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Full Length Research Article

THE EFFECT OF METHANOLIC CRUDE EXTRACT OF *OCIMUM GRATISIMUM* LEAVES ON INSULIN RESISTANCE AND GLUT-4 GENE EXPRESSION IN MONOSODIUM GLUTAMATE INDUCED OBESE RATS

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ABSTRACT
Obesity is a complex chronic global disease affecting people worldwide across all ages, sexes, ethnicities and nationalities. It is accompanied by remodeling of adipocyte, insulin resistance (IR) and type 2 diabetes. The present study was aimed to determine the effect of methanolic crude extract of *Ocimum gratisimum* leaves on insulin resistance and GLUT-4 gene expression in Monosodium induced obese Rats. Phytochemical screening of the crude extract of *Ocimum gratisimum* leaves was carried out before the grouping of animals. The study was conducted using thirty 30 male Wistar rats weighing between 100.0 – 150.0 g. The animals were divided into five groups of six each; Normal control (NC) rats, Obese control (DC) rats, Obese rats treated with *Ocimum gratissimum* (OG) 100 mg/kg B.W (OG-100), Obese rats treated with OG 200 mg/kg B.W (OG-200), Obese rats treated with orlistat 50 mg/kg B.W (OR-50). Obesity was induced by oral administration of 8 mg/g MSG for 7 days and animals were treated with respective doses orally for 1 week. The phytochemical screening of the crude extract of *Ocimum gratisimum* leaves revealed the presence of saponins, tannins, flavonoids, glycosides and the results obtained after induction of obesity with MSG showed significant ($P<0.05$) increase in weight of the rats. After 1 week of treatment with the extract, the weight, non-fasting blood glucose (NFBG) and HOMA-IR level of the rats decreased significantly ($P<0.05$) when compared to obese control rats. In addition, the level of serum insulin was increased significantly in all groups while fold expression of GLUT-4 gene was increased significantly ($P<0.05$) in OG-200 only. In conclusion, the use of methanolic crude extract of *Ocimum gratissimum* leaves can be a therapy in the treatment of obesity due to its significant hypoglycemic, anti hyperlipidemic and insulin resistance lowering properties.

Keywords: Glucose, Monosodium glutamate, Obesity, *Ocimum gratisimum*

1.0 INTRODUCTION

Obesity is a complex chronic global disease affecting people worldwide across all ages, sexes, ethnicities and nationalities, and it is the fifth leading cause of mortality globally. According to WHO, the prevalence of obesity worldwide, has doubled in more than 70 countries since 1980 (Chooi *et al.,* 2019; Song *et al,* 2019). In 2008, 1.4 billion adults aged 20 years and above were overweight or obese, of whom approximately 200 million men and 300 million women were estimated to be obese (Akarolo-Anthony *et al.,* 2014). Despite the fact that obesity and overweight are problems of high-income countries, low- and middle-income countries (LMICs), in particular urban settings of sub-Saharan African countries, face the challenge of an increasing trend. Obesity does not affect adults only but children are also affected (Biadgilign *et al.,* 2017). In Africa, the number of children who are overweight or obese has nearly doubled from 5.4 million in 1990 to 10.6 million in 2014 (Biadgilign *et al.,* 2017). The clear picture of obesity and overweight is that it can affect a child's immediate health, educational attainment and quality of life (Danquah *et al.,* 2019). According to the 2010 World health organization survey data on Nigeria, the prevalence of overweight was 26% and 37% in men and women respectively, while the prevalence of obesity was 3% and 8.1% in men and women respectively (Akarolo-Anthony *et al.,* 2014).

Obesity is a condition of increased adipose tissue mass and can also be defined as an increase in body weight beyond the limits of physical requirement, as a result of an excessive accumulation of fat (Sikaris, 2004; Thaler *et al.,* 2013; Sears and Perry, 2015). It is accompanied by remodeling of the adipocyte by advanced glycated end products which leads to free radical generation, inflammation and apoptosis (Sun *et al.,*2011; Sears and Perry, 2015). These causes recruitment of primary inflammatory cells such as macrophages, lymphocytes and monocytes to the site of action for resolution and repair (Caminos *et al.,* 2005). Recruited immune cells releases proinflammatory cytokines which binds to their receptors at the cell surface and activate the Jak-Stat and Nuclear Factor Kappa B (NF-κB) signaling pathway that leads to synthesis of more cytokines (Bako *et al.,* 2019; Lan *et al.,* 2019). As the level of cytokines rises due to increase in fat accumulation, the level of suppressor of cytokines increases which leads to insulin resistance through inhibiting the binding of insulin receptor substrate to insulin receptor, thereby preventing the translocation of GLUT-4 from its vesicle to the cell surface for glucose absorbtion (Rawlings *et al.,* 2004; Sears and Perry, 2015; Bako *et al.,* 2019).

