* in a High-Fat Diet-Induced Obese C57BL/6J Mouse Model. *Foods*. 2021;10(8):1914.
 45. Inui A, Asakawa A, Bowers CY, Mantovani G, Laylano A, MeguidMM, Fujimiya M, et al. the emerging role of the stomach as an endocrine organ. *The FASEB Journal*. 2004;18(3):439-56.
 46. Izquierdo AG, Crujeiras AB, Casanueva FF, Carreira MC. Leptinobesity and leptin resistance: Where are we 25 years later? *Nutrients*. 2019;11: 2704.
 47. Ghadge AA, Khaire AA, Kuvalekar AA. Adiponectin: a potential therapeutic target for metabolic syndrome. *Cytokine & growth factor reviews*. 2018;39:151-8.
 48. Nigro E, Scudiero O, Monaco ML, Palmieri A, Mazzarella G, Costagliola C, Bianco A and Daniele A, et al. New insight into adiponectin role in obesity and obesity related diseases. *Biomed Res Int*. 2014;658913.
 49. Stoica L, Gadea R, Navolan DB, Lazar F, Duta C, Stoian D, Tarta C, Olaru F, Isaic A, Dobrescu A. Plasma ghrelin, adiponectin and leptin levels in obese rats with type 2 diabetes mellitus after sleeve gastrectomy and gastric plication. *Experimental and Therapeutic Medicine*. 2021;21(3):1.
 50. Giammanco M, Lantieri L, Leto G, Plescia F and Di Majo D. Nutrition, obesity and hormones. *J. Biol. Res*. 2018;91:108-18.
 51. Hernández Morante JJ, Díaz Soler I, Muñoz JS, Sánchez HP, Barberá Ortega M, Martínez CM, et al. Moderate Weight Loss Modifies Leptin and Ghrelin Synthesis Rhythms but Not the Subjective Sensations of Appetite in Obesity Patients. *Nutrients*. 2020;12(4):916.