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Research Article

Amelioration of thyroidectomy-induced glucose intolerance by *Ocimum gratissimum* leaf extract in male Wistar rat

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Abstract

Derailed glucose homeostasis is well documented in hypothyroidism and studies have found *Ocimum gratissimum* leaf extract (OG) to be beneficial in other conditions such as diabetes mellitus and metabolic syndrome characterized by imbalance glucose homeostasis. There is however dearth of information on the effect of OG in hypothyroid condition. This study therefore investigated the effect of OG on glucose metabolism in thyroidectomy-induced hypothyroid male Wistar rats.

Thirty-six male Wistar rats were randomly divided into six (6) groups (n=6) as 1 = control, 2 = control + OG, 3 =sham operated (Sham), 4 = Sham + OG, 5 = Thyroidectomized (Thyrd) and 6 = Thyrd + OG. Groups 1, 3 and 5 received 0.2 mL/kg of distilled water while 100 mg/kg of OG was administered to groups 2, 4 and 6 for 28 days post healing of the surgical site. Oral Glucose Tolerance Test was carried out, lactate level, lipid profile and markers of oxidative stress were assessed in the plasma while liver and muscle samples were obtained for the determination of glycogen content and hepatic hexokinase activities. Glucose intolerance was observed with increased AUC (18274 ± 442.55 mg.min/dL) in Group 5 and reversed in group 6 (15228±184.97 mg. min/dL) relative to control (14421±515.58 mg.min/dl). Hepatic hexokinase activity and plasma lactate level were significantly reduced in group 5. Muscle glycogen decreased in groups 2, 4, 5 and 6 while hepatic glycogen increased in groups 3, 5 and 6. Triglycerides and HDL decreased while LDL increased in group 5 and were reversed in group 6. Malondialdehyde level was increased, and catalase activity decreased in group 5 while they were reversed in group 6. Findings from this study suggest that OG may prevent suppression of hepatic hexokinase activity and thus promote glucose utilization in thyroidectomized male Wistar rats.

Key Words: Glucose intolerance, Hypothyroidism, Lipid profile, Oxidative stress, Rat

INTRODUCTION

Hypothyroidism is associated with glucose intolerance and has been implicated by several studies to predisposes type 2 diabetes and metabolic syndrome (Li *et al*., 2022; Roa Dueñas *et al*., 2022). For instance, hypothyroid Danish population was monitored for an average of 6 years and diabetes mellitus was identified as one of the major co-morbidities (Thvilum *et al*., 2013). In another large cohort study among Israeli, hypothyroidism and subclinical hypothyroidism was found as risk factors for diabetes mellitus (Gronich *et al*., 2015). In fact, among Nigerian diabetic patients, the incidence of abnormal thyroid hormone levels was found to be high with hypothyroidism accounting for the greatest percentage (Udiong *et al*., 2007). Experimental-induced maternal hypothyroidism in mice was found to cause altered glucose metabolism and increased susceptibility to type 2 diabetes mellitus in adult offspring up to the second filial generation (Kemkem *et al*., 2020). Decreased mitochondrial oxidative capacity, increased lipid accumulation and decreased ATP synthesis through the PGC1 dependent pathway is a link between hypothyroidism, insulin resistance and risk of diabetes mellitus (Crunkhorn *et al*., 2008). It is therefore not surprising that the use of some certain antidiabetic drugs in patients with type 2 diabetes mellitus may influence thyroid function (Eom *et al*., 2022).

Among numerous medicinal plants with antidiabetic properties, *Ocimum gratissimum* (OG) Linn. leaf extract has received considerable attention from several groups (Aguiyi *et al*., 2000; Owoyele *et al*., 2005; Egesie *et al*., 2006; Mohammed *et al*., 2007; Onaolapo *et al*., 2011; Oguanobi *et al*., 2012; Casanova *et al*., 2014; Shittu *et al*., 2016; Okoduwa *et al*., 2017) using animal models of diabetes mellitus to consistently show its hypoglycemic efficacy at the systemic level. We documented that OG inhibits hepatic glycogen phosphorylase activity in streptozotocin-induced diabetic rats (Shittu *et al*., 2018), reversed diabetes-induced intestinal hyperplasia in alloxan diabetic rats (Shittu *et al*., 2019) and recently showed that it enhances insulin sensitivity in rats with metabolic syndrome (Shittu *et al*., 2021). Our observation in the metabolic syndrome rats (Shittu *et al*., 2021) supported the earlier postulation of Egesie *et al*., (2006) that OG may promotes peripheral glucose utilization.

Physiologically, thyroid hormone increases glucose and energy metabolism by regulating genes that facilitate entry and utilization of glucose by several tissues (Alkemade, 2010). A question that then comes to mind is, could *Ocimum gratissimum* mimic thyroid hormones' effect on glucose

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metabolism in hypothyroid animals? Consequently, it is imperative to investigate the effect of *Ocimum gratissimum* leaf extracts on glucose metabolism in the absence of thyroid hormone. The effect of OG on glucose metabolism in thyroidectomized male Wistar rats was therefore studied.

