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Research Article

Preventive Effect of *Bridelia micrantha* Leaf Extract on Insulin Resistance and Dyslipidaemia in Rats Fed with High Salt Diet

Ajao F.O, Olatunji L.A, Soladoye A.O

¹Cardiovascular Research Unit, Department of Physiology, University of Ilorin, Ilorin, Nigeria.

²Department of Physiology, Ladoké Akintola University of Technology, Ogbomosho, Oyo State. Nigeria

ABSTRACT

The preventive effect of *Bridelia micrantha* leaf extract on insulin resistance and dyslipidaemia in high-salt diet rats were investigated. Twenty-four Wistar rats were divided into four groups of six rats each. Group one serves as control and was fed with normal feed for 6 weeks, group 2 was fed with high salt diet only for 6 weeks while groups 3 and 4 were fed with high-salt diet and orally administered methanol extract of *Bridelia micrantha* leaf at a dose of 50 and 200 mg/kg/day simultaneously for 6 weeks. At the end of the study, fasting blood glucose level, fasting blood insulin level and lipid profile and were measured. Homeostasis model of assessment for IR (HOMA-IR), triglyceride-glucose (TyG) index triglyceride (TG)/HDL-cholesterol (HDL-C) and total cholesterol (TC)/HDL-C ratios were estimated. Fasting insulin, HOMA-IR and TyG levels of rats given high-salt diet only significantly increased compared with control showing induction of insulin resistance. There was also impaired glucose tolerance and dyslipidaemia in the high-salt diet only rats. The high-salt diet rats administered *Bridelia micrantha* at doses of 50 and 200 mg/kg/day lower the above parameters significantly when compared with the group given only high-salt diet, with stronger impact by the methanolic extract. In Conclusion, oral administration of *Bridelia micrantha* leaf extract could prevent the development of insulin resistance and hyperlipidaemia in high-salt diet-fed rat.

Keywords: *Bridelia micrantha*, high-salt diet, insulin resistance, dyslipidemia

*Author for correspondence: E-mail: - folatun99@yahoo.com ; Tel. +2348034659049

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INTRODUCTION

The incidence of type 2 diabetes has been on the increase in the last few decades. Type 2 diabetes is a progressive disease usually predisposed by a cluster of conditions in the metabolic syndrome, including insulin resistance, dyslipidaemia, visceral obesity and hypertension (Alberti *et al.*, 2005; Wilson *et al.*, 2005). Studies have shown that the development of metabolic syndrome is mainly determined by insulin resistance. Therefore, prevention of insulin resistance is essential to halt the progression of the disease. Insulin resistance prevents blood glucose from entering the target cells, resulting in increase of blood glucose. This causes the pancreatic beta cells to increase its insulin secretion in order to maintain euglycemia, resulting in hyperinsulinemia state (Basciano *et al.*, 2005; Antuna-Puente *et al.*, 2011). Excessive salt intake is linked with inflammation, lipid disorders, end-organ injury, hypertension, decrease insulin sensitivity (Ogihara *et al.*, 2001). Studies have also shown that obesity,

dyslipidemia, and oxidative stress are important to the development of insulin resistance (Altaş *et al.*, 2010; Henriksen *et al.*, 2011; Samuel *et al.*, 2011; Tiganis, 2011). Approach to prevent insulin resistance by using natural ingredients containing potent antioxidant and anti-dyslipidemia effect is a safe alternative because it rarely cause unwanted side effects.

In South Western Nigeria, one of the herbs with desired effect is *Bridelia micrantha*. A leaf decoction of *Bridelia micrantha* is used traditionally as part of recipe for the management of diabetes mellitus (Abo and Jaiyesimi, 2008). *Bridelia micrantha*. (Euphorbiaceae), commonly known in English as “coast gold leaf” or “ogaofia” (the boss of the bush) in Igbo or “igi-ira” in Yoruba, is a deciduous tree of about 20 meters tall with a dense rounded crown. The plant is indigenous to southern part of Nigeria. The leaf extract of the plant *Bridelia micrantha* has been reported to have several beneficial metabolic effects in animal models, including blood glucose lowering and antioxidant effects (Adika *et al.*, 2012).

