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Effects of *Telfairia Occidentalis* Leaf Extract on Plasma Lactate and Liver Glycogen in Rats

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Summary: *Telfairia occidentalis* is a green vegetable popularly consumed among the native of Africa and it is generally believed to be of medicinal and nutritional value. Studies have reported its hypoglycaemic and hyperglycaemic effects in rats. In addition to these conflicting reports, the mechanisms for its effects on blood glucose remain inconclusive. The objective of this study was to investigate the mechanism involved in the increased blood glucose following treatment with *T. occidentalis*. Twenty five (25) male albino rats (200-250g) were randomly divided into 5 groups (n=5/group). Rats in the control group received normal saline while rats in other groups were orally treated with 100 or 200 mg/kg body weight of the extract for either 1 or 2 weeks. At the end of the treatment, the rats were anaesthetized and blood samples were collected for the estimation of some biochemical parameters. The results showed significant decreases in plasma glucose after 1 week of treatment with 100 mg/kg and 200 mg/kg. However, after 2 weeks of treatment with both doses, plasma glucose levels increased significantly and were higher than those of the control and the rats treated for 1 week with both doses. There were also dose- and duration-dependent decreases in glycogen concentration in the treated rats, especially those treated for two weeks. Glucose-6-phosphatase activity and liver glycogen concentration were lower in rats treated for 2 weeks when compared with those treated for 1 week with both doses. Moreover, plasma lactate concentration was lower in the treated groups when compared with control. The results suggest that *Telfairia occidentalis*-induced lowering of plasma glucose after one week of treatment probably favoured lactate oxidation/gluconeogenesis and elicited breakdown of liver glycogen which resulted in increased plasma glucose after two weeks of treatment.

Keywords*:* Blood glucose, Lactate, Liver glycogen, Glucose-6-phosphatase, *Telfairia occidentalis*

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INTRODUCTION

Telfairia occidentalis is a tropical vine grown in West Africa as a leaf vegetable and for its edible seeds. Common names for the plant include fluted gourd, fluted pumpkin, and Ugwu. *Telfairia occidentalis* is a member of the Curcurbitaceae family and is indigenous to southern Nigeria (Akoroda, 1990). There is paucity of information on the phytochemical constituents of the leaf of *T. occidentalis*. However, it has been reported that the leaf contains tannins, flavonoids, alkaloids, saponins, steroids, anthraquinones, and glycosides (Eseyin *et al*., 2000; Oboh, 2006). The presence of long chain n-3-unsaturated fatty acid in the leaf has also been reported. Palmitoleic acid (16.62%) and elaidic acid (0.85%) are the predominant omega 9 fatty acids present in the leaf (Inuwa *et al*., 2012). The carbohydrate content of the leaf is 25% (Oyenuga, 1968; Akwaowo *et al*., 2000). The amino acid profile of *Telfairia occidentalis* had also been shown to be very rich and includes alanine, aspartate, leucine, glycine, glutamine, histidine, lysine, methionine, tryptophan, arginine, cystine, serine, threonine, phenylalanine, isoleucine, valine and tyrosine (Tindall, 1968; Fasuyi, 2006).

Medicinal and Nutritional values of *Telfairia occidentalis* have also been reported; its shoots contain high levels of potassium and iron, while seeds are composed of 27% crude proteins and 53% fats (Aiyelaagbe and Kintomo, 2002). Its anxiolytic and sedatives properties (Akindele and Ajao, 2013), blood coagulation (Nubila *et al*., 2013), immunomodulatory (Egba *et al*., 2013a; Egba *et al*., 2013b), phytoextraction (Iyagba and Offor, 2013), testiculoprotective (Akang *et al*., 2010; Saalu *et al*., 2010), amelioration of radiation-induced testicular injury (Adejuwon *et al*., 2014), cancer chemopreventive (Iweala and Obidoa, 2009), anti-oxidant and anti-microbial properties (Oboh *et al*., 2006; Iweala and Obidoa, 2009), hepatoprotective (Ekpenyong *et al*., 2012), anti-anaemic (Alada, 2000; Dina *et al*., 2000; Oboh, 2004) anti-convulsant (Gbile, 1986), anti-inflammatory (Oluwole *et al*., 2003) and

purgative (Dina *et al*., 2001) properties have also been reported.

