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# Effect of *Ocimum gratissimum* L. leaf extract on muscular atrophy in diabetic male wistar rats

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**ABSTRACT**

Oxidative stress and inflammation precipitate muscular atrophy in diabetes mellitus. The antioxidant and anti-inflammatory activities of *Ocimum gratissimum L.* (OG) is well documented. Thus, the effect of OG on muscular atrophy in diabetic rats was investigated. Twenty rats grouped as Control, OG, Diab and Diab+OG were administered distilled water or OG for 28 days. Glycogen content was determined in gastrocnemius muscle, oxidative stress biomarkers were assessed in right rectus femoris and extensor digitorum longus muscles while histomorphometry analysis was done in the contralateral muscles. Elevated blood glucose and depleted glycogen content were observed in the Diab (204.75 ± 9.95mg/dl and 0.09 ± 0.03mg/ml) and reversed in Diab+OG (90.01 ± 26.50mg/dl and 0.36 ± 0.05mg/ml). The lengths of the rectus femoris and extensor digitorum longus muscles were reduced while malonaldehyde increased in Diab (0.420 ± 0.031µmol/l) compared with Diab+OG (0.370 ± 0.01µmol/l). In conclusion, OG prevented atrophy and promoted glycogenesis by decreasing lipid peroxidation in the skeletal muscles of diabetic rats.

**Keywords:** muscular atrophy, diabetes mellitus, oxidative stress, *Ocimum gratissimum* L.

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## INTRODUCTION

Onset, progression and complications of diabetes mellitus are closely associated with oxidative stress resulting from prolonged hyperglycemia[1]. The characteristic uncontrolled hyperglycemia can directly increase free radical production through autoxidation of glucose[2], nonenzymatic reaction of glucose with protein leading to formation of Advanced Glycation End products[3], enhanced glucose channeling to the sorbitol pathway[4] and enzymatic induction of Nitric Oxide Synthase[5] among other pathways generating free radicals at different levels[6]. The increased expression of inducible nitric oxide synthase (iNOS) secondary to hyperglycemia is activated by inflammation-sensitive Nuclear Factor κappa B (NF-κB)[7] which contributes to muscle atrophy in diabetes mellitus[8].

Muscle atrophy results from imbalanced protein synthesis and degradation which is a stray in maintaining muscle homeostasis crucial for preserving the body's integrity and function[9,10]. Diabetic muscular atrophy (DMA) is a complication of diabetes mellitus characterized by proximal lower extremity muscle weakness, atrophy, pain, sensory disturbances, and even quadriplegia in severe cases[11]. Denervation associated with peripheral neuropathy in diabetes plays a significant role in the development of muscular atrophy[12]. The prevalence of diabetic neuropathy in Africa is 46% among diabetic patients[13] thus, the prevalence of diabetic muscle atrophy may equally be high given that Andreassen et al.[14] had demonstrated that the severity of neuropathy correlated directly with muscle atrophy in diabetic patients. Animal model of diabetes mellitus induced by streptozotocin were also shown to manifest DMA[15,16].

Muscle atrophy is closely related to two major protein degradation pathways, the ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway (ALP). It is also related to the protein synthesis pathways, such as the insulin-like growth factor 1– phosphoinositide-3-kinase–Akt/protein kinase B–mammalian target of rapamycin (IGF1–PI3K–Akt/PKB–mTOR) pathway and IGF-1-AKT- Forkhead box O (FoxO) pathways[17,18]. Insulin resistance in type 2 diabetes mellitus inhibits protein synthesis by inhibiting the IGF-1-PI3K-AKT/PKB-mTOR pathway and activates the UPS and ALP through the IGF-1-AKT-FoxO signaling pathway thereby promoting muscle atrophy while muscle atrophy in type 1 diabetes mellitus is mediated by the FoxO-driven protein degradation pathway[10]. The links between all these pathways are inflammation and oxidative stress hence, therapeutic agents targeting any of these links in addition to glycemic control have been found beneficial in ameliorating diabetic muscular atrophy. For instance, Punkt et al.[15], Brocca et al.[19], and Ono et al.[20] explored the antioxidants target while

Bako et al.[21] and Cea et al.[22] documented the role of inflammation. Exploration of agents with antioxidants and anti-inflammatory properties would therefore be a desirable progress in ameliorating DMA. A good candidate with such property is *Ocimum gratissimum* (OG), Africa basil/sweet basil, a plant belonging to *Lamiaceae* family native to Africa, Asia and South America. In Nigeria, it is known as *efinrin*, *Nehonwu*, and *ai daya ta guda* by the Yoruba, Igbo and Hausa, respectively[23]. Its bioactive compounds include phytochemicals (oleanolic acid, caffeic acid, ellagic acid, epicatechin, sinapic acid, rosmarinic acid, chlorogenic acid, luteolin, apigenin, nepetoidin, xanthomicrol, nevadensin, salvigenin, gallic acid, catechin, quercetin, rutin, and kaempferol) and essential oils (camphene, β-caryophyllene, α- and β-pinene, α-humulene, sabinene, β-myrcene, limonene, 1,8-cineole, trans-β-ocimene, linalool, α- and δ-terpineol, eugenol, α-copaene, β-elemene, p-cymene, thymol, and carvacrol)[24]. Several studies have documented its hypoglycaemic[25,26], antioxidant[27] and anti-inflammatory[28,29] activities. This study therefore investigated the effect of aqueous leaf extract of OG on skeletal muscle atrophy in streptozotocin-induced diabetic male Wistar rats.