Nevertheless, there is no report on *Bridelia micrantha* preventing insulin resistance. In this present study, we therefore investigated the preventive effect of methanolic extracts of *Bridelia micrantha* leaf on insulin resistance and dyslipidaemia in high-salt diet rats.

MATERIALS AND METHODS

Plant material and extract preparation

Fresh leaves of *Bridelia micrantha* were collected from a farm settlement in Ogbomoso, Oyo State, Nigeria. The plant was identified, authenticated and registered with voucher specimen number (LHO 376) at the herbarium unit of the Biology department, Ladoké Akintola University of Technology, Ogbomoso. The *Bridelia micrantha* leaves were collected washed in tap water and air dried at room temperature. The dried leaves were ground into powder and the powdered sample was extracted using water and methanol as solvent (Adika *et al.*, 2011). The powder (800g) was macerated in 100% methanol at room temperature for 72 hrs. This was then filtered using a filter paper (Whatmann size no.1) and the filtrate was evaporated to dryness in water bath at 40°C to a brown dried residue of 80.6g and kept in an air tight bottle until used.

Preliminary Phytochemical screening

Adopting the methods of Trease and Evans (1983) and Sofowora (1993), preliminary phytochemical screening of the methanolic extract of *Bridelia micrantha* leaf was carried out.

Animals and treatment

The investigation was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the University of Ilorin Ethical Review Committee (Reference no: UIL/UERC/12/68BK001). Every effort was made to minimize both the number of animals used and their suffering. Twenty four Wistar rats weighing between 150-200g were randomly assigned into 4 groups (n=6/group).

Group 1 - Control received normal feed and distilled water for 6 weeks (NO)

Group 2- received high-salt (8%) in feed for 6 weeks (HS)

Group 3- received high salt + 50mg/kg *Bridelia micrantha* extract *p.o* simultaneously for 6weeks (HS +BMLD)

Group 4 - received high salt + 200mg/kg *Bridelia micrantha* extract *p.o* simultaneously for 6 weeks (HS + BMHD).

Sample preparation

At the end of treatment period, the rats were anesthetized with sodium pentobarbital. Blood was collected by cardiac puncture into heparinized bottle and was centrifuged at 3000g for 5min. Plasma was stored frozen until needed for biochemical assay.

Oral glucose tolerance test (OGTT)

Glucose challenge test was performed 24 hrs before the end of the experiment. The rats had 12 hrs overnight fast. Glucose (2 g/kg *bw*) was given (*po*). Blood sample was obtained from the tail before glucose load and then sequentially after 30, 60, 90

and 120 min. Blood glucose levels were determined with a glucometer (ACCU-CHEK Active- Roche Diagnostics, Germany). Glucose tolerance was expressed as a function of the area under the OGTT curve (AUC) as previously described (Olatunji *et al.*, 2016). Elevated 1-hr postload glucose level is also used as a reliable predictor of IR, pancreatic β -cell function, atherosclerotic CVD and renal dysfunction (Succurro *et al.*, 2010; Sciacqua *et al.*, 2011; Bianchi *et al.*, 2013).

Biochemical assays and IR

Plasma insulin was determined using enzyme linked immunoassay (ELISA) kit from Ray Biotech, Inc. (Georgia, USA). Fasting plasma levels of total cholesterol (TC) and triglyceride (TG) were measured by standardized enzymatic colorimetric methods using assay kit obtained from Fortress Diagnostics Ltd. (Antrim, UK). High-density lipoprotein-cholesterol (HDL-C) was measured by enzymatic clearance assay (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) whereas low-density lipoprotein-cholesterol (LDL-C) was estimated using modified Friedewald's formula (Friedewald *et al.*, 1972). TC/HDL-C and TG/HDL-C ratios were estimated as marker of atherogenic lipid indices. TyG index: $\ln [TG (mg/dl) \times FPG (mg/dl)/2]$ (Du *et al.*, 2014; Lee *et al.*, 2014). IR was estimated using the homeostasis model assessment for IR (HOMA-IR). HOMA-IR is expressed as fasting glucose (mmol/l) * fasting insulin (μ U/l)/22.5.