Until recently, the effects of *T. occidentalis* on blood glucose has been controversial with some studies reporting a reduction (Aderibigbe *et al*., 1999; Eseyin *et al*., 2000; Emudianughe and Aderibigbe, 2002; Nwozo *et al*., 2004; Salman *et al*., 2008; Eseyin *et al*., 2010; Eseyin *et al*., 2014) while others reported an increase in blood glucose (Adisa *et al*., 2012) following short-term and long-term treatment respectively. Salman *et al.* (2013) recently reported a decrease in blood glucose after one week of treatment with *T. occidentalis* and an increase in blood glucose after two weeks of treatment. While Salman *et al*. (2013) reported that the blood glucose-lowering effect of *T. occidentalis* observed after one week of treatment could be insulin-dependent as the authors observed an increase in plasma insulin while plasma insulin returned to normal after two weeks of treatment following an increase in blood glucose, the mechanism(s) involved in the increased blood glucose following two weeks of treatment with *T. occidentalis* remains poorly understood

It is hoped that the present study aimed at investigating the effects of *T. occidentalis* on plasma lactate and liver glycogen will throw more light on the probable biphasic effects of *T. Occidentalis* with respect to its role in glucose metabolism.

MATERIALS AND METHODS

Animals and Grouping

Twenty-five (25) male albino rats (200-250g) from the Central Animal House of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Nigeria were used for this study. The animals were housed in a well-ventilated room at a temperature of 23-27ºC on a 12hour light/dark cycle. The principles of laboratory animal care (NIH publication No. 85-23, revised in 1985) were followed. All experiments were examined and approved by the University of Ilorin ethics committee. All necessary protocols were followed to ensure the humane treatment of the animal.

The rats were divided into five groups (A-E, n=5/group) as follows:

Group A- control: received only standard feeds and 0.2ml normal saline for 2 weeks.

Group B- lower dose 1 (LD1): received 100 mg/kg of *Telfairia occidentalis* for 1 week.

Group C- lower dose 2 (LD2): received 100 mg/kg of *Telfairia occidentalis* for 2 weeks.

Group D- higher dose 1 (HD1): received 200 mg/kg of *Telfairia occidentalis* for 1 week.

Group E- higher dose 2 (HD2): received 200 mg/kg of *Telfairia occidentalis* for 2 weeks.

A day after the last treatment in each group, blood sample from each rat was collected by cardiac puncture into lithium heparinised capillary tubes. Plasma was collected from each sample and preserved at -20ºC.

Plant Materials, Preparation and Extraction

Fresh leaves of *Telfairia occidentalis* were bought at Oja Tuntun, Ilorin, Kwara State, Nigeria and authenticated by Mr Bolu Ajayi of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. Its voucher number (UIH1063) was deposited in the departmental herbarium. The leaves were washed with water to remove sand and other debris, chopped into smaller bits, air-dried and ground with a mortar and pestle. The leaf material weighing 220g was macerated in 2.5 litres of distilled water for 72 hours and stirred at intervals. The extracts obtained were filtered and the filtrate was dried at 40ºC to obtain a solid extract of 50g.

Measurement of Plasma Biochemical Parameters:

Plasma glucose

Plasma glucose was measured by standard laboratory procedure using the glucose oxidase method (Trinder, 1969).

Plasma lactate

Plasma lactate was assayed by an enzyme linked immunosorbent method using a clinical kit supplied by Monobind Inc., Lake Forest, CA, USA. The protocol was carried out using the manufacturer's instructions. Spectrophotometric readings were taken on a microplate reader at 550 nm wavelength.

Glycogen

The glycogen was extracted from rats' crude liver homogenate and made to react with Anthrone reagent to form a blue-colour solution that was compared spectrophotometrically with that formed by a known amount of glycogen (Seifter *et al*., 1950).

Glucose-6-phosphatase activity

Glucose-6-phosphatase activity was determined using kit from Elabscience Biotechnology Co. Ltd, Wuhan, Hubei Province, China. The ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in the kit was pre-coated with an antibody specific to Glucose-6-phosphatase. Standards or samples were added to the appropriate micro-ELISA plate wells and combined to the specific antibody. Then a biotinylated detection antibody specific for Glucose-6-phosphatase and Avidin-Horseradish Peroxidase (HRP) conjugate were added to each microplate well and incubated. Free components were washed away. The substrate was added to each well. Only those wells that contain Glucose-6-phosphatase, biotinylated detection antibody and Avidin-HRP conjugate appeared blue in colour and were read spectrophotometrically at 450 nm.

Statistical Analysis

Data were analyzed using SPSS version 20.0 for windows (IBM Corporation, Armonk, NY, USA). All

values given were the Mean ± S.E.M. of the variables measured. Significance was assessed by the one-way analysis of variance (ANOVA), followed by a post-hoc Least Significance Difference (LSD) test for multiple comparisons. *p*-Values of 0.05 or less were taken as statistically significant.

RESULTS

Effects of *Telfairia occidentalis* on plasma glucose level.