## METHODOLOGY

### Animal

Twenty male Wistar rats weighing between 180 and 230 g were used for this study. They were housed in the Department of Physiology Postgraduate Animal House, College of Medicine, University of Ibadan, Ibadan, Nigeria. The rats were housed in plastic cages with perforated lids and allowed to acclimatize for two weeks at a standard room temperature, humidity, and a natural photoperiod of 12 hours of light/dark cycle. They were allowed free access to standard rat chow (Ladokun Feeds®) and tap water. All experimental protocols were conducted in strict adherence to institutional guideline on the use of laboratory animals for experiments based on the NIH publication No. 85-23 guidelines.

### Preparation of aqueous leaf extract *of Ocimum gratissimum L.*

The fresh leaves of *Ocimum gratissimum L.* 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, 1 kg 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 a temperature of 40°C to yield 13.26 % aqueous extract.

**Induction of diabetes mellitus**

Diabetes mellitus was induced by a single intraperitoneal dose of 65 mg/kg of freshly prepared Streptozotocin, STZ (Sigma®, St Loius, USA) in cold 50 mM sodium citrate buffer, pH 4.5. Fasting Blood Glucose was assessed after 72 hours of induction and animals with Fasting Blood Glucose level ≥ 200 mg/dL were confirmed as diabetic.

**Experimental design**

The animals were randomly divided into 4 groups (n=5) as follows:

I - Control, Normal rats administered distilled water

II - OG, Normal rats administered *Ocimum gratissimum* (400 mg/kg bwt)

III - Diab, Diabetic rats administered distilled water

IV - Diab+OG, Diabetic rats administered *Ocimum gratissimum* (400 mg/kg bwt)

The administered 400 mg/kg bwt of OG was established in earlier studies as being the effective therapeutic dose[27]. All administrations were done by oral gavage for 28 consecutive days. The weight and fasting blood glucose of all the rats were taken before and after the experiment. Blood was obtained by once-off tail prick and glucose levels determined using Accu-Chek® Glucometer (Roche, Germany).

**Sample collection**

Following an overnight fast, the animals were anaesthetized by 100 mg/kg Ketamine, *i.p.* (Ketanir®, Aculife Healthcare Pvt Ltd, India) and muscle samples were obtained. The gastrocnemius muscle was obtained for the determination of glycogen content, the right rectus femoris and extensor digitorum longus muscles were obtained separately for homogenization in phosphate buffer saline, PBS (pH 7.4) for markers of oxidative stress while their left counterparts were fixed separately in 10% formalin for histology and histomorphometry analysis.

**Determination of muscle glycogen content**

The glycogen content of the gastrocnemius muscle was determined by the Anthrone method as previously reported[30]. Briefly, gastrocnemius muscle was digested in 30% KOH overheat, 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°C for 10 min-

utes 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 oxidative stress biomarkers**

Oxidative stress biomarkers, Malondialdehyde, Superoxide Dismutase (SOD) and Catalase activity were determined in the supernatant of the homogenized right rectus femoris and extensor digitorum longus muscles.

Malondialdehyde (MDA) was determined according to the method described by Hagege et al.[31]. Briefly, 0.5ml of supernatant 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°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 $1.56\times.10^{5}m^{-1}cm^{-1.}$

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

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×; 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[32]. Briefly, 0.2 ml of supernatant (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 420 nm within 3 minutes.

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

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[33]. Briefly, 0.2 ml of supernatant 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 12.5 mM $H_2SO_4$ and read at 305 nm wavelength.

### Histomorphometry analysis

The fixed left rectus femoris and extensor digitorum longus muscles were taken for heamatoxylin and Eosin histological preparation using standard methods. Diameters of longitudinal and cross sections of the muscles were measured in microns using the measurements plug-in on AmScope 3.7, a computer enabled digital camera and software.

### Statistical analysis

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

## RESULTS and DISCUSSION

### Effects of *Ocimum gratissimum* L. leaf extract on body weight in normal and diabetic rats

As shown in Figure 1, Diabetes caused significant weight loss in Diab group when their initial weight (200.5 ± 2.52 g) was compared with their final weight (174.5 ± 9.18 g). The weight loss effect of diabetes was however abolished by OG treatment in Diab+OG group (Initial= 202.25 ± 4.33 g, Final = 195.5 ± 9.05 g).