Data analysis and statistics

All data were expressed as means \pm standard error of mean (SEM). Statistical group analysis was performed with SPSS statistical software. One-way analysis of variance (ANOVA) was used to compare the mean values of variables among the groups. Bonferroni's test was used to identify the significance of pair wise comparisons of mean values among the groups. Statistically significant differences were accepted at $p < 0.05$.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening results of the methanolic extract of *Bridelia micrantha* showed the presence of alkaloids, cardenolides, flavonoids, anthraquinones, tannins, and Saponins.

Physiological parameters

In the high salt only treated rats, high-salt diet only caused a significant decrease in body weight (Table 1). Treatment with the extracts from day 1 of high-salt loading prevented the decrease in body weight. High-salt diet only led to significant decrease in food intake (Table 1). The high-salt diet only rats consumed significantly more water but less food compared to the control (Table 1). Treatment with both doses of the extract from day 1 of high-salt loading prevented these effects.

Glucose regulation

Fasting glycemia was not affected in all experimental groups (Fig.1). High-salt diet only led to significant elevated glycemia after ½-hour of glucose load. High salt diet only still

caused elevated glycemia after 1-hour and 1½-hour of glucose load. However, treatment with HS + BMLD and HS + BMHD led to significant reduction in glycemia after 1-hour and 1½-hour of glucose load in the high-salt diet rats (Fig. 1). Glucose tolerance was estimated by the area under the curve (AUC) of

oral glucose tolerance test (OGTT). The values of AUC were significantly higher in high-salt diet only rats when compared to the control, whereas AUC was significantly lower in HS +BMLD and HS + BMHD treated rats when compared to high-salt diet only rats (Fig. 1).

Table 1:

Effect of methanolic extract of *Bridelia micrantha* leaf on body weight, food and water intake during high salt-induced IR

PARAMETERS	NO	HS	HS+BMLD	HS+BMHD
Body weight change (g)	9.0±0.3	3.2±0.6**	8.0±0.2##	8.3±0.2##
Food intake (g/kg)	136.1±3.1	123.7±2.4*##	131.5±2.3*##	130.5±1.4*##
Water intake (ml/kg)	124.2±3.1	130.7±3.4##	122.5±2.2##	123.4±2.6##

Values are expressed as mean ± SEM (n=6). *- significant at $p < 0.05$ compared with control, ** - significant at $p < 0.01$ compared with control, # - significant at $p < 0.05$ compared with high salt-induced IR group; ## - significant at $p < 0.01$ compared with high salt-induced IR group).