MATERIALS AND METHODS

Animals: Thirty-six male Wistar rats (110 ± 20 g) were purchased from the central animal house, College of Medicine, University of Ibadan, Ibadan. They were housed and acclimatized for two weeks in the Department of Physiology animal house, in strict adherence to the University of Ibadan Animal care and use research ethics and in compliance with institutional ethical regulation. They were allowed unhindered access to food (Ladokun feeds®) and water. All experimental protocol and handling of the rats were done in accordance with standard ethical guidelines as contained in the NIH publication No. 85-23 guidelines.

Preparation of aqueous leaf extract of Ocimum gratissimum: The fresh leaves of *Ocimum gratissimum* were collected from Ibadan metropolis and identified and authenticated at the Forest Research Institute of Nigeria (FHI.110026). The leaves were separated from their stalk, rinsed with water to remove dirt, and air-dried for three (3) weeks at room temperature. They were pulverized to fine powder. One kilogram of the pulverized leaves was macerated for 24 hours in distilled water, filtered and the filtrate was collected in a round bottom flask for concentration in a rotary evaporator set at temperature of 40 0C to yield 13.26% aqueous extract.

Thyroidectomy Procedure: Total thyroidectomy was carried out as described by Shan and Iwao (2021) with a modification in the choice of anesthetic agent. Briefly, anaesthetized [100 mg/kg Ketamine, i.p. (Ketanir®, Aculife Healthcare Pvt Ltd, India) and 5 mg/kg Xylazine, i.m. (Xylased®, Bioveta, Czech Republic)] rat was firmly secured on a dissecting board, the neck regions was shaved and a longitudinal incision of the neck skin and subcutaneous connective tissue was made. The trachea sternohyoid muscle was bluntly separated along the midline to expose the trachea and the thyroid glands. The superior thyroid arteries on both sides were ligated, the isthmus was cut with ophthalmic scissors, the recurrent laryngeal nerve was carefully stripped, and each thyroid gland was resected one at a time. Care was taken to avoid damage to the blood vessels and the sternohyoid muscle was reset followed by a layer-by layer suturing of the muscle membrane and the skin. The site of the suture was covered with penicillin (DBT fortified Procaine Penicillin®, Azhui Chengshi Pharmaceutical Co. Ltd, China) for disinfection and the animals were kept in a warm and well illuminated environment until recovery. In another set of rats, similar incision and exposure of the thyroid gland was carried out except that the thyroid glands were neither devascularised nor extirpated to serve as the sham operated groups. After complete healing surgical site, thyroid hormones (T3 and T4) levels were determined in the thyroidectomized and compared with the control, significant reduction in the hormones confirmed the effectiveness of the thyroidectomy-induced hypothyroidism. They were then assigned into their respective groups.

Experimental design: The rats were randomly divided into 6 groups (n = 6) and treated per os for 28 days as follows:

Control: Normal animal treated with 0.2 ml/kg Distilled water
Control+OG: Normal animal treated with 100 mg/kg *Ocimum gratissimum* leaf extract
Sham: Sham operated animal treated with 0.2 ml/kg Distilled water
Sham+OG: Sham operated animal treated with 100 mg/kg *Ocimum gratissimum* leaf extract
Thyrd: Thyroidectomized animal treated with 0.2ml/kg Distilled water
Thyrd+OG: Thyroidectomized animal treated with 100 mg/kg *Ocimum gratissimum* leaf extract

The body weight was monitored before and after the 28 days administrations.

Oral Glucose Tolerance Test: On the 28th day of administration, Oral Glucose Tolerance Test (OGTT) was carried out following an overnight fast. Basal/fasting glucose level was determined and the rats were orally administered D-Glucose (2 g/kg body weight) and the blood glucose level was determined at 30-, 60-, 90- and 120-minutes blood sampled from the tail vein using ACCU-CHEK® glucometer. The blood glucose level at each point was plotted in a line graph and the Area Under the Curve was calculated according to the mathematical model of Tai (1994). The animals were further administered OG or distilled water then fed after the OGTT to complete the 28 days administration.

Sample collection: At the end of 28 days of treatment, the animals were fasted overnight and euthanized by cervical dislocation. Blood samples were obtained by cardiac puncture into Eppendorf tube containing Fluoride/oxalate for determination of plasma lactate and into lithium EDTA bottle for determination of lipid profile [Cholesterol, Triglycerides (Trig), High Density Lipoprotein (HDL) while Low Density lipoprotein (LDL) was calculated] and oxidative stress biomarkers [Malondialdehyde (MDA), Superoxide Dismutase activity (SOD) and catalase activity]. The liver and soleus muscles were harvested and weighed immediately. One gram each of liver and soleus muscle was taken for glycogen assay while the remaining liver tissue was stored in Phosphate buffer Saline, PBS (0.5 mMol, pH 7.4). Samples collected into PBS were homogenized and centrifuged at -4ºC to obtain supernatant that was used to assay hexokinase activity.