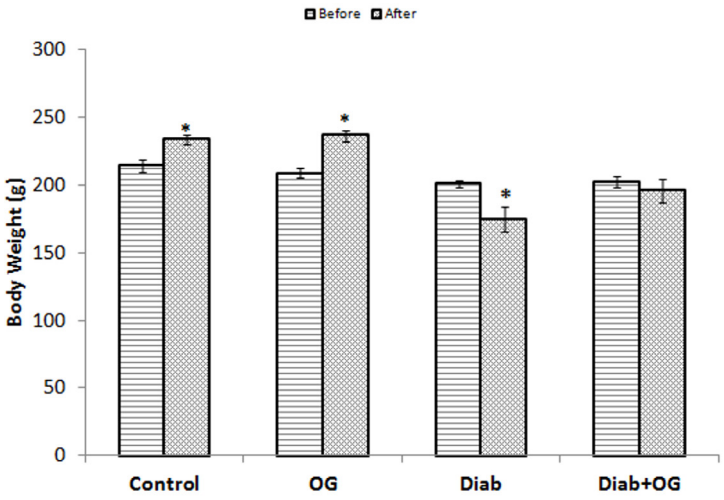


**Figure 1.** Effect of *Ocimum gratissimum* leaf extract on body weight in streptozotocin-induced diabetic male Wistar rats. *p<0.05 Before vs After

### Effects of *Ocimum gratissimum* L. leaf extract on fasting blood glucose in normal and diabetic rats

*Ocimum gratissimum* leaf extract caused significant reduction in the fasting blood glucose level of animals in the Diab+OG (90.01 ± 26.50 mg/dl) when compared with the Diab (204.75 ± 9.95 mg/dl), Figure 2.

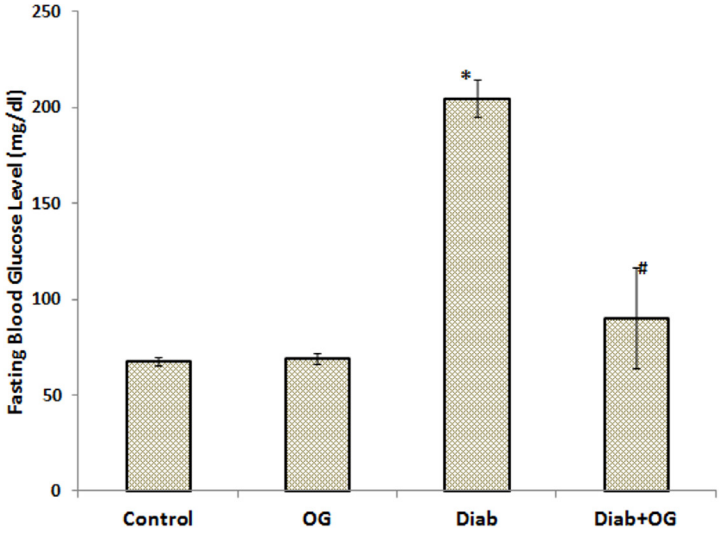


**Figure 2.** Effect of *Ocimum gratissimum* leaf extract on fasting blood glucose in normal and streptozotocin-induced diabetic male Wistar rats. *p<0.05 vs Control, #p<0.05 vs Diab

### Effects of *Occimum gratissimum* L. leaf extract on glycogen content of the gastrocnemius muscles of normal and diabetic rats

Glycogen content was significantly depleted in the gastrocnemius muscle of the Diab group (0.09 ± 0.03 mg/ml) compared with the control (0.37 ± 0.07 mg/ml). The glycogen depletion was however reversed in the Diab+OG group (0.36 ± 0.05 mg/ml) compared with the Diab group (0.09 ± 0.03 mg/ml), as shown in Figure 3.

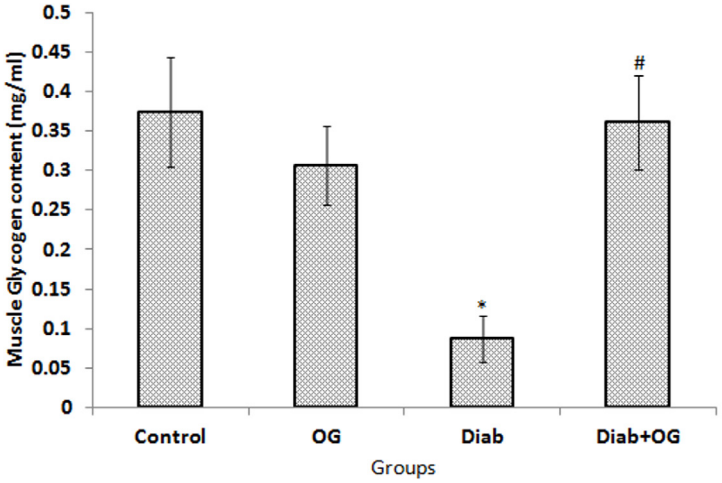


**Figure 3.** Effect of *Ocimum gratissimum* leaf extract on muscle glycogen content in normal and streptozotocin-induced diabetic male Wistar rats. *p<0.05 vs Control, #p<0.05 vs Diab