Ocimum gratissimum (OG) is an aromatic medicinal plant which belongs to the Lamiaceae family. It is popularly known as scent leaf. It is used in cooking due to its minty aromatic flavour. In Nigeria, the plant is called "Effinrin-na" by the Yoruba speaking tribe, "Alumokho" in Esan, "Nchanwu" in Igbo, "Aramogbo" in Edo, and in the northern part of Nigeria, the Hausa's call it "Doidoya". Traditionally, OG has been used for the treatment of headache, diarrhoea, warts, worms, kidney infections and diabetes mellitus (Okoduwa *et al.,* 2017). The crushed leaf juice is used in the treatment of convulsion, stomach pain, and catarrh while oil from the leaf has been shown to possess antiseptic, antibacterial, and antifungal activities (Shittu *et al.,* 2016). *Ocimum gratissimum crude* leaves extract have low toxicity and could be well tolerated at low to moderate doses. Rats administered with 800mg/kg of the

extract showed no sign of distress or toxicity which accounts for its use in most part of the world for the management of diseases. The LD_{50} has been reported to be 2075mg/kg (Udoha *et al.,* 2019). The plant was also reported to have antioxidant, hypolipidemic and hematological properties (Shittu *et al.,* 2016) due the presence of certain phytochemical constituent (Ayinla *et al.,*2011). Major complications associated with obesity are hyperglycemia, insulin resistance and hyperlipidemia, OG have been reported to have hypoglycemic activity but its effect on GLUT-4 gene in obese rats is yet to be reported, therefore investigating the potentials of this plant in reducing complications associated with obesity through targeting GLUT-4 gene will be of importance.

2.0 MATERIALS AND METHODS

2.1 Preparation of plant material

Fresh leaves of *O. gratissimum* (OG) were collected from Kaduna central market, Kaduna State and were identified and authenticated in biological science, Kaduna State University by Mal. U S Gallah who assigned the plant the voucher specimen no. 1120. The leaves were air dried and crushed to powdered form using mortar and pestle, which was soaked in methanol for 48 h. The extract was obtained by filtration using whatman filter paper and the methanol was evaporated using rotary evaporator.

2.2 Phytochemical Screening

The phytochemical screening of the crude extract of *Ocimum gratissimum* leaves was carried out in order to ascertain the presence of its constituents utilizing standard methods.

2.2.1 Test for Saponins

Crude extract of *Ocimum gratissimum* leaves 1.0 g was boiled with 5.0 ml of distilled water, then filtered. To the filtrate, about 3.0 ml of distilled water was further added and shaken vigorously for about 5 min. Frothing which persisted on warming was taken as an evidence for the presence of saponins (Sofowora, 1993).

2.2.2 Test for Tannins

About 0.5 g of the extract was stirred with about 10.0 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution was added to 2.0 ml of the filtrate. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins (Trease and Evans, 2002).

2.2.3 Test for flavonoids

The extract, 0.5 g was boiled with distilled water and then filtered. To the filtrate 2.0 mL, few drops of 10% ferric chloride solution were then added. A green-blue or violet colouration indicated the presence of a phenolic hydroxyl group (Trease and Evans, 2002).

2.2.4 Test for Glycosides

To 2.0 mL of the extract, 3.0 mL of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides (Sofowora, 1993).

2.3 Experimental Animals

Thirty male Wistar rats weighing between 100.0 to 150.0 g were used for the study. They were housed 6 per cage and acclimatized for two weeks in the Animal house of Biochemistry Department, Kaduna State University, prior to the conduction of the experiment. They were fed on standard rat pellet diet and were allowed water *ad libitum*. The animals were maintained under standard laboratory conditions of (25-35^{o}C), and were subjected to12 h light and dark cycle.