Biochemical assays: Determination of plasma lactate and lipid profile: Plasma lactate, Cholesterol, Trig and HDL were assayed using commercially available kits (Fortress® Diagnostics Limited, United Kingdom) according to the manufacturers' instruction while LDL was calculated as: Total cholesterol – HDL – Trig/5

Determination of glycogen content: Liver and muscle glycogen contents were determined by the Anthrone method as previously reported (Shittu *et al*., 2018). Briefly, the liver or muscle was digested in 30 % KOH over heat, washed twice by 95 % ethanol and centrifuged to obtain glycogen precipitate. The precipitate was reconstituted with distilled water followed by stepwise addition of concentrated HCl, 88

% formic acid and anthrone reagent then mixed thoroughly. It was incubated at 100 ^{0}C for 10 minutes to obtain a blue colored solution. Absorbance of the solution was recorded at 630 nm against a reagent blank. Several dilutions of 0.2 mg/mL of glycogen standard were similarly treated to obtain a standard curve from which the glycogen concentrations of the samples were determined.

Determination of hepatic hexokinase activities: Hexokinase activities was determined in the PBS homogenized liver samples using the Branstrup *et al* (1957)'s method as previously reported (Shittu *et al*., 2018). Briefly, 2 mL of a buffer containing 0.0025 M glucose, 0.0025 M MgCl2, 0.01 M K2HP04, 0.077 M KCl, and 0.03 M Tris (Hydroxy-methyl) aminomethane (pH 8) was pipetted into a test tube followed by 0.1 ml of 0.18 M ATP solution and 0.9 ml of distilled water. The mixture was preheated in water for 5 minutes at 38 ^{0}C, then 1 mL of liver or muscle homogenate was added and the initial glucose concentration was determined immediately. The mixture was then incubated at 38 ^{0}C for 30 minutes and the final glucose concentration was determined. The difference in the level of glucose was calculated and hexokinase activity was expressed as glucose metabolised/mg. pr/30min. All assays were carried out in duplicates. In this assay, glucose was assayed using a commercially available Glucose GOD-PAP kit (Fortress Diagnostic®, United Kingdom).

Determination of Oxidative stress markers: Malondialdehyde (MDA) was determined according to the method described by Hagege *et al*. (1995). Briefly, 0.5ml of plasma sample was aliquoted into 1ml of TCA-TBA-HCL solution (15 g of trichloroacetic acid and 0.375 g of thiobarbituric acid dissolved in 100 ml of 0.25 N hydrochloric acid) and incubated for 15 minutes in boiling water (100 ^{0}C). After cooling, the mixture was centrifuged at 1000 g for 10 minutes and supernatant was read at 535 nm against the blank. The malondialdehyde concentration of the sample can be calculated using extinction coefficient of $1.56\times10^5 m^{-1}cm^{-1}$

MDA concentration = $\frac{O.D \times V \times 1000}{a \times v \times I \times Y}$

Where O.D= absorbance of sample test at 535 nm; V= total volume of the reaction=1.5 ml; a= molar estimation coefficient of product= $1.56\times10^5 m^{-1}cm^{-1}$; I= light path=1 cm; v= volume of sample used = 0.5 ml.

Superoxide Dismutase (SOD) activity was determined according to the method of Misra and Fridovich (1972). Briefly, 0.2 ml of plasma sample (test) or distilled water (reference) was added to 2.5 ml of 0.05 M Carbonate buffer (pH 10.2) was then added and incubated at room temperature. 0.3 ml of 0.3 mM adrenaline solution was added to the test and each of the reference solutions and were mixed by inversion and read using the spectrophotometer at 420nm within 3 minutes.

Inhibition= $\frac{O.D_{Ref} - O.D_{Test}}{O.D_{Ref}} \times 100$

1 unit of SOD activity was taken as the amount of SOD required to cause 50% inhibition of the auto-oxidation of adrenaline to adrenochrome.

Catalase activity was determined using a method based on the reaction of undecomposed hydrogen peroxide with ammonium molybdate to produce a yellowish color (Goth *et al*., 1991). Briefly, 0.2 ml of plasma sample was incubated with 1 ml of substrate solution (65 mmol/ml hydrogen peroxide in 60 mmol/l sodium–potassium phosphate buffer, pH 7.4) at 37 °C for three minutes. The reaction was stopped with 1 ml of 4% ammonium molybdate in 12.5mM H2SO4 and read at 305nm wavelength.