### Effects of *Occimum gratissimum* L. leaf extract on transverse and longitudinal dimensions of rectus femoris and extensor digitorum longus muscles in normal and diabetic rats

As shown in Table 1, the size of the rectus femoris muscle was significantly decreased in the Diab group (Transverse dimension=248.97 ± 7.36 μm; Longitudinal dimension=250.93 ± 7.82 μm) when compared with the control (Transverse dimension=372.03 ± 18.23 μm; Longitudinal dimension=327.97 ± 14.22 μm). The reduction in size was however reversed by treatment with OG in the Diab+OG group when compared with the Diab group (Transverse dimension=297.18 ± 12.36 μm vs 248.97 ± 7.36 μm; Longitudinal dimension=317.37 ± 10.12 μm vs 250.93 ± 7.82 μm).

**Table 1.** Effects of *Ocimum gratissimum* L. leaf extract on transverse and longitudinal dimensions of rectus femoris and extensor digitorum longus muscles in normal and streptozotocin-induced diabetic rats

| | RECTUS FEMORIS length (µm) | | EXTENSOR DIGITORIS length (µm) | |
|---|---|---|---|---|
| | Transverse | Longitudinal | Transverse | Longitudinal |
| Control | 372.03 ± 18.23 | 327.97 ± 14.22 | 283.44 ± 13.72 | 289.72 ± 9.74 |
| OG | 372.56 ± 32.49 | 333.19 ± 11.29 | 273.66 ± 13.44 | 273.63 ± 9.47 |
| Diab | 248.97 ± 7.36* | 250.93 ± 7.82* | 227.59 ± 10.85* | 261.58 ± 9.83* |
| Diab+OG | 297.18 ± 12.36*# | 317.37 ± 10.12# | 238.88 ± 5.83* | 262.04 ± 10.09* |

*p<0.05 vs Control; #p<0.05 vs Diab

In the extensor digitorum longus muscle, both the transverse (227.59 ± 10.85 µm) and longitudinal (261.58 ± 9.83 µm) dimensions were significantly reduced in the Diab group compared with the control (283.44 ± 13.72 µm; 289.72 ± 9.74 µm). Treatment with OG in the Diab+OG had no effect on the diabetes-induced size reduction of the extensor digitorum muscle, Table 1.

### Effect of *Ocimum gratissimum* L. leaf extract on oxidative stress biomarkers in the rectus femoris muscle of normal and diabetic rats

As shown in Table 2, MDA level in the Rectus Femoris muscle was significantly reduced in the Diab+OG group compared with the Diab group. While SOD activity was not different across all the groups, catalase activity was significantly decreased in the Diab group (0.008 ± 0.0004 Activity/mg protein) compared with control (0.010 ± 0.0002 Activity/mg protein) and the Diab+OG group (0.009 ± 0.0005 Activity/mg protein).

**Table 2.** Effect of *Ocimum gratissimum* L. leaf extract on Oxidative stress biomarkers in rectus femoris in normal and streptozotocin-induced diabetic male Wistar rats

| | Control | OG | Diab | Diab+OG |
|---|---|---|---|---|
| MDA (µmol/l) | 0.402 ± 0.019 | 0.414 ± 0.019 | 0.420 ± 0.031 | 0.370 ± 0.01# |
| SOD (Activity/mg protein) | 2.03 ± 0.03 | 1.98 ± 0.04 | 2 ± 0.06 | 2 ± 0.04 |
| Catalase (Activity/mg protein) | 0.01 ± 0.0002 | 0.011 ± 0.0006 | 0.008 ± 0.0004* | 0.009 ± 0.0005# |

*p<0.05 vs Control; #p<0.05 vs Diab

### Effect of *Ocimum gratissimum* L. leaf extract on oxidative stress biomarkers in the extensor digitorum longus muscle of normal and diabetic rats

The MDA level of the Extensor Digitorum Longus Muscle of the Diab group (0.485 ± 0.025 μmol/l) was significantly elevated when compared with the control (0.388 ± 0.004 μmol/l). The elevated MDA was significantly reduced in the Diab+OG group (0.403 ± 0.03 μmol/l) when compared with the Diab group (0.485 ± 0.025 μmol/l). Extensor Digitorum Longus Muscle SOD and catalase activities were however not different across all the groups, Table 3.