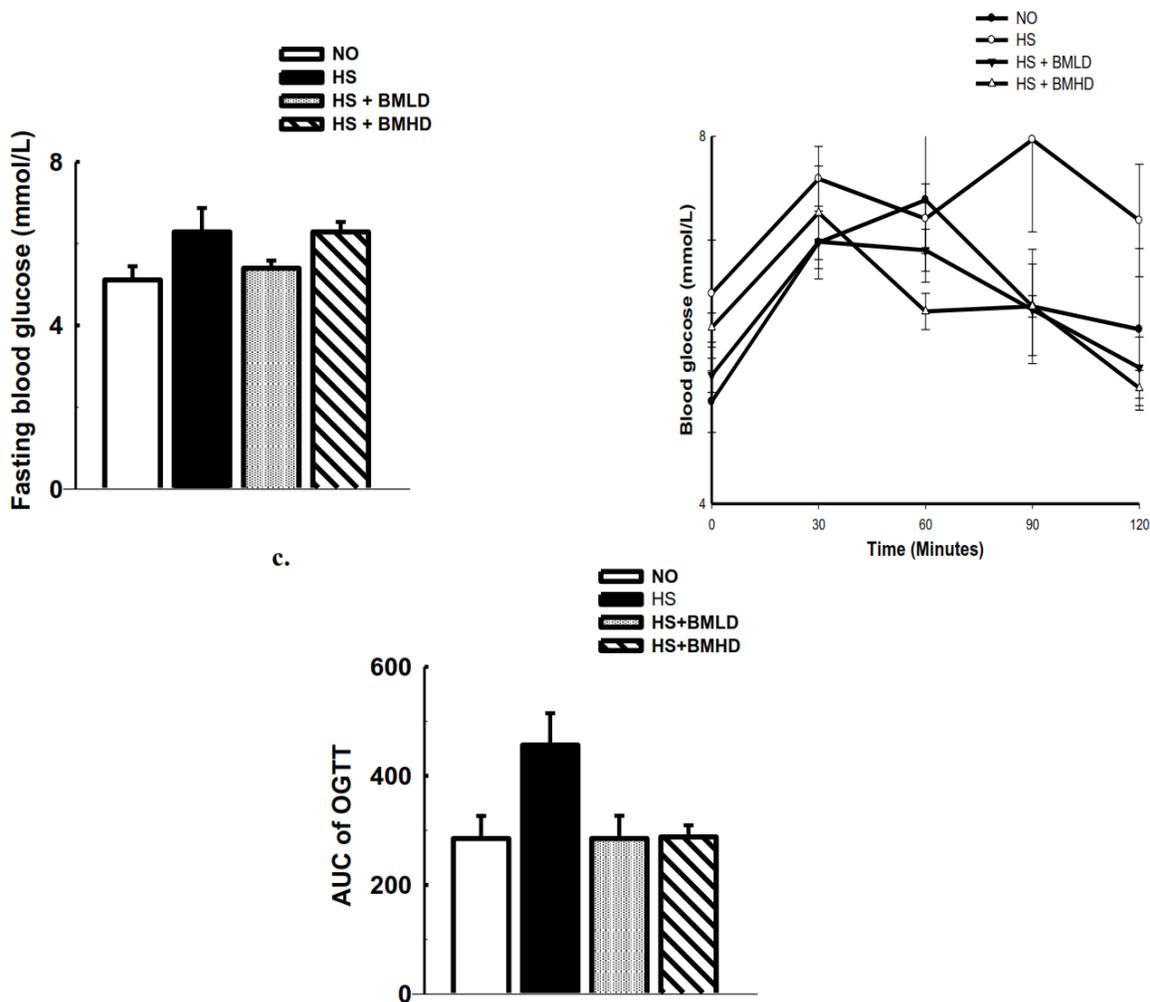


Figure 1:

Effect of methanolic extract of *Bridelia micrantha* on (a) fasting blood glucose, (b) oral glucose tolerance test (OGTT) and (c) area under curve (AUC) of OGTT during high-salt induced IR. High salt diet only led to increase in 1hr postload glucose and AUC that was attenuated in HS + BMLD and HS + BMHD rats. There was however no difference in the fasting blood glucose between groups. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean ± SEM of 6 rats per group (* $p < 0.05$ vs CON; ** $p < 0.01$ vs CON; # $p < 0.01$ vs HS; ## $p < 0.01$ vs HS).