Statistical analysis: The data from each group was expressed as mean ± standard error of the mean (mean ± SEM). The data was analyzed using ANOVA and Tukey's post hoc test. $P < 0.05$ was considered significant. All analyzes were performed using GraphPad prism®, version 7.

RESULTS

Effect of OG on the body weight of normal and thyroidectomized male Wistar rats: All the groups had significant increase in body weight when the weight before the experiment was compared with the weight after experiment. Thyroidectomy did not produce any significant change in the body weight when the thyroidectomized rats were compared with control. Similarly, OG had no effect on the weights of animals in control + OG (188.9 ± 11.363 g), sham + OG (218.16 ± 4.102 g), and Thyrd + OG (214.6 ± 11.985 g) when the final weights were compared with the control and their untreated counterparts (Fig 1).

Effect of OG on oral glucose tolerance of normal and thyroidectomized male Wistar rats: The effects of OG on oral glucose tolerance of the normal and thyroidectomized male Wistar rats are shown in figure 2. Following the oral load of 2 g/kg body weight of glucose, blood glucose reached its peak at 30 minutes and declined gradually for the 120 minutes observation period in all groups. When the area under the curve was calculated, thyroidectomy caused significant increased AUC (18274 ± 442.55 mg.min/dl) when compared with the control (14421 ± 515.58 mg.min/dl).

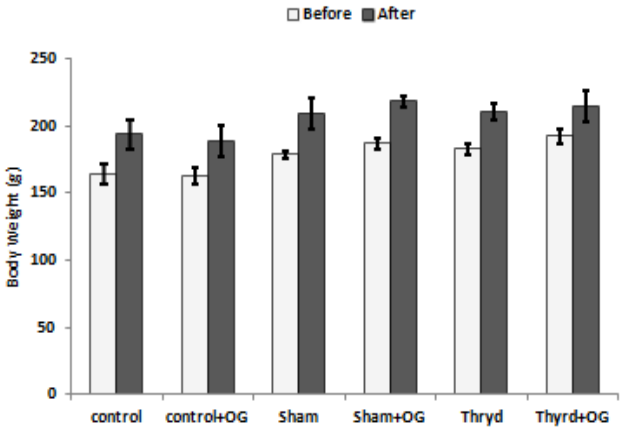

Figure 1:
Effect of *Ocimum gratissimum* aqueous leaf extract on the body weight of thyroidectomized male Wistar rats.

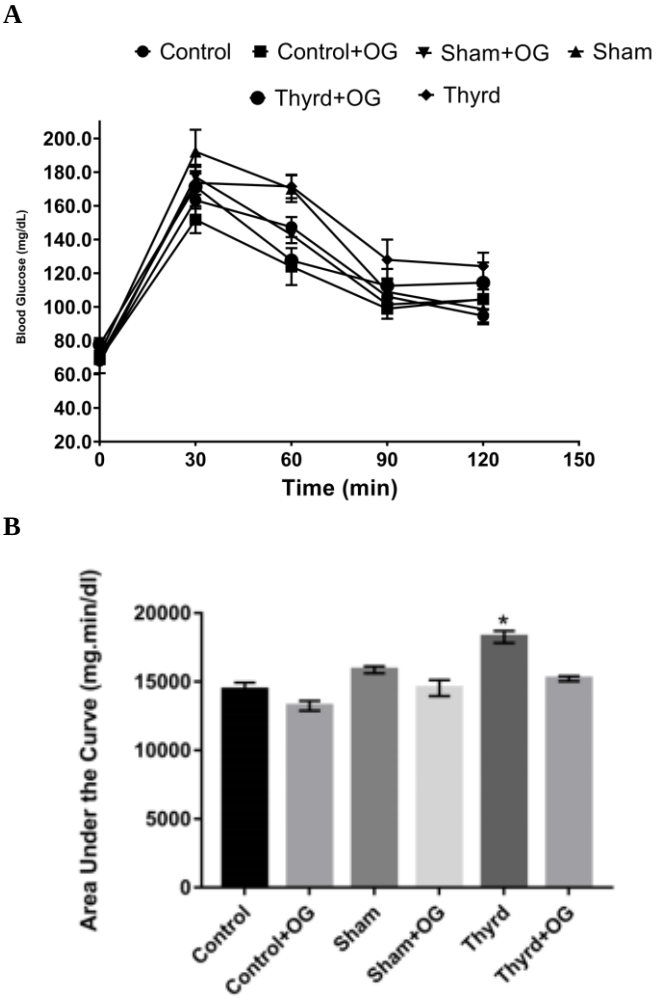

Figure 2:
Effects of *Ocimum gratissimum* on (A) Oral glucose tolerance test and (B) Area under the Curve of the Oral Glucose Tolerance Test in normal and thyroidectomized male Wistar rat. *P < 0.05 Thyroidectomized group Vs Thyroidectomized +OG

impaired glucose tolerance was reversed by OG treatment in the Thyrd+OG group (15228 ± 184.97 mg.min/dl). It is also worthy to note that OG treatment in the normal rats caused reduced AUC when compared with their corresponding untreated normal animals [i.e Control = 14421 ± 515.58 mg.min/dl Vs Control+OG =13247 ± 355.79 mg.min/dl; Sham = 15869 ± 251.30 mg.min/dl Vs Sham + OG = 14543 ± 584.35 mg.min/dl].