**Table 3.** Effect of *Ocimum gratissimum* L. on Oxidative Stress Biomarkers in Extensor Digitorum Longus Muscle in normal and streptozotocin-induced diabetic male Wistar rats

| | Control | OG | Diab | Diab+OG |
|---|---|---|---|---|
| MDA (μmol/l) | 0.388 ± 0.004 | 0.394 ± 0.018 | 0.485 ± 0.025* | 0.403 ± 0.03# |
| SOD (Activity/mg protein) | 2.124 ± 0.04 | 2.131 ± 0.02 | 2.117 ± 0.03 | 2.287 ± 0.12 |
| Catalase (Activity/mg protein) | 0.01 ± 0.0006 | 0.00859 ± 0.0004 | 0.0089 ± 0.0005 | 0.00944 ± 0.0002 |

*$p<0.05$ vs Control; #$p<0.05$ vs Diab

This study was designed to examine the effect of *Ocimum gratissimum* leaf extract on diabetes-induced muscular atrophy in male Wistar rats. The abolishment of weight loss and reduction in fasting blood glucose level observed in the OG treated diabetic animals in this study are in agreement with the well documented effects of OG[25,26].

Skeletal muscle is the largest reservoir of glycogen, containing about fourfold of what is contained in the liver postprandially and is depleted by 65% in type 1 or 25% in type 2 diabetes mellitus patients[34,35]. The observed significant depletion in the glycogen content of the gastrocnemius muscle in the present study agrees with these earlier reports. Inhibition of glycogen synthase[36] and stimulation of glycogen phosphorylase[37] activities are contributing factors in the diabetes-induced glycogen depletion and are major therapeutic targets. Earlier on, Shittu et al.[30] reported that OG inhibited glycogen phosphorylase activity in liver of streptozotocin-induced diabetic rats and the restoration of glycogen

content in the gastrocnemius muscle of OG-treated diabetic rats in the current study is in conformity with this earlier finding. Other studies also showed that stimulating glycogen synthase activity by inhibiting its phosphorylating enzyme, glycogen synthase kinase 3β (GSK-3β) promotes muscle glycogen and insulin sensitivity[38,39]. The restoration of muscle glycogen in the OG treated rats in this study may be linked with the absence of diabetes-induced weight loss given that a recent study in human documented that lowered muscle glycogen is associated with reduction in body mass[40].

The reduction in glycogen synthesis by GSK-3β activity occurs through the PI3K/Akt signaling pathway[41] which is also implicated in free radical production/oxidative stress induction[42] therefore, the increased MDA level in Extensor Digitorum Longus Muscle matching glycogen depletion in the gastrocnemius muscle observed in the diabetic animals of the present study may not be surprising. Elevated skeletal muscle oxidative stress is a well-documented phenomenon in diabetes mellitus[16,43-45] and treatment with antioxidants were found to be ameliorative[15,46]. The decreased MDA level and increased Catalase activity observed in the OG treated diabetic animals of this study are consistent with documented antioxidant property of OG[27]. The high phenol content of OG can be adduced to its antioxidant effect[24] and studies using phenol-rich fraction of OG reported superior antioxidants potentials compared to other fractions[47,48].

Histomorphometry analysis in this study showed significant muscular atrophy in both the rectus femoris and extensor digitorum longus muscles of the diabetic rats. Treatment with OG reverses the observed diabetes-induced muscular dystrophy. Such reversal by antioxidant treatments had been documented to increase skeletal muscle weights, strengths and fiber size in type1 diabetic rats[49] which corroborated with the effect of *OG* in the transverse and longitudinal dimensions of the rectus femoris and extensor digitorum longus muscles of diabetic rats. The anti-inflammatory property of OG[28,29] may also be involved in the prevention of muscular atrophy given that plants with anti-inflammatory properties reverse diabetes-induced muscle atrophy via the MPK/SIRT1 pathway[50].

O*cimum gratissimum* leaf extract prevented atrophy and promoted glycogenesis by decreasing lipid peroxidation in the skeletal muscles of STZ-induced diabetic male Wistar rats. The interaction between OG and glycogen synthase activity requires further elucidation.

## STATEMENT OF ETHICS

All experimental protocols were conducted in strict adherence to institutional guideline on the use of laboratory animals for experiments based on the NIH publication No. 85-23 guidelines. The ethical approval number is UI-ACUREC/100-1023/13.

## CONFLICT OF INTEREST STATEMENT

No conflict of interest was declared by the authors.

## AUTHOR CONTRIBUTIONS

Design – Shittu ST, Lasisi T; Acquisition of data – Shittu ST, Bello I; Analysis of data – Shittu ST, Shittu SA, Bello I; Drafting of the manuscript – Shittu ST, Bello I; Critical revision of the manuscript – Akor-Dewu M, Lasisi T; Statistical analysis – Shittu ST, Bello I, Shittu SA; Technical or financial support – Shittu ST, Bello I, Shittu SA, Akor-Dewu I, Lasisi T; Supervision – Akor-Dewu I, Lasisi T.