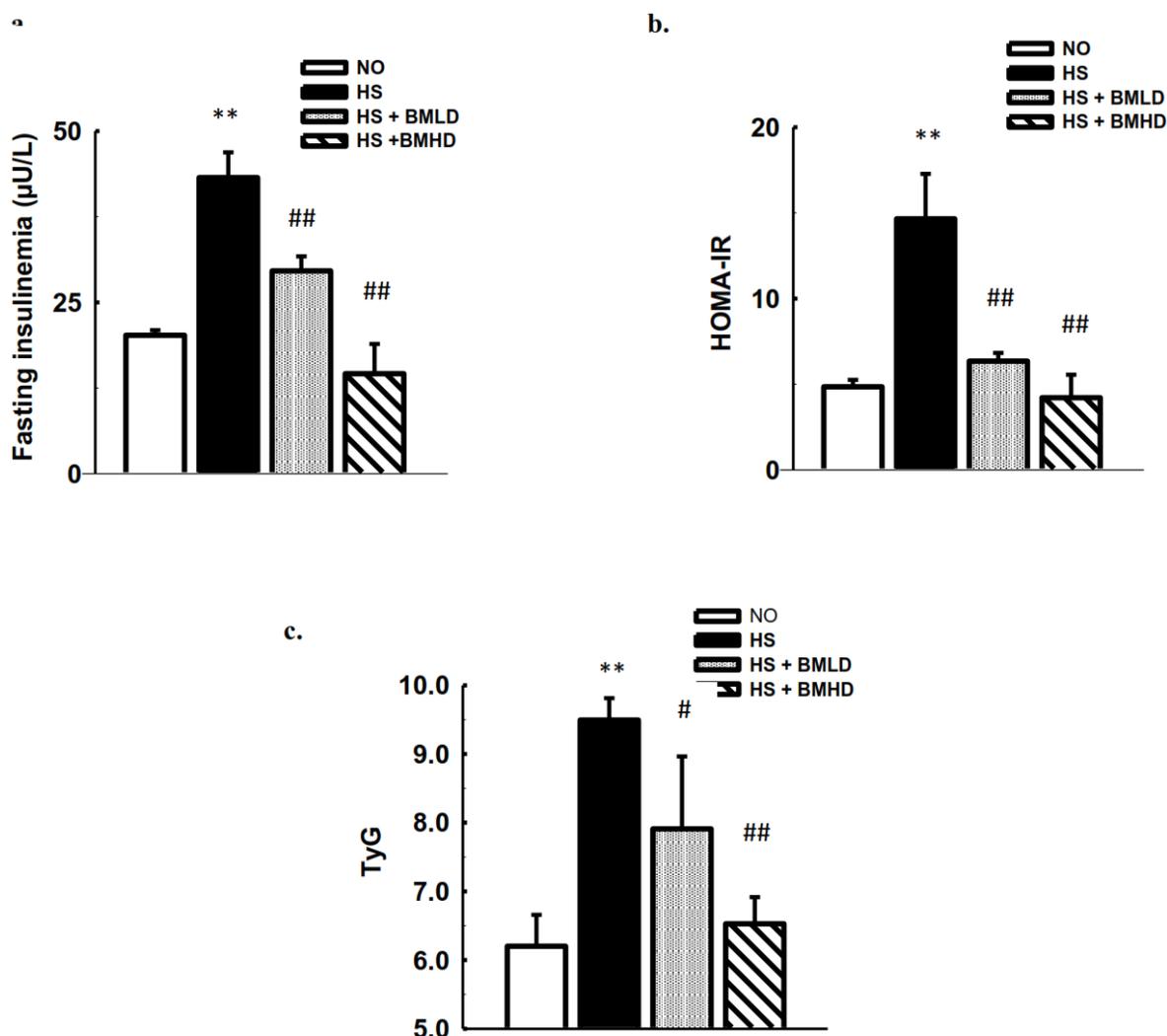


Figure 2: Effect of methanolic extract of *Bridelia micrantha* on insulin resistance markers- (a) fasting insulin level, (b) HOMA-IR and (c) TyG index during high-salt induced IR. High salt diet only led to significant increases in fasting insulin level, HOMA-IR and TyG index. The fasting insulin, HOMA-IR and TyG levels were significantly lower in the HS + BMLD and HS + BMHD rats when compared with the high-salt diet only rats (HS). Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean \pm SEM of 6 rats per group (* p <0.5 vs CON; # p <0.05 vs HS).