There were significant decreases in plasma glucose levels following treatment with 100 mg/kg ($p<0.01$) and 200 mg/kg ($p<0.05$) of *Telfairia occidentalis* for 1 week when compared with the control. However, after 2 weeks of treatment with both doses, plasma glucose was significantly higher than the control and the groups treated with 100- and 200 mg/kg for 1 week (Figure 1).

Effects of *Telfairia occidentalis* on lactate concentration

The plasma lactate concentration decreased significantly ($p<0.05$) in the group of rats treated with 200 mg/kg for 1 week while there was no change in those treated with 100 mg/kg for 1 week when compared with the control. However, plasma lactate decreased significantly after treatment with both 100- and 200 mg/kg for two weeks (Figure 2).

Effects of *Telfairia occidentalis* on liver glycogen concentration

The *T. occidentalis* treatments caused significant reductions in glycogen concentration in all the treated groups except LD1 compared to the control. However, glycogen concentration was significantly lower in rats treated for 2 weeks when compared with those treated for 1 week with the same doses. Similarly, glycogen concentration was also higher in rats treated with 100 mg/kg for 1 week compared with those treated with 200 mg/kg for 1 week. That is, the effect of *T. occidentalis* on glycogen is both dose- and duration-dependent (Figure 3).

Effects of *Telfairia occidentalis* on Glucose-6-phosphatase (G6Pase) activity

There were significant reductions in G6Pase activity in all the rats treated for two weeks while there was no significant change in those treated for one week when compared with the control. Moreover, the two doses of *T. occidentalis* caused significant reductions in G6Pase activity after two weeks of treatment compared to one week of treatment. Furthermore, G6Pase activity was significantly higher in rats treated with 200 mg/kg for 2 weeks when compared with those treated with 100 mg/kg for 2 weeks (Figure 4).

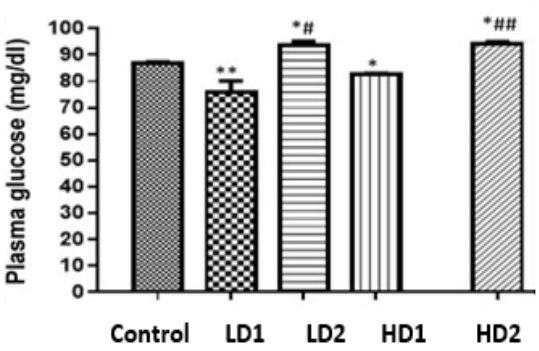

Figure 1: Effects of *Telfairia occidentalis* on blood glucose**.** *$p<0.05$ vs control, **$p<0.01$ vs control; #$p<0.05$ vs 1 week of the same dose, ##$p<0.01$ vs one week of the same dose.

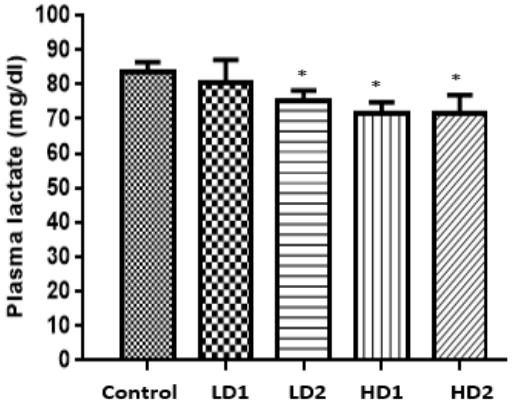

Figure 2: Effects of *Telfairia occidentalis* on plasma lactate concentration. *$p<0.05$ vs control.

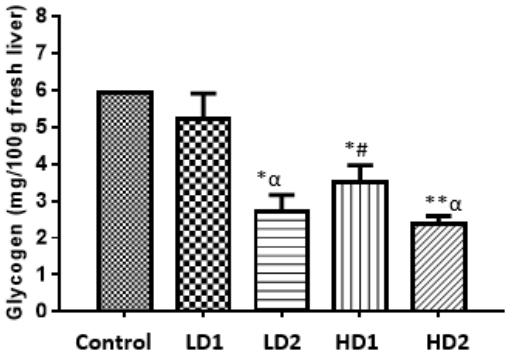

Figure 3: Effects of *Telfairia occidentalis* on liver glycogen concentration. α$p<0.01$ compared to the respective 1 week treatment. *$p<0.01$ vs control, **$p<0.0001$ vs control, #= $p<0.05$ vs LD1.