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## REFERENCES


1. Rösen P, Nawroth PP, King G, Möller W, Tritschler HJ, Packer L. The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a congress series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. Diabetes Metab Res Rev, 2001;17(3):189-212. Doi:10.1002/dmrr.196

2. Turko IV, Marcondes S, Murad F. Diabetes-associated nitration of tyrosine and inactivation of succinyl-CoA:3-oxoacid CoA-transferase. Am J Physiol Heart Circ Physiol, 2001;281(6):H2289-2294. Doi: 10.1152/ajpheart.2001.281.6.H2289

3. Gillery P. Produits avancés de glycation (AGE), radicaux libres et diabète [Advanced glycation end products (AGEs), free radicals and diabetes]. J Soc Biol, 2001;195(4):387-390.

4. Lorenzi M. The polyol pathway as a mechanism for diabetic retinopathy: attractive, elusive, and resilient. J Diabetes Res, 2007;10. Doi:10.1155/2007/61038

5. Johansen JS, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. Cardiovasc Diabetol, 2005;4:5. Doi: 10.1186/1475-2840-4-5

6. Robertson RP. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. J Biol Chem, 2004;279(41):42351-42354. Doi: 10.1074/jbc.R400019200

7. Jia J, Liu Y, Zhang X, Liu X, Qi J. Regulation of iNOS expression by NF-κB in human lens epithelial cells treated with high levels of glucose. Invest Ophthalmol Vis Sci, 2013; 54(7):5070-5077. Doi: 10.1167/iovs.13-11796

8. Perry BD, Caldow MK, Brennan-Speranza TC, Sbaraglia M, Jerums G, Garnham A, et al. Muscle atrophy in patients with type 2 diabetes mellitus: roles of inflammatory pathways, physical activity and exercise. Exerc Immunol Rev, 2016;22:94-109.

9. Yin L, Li N, Jia W, Wang N, Liang M, Yang X, et al. Skeletal muscle atrophy: from mechanisms to treatments. Pharmacol Res, 2021;172:105807. Doi: 10.1016/j.phrs.2021.105807

10. Shen Y, Li M, Wang K, Qi G, Liu H, Wang W, et al. Diabetic muscular atrophy: molecular mechanisms and promising therapies. Front Endocrinol, 2022;13:917113. Doi: 10.3389/fendo.2022.917113

11. Mahashabde M, Chaudhary G, Kanchi G, Rohatgi S, Rao P, Patil R, et al. An unusual case of critical illness polyneuromyopathy. Indian J Crit Care Med, 2020;24:133-135. Doi: 10.5005/jp-journals-10071-23346

12. Parasoglou P, Rao S, Slade JM. Declining skeletal muscle function in diabetic peripheral neuropathy. Clin Ther, 2017;39(6):1085-1103. Doi: 10.1016/j.clinthera.2017.05.001

13. Shiferaw WS, Akalu TY, Work Y, Aynalem YA. Prevalence of diabetic peripheral neuropathy in Africa: a systematic review and meta-analysis. BMC Endocr Disord, 2020;20(1):49. Doi: 10.1186/s12902-020-0534-5

14. Andreassen CS, Jakobsen J, Ringgaard S, Ejskjaer N, Andersen H. Accelerated atrophy of lower leg and foot muscles—a follow-up study of long-term diabetic polyneuropathy using magnetic resonance imaging (MRI). Diabetologia, 2009;52(6):1182-1191. Doi: 10.1007/s00125-009-1320-0

15. Punkt K, Psinia L, Welt K, Barth W, Asmussen G. Effects on skeletal muscle fibres of diabetes and *Ginkgo biloba* extract treatment. Acta Histochem, 1999;101:53-69. Doi: 10.1016/S0065-1281(99)80008-7

16. Aughsteen AA, Khair AB, Suleiman AA. Quantitative morphometric study of the skeletal muscles of normal and streptozotocin-diabetic rats. J Pancreas, 2006;7(4):382-389.

17. Pohl C, Dikic I. Cellular quality control by the ubiquitin-proteasome system and autophagy. Science, 2019;366(6467):818-822. Doi: 10.1126/science.aax3769

18. Milan G, Romanello V, Pescatore F, Armani A, Paik JH, Frasson L, et al. Regulation of autophagy and the ubiquitin-proteasome system by the FoxO transcriptional network during muscle atrophy. Nat Commun, 2015;6:6670. Doi: 10.1038/ncomms7670

19. Brocca L, D'Antona G, Bachi A, Pellegrino MA. Amino acid supplements improve native antioxidant enzyme expression in the skeletal muscle of diabetic mice. Am J Cardiol, 2008;101(11A):57E-62E. Doi: 10.1016/j.amjcard.2008.03.002

20. Ono T, Takada S, Kinugawa S, Tsutsui H. Curcumin ameliorates skeletal muscle atrophy in type 1 diabetic mice by inhibiting protein ubiquitination. Exp Physiol, 2015;100:1052-1063. Doi: 10.1113/EP085049