Table 2: Effect of methanolic extract of *Bridelia micrantha* leaf on lipid profile during high salt-induced IR

PARAMETERS	NO	HS	HS + BMLD	HS + BMHD
Triglyceride (mg/dl)	35.2 \pm 2.5	61.5 \pm 6.3**	53.5 \pm 3.3##	50.9 \pm 4.1##
Total cholesterol (mg/dl)	51.5 \pm 3.8	64.6 \pm 2.8*	51.3 \pm 0.7##	51.4 \pm 2.4##
HDL-cholesterol (mg/dl)	22.6 \pm 1.2	15.5 \pm 2.6*	26.8 \pm 0.8###	24.3 \pm 1.3##
LDL- cholesterol (mg/dl)	21.9 \pm 2.8	36.8 \pm 4.2*	15.1 \pm 1.3###	16.4 \pm 1.2##

Data are expressed as mean \pm SEM of 6 rats per group. Data were analyzed by one-way ANOVA followed by Bonferroni *posthoc* test (* p <0.05 vs CON; ** p <0.01 vs CON; # p <0.05 vs IR, ## p <0.01 vs IR)

Insulin sensitivity

High-salt diet only led to significant increase in HOMA-IR, whereas treatment of the high-salt fed rats with 50mg/kg bw and 200 mg/kg bw methanolic extracts of *Bridelia micrantha* leaf prevented the induction of IR (Fig. 2). High-salt diet only resulted in significantly increased fasting plasma insulin level compared to control (Fig. 2). After the experiment, the high-salt diet rats that received 50 mg/kg bw and 200 mg/kg bw methanolic extracts of *Bridelia micrantha* leaf from day 1 of high-salt loading had significantly reduced plasma insulin levels compared with the high-salt diet only. High-salt diet only led to significant increase in TyG index, whereas the high-salt fed rats that received 50 mg/kg bw and 200 mg/kg bw methanolic extracts of *Bridelia micrantha* leaf from day 1 of high-salt loading had significantly reduced TyG index comparable with the high-salt diet only (Fig. 2).