2.4 Animal grouping and induction of obesity

Animals were randomly divided into five groups of six animals each namely:
Group 1: Normal control (NC) rats,
Group 2: Obese control (OC) rats,
Group 3: Obese rats treated with methanolic leaves extract of *Ocimum gratissimum* 100.0 mg/kg B.W (OG-100),
Group 4: Obese rats treated with methanolic leaves extract *Ocimum gratissimum* 200.0 mg/kg B.W (OG-200),
Group 5: Obese rats treated with Orlistat 50.0 mg/kg B.W (OR-50),
Obesity was induced by oral administration of 8.0 mg/g monosodium glutamate (MSG) for 7 days. All animals were treated with respective doses orally for 1 week while NC and OC groups were administered with vehicle only.

2.5 Collection of blood

Animals were first euthanized with chloroform, then blood samples were collected via cardiac puncture and the blood was immediately centrifuged at 3,000 rpm for 10 min to obtain serum which was preserved at –30 °C prior to analysis. Skeletal muscle was harvested, preserved in liquid nitrogen and stored at −80°C before RNA extraction.

2.6 Analytical methods

The serum insulin, high density lipoprotein cholesterol, total cholesterol, triglyceride, low density lipoprotein cholesterol concentration was measured at the end of the experimental period according to the protocol described by the manufacturers. Homeostatic model assessment for insulin resistance (HOMA-IR) was also calculated from fasting serum insulin and fasting blood glucose concentrations obtained at the end of the experimental period using the following formula:

$$\text{HOMA} - \text{IR} = \frac{\text{Serum insulin in} \frac{\text{U}}{\text{L}} \text{X Blood glucose in mmol/L}}{22.5}$$

Bako *et al.,* 2019

2.7 GLUT-4 gene expression studies

2.7.1 Tissue RNA extraction

Total RNA in muscle tissue was extracted using RNA extraction kit according to manufacturer's recommendation (Bioneer Accupower, Korea). Briefly, 150 mg of the samples were added to 400µL of the binding buffer (guanidine HCl) and homogenized before the addition of 10µL of proteinase K. The mixture was vortexed for 10 sec and incubated at 60 ^{o}C for 10 min. Thereafter, a volume of 100µL of isopropanol was added and vortexed for 10 sec. and the samples were transferred to a binding column and centrifuged for 1min at 8000rpm. The binding columns were transferred into 2.0 mL collection tubes and 500 µL of wash buffer 1 (containing chaotropic salts) was added and re-centrifuged for 1.0min at 8000rpm. Then, the binding columns were transferred to another 2.0mL collection tubes and 600 µL of another wash buffer (containing ethanol) was added. Subsequently, the binding tubes were transferred into 1.5mL collection tubes and 50µL of elution buffer (containing 10 mMTris) was added. The eluted RNA solutions were used for cDNA synthesis (Bako *et al.,* 2019).

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2.7.2 Reverse transcription and quantitative polymerase chain reaction

Exactly 15µL of RNA, 2 µL of forward and reverse primer and 3 µL of deionised water were added to PCR tube packaged with reverse transcriptase. The mixture was inserted into PCR machine and subjected to the following cycling conditions; 95°C for 5min (denaturation), 42°C for 60 min (reverse transcription) to synthesise cDNA. Subsequently, 16 µl of PCR master mix (containing DNA polymerase, dNTPs and SYBR green) was added to the tubes and equal volumes of primer and the cDNA (2 µl) were added. The tubes were inserted into RT-PCR machine (Rotor-gene Q, Qiagen) and subjected to the following cycling condition; 95°Cfor 10min pre-denaturation, 95°C for 10sec denaturation, 50°C for 15sec annealing and 72°C for 20min extension for 40 cycles. Melting curve was within 65 to 95°Cat 5°C increment per 5sec. The mRNA expression of the genes of interest was first normalized against the reference gene (GAPDH) and the expression of the treated groups was expressed as fold change from their respective control groups using the Livak method. The PCR primer used were 5'-AGA GTC TAA AGC GCC T-3' forward 5'-CCG AGA CCA ACG TGA A-3' reverse for GLUT-4 (Bako *et al.,* 2019).

2.8 Statistical analysis

All data obtained during the study were presented as the mean ± SEM. Analysis of data was done using a statistical software package (SPSS for Windows, version 22, IBM Corporation, NY, USA) using Tukey's-HSD multiple range *post-hoc* test. Values were considered significantly different at P< 0.05.