21. Bako HY, Ibrahim MA, Isah MS, Ibrahim S. Inhibition of jak-stat and NF-κB signalling systems could be a novel therapeutic target against insulin resistance and type 2 diabetes. Life Sci, 2019;239:117045. Doi: 10.1016/j.lfs.2019.117045

22. Cea LA, Vásquez W, Hernández-Salinas R, Vielma AZ, Castillo-Ruiz M, Velarde V. Skeletal muscle atrophy induced by diabetes is mediated by non-selective channels and prevented by boldine. Biomolecules, 2023;13:708. Doi: 10.3390/biom13040708

23. Ayinla MT, Dada SO, Shittu ST, Olayaki LA, Akiode AO, Ojulari SL. Anti-hyperlipidemic effect of aqueous leaf extract of *Ocimum gratissimum* in alloxan induced diabetic rats. J. Med. Med. Sci, 2011;3(12):360-363. Doi: 10.5897/IJMMS.9000022

24. Ugbogu OC, Emmanuel O, Agi GO, Ibe C, Ekweogu CN, Ude VC, et al. A review on the traditional uses, phytochemistry, and pharmacological activities of clove basil (*Ocimum gratissimum* L.). Heliyon, 2021;7(11):e08404. Doi: 10.1016/j.heliyon.2021.e08404

25. Aguiyi JC, Obi CI, Gang SS, Igweh AC. Hypoglycaemic activity of *Ocimum gratissimum* in rats. Fitoterapia, 2000;71:444446. Doi: 10.1016/s0367-326x(00)00143-x

26. Okoduwa SIR, Umar IA, James DB, Inuwa HM. Anti-diabetic potential of *Ocimum gratissimum* leaf fraction in fortified diet fed streptozotocin-treated diabetic rats. Medicines, 2017;4:E73. Doi: 10.3390/medicines4040073

27. Shittu ST, Oyeyemi WA, Lasisi TJ, Shittu SA, Lawal TT, Olujobi ST. Aqueous leaf extract of *Ocimum gratissimum* improves hematological parameters in alloxan-induced diabetic rats via its antioxidant properties. Int J App Basic Med Res, 2016;6:96-100. Doi: 10.4103/2229-516X.179016

28. Ajayi AM, Ologe MO, Ben-Azu B, Okhale SE, Adzu B, Ademowo OG. *Ocimum gratissimum* Linn. leaf extract inhibits free radical generation and suppressed inflammation in carrageenan-induced inflammation models in rats. J Basic Clin Physiol Pharmacol, 2017;28(6):531-541. Doi: 10.1515/jbcpp-2016-0096

29. Anusmitha KM, Aruna M, Job JT, Narayanankutty A, Benil PB, Rajagopal R, Alfarhan A, et al. Phytochemical analysis, antioxidant, anti-inflammatory, anti-genotoxic, and anticancer activities of different Ocimum plant extracts prepared by ultrasound-assisted method. Physiol Mol Plant Pathol, 2022;117:101746. Doi: 10.1016/j.pmpp.2021.101746

30. Shittu ST, Oyeyemi WA, Shittu SA, Lasisi TJ. *Ocimum gratissimum* inhibits glycogen phosphorylase activity without changes in hepatic nuclear factor kappa B (NF-κB) and inducible nitric oxide synthase (iNOS) in streptozotocin-induced diabetic rats. Nig Med Pract, 2018;73(1-2):10-17

31. Feutrys HD, Krsnik-Rasol M, Poder D, Menez JF. Estimation of free and bound MDA in plant extracts. Comparison between spectrophotometric and HPLC methods. In: Chen T, Zhang B, editors. Plant lipid metabolism [Internet]. Dordrecht: Springer; 1995 [cited 2023 Nov 01]. p. 259-261.

32. Misra HP. Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Bio Chem, 1972;247(10):3170-3175. Doi: 10.1016/s0021-9258(19)45228-9

33. Góth L. A simple method for determination of serum catalase activity and revision of reference range. Clin Chim Acta, 1991;196(2-3):143-151. Doi: 10.1016/0009-8981(91)90067-m

34. Roch-Norlund AE, Bergstrom J, Castenfors H, and Hultman E. Muscle glycogen in patients with diabetes mellitus. Acta Med Scand, 1970;187:445-453. Doi: 10.1111/j.0954-6820.1970.tb02969.x

35. He J, Kelley DE. Muscle glycogen content in type 2 diabetes mellitus. Am J Physiol – Endocrinol, 2004;287(5): E1002-E1007. Doi: 10.1152/ajpendo.00015.2004

36. Svetlana EN, Theodore PC, Leslie C, Sunder M, Kyong SP, Robert RH. Impaired muscle glycogen synthase in type 2 diabetes is associated with diminished phosphatidylinositol 3-kinase activation. J Clin Endocrinol Metab, 2001;86(9):4307-4314. Doi: 10.1210/jcem.86.9.7872