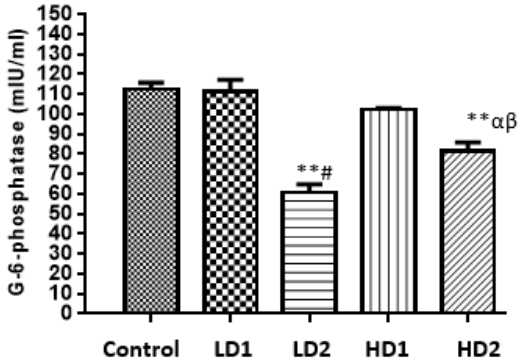

Figure 4: Effects of *Telfairia occidentalis* on Glucose-6-phosphatase activity**.** **$p<0.001$ vs control, #$p<0.01$ vs LD1, α$p<0.01$ vs HD1, β$p<0.01$ vs LD2.

DISCUSSION

The present study showed that *Telfairia occidentalis* caused significant reduction in plasma glucose levels after one week of treatment and significant increase after two weeks of treatment. These findings are consistent with the previously reported hypoglycaemic (Emudianughe and Aderibigbe, 2002; Nwozo *et al*., 2004; Salman *et al*., 2008; Eseyin *et al*., 2010; Salman *et al*., 2013) and hyperglycaemic effects (Adisa *et al*., 2012; Salman *et al*., 2013) of *T. occidentalis*.

The decrease in blood glucose after one week of treatment could be as a result of increase in plasma insulin following treatment with *T. occidentalis* as previously reported (Salman *et al*., 2013). Insulin is a well-known hypoglycaemic agent which regulates glucose metabolism by direct and indirect actions. Through binding to its receptors in the liver, kidney, muscle, and adipose tissue, insulin activates its signaling pathway which involves a complex cascade of protein kinases and regulatory proteins of which insulin receptor substrate 1 (IRS-1) and insulin receptor substrate 2 (IRS-2) are the most important. This causes (1) suppression of glucose release from liver and kidney, (Meyer*et al.*, 1998), (2) translocation of glucose transporters in muscle and adipose tissue to increase their glucose uptake (Oster-Jorgensen *et al*., 1990).

Of great interest is the dose- and duration-dependent decrease in liver glycogen concentration in the treated animals. A cursory look at the data on liver glycogen concentration showed that there was a decrease of about 50% and 60% respectively in liver glycogen concentration in the rats treated with 100- and 200 mg/kg for two weeks when compared with the control. In the liver, glycogen can be degraded by a hydrolytic as well as a phosphorolytic pathway (Vandebroeck *et al*., 1985). However, the hydrolysis of glycogen by α-glucosidase in the lysosomes is only a manifestation of autophagocytosis and is quantitatively unimportant in the overall process of glycogen mobilization. The regular phosphorolytic pathway of glycogenolysis is catalysed by phosphorylase-α and results in the release of glucose-1-phosphate, which is in equilibrium with glucose 6-phosphate by the action of phosphoglucomutase. In the periportal zone, which is rich in glucose 6-phosphatase, glucose-6-phosphate is mainly converted into glucose when the circulating glucose concentrations are falling. In the perivenous zone, on the other hand, glucose 6-phosphate is mainly converted into lactate in the post-absorptive phase (Jungermann and Kietzmann, 1996). Thus, the breakdown of liver glycogen can either lead to the production of glucose or lactate. However, since plasma glucose increased significantly while lactate decreased, it is tempting to suggest that the glycogen was probably converted to glucose.

A question that readily comes to mind is, what was the fate of the glucose removed from the blood after one week of treatment. Was it phosphorylated or converted to glycogen? Liver glycogen was measured because the liver is the main organ involved in glucose homeostasis which has the capacity to store excess glucose as glycogen. Interestingly, there was no any increase in liver glycogen in any of the treated groups in this study. However, since the fate of glucose taken up by cells is either to be oxidized and converted to pyruvate/lactate or stored as glycogen, it will not be unreasonable to speculate that *T. occidentalis* could have the potential of increasing glycolysis since there was no increase in glycogen or lactate concentration after one week of treatment. This is reasonable because glycogenesis can only be favoured when there is hyperglycaemia but on the contrary, *T. occidentalis* actually caused a reduction in plasma glucose after one week of treatment.

The physiological significance of the reduction in lactate concentration observed in this study and the probability of lactate contributing to the increased blood glucose seen after two weeks of treatment with *T. occidentalis* is not clear. However, in resting mammals, oxidation accounts for approximately half lactate disposal and gluconeogenesis approximately 20% (Stanley *et al*., 1986; Brooks *et al*., 1991). Thus, the significant reduction in plasma lactate in this study suggests that the lactate was probably used for oxidation, gluconeogenesis or glycogenesis via the indirect pathway.