Effect of OG on hepatic hexokinase activities and plasma lactate level of normal and thyroidectomized male Wistar rats: Hexokinase activity was significantly reduced in the Thyrd (0.04 ± 0.004 activity/mg.pr) compared with the control (0.06 ± 0.006 activity/mg.pr). Although, there was an apparent increase in hepatic hexokinase activity in control + OG group (0.07 ± 0.006 activity/mg.pr), it was not statistically significant when compared with control group (0.06 ± 0.006 activity/mg.pr). A decrease in hexokinase activity was observed in Thyrd + OG (0.04 ± 0.008 activity/mg.pr), when compared with the control group (0.06 ± 0.006 activity/mg.pr), but had no significant difference (Fig 3A). Plasma lactate concentration of the thyroidectomized rats (20.69 ± 0.46 mg/dl) was significantly reduced when compared with the control (40.10 ± 2.57 mg/dl) and this was significantly reversed by OG treatment in the Thyrd+OG (36.33 ± 2.28 mg/dl) (Fig 3B).

Effect of OG on the hepatic and muscle glycogen content of normal and thyroidectomized male Wistar rats: Muscle glycogen was decreased in control + OG, Sham + OG, Thyrd group and Thyrd + OG (0.73 ± 0.067 mg/ml; 0.74 ± 0.14 mg/ml; 0.86 ± 0.1.11mg/ml; 0.69 ± 0.08mg/ml) respectively when compared with the control (0.97 ± 0.04 mg/ml). However, an increase was observed in the Sham group (1.04 ± 0.04 mg/ml) when compared with the control group (0.97 ± 0.04 mg/ml). Hepatic glycogen content was significantly increased in the sham group (55.04 ± 5.83 mg/ml/100g tissue), Thy group (48.3 ± 9.86 mg/ml/100g tissue), and Thyrd + OG (54.59 ± 9.35 mg/ml/100g tissue) compared to control (23.42 ±3.12 mg/ml/100g tissue). However, treatment with OG had no significant increase in liver glycogen content of control + OG (28.57 ± 2.35 mg/ml/100g tissue) and Sham + OG (33.55 ± 7.42 mg/ml/100g tissue), when compared with the control group (23.42 ± 3.12 mg/ml/100g tissue).

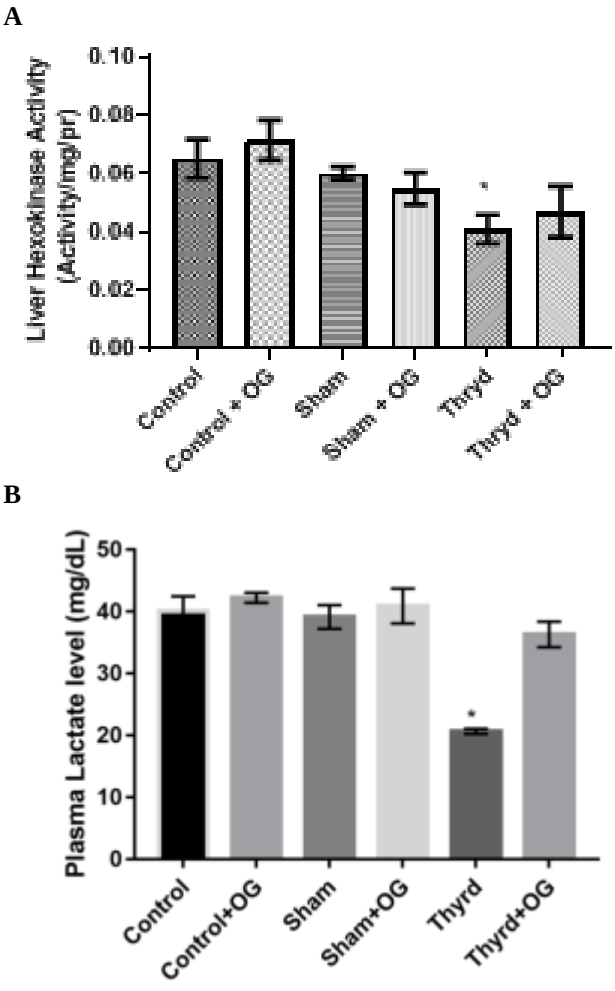

Figure 3.
Effect of *Ocimum gratissimum* aqueous leaf extract on (A) hepatic hexokinase activity and (B) plasma lactate level in normal and thyroidectomized male Wistar rats. *P< 0.05