3.0 RESULTS

Phytochemical screening of the methanolic crude extract of *Ocimum gratissimum* leaves result showed the presence of saponins, tannins, flavonoids and glycosides. The weekly weight of obese rats treated with methanolic crude extract of *Ocimum gratissimum* leaves was shown in Figure 1. The weight of the rats increases significantly (P<0.05) after induction of obesity with monosodium glutamate, and after one week of treatment with the extract, the weight of the rats decreases significantly (P<0.05) when compared to obese control rats. Also, the weekly blood glucose of obese induced rats treated with the methanolic extract was also measured and the result was shown in Figure 2. The non-fasting blood glucose (NFBG) level was elevated a week after the induction of obesity in all groups with the exception of normal control group. After one week of treatment, the NFBG level reduced significantly (P<0.05) in all groups when compared to obese control group. In addition, the level of insulin was measured and the result was presented in Figure 3 which shows significant (P<0.05) increase in the level of insulin in all groups when compared to obese control group. The insulin level and fasting blood glucose measured was used in calculating the level of insulin resistance which was presented as HOMA-IR in Figure 4. The HOMA-IR values were reduced significantly in all groups when compared to obese control group. These shows the effectiveness of the plant extract in reducing the level of insulin resistance. In order to know the mechanism by which the plant extract exert its hypoglycemic effect, gene expression studies was carried out to measure the fold expression of GLUT-4 in obese rats treated with the extract and the result was shown in Figure 5. A significant fold increase in GLUT-4 gene expression in OG-200 only was obtained when compared to obese control group, showing the effect of higher doses of the plant in increasing the expression of GLUT-4, thereby reducing blood glucose and insulin resistance level. Moreover, the lipid profile parameters were measured to evaluate the effect of the methanolic extract on the level of total cholesterol, triglyceride, LDL and HDL of obese rats and the results were presented in Table 1. A significant (P<0.05) reduction in the level of T-CHOL and LDL in all groups when compared to OC group. However no significant decrease was observed in the level of TRIG in OG-100 group when compared to obese control group, also the level of HDL in OG-100 group did not increased significantly (P<0.05) when compared to obese control group.

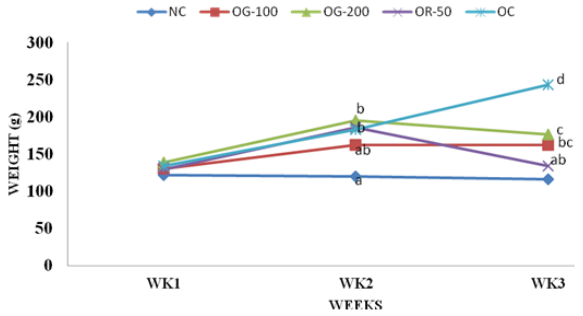

Figure 1: Effect of methanolic crude extract of *Ocimum gratissimum* leaves on weight of MSG induced obese rats. The results are expressed as the mean ± SE. Different alphabets indicate significant difference (Tukey's-HSD multiple range *post hoc* test, P<0.05). NC= normal control, OG-100= obese rats treated with 100 mg/kg b.w methanolic extract of *Ocimum gratissimum*, OG-200= obese rats treated with 200 mg/kg b.w methanolic extract of *Ocimum gratissimum* , OR-50=obese rats treated with 50 mg/kg b.w orlistat, OC= obese control.

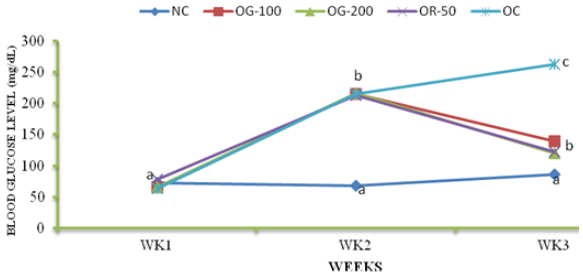

Figure 2: Effect of methanolic crude extract of *Ocimum gratissimum* leaves on weekly blood glucose of MSG induced obese rats. The results are expressed as the mean ± SE. Different alphabets indicate significant difference (Tukey's-HSD multiple range *post hoc* test, P<0.05).NC= normal control rats, OG-100= obese rats treated with 100 mg/kg b.w methanolic extract of *Ocimum gratissimum*, OG-200= obese rats treated with 200 mg/kg b.w methanolic extract of *Ocimum gratissimum* , OR-50=obese rats treated with 50 mg/kg b.w orlistat, OC= obese control.