For much of the 20th century, lactate was largely considered a dead-end product of glycolysis due to hypoxia, the primary cause of the oxygen debt following exercise, a major cause of muscle fatigue, and a key factor in acidosis-induced tissue damage. Since the 1970s, a lactate revolution has occurred. At present, we are in the midst of a lactate shuttle era; the lactate paradigm has shifted. It now appears that increased lactate production and concentration as a result of anoxia or dysoxia are often the exception rather than the rule (Gladden, 2004). What is now known as the cell-to-cell lactate shuttle was introduced by Brooks (1985) simply as the lactate shuttle. Since its introduction, this hypothesis has been repeatedly supported by studies using a wide variety of experimental approaches. It posits that lactate formation and its subsequent distribution throughout the body is a major mechanism whereby the coordination of intermediary metabolism in different tissues, and cells within those tissues, can be accomplished.

The importance of lactate as a carbohydrate fuel source is underscored by the fact that during moderate intensity exercise, blood lactate flux may exceed glucose flux (Brooks, 2000). Because of its large mass and metabolic capacity, skeletal muscle is probably the major component of the lactate shuttle, not only in

terms of lactate production but also in terms of net lactate uptake and utilization as well. At rest, muscles slowly release lactate

into the blood on a net basis, although at times they may show a small net uptake. During

exercise, particularly short-term, high-intensity exercise, muscles produce lactate rapidly while lactate clearance is slowed. This results in an increased intramuscular lactate concentration and an increased net output of lactate from muscles into the blood. Later, during recovery from short-term exercise, or even during continued, prolonged exercise, there is net lactate uptake from the blood by resting muscles or by other muscles that are exercising at a low to moderate intensity (Richter et al. 1988; Brooks, 2000; Gladden, 2000).

Recently, Miller et al. (2002a,b) investigated subjects exercising at a moderate exercise intensity with lactate infusion to maintain lactate concentration at approximately 4mM. Overall, they (Miller et al. 2002a) found a significant increase in lactate oxidation accompanied by a decrease in glucose oxidation; the interpretation is that lactate competes successfully with glucose as a carbohydrate fuel source, thus sparing blood glucose for use by other tissues. For instance, lactate, which is a substrate for lactate dehydrogenase, has been shown to maintain synaptic formation and sustain synaptic adaptation during hypoglycaemia in the mammal hippocampus (Schurr *et al*., 1988; Sakurai *et al*., 2002). Since the fate of lactate taken up is to be oxidized or converted to glycogen or glucose and there is no increase in liver glycogen, it probably means the reduction in plasma lactate observed in this study was due to its oxidation or conversion to glucose. However, lack of data on plasma pyruvate is a major limitation of this study which makes it difficult to conclude that the reduction in plasma lactate was actually due to its oxidation. Further studies (acute and chronic) involving simultaneous measurement of lactate and pyruvate, glycolytic enzymes, hormones and enzymes of glycogen and lactate metabolism will shed more light into the mechanism of *T. occidentalis*-induced reduction in plasma lactate and liver glycogen and the resultant increase in plasma glucose.

Although, there may be no direct evidence yet, the present study has provided an insight into the possibility of lactate gluconeogenesis/oxidation contributing to the increased blood glucose observed after two weeks of treatment. However, overall contribution of gluconeogenesis to the increase in blood glucose seen following *T. occidentalis*-induced lowering of blood glucose could not be ascertained or quantified in this study since there are many other gluconeogenic substrates apart from lactate such as alanine and glutamine that might have been used for gluconeogenesis. It should be noted that *T. occidentalis* itself is rich in gluconeogenic amino acids such as alanine and glutamine. Thus, the role of amino acid constituents of *T. occidentalis* in the increased blood glucose after two weeks of treatment may also need further investigation in order to be able to quantify the overall contribution of gluconeogenesis to the increased blood glucose after two weeks of treatment.

It is also noteworthy that blood glucose in rats treated for two weeks rose to values above the control in response to *T. occidentalis* -induced lowering of blood glucose. This probably suggests there was overactivation of the counterregulatory response. For instance, insulin hypoglycaemia has been shown to accelerate the release of blood-sugar-raising hormones from the adrenal-pituitary system, setting off a running contest between the latter and excessive insulin action. Under the recurrent stress of hypoglycaemia, the insulin-opposing factors can gain ascendency over insulin action and thus can produce hyperglycaemia despite hyperinsulinism (Somogyi *et al*., 1959). Further investigation on the role of counterregulatory hormones such as glucagon and adrenaline in the response to the blood-glucose-lowering effect of *T. occidentalis* will shed more light into the mechanisms involved in the increased blood glucose levels after two weeks of treatment.