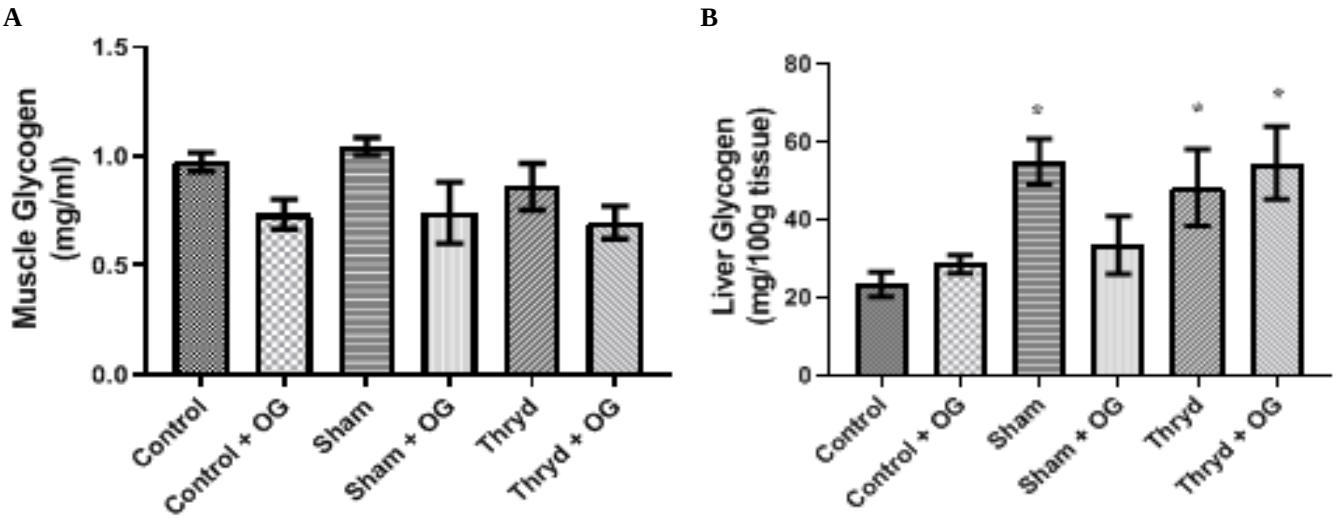

Figure 4:
Effect of *Ocimum gratissimum* aqueous leaf extract on (A) Muscle Glycogen content and (B) liver Glycogen content of normal and thyroidectomized male Wistar rats. *P<0.05

Table 1:
Effect of *Ocimum gratissimum* aqueous leaf extract on lipid profile in normal and thyroidectomized male Wistar rats.

Lipid profile (mg/dL)	Control	Control+OG	Sham	Sham+OG	Thyrd	Thyrd+OG
Chol	46.23±3.61	53.77±4.94	51.28±3.81	50.39±2.75	43.90±2.30	51.84±3.07*
Trig	40.48±2.99	45.86±8.68	33.85±5.14	22.61±2.55*	4.14±1.28*	18.21±3.88*#
HDL	22.84±1.19	28.09±1.97	28.16±4.27	25.72±0.45	10.12±3.29*	27.49±3.88#
LDL	15.29±2.42	16.51±2.32	16.35±1.62	20.15±2.54	32.95±4.51*	20.71±3.21#

* P<0.05 Vs Control; # P<0.05 Vs Thyroidectomized

Table 2:
Effect of *Ocimum gratissimum* aqueous leaf extract on markers of oxidative stress in normal and thyroidectomized male Wistar rats

	Control	Control+OG	Sham	Sham+OG	Thyrd	Thyrd+OG
MDA ($x10^{-3}$ mMol/ml)	6.68±0.11	6.77±0.16	6.65±0.10	6.55±0.05	7.24±0.22*	6.62±0.10
SOD (U/mL)	1.32±0.13	1.45±0.715	1.27±0.09	1.15±0.23	0.95±0.10*	1.47±0.26
Catalase ($x10^{-3}$U/mL)	3.07±0.22	4.22±0.65	3.06±0.23	2.82±0.21	1.67±0.11*	2.51±0.40

* P<0.05 Vs Control

Effect of OG on the lipid profile of normal and thyroidectomized male Wistar rats: Total cholesterol, Trig and HDL were significantly decreased in the thyroidectomized untreated rats when compared with the control. These were however reversed by OG treatment in the Thyrd+OG group. Low Density Lipoprotein was significantly elevated in the thyroidectomized group (32.95 ± 4.55 mg/dl) when compared with the control (15.3 ± 2.44 mg/dl) and the OG treated thryroidectomized rats (20.71 ± 3.24 mg/dl) Table 1.