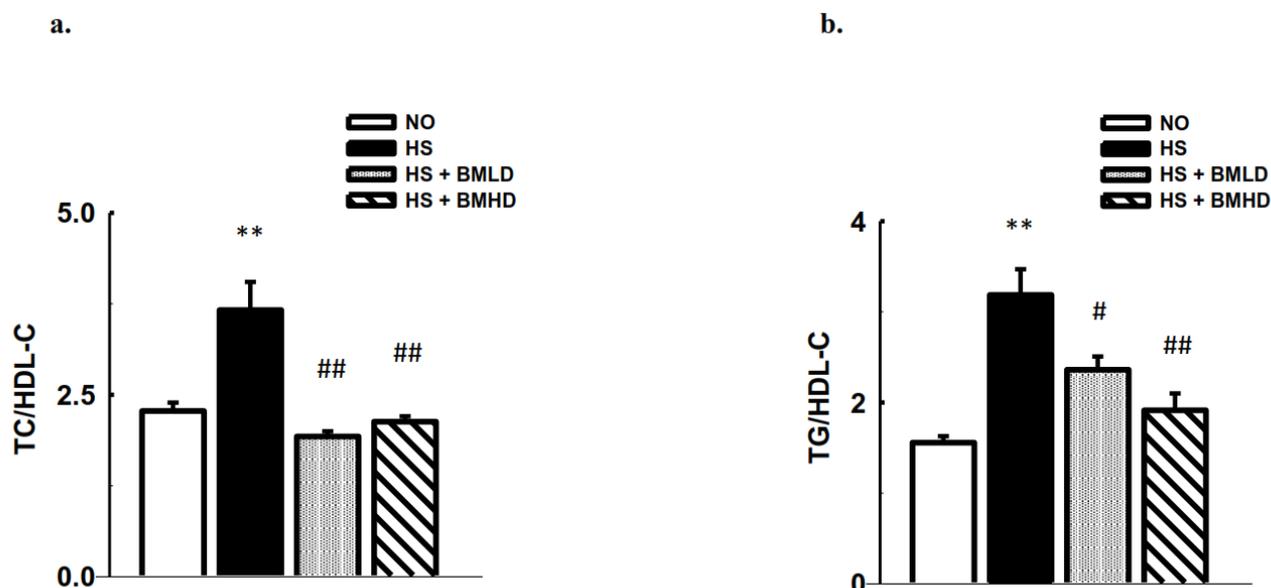


Figure 3:

Effect of methanolic extract of *Bridelia micrantha* on atherogenic indices (a & b) during high-salt induced IR. High salt diet led to an increase in TC/HDL-C and TG/HDL-C ratios. The TC/HDL-C and TG/HDL-C ratios were significantly lower in the HS + BMLD and HS + BMHD rats when compared with the high-salt diet only rats (HS). Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean \pm SEM of 6 rats per group (* $p < 0.05$ vs CON; # $p < 0.05$ vs HS, ## $p < 0.01$ vs HS).

Circulating lipids and atherogenic dyslipidemia

Table 2 depicts the effect of aqueous and methanolic extract of *Bridelia micrantha* leaf and metformin on TG, TC, HDL-C and LDL-C in IR rats. High-salt diet only led to significant increase in plasma levels of TC, TG and LDL-C in all experimental groups when compared with the control whereas plasma HDL-C level was significantly decreased in all experimental groups compared to control. The atherogenic indices were significantly increased in high-salt diet only rats compared to control, TC/HDL-C and TG/HDL-C ratios reduced significantly in the HS +BMLD and HS + BMHD groups when compared with the HS rats (Fig. 3a & b).

DISCUSSION

The use of medicinal plants in the management of diseases is on the increase worldwide. (Adewunmi and Ojewole, 2004; Aliyu et al., 2007). Nigeria has thousands of plant species that are claimed to have medicinal properties and used in the treatment of diseases (Iweala and Oludare, 2011). Studies in both humans and experimental animals have validated that high sodium intake apart from causing hypertension leads to insulin resistance (Donovan et al., 1993, Ogihara et al., 2001; Ogihara et al., 2002; Qin et al., 2007). High salt intake increases the risk of developing of type 2 diabetes mellitus, independent of hypertension or physical inactivity (Hu et al., 2005).

In the present study, we investigate the preventive properties of methanolic extract of *Bridelia micrantha* leaf in high salt-induced IR female rats. Fasting insulin, 1-h postload glucose level, HOMA-IR and TyG index are commonly used surrogate markers of IR (Bianchi et al., 2013; Du et al., 2014). Our data showed an increase in fasting insulin, 1-h postload glucose level, HOMA-IR and TyG index in the high salt fed only rats, suggesting induction of IR. Interestingly, rats fed with high-salt diet along with *Bridelia micrantha* leaf extract had lower fasting plasma insulin level, HOMA-IR, and TyG index than the group given high-salt diet alone. These results indicated that methanolic extract of the *Bridelia micrantha* leaf was able to prevent the occurrence of insulin resistance in high salt diet rats. Indeed, insulin sensitizers have been shown to improve insulin sensitivity by exerting its effect on muscle insulin sensitivity, regulation of leptin expression and stimulation of insulin-sensitive fat cells (Vos et al., 1996). The mechanisms by which *Bridelia micrantha* improves insulin sensitivity either by its direct effects on target tissues or indirectly through endogenous substances, requires further study. Administration of the extract at doses of 50 and 200 mg/kg bw significantly prevented the increased insulin resistance markers with the high dose being the most effective suggesting that methanolic extract of *Bridelia micrantha* prevention of insulin resistance works in a dose-dependent manner. The result was consistent with the study of Andraini and Yolanda (2014) in which *Hibiscus sabdariffa* linn. prevent the occurrence of insulin resistance in a high-fructose diet model.