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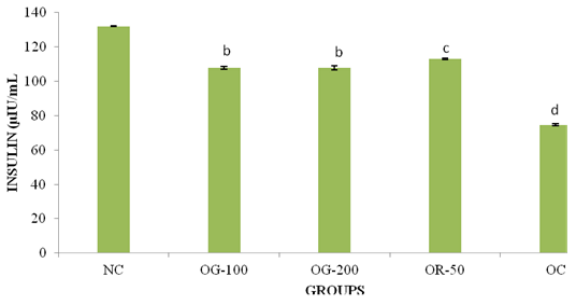

Figure 3: Effect of methanolic crude extract of *Ocimum gratissimum* leaves on insulin level of MSG induced obese rats. The results are expressed as the mean ± SE. Different alphabets indicate significant difference (Tukey's-HSD multiple range *post hoc* test, P<0.05).NC= normal control rats, OG-100= obese rats treated with 100 mg/kg b.w methanolic extract of *Ocimum gratissimum*, OG-200= obese rats treated with 200 mg/kg b.w methanolic extract of *Ocimum gratissimum*, OR-50=obese rats treated with 50 mg/kg b.w orlistat, OC= obese control.

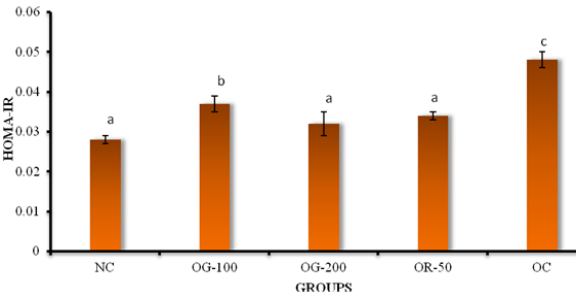

Figure 4: Effect of methanolic crude extract of *Ocimum gratissimum* leaves on insulin resistance level of MSG induced obese rats. The results are expressed as the mean ± SE. Different alphabets indicate significant difference (Tukey's-HSD multiple range *post hoc* test, P<0.05).NC= normal control rats, OG-100= obese rats treated with 100 mg/kg b.w methanolic extract of *Ocimum gratissimum*, OG-200= obese rats treated with 200 mg/kg b.w methanolic extract of *Ocimum gratissimum*, OR-50=obese rats treated with 50 mg/kg b.w orlistat, OC= obese control.

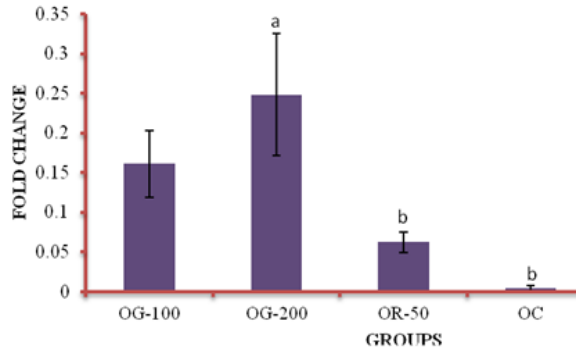

Figure 5: Effect of methanolic crude extract of *Ocimum gratissimum* leaves on GLUT-4 relative gene expression of MSG induced obese rats. The results are expressed as the mean ± SE. Different alphabets indicate significant difference (Tukey's-HSD multiple range *post hoc* test, P<0.05). OG-100= obese rats treated with 100 mg/kg b.w methanolic extract of *Ocimum gratissimum*, OG-200= obese rats treated with 200 mg/kg b.w methanolic extract of *Ocimum gratissimum*, OR-50=obese rats treated with 50 mg/kg b.w orlistat, OC= obese control.

Table 1: Effect of methanolic crude extract of *Ocimum gratissimum* leaves on lipid profile of Monosodium induced obese rats

GROUP	T-CHOL	TRIG	LDL	HDL
NC	122.17±5.51^{a}	54.49±7.36^{a}	10.58±3.97^{a}	100.09±1.32^{a}
OG-100	174.32±2.73^{c}	165.69±20.28^{bc}	77.43±4.10^{b}	63.75±3.94^{b}
OG-200	162.79±1.30^{cd}	148.25±2.48^{cd}	55.11±3.14^{c}	78.04±3.90^{c}
OR-50	155.06±2.36^{d}	113.46±5.75^{d}	39.27±0.47^{c}	93.10±1.61^{ac}
OC	212.07±1.62^{e}	202.49±1.48^{b}	113.08±4.40^{d}	58.50±5.64^{b}

T-CHOL: total cholesterol, TRIG: triglyceride, LDL: low density lipoprotein, HDL: high density lipoprotein.