Effect of OG on plasma oxidative stress biomarkers of normal and thyroidectomized male Wistar rats: As shown in table 2, plasma Malondialdehyde level of the thyroidectomized untreated rats (7.24 ± 0.22 $x10^{-3}$ nMol/ml) was significantly elevated when compared with the control rats (6.68 ± 0.11 $x10^{-3}$ nMol/ml) and this was reversed significantly in the thyroidectomized rats treated with OG (6.62 ± 0.10 $x10^{-3}$ nMol/ml). Both catalase and SOD activities were significantly reduced in the thyroidectomized untreated when compared with the control and the OG treated thyroidectomized groups. It is worthy to note that OG treatment to normal animal also showed significant increase in catalase activities (Control = 3.07 ± 0.22 Activity/ml Vs Control+OG = 4.22 ± 0.65 Activity/ml).

DISCUSSION

Weight increase has been associated with thyroidectomy and has been a major cause of dissatisfaction in human with hyperthyroidism treated with thyroidectomy (O'Malley *et al*., 2000; Rotondi *et al*., 2014; Glick *et al*., 2018; Huynh *et al*.,

2021). However, a recent follow up study among Koreans could not find any significant effect of thyroidectomy on weight gain (Jin *et al*., 2022). Such observation has been consistently demonstrated in rodent studies (Soukup *et al*., 2001; Ahmed *et al*., 2012; Nida *et al*., 2016), accordingly, we did not find any significant effect of thyroidectomy on the weight gained by the male Wistar rats in this study. The discrepancy in the animal studies and the earlier human studies was adduced to the difference in the mechanism by which energy is dissipated. The presence of Brown Adipose Tissue in rodents could be correlated to the possible mechanism of decreased weight gained in rats when compared to humans because it is activated and thus resulted in decreased weight gain in hypothyroid rats (Curico *et al*., 1999).

Impaired glucose tolerance is a well-known phenomenon during hypothyroidism caused by different agent including thyroidectomy in human (Kosovskiĭ *et al*., 1992; Jing *et al*., 2014) and rats (Cettour-Rose *et al*., 2005; Arigi *et al*., 2014; Kent *et al*., 2022). The observed increased AUC in the thyroidectomized rats in this study is in tandem with the impaired glucose tolerance reported by several workers (Kosovskiĭ *et al*., 1992; Jing *et al*., 2014; Arigi *et al*., 2014; Kent *et al*., 2022; David *et al*., 2021). Decreased peripheral glucose utilization and an exaggerated glucose-fatty acid cycle through the PGC1 dependent pathway are probable mechanisms by which hypothyroidism induces glucose intolerance (Cettour-Rose *et al*., 2005; Crunkhorn and Patti, 2008). The observed decreased AUC cum improved glucose tolerance following OG treatment in the thyroidectomized rats may be linked to the ability of OG to decrease hepatic cholesterol synthesis and increase insulin sensitivity taking cue from Shittu *et al* (2021)'s report that OG reduces hepatic cholesterol synthesis and increases insulin sensitivity of dexamethasone-induced metabolic syndrome rats.

Thyroidectomy caused significant inhibition of hexokinase activities in the current study. This observation has been well documented in several tissues obtained from hypothyroid animals. For instance, Nehal and Baquer (1989) and Al-Jamal (2004) showed that hexokinase activity was significantly reduced in rat red blood cell and rabbit isolated brain tissue respectively as a consequence of hypothyroidism and the activities were reversed by administration of thyroid hormone thus underscoring the importance of the thyroid hormone on the regulation of hexokinase activity. Thyroid hormones contribute to AKT phosphorylation which in turn firstly phosphorylates hexokinase to increase its association with the mitochondria and secondly phosphorylates and thus inactivates GSK3β. The inactivated GSK3β may decrease VDAC phosphorylation which allows further binding of hexokinase to mitochondria (Peçanha *et al*., 2017). Therefore, phosphorylation of both AKT and GSK3β are hampered in the absence of thyroid hormones thereby accounting for the reduced hexokinase activity reported during hypothyroidism and as observed in the current study. The activity of hexokinase in the thyroidectomized rats treated with OG was however not different from the control animals of this study indicating OG treatment suppressed the inhibitory effect of thyroidectomy in thyroidectomized rats. Plasma lactate concentration was evaluated in the current study to correlate its level with hepatic hexokinase activities. A linear relationship was observed in the hepatic hexokinase activities and the plasma lactate level in the thyroidectomized rats of this study, that is, decreased hepatic hexokinase activity was matched by decreased plasma lactate level in the thyroidectomized rats and these were reversed by treatment with OG in the Thyrd+OG group. Hexokinase is a rate limiting enzyme in glycolysis while lactate is an end product, thus decreased hexokinase activity profoundly affected lactate production through glycolysis. This may not be surprising as hypothyroidism was recently shown to reduce lactate production and inhibits glycolysis in rats' brain (Glombik *et al*., 2020).