Baudrand et al. (2014) reported that a high sodium intake was associated with dyslipidemia and insulin resistance. The dyslipidemia observed in high salt diet only rats was reflected by significantly enhanced plasma TG, TC, LDL-C and VLDL-C with significantly decreased HDL-C. Thus high salt diet rats showed higher atherogenic index, which is the risk factors for coronary heart disease. Methanolic extract of *Bridelia micrantha* leaf also prevents the development of dyslipidemia which was reflected by significant decreases in TC, TG and LDL-C levels and a significant increase in HDL-C level of HS + BMLD and HS + BMHD rats respectively when compared with the HS rats. The atherogenic lipid (TC/HDL-C and TG/HDL-C) ratios were also significantly increased in the HS + BMLD and HS + BMHD rats respectively when compared with the HS rats. The preventive effects of the extract on increased lipid and atherogenic lipid ratios also suggest its ability to reduce cardiovascular risks. As insulin resistance and reduced insulin binding have been reported in hypertriglycerolemic persons (Kelly et al., 2004), this may be one mechanism by which high salt diet promotes insulin resistance. Since *Bridelia micrantha* treatment prevented high salt-induced hyperinsulinemia in the HS + BMLD and HS + BMHD groups, this positive effect could be attributed to prevention of hypertriglyceridemia in these rats.

Saponins and tannins have been reported to contribute to the ability of plants to improve dyslipidemia (Nimenibo-uadia, 2003; Rotimi et al., 2011). Preliminary phytochemical screening of *Bridelia micrantha* leaf extract revealed the presence of saponin among other polyphenolic compounds which may be responsible its lipid-lowering effect. Saponins have also been reported to have hypocholesterolic effect (James et al., 2010). Saponins lowers cholesterol level either by binding with cholesterol in the intestinal lumen, preventing its absorption or by binding with bile acids, resulting in a reduction in the enterohepatic circulation of bile acids and increase its fecal excretion (Nimenibo-uadia, 2003; James et al., 2010; Rotimi et al., 2011). Increased bile acid excretion is compensated for by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol (Rotimi et al., 2011).

The observed hypolipidemic effect of *Bridelia micrantha* leaf extract can be therefore, linked to the synergistic actions of phytochemicals like saponins and polyphenolic compounds present in the plant extract. Studies have shown that derangement of glucose, fat and protein metabolism during diabetes also result in the development of hyperlipidemia (Brown and Goldstein, 1983; Austin and Hokanson, 1994). The significantly lowered cholesterol level may have contributed to the observed significant high serum high-density lipoprotein cholesterol in the animals. About 30% of blood cholesterol is carried in the form of high-density lipoprotein cholesterol. A significant decrease in total cholesterol and significant increase in high-density lipoprotein cholesterol is a very good biochemical state for the prevention of atherosclerosis and ischaemic conditions (Schwenke and Carew, 1989; Luc and Fruchart, 1991; Mitra et al., 1995). High level of high-density lipoprotein cholesterol (HDL-C) protects against cardiovascular disease since HDL-C removes cholesterol antheroma within arteries and transport it to the liver for excretion or reutilization (Kwiterovich, 2000; James

et al., 2010). Therefore, the observed significantly increase serum HDL-C level in the HS + BMLD and HS + BMHD rats demonstrates the HDL-C boosting effect of the extract.

In conclusion, the present study suggests that the administration of methanolic extract of *Bridelia micrantha* leaf can prevent insulin resistance associated with high-salt diet, the effect is dose dependent. Further studies are needed to determine the mechanisms of action of *Bridelia micrantha* for the prevention of insulin resistance.

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