Therefore, the increase in blood glucose to values above the control in rats treated for two weeks could have resulted in the reduction in the glucose-6-phosphatase activity presumably to shut down glycogenolytic and gluconeogenic activities in order to maintain glucose homeostasis. However, since glucose-6-phosphatase catalyzes the final step of both glycogenolysis and gluconeogenesis which are the counterregulatory responses to hypoglycaemia, it will not be unreasonable to suggest that an increase in the activity of the enzyme could have probably occurred much earlier (i.e. between the 8^{th} and 14^{th} day of treatment) at a time when the activity of the enzyme was not determined in this study. The increase could not be observed probably because the estimation of glucose-6-phosphatase activity was done 24 hours after the last day of treatment with *T. occidentalis* when blood glucose had risen to values above that of the control rats; a situation that could lead to a reduction in glucose-6-phosphatase activity and consequently a reduction in glycogenolytic and gluconeogenic activities in order to prevent further rise in blood glucose and maintain glucose homeostasis. Therefore, estimation of the activity of this enzyme between the 8^{th} and 14^{th} day of treatment will shed more light into the activities of the enzyme during *T. occidentalis*-induced lowering of the plasma glucose.

In conclusion, the present study has shown that *T. occidentalis*-induced reduction in plasma glucose after one week of treatment elicited dose- and duration-dependent reduction in liver glycogen and plasma

lactate which resulted in the increased plasma glucose after two weeks of treatment.

REFERENCES

Adejuwon, S.A., Omirinde, J.O., Ebokaiwe, A.P., Aina, O.O.,Adenipekun, A., Farombi, E.O. (2014). Radiation-induced Testicular Injury and Its Amelioration by*Telfairia occidentalis*.*British Journal of Medicine and Medical Research*.4(12): 2431-2445.

Aderibigbe, A., Lawal, B., Oluwagbemi, J. (1999). The antihyperglycaemic effect of *Telfaria occidentalis* in mice. *African Journal of Medicine and Medical Sciences*. 28(3-4): 115-171.

Adisa, W.A., Otamere, H.O., Osifo, C.U., Ediangbe, A.P., Ogarah, P.A., Aigbogun, T.O. (2012). Plasma glucose in *Telfairiaoccidentalis* treated rats. *Advances in Applied ScienceResearch*. 3: 1551-1553.

Aiyelaagbe, I.O.O. and Kintomo, A.A. (2002). Nitrogen Response of Fluted Pumpkin (*Telfairia occidentalis*Hook. F.) Grown Sole or Intercropped with Banana.*Nutrient Cycling in Agroecosystems*. 64: 231-235.

Akang, E.N., Oremosu, A.A., Dosumu, O.O., Noronha, C.C., Okanlawon, A.O.(2010). The effect of fluted pumpkin (*Telfairia occidentalis*) seed oil (FPSO) on testis and semen parameters.*Agriculture and Biology Journal of North America*.1(4): 697-703.

Akindele, A.J. and Ajao, M.Y. (2013). Anxiolytic and sedative properties of hydroethanolicextract of *Telfairia occidentalis* leaves in mice.*RevistaBrasileira de Farmacognosia*. 23(2):301-309.

Akoroda, M.O. (1990). Ethnobotany of *Telfairia occidentalis* (Cucurbitaceae) among Igbos of Nigera. *Economic Botany*. 44(1): 29-39.

Akwaowo, E.U., Ndon, B.A., Etuk, E.U. (2000). Minerals and antinutrients in fluted pumpkin (*Telfairia occidentalis*Hook. f.). *Food Chemistry*.70(2):235-40.

Alada, A.R.A. (2000). The haematological effects of *Telfairiaoccidentalis* diet preparation. *African Journal of BiomedicalResearch*. 3: 185-186.

Brooks, G.A. (1985a). Lactate: glycolytic product and oxidativesubstrate during sustained exercise in mammals- the 'lactate shuttle.' In Comparative Physiology and Biochemistry:Current Topics and Trends, vol. A. In: Gilles, R. (ed.), Respiration-Metabolism-Circulation, Springer, Berlin.208-218.

Brooks, G.A. (2000). Intra- and extra-cellular lactate shuttles.*Medicine and Science in Sports and Exercise*.32:790-799.

Brooks, G.A., Butterfield, G.E., Wolfe, R.R., Groves, B.M., Mazzeo, R.S., Sutton, J.R., Wolfel, E.E., Reeves, J.T.(1991). Decreased reliance on lactate during exercise after acclimatization to 4,300 m. *Journal of Applied Physiology*.71: 333-341.

Dina, O.A., Adedapo, A.A., Oyinloye, O.P., Saba, A.B.(2000). Effect of *Telfairia occidentalis* extract on experimentally induced anaemia in domestic rabbits. *African Journal of BiomedicalResearch*. 3: 181-183.

Dina, O.A., Saba, A.B., Akhiromen, I.O., Adedapo, A.A., Ola, O.E.(2001). The effects of aqueous leaf extract of *Telfairiaoccidentalis* on isolated guinea pig ileum. *African Journal ofBiomedical Research*. 4: 53-54.