37. Kantsadi AL, Parmenopoulou V, Bakalov DN, Snelgrove L, Stravodimos GA, Chatzileontiadou DS et al. Glycogen phosphorylase as a target for type 2 diabetes: synthetic, biochemical, structural and computational evaluation of novel N-acyl-N´-(β-D-glucopyranosyl) urea inhibitors. Curr Top Med Chem, 2015;15(23):2373-2389. Doi: 10.2174/1568026615666150619142253

38. Kaidanovich-Beilin O, Eldar-Finkelman H. Long-term treatment with novel glycogen synthase kinase-3 inhibitor improves glucose homeostasis in ob/ob mice: molecular characterization in liver and muscle. J Pharmacol Exp Ther, 2006;316(1):17-24. Doi: 10.1124/jpet.105.090266

39. Ponnusamy R, Giriraj K, Magesh Selvakumar AM, Thangaraj P, Sathyanarayanan S, Antunes de Souza Araújo A, et al. Glycogen synthase kinase 3β inhibition and insulin-receptor binding enhancement of compounds isolated from wild leafy vegetable Acalypha alnifolia, Phytomed Plus, 2022;2(1):100216. Doi: 10.1016/j.phyplu.2022.100216

40. Schytz CT, Ørtenblad N, Birkholm TA, Plomgaard P, Nybo L, Kolnes KJ, et al. Lowered muscle glycogen reduces body mass with no effect on short-term exercise performance in men. Scand J Med Sci Sports, 2023;33:1054-1071. Doi: 10.1111/sms.14354

41. Dokken BB, Sloniger JA, Henriksen EJ. Acute selective glycogen synthase kinase-3 inhibition enhances insulin signaling in prediabetic insulin-resistant rat skeletal muscle. Am J Physiol Endocrinol Metab, 2005;288:E1188-E1194. Doi: 10.1152/ajpendo.00547.2004

42. Le Belle JE, Orozco NM, Paucar AA, et al. Proliferative neural stem cells have high endogenous ROS levels that regulate self-renewal and neurogenesis in a PI3K/Akt-dependant manner. Cell Stem Cell, 2011;8(1):59-71. Doi: 10.1016/j.stem.2010.11.028

43. Klueber KM, Feczko JD, Schmidt G, Watkins JB. Skeletal muscle in the diabetic mouse: histochemical and morphometric analysis. Anat Rec, 1989;225:41-45. Doi: 10.1002/ar.1092250107

44. Medina-Sanchez M, Rodriguez-Sanchez C, Vega-Alvarez JA, Menedez-Pelaez A, Perez-Casas A. Proximal skeletal muscle alterations in streptozotocin-diabetic rats: a histochemical and morphometric analysis. Am J Anat, 1991;191:48-56. Doi: 10.1002/aja.1001910105

45. Ozaki K, Matsuura T, Narama I. Histochemical and morphometrical analysis of skeletal muscle in spontaneous diabetic WBN/Kob rat. Acta Neuropathol, 2001;102:264-270. Doi: 10.1007/s004010100363

46. Call JA, Chain KH, Martin KS, Lira VA, Okutsu M, Zhang M, et al. Enhanced skeletal muscle expression of extracellular superoxide dismutase mitigates streptozotocin-induced diabetic cardiomyopathy by reducing oxidative stress and aberrant cell signaling. Circ Heart Fail, 2015; 8(1):188-197. Doi: 10.1161/CIRCHEARTFAILURE.114.001540

47. Ajayi AM, Umukoro S, Ben-Azu B, Bulus A, Ademowo OG. Toxicity and protective effect of phenolic-enriched ethylacetate fraction of *Ocimum gratissimum* (Linn.) leaf against acute inflammation and oxidative stress in rats. Drug Dev Res, 2017;78:135-145. Doi: 10.1002/ddr.21384

48. Alabi QK, Akomolafe RO, Omole JG, Aturamu A, Ige MS, Kayode OO, et al. Polyphenol-rich extract of *Ocimum gratissimum* leaves prevented toxic effects of cyclophosphamide on the kidney function of Wistar rats. BMC Complement Med Ther, 2021;21:274. Doi: 10.1186/s12906-021-03447-3

49. Oyenihi AB, Langa SOP, Mukaratirwa S, Masola B. Effects of *Centella asiatica* on skeletal muscle structure and key enzymes of glucose and glycogen metabolism in type 2 diabetic rats. Biomed Pharmacother, 2019;112:108715. Doi: 10.1016/j.biopha.2019.108715

50. Li J, Zhang Y, Yu F, Pan Y, Zhang Z, He Y, et al. Proteoglycan extracted from *Ganoderma lucidum* ameliorated diabetes-induced muscle atrophy via the AMPK/SIRT1 pathway in vivo and in vitro. ACS Omega, 2023;8(33):30359-30373. Doi: 10.1021/acsomega.3c03513