The results are expressed as the mean ± SE. Different alphabets indicate significant difference (Tukey's-HSD multiple range *post hoc* test, P<0.05). NC= normal control, OG-100= obese rats treated with 100 mg/kg b.w methanolic extract of *Ocimum gratissimum*, OG-200= obese rats treated with 200 mg/kg b.w methanolic extract of *Ocimum gratissimum*, OR-50=obese rats treated with 50 mg/kg b.w orlistat, OC= obese control.

4.0 DISCUSSION

Throughout the past few years, many medications have been introduced and approved by the United States Food and Drug Administration (FDA) for the treatment of obesity. However, most of them have subsequently been withdrawn due to various serious adverse effects. The most commonly used drug (orlistat) causes gastrointestinal side effects, such as diarrhea, flatulence, bloating, abdominal pain, and dyspepsia (Cheung *et al.,* 2013). Numerous plants in Africa are used in the treatment of obesity, however only few of them have received scientific evaluation to assess their mechanism of action, safety and efficacy. *Ocimum gratissimum* is one of the plant still under investigation due to its numerous therapeutic effects.

Increase in weight of the animals caused by obesity occur due to accumulation of fat in the adipose tissues that leads to inflammation (Bueno *et al.,* 2005). This results in recruitment of immune cells, generation of free radicals and continuous propagation of proinflammatory cytokines through the JAK-STAT and NF-Kβ signaling pathways. Activation of these signaling pathways eventually lead to insulin resistance, that prevent the translocation of GLUT-4 to the cell surface from its vesicle for glucose absorption (Bako *et al.,* 2019). Treatment with methanolic crude leaves extract of the plant has the therapeutic potential of reducing the body weight of the rats within short period of one week and the drastic decrease can be due to the ability of the plant to increase the level of insulin which inhibit hormone sensitive lipase and denovo synthesis of cholesterol by the liver (Ajayi *et al.*, 2014). This prevent fat accumulation, reduce the level of inflammation and also enhance the binding of insulin to its receptors thereby reducing the level of insulin resistance and increasing translocation of GLUT-4 from its vesicle to the cell surface for glucose transport in to the cells (Bako *et al.,* 2019). It was reported that OG reduced hyperglycaemia in diabetic rats by 29.3% at 4 h post oral administration. This finding was consistent with the report of the 25% reduction criteria suggested by Oguanobi *et al.* (2012). The hypoglycemic potency of OG crude leaves extract has been attributed to some basic phytochemical constituents present in the

plant which include saponins, tannins, flavonoids and glycosides. Saponins possess insulin-like properties, which stimulate glucose uptake by enhancing Glut4 expression and contributing to storage of glucose as glycogen in adipocytes (Elekofehinti *et al.,* 2014; Okoduwa *et al.,* 2017).

More also, the increase in level of insulin which inhibit hormone sensitive lipase responsible for lipolysis caused by high blood glucose level, leads to low synthesis of triglyceride, total cholesterol, LDL and increase synthesis of HDL that decreases further risk of cardiovascular disease (Nwogor, 2016). Treatment of the obese groups with *Ocimum gratissimum* crude leaves extract shows reduction in TG, LDL, Total cholesterol and increase in HDL levels, and these results were in line with the finding reported by Ayinla *et al.* (2011) that administration of the plant extract has anti hyperlipidemic effect. In addition, saponins present in the plant may decrease the level of cholesterol by binding with cholesterol in the intestinal lumen, thereby inhibiting its absorption and/or by binding with bile acids and causing a decline in the enterohepatic circulation of bile acids and increase in its faecal excretion (Rotimi *et al.,* 2011). The increased bile acid excretion is equalized by enhanced bile acid production from cholesterol in the liver and consequent lowering of the plasma cholesterol (Rotimi *et al.,* 2011). In conclusion, the use of methanolic leaves extract of *Ocimum gratissimum* crude leaves extract can be a significant therapy in the treatment of obesity due to its significant hypoglycemic and anti hyperlipidemic properties obtained after treatment in MSG induced obese rats. Also, its therapeutic effect can be obtained within short period of administration and its dose dependent

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