Egba, S.I, Ikechukwu, G.C., Njoku, O.U.(2013a). Aqueous extracts of *Telfairia occidentalis*leaf reverses pyrogallol induced leucopenia and stimulates the immune system in Wistar albino rats. *Journal of Chemical and Pharmaceutical Research*. 5(4):149-153.

Egba, S.I., Ikechukwu, G.C., Njoku, O.U.(2013b). Immunomodulatory activity of ethanol extract of *Telfairia occidentalis* in Wistar albino rats. *The Experiment*. 12(3): 791-800.

Ekpenyong, C.E., Akpan, E.E., Udoh, N.S. (2012). Phytochemistry and toxicity studies of *Telfairia occidentalis* aqueous leaves extract on liver biochemical indices in Wistar rats. *American Journal of Medicine and Medical Science*. 2: 103-110.

Emudianughe, T.S. and Aderibigbe, A.O. (2002). The hypoglycaemic effect of *Telfairia occidentalis* aqueous leaf extract in rats. *West African Journal of Pharmacology andDrug Research*. 18: 14-16.

Eseyin, O., Ebong, P., Eyong, E., Awofisayo, O., Agboke, A.(2010). Effect of *Telfairia occidentalis* on oral glucosetolerance in rats. *African Journal of Pharmacy and Pharmacology*. 4: 368-372.

Eseyin, O.A., Oforah, E., Dooka, B.D. (2000). Preliminary study of the hypoglycaemic action of the extract of leaf of *Telfairia occidentalis* in normoglycaemic guinea pigs. *GlobalJournal of Pure and Applied Science*. 6: 639-641.

Eseyin, O.A., Sattar, M.A., Rathore, H.A., Ahmad, A., Afzal, S., Lazhari, M., Ahmad, F., Akhtar, S. (2014). Hypoglycaemic Potential of Polysaccharides of the Leaf Extract of *Telfairia occidentalis*. *Annual Research and Review in Biology*. 4(11): 1813-1826.

Fasuyi, A.O. (2006). Nutritional potentials of some tropical vegetable leaf meals: chemical characterization and functional properties. *African Journal of Biotechnology*.; 5: 49-53.

Gbile, Z.O.(1986). Ethnobotany, Taxonomy and Conservation of Medicinal Plants. In: Sofowora, A. (ed.), The State of Medicinal PlantsResearch in Nigeria, University of Ibadan Press, Ibadan, Nigeria.

Gladden, L. B. (2004). Lactate metabolism: a new paradigmfor the third millennium. The *Journal of Physiology.* 558(1): 5-30.

Gladden, L.B. (2000). Muscle as a consumer of lactate.

Medicine and Science in Sports and Exercise.32:764-771.

Inuwa, H.M., Aimola, I.A., Muhammad, A., Habila, N., Okibe, P., Latayo, M., Ahmed, Z.(2012). Isolation anddetermination of omega-9 fatty acids from *Telfairia occidentalis*. *International Journal of Food Nutrition and Safety*.1(1):9-14.

Iweala, E.E.J. and Obidoa, O. (2009). Some biochemical, haematological and histological responses to a long-term consumption of *Telfairia occidentalis*-supplemented diet in rats. *Pakistan Journal of Nutrition*. 8: 1199-1203.

Iyagba,A.G. and Offor, U.S.**(**2013). Phytoextraction of heavy metals by fluted pumpkin *(Telfaria occidentalis* Hooker *F.)* planted on oil contaminated soil amended with sawdust and siam weed (*Chromolaena odorata*) (L.) R.M. King and Robinson) leaves. International Research Journal of Agricultural Science and Soil Science. 3(12):422-426.

Jungermann, K. and Kietzmann, T. (1996).*Annual Review of Nutrition*. 16: 179-203.

Meyer, C., Dostou, J., Nadkarni, V., Gerich, J. (1998). Effects of physiological hyperinsulinaemia on systemic, renal and hepatic substrate metabolism. *American Journal of Physiology*.275: F915-F921.

Miller, B.F., Fattor, J.A., Jacobs, K.A., Horning, M.A., Navazio, F.,Lindinger, M.I., Brooks, G.A. (2002a). Lactate and glucoseinteractions during rest and exercise in men: effect ofexogenous lactate infusion. The *Journal of Physiology*.544:963-975.

Miller BF, Fattor JA, Jacobs KA, Horning MA, Suh, S.H.,Navazio, F., Brooks, G.A. (2002b). Metabolic and cardiorespiratory responses to 'the lactate clamp'.*American Journal of Physiology*.283:E889-E898.