Glycogen, an essential component of glucose homeostasis and metabolism in both skeletal muscles and liver is produced during postprandial state (glycogenesis) from glucose and fasting period state (gluconeogenesis) from other carbohydrates and amino acid sources (He *et al*., 2013). This prandial state of glucose homeostasis and metabolism is regulated by glycogen synthase and phosphorylase (Li *et al*., 2018). Glycogen synthase synthesizes glycogen, thereby increasing the glycogen content of liver, muscle and peripheral tissues. In the risk factors of metabolic syndrome most especially insulin resistance and diabetes mellitus, there is decreased formation of glycogen along with reduced glycogen content and disposition (Priest and Tontonoz, 2019). In thyroidectomized chicks, liver glycogen was significantly increased while muscle glycogen decreased (Nobukuni *et al*., 1989). The observed increase in liver glycogen content and decreased muscle glycogen in the current study is in tandem with the earlier report of Nobukuni *et al* (1989). Such decrease muscle glycogen was reported by Chu *et al* (1985) in rat skeletal muscle and this was adduced to reduction in the responsiveness of skeletal muscle to insulin action in hypothyroid rats. In fact, Czech *et al* (1980) has shown that hypothyroidism suppresses insulin-induced glucose transport and conversion into glycogen in skeletal muscles. Although, insulin level was not determined in this study, current evidences in animals have shown that islet insulin secretion is greatly impaired during hypothyroidism (Godini *et al*.,2014;2015). The observed trend in glycogen content of the thyroidectomized animals was not reversed by OG in this study.

Hypothyroidism is relatively associated with lipid dysfunction (Rizos *et al*., 2011). Dyslipidemia, arterial hypertension, endothelial dysfunction, and insulin resistance play a pivotal role in the development of atherosclerosis in patients with hypothyroidism (Biondi and Wartofsky, 2014). Thyroid hormone influences cholesterol metabolism by controlling the activity of cholesterol ester transfer protein, the hepatic lipase, and the lipoprotein lipase; it also controls the flow of bile acid and the low density lipoprotein (LDL) receptor activity in the liver (Duntas and Brenta, 2012). The observed dyslipidemia in the thyroidectomized rats of the current study is in tandem with the well documented effect of hypothyroidism on lipid metabolism. *Ocimum gratissimum* treatment caused significant reversal of the dyslipidemia in the thyroidectomized rats of this study, this effect of OG on dyslipidemia has been documented in diabetic rats (Ayinla *et al*.,2011).

The increased plasma Malondialdehyde level of the thyroidectomized untreated animals in the current study is consistent with the reported effect of hypothyroidism on oxidative stress in human (Baskol *et al*., 2007; Kaçmaz *et al*., 2015; Chakrabarti *et al*., 2016) and in rat (Sarandöl *et al*., 2005; Taş *et al*., 2006). Increased MDA level in hypothyroidism has been linked to the elevation of thyroid

stimulating hormone (Nanda *et al*., 2008). Treatment of the underlining factor or thyroid replacement therapy was shown to reverse the elevated MDA levels during hypothyroidism (Chakrabarti *et al*., 2016; Sarandöl *et al*., 2005) accordingly; the MDA level of the thyroidectomized rats treated with OG was significantly reduced when compared with the untreated thyroidectomized rats. Antioxidant supplementation to hypothyroid human caused significant reduction in the MDA level (Baskol *et al*., 2007; Chakrabarti *et al*., 2016; Sarandöl *et al*., 2005; Taş *et al*., 2006) thus the observed effect in the MDA level of OG treated thyroidectomized rats may be due to the well documented antioxidant potentials of OG (Shittu *et al*., 2016).

Catalase activity was significantly reduced in the thyroidectomized rats of this study. This is consistent with earlier reports (Pasupathi and Latha, 2008; Sahoo *et al*.,2008) in patients with hypothyroidism. Some other studies are at variance to the observation on catalase activity in the current study. For instance, Naazeri *et al*. (2014) reported increased while Carmeli *et al* (2008) reported decreased catalase activity in both hypothyroidism and hyperthyroidism. Nonetheless, catalase activity in the OG-treated thyroidectomized rats increased when compared with the untreated thyroidectomized and was not different from the control. This effect of OG on the catalase activity of the thyroidectomized rats cannot be dissociated from the well documented antioxidant capability of Ocimum gratissimum. Superoxide dismutase activity was not different across all groups. Earlier study has also reported no effect of thyroidectomy-induced or methimazole-induced hypothyroidism on SOD level in rats (Cano-Europa *et al*., 2010).

It is thus concluded that Ocimum gratisimum leaf extract decreases glucose intolerance through mechanism that may involve the promotion of hexokinase activity and suppression of oxidative stress in thyroidectomized male Wistar rats.

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