Nubila, T., Ukaejiofo, O.E., Nubila, I.N., Shu, N.B., Okwuosa, U.C., Ukaejiofo, C.A., Iyare, E.E., Ogbuta, O.I. (2013). Effects of methanolic seed extract of *Telfairia occidentalis* on blood coagulation in Albino rats. *Nigerian Journal of Experimental and Clinical Biosciences*. 1:10-13.

Nwozo, S.O., Adaramoye, O.A., Ajaiyeoba, E.O.(2004). Antidiabetic and hypolipidaemic studies of *Telfairiaoccidentalis* on Alloxan-induced diabetic rats. *NigerianJournal of Natural Product and Medicine*.8: 45-47.

Oboh, G. (2006). Tropical green leafy vegetables prevent garlic-induced hepatotoxicity in the rat. *Journal of Medicinal Food*. 9(4):545-51.

Oboh, G.(2004). Prevention of garlic induced haemolytic anaemic by some tropical green leaves vegetable. *Biomedical Research*. 15: 134-137.

Oboh, G., Nwanna, E.E., Elusiyan, C.A. (2006). Antioxidant and Antimicrobial Properties of *Telfairia occidentalis* (Fluted pumpkin) Leaf Extracts.*Journal of Pharmacology and Toxicology*. 1: 167-175.

Oluwole, F.S., Falode, A.O., Ogundipe, O.O.(2003). Anti-inflammatory effect of some common Nigeria vegetables. *Nigerian Journal of Physiological Sciences*. 18: 35-38.

Oster-Jorgensen, E., Pedersen, S.A., Larsen, M.L. (1990). The influence of induced hyperglycaemia on gastric emptying rate in healthy humans. *Scandinavian Journal of Clinical and Laboratory Investigation*.50: 831-836.

Oyenuga, V.A. (1968). Nigeria's food and feeding-stuffs: their chemistry and nutritive value. Ibadan University Press, Ibadan.1116-1120.

Richter, E.A., Kiens, B., Saltin, B., Christensen, N.J., Savard, G. (1988). Skeletal muscle glucose uptake during dynamicexercise in humans: role of muscle mass. *American Journal of Physiology*.254:E555-E561.

Saalu, L.C., Kpela, T., Benebo, A.S., Oyewopo, A.O., Anifowope, E.O., Oguntola, J.A.(2010). The Dose-Dependent Testiculoprotective and Testiculotoxic Potentials of *Telfairia occidentalis* Hook. f. Leaves Extract in Rat. *International Journal of Applied Research in Natural Products*. 3(3): 27-38.

Sakurai, T., Yang, B., Takata, T., Yokono, K.(2002). Synaptic adaptation to repeated hypoglycaemia depends on theutilization of monocarboxylates in guinea pig hippocampalslices. *Diabetes*. 51: 430-438.

Salman, T.M., Olayaki, L.A., Oyeyemi, W.A.(2008). Aqueous extract of *Telfairia occidentalis* leaves reduces blood sugar and increases haematological and reproductive indices in male rats. *African Journal of Biotechnology*.7:2299-2303.

Salman, T.M., Isiaka,A.A., Sikiru, A.B., Olusegun,A.A., Opeyemi, K.O., Olanrewaju, A.A. (2013).Blood glucose-lowering effect of *Telfairia occidentalis*: A preliminary study on the underlying mechanism and responses. *Biokemistri*. 25(3): 133-139.

Schurr, A., Dong, W.Q., Reid, K.H., West, C.A., Rigor, B.M. (1988). Lactic acidosis and recovery of neuronal functionfollowing cerebral hypoxia in vitro. *Brain Research*. 438: 311-314.

Schurr, A., West, C.A., Rigor, B.M.(1988). Lactate-supported synaptic function in the rat hippocampal slice preparation. *Science*. 240: 1326-1328.

Seifter, S., Dayton, S., Novic, B., Muntwryler, E. (1950). Isolation and Purification of Glycogen: The estimation of glycogen with anthrone reagent. *Archives of Biochemistry*. 25: 191-199.

Somogyi, M. (1959). Exacerbation of diabetes by excess insulin action. *The American Journal of Medicine*. 26(2): 169-191.

Stanley, W.C., Gertz, E.W., Wisneski, J.A., Morris, D.L., Neese, R.A., Brooks, G.A.(1986). Lactate extraction during net lactate release in legs of humans during exercise. *Journal of Applied Physiology*. 60(4): 1116-1120.

Tindall, H.D. (1968). Commercial vegetable growing. 1st Edn., Oxford University Press, Oxford, UK.

Trinder, P.(1969). Determination of glucose in blood using glucose oxidase with analternative oxygen acceptor. *Annals of Clinical Biochemistry*. 6: 158-161.

Vandebroeck, A., Bollen, M., De Wulf, H., Stalmans, W. (1985).*Europian Journal of Biochemistry*.153: 621-628.