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EFFECTS OF ETHANOLIC SEED EXTRACT OF *TETRACARPIDIUM CONOPHORUM* ON BODY WEIGHT, FOOD-INTAKE, WATER-INTAKE AND BLOOD GLUCOSE LEVEL OF FEMALE WISTAR RATS

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ABSTRACT

Objective: This study was carried out to investigate the effects of ethanolic seed extract of *Tetracarpidum conophorum* on blood glucose level, food-intake, water-intake and body weight of normal female wistar rats. **Methodology:** Thirty-two female wistar rats weighing between 140- 200g were purchased from the Animal house of Human Physiology Department Nnamdi Azikiwe University Nnewi. They were acclimatized for two weeks after which they were divided into 4 groups of 8 rats each and housed in cages. The groups were designated as groups A, B, C and D. Group I served as control group that received only distilled water while the other groups B-D served as test groups that were administered ethanolic seed extract of *T. conophorum* orally throughout a period of 14 days. The extract was administered using oral cannula at doses of 200mg/kg, 400mg/kg and 600mg/kg to groups B, C and D respectively. **Results:** There was a significant ($P<0.05$) decrease in body weight in group D when compared with the control. There was insignificant ($P>0.05$) decrease in food intake in all test groups when compared with the control. There was insignificant ($P>0.05$) increase in water intake in all test groups when compared with the control. There was a significant ($P<0.05$) decrease in blood glucose level in group D when compared with the control. **Conclusion:** This study has revealed that *Tetracarpidium conophorum* may have control effect on hyperglycemia and obesity hence its consumption by diabetic and overweight patient is highly recommended. However, this should be done with caution to avoid toxicity.

KEYWORDS: *Tetracarpidium conophorum*, Body weight, Food-intake, water-intake, Blood glucose, Toxicity.

1. INTRODUCTION

The important goal of researchers in the world is to discover new drugs for the development of new products with high therapeutic efficacy and low toxicity profile.[1] In order to accomplish this, serious attention has been focused on medicinal plants in recent years. Of late, traditional medicine has been used in complementing conventional medicine in the healthcare system in Nigeria.[2] Synthetic drug consumers in developed countries are becoming disappointed with modern health-care and are therefore seeking for alternatives.[3]

Nuts have been found to exhibit both hypoglycemic and anti-obesity properties.[4] African walnut and other nutrient-rich nuts have been found to contribute to satiety, which can help control appetite and caloric intake.[5,6] *Tetracarpidium conophorum* also called *Plukenitia conophorum* (Nigerian walnut) and commonly called the African walnut is a perennial climbing shrub found in the moist forest zones of sub-sahara Africa.[7,8] It is commonly called Ukpa in Igbo, Asala or Awusa in Yoruba, Gawudi bairi in Hausa and Okhue or Okwe in Edo.[9] This plant is cultivated principally for the nuts which are cooked and consumed as snack.[10] The fruits are edible, the plant is medicinal and used for various purposes, including masticatory, thrush, antihelminth, syphilis, dysentery and as an antidote to snake bites.[10] Walnuts are considered to be an herb in Traditional Chinese medicine. They are said to tonify kidneys, strengthen the back and knees, and moisten the intestines. Its anticholesterolic,[11] antihyperglycemic,[12] Antioxidant,[13] and anti-infertility[10] activities have been

reported. A study carried out by Nwaoguikpe *et al.*,[14] reported that dried powdered seed of *T. conophorum* is composed of; Flavonoids (2.70±0.1%), Alkaloids (0.35±0.01%), Saponins (5.03±0.01%), Tannins (0.45±0.01%) and Phenols (1.51±0.01%) after a phytochemical analysis study.

The prevalence of overweight individuals in Nigeria has been recorded to be 20.3% - 35.1% while the prevalence of obesity ranged from 8.1- 22.2% with 95% of the individuals involved being women.[15] The current prevalence of diabetes in Nigeria is not known but guestimates may likely be in the region of 8 – 10%.[16] There is an unintentional continuous rise in the total body weight of individuals particularly females and this has been associated with uncontrolled feeding.[17] This has led to the sort for possible food supplements that can help reduce blood glucose, control body weight, water in-take, appetite and food in-take.

Therefore, this research is focused on the effects of ethanolic seed extract of African walnut (*Tetracarpidum conophorum*) on blood glucose level, food intake, water intake and body weight of female wistar rat as there is paucity of data regarding these.

2. MATERIALS AND METHODS

2.1. Collection and Identification of Plant Material

Fresh matured seeds of *Tetracarpidium conophorum* were purchased from a market in Nnewi, Anambra State, Nigeria. The seeds were identified by the herbarium curator in the Department of Botany, Nnamdi Azikiwe University Awka and a voucher specimen was deposited at the herbarium with a voucher number (N.A.U-145A). It was classified under the family *Euphorbiaceae.*

2.2 Extract Preparation

The seeds were washed then dehulled and placed under running tap water in a basin to remove dirt, cut into pieces and were air dried under ambient temperature for 72hours after which they were oven dried at a considerable temperature. Upon complete dryness, the dried seed were milled into coarse powder using Local grinder. 50g of the dried seed was macerated in 95% ethanol for 48hours. It was then filtered using a clean handkerchief and further filtration using Whatman No 1 filter paper. The filtrate was concentrated using a rotatory evaporator and was further dried using a laboratory oven at 45^{o}C into a gel-like form. The extract was preserved in a refrigerator at 4^{o}C for further usage.

2.3 Acute Toxicity Study

The median lethal dose (LD_{50}) of Ethanolic extraction of Walnut seed in rats was carried out in the department of Human Physiology, Faculty of Basic Medical Science, Nnamdi Azikiwe University, Okofia Campus. This was determined using the method of Dietrich Lorke.[18] In this study, a total of 13 rats were used. They received the extract via oral route and it was carried out in two phases.

PHASE I

Nine (9) rats were used and they were grouped into three groups of 3 rats each.
Group 1 received 10mg/kg
Group 2 received 100mg/kg
Group 3 received 1000mg/kg

The animals were observed over a period of 24hrs for mortality. From the result of phase I, the second phase was carried out. In this phase, three rats were used and they were grouped into three groups of one animal each per group.

PHASE II

Group 1 received 1600mg/kg
Group 2 received 2900mg/kg
Group 3 received 5000mg/kg

The animals were monitored over a period of another 48hrs for mortality. The median lethal dose was then calculated thus:
$LD_{50} = \sqrt{a \times b}$
A= maximum dose with 0% mortality
B= minimum dosed with 100% mortality

2.4 Experimental Research Design and Laboratory Testing

2.5 Animal Housing and Grouping

Thirty-two inbreed female rats weighing 140-200g were purchased from the Animal house, Department of Human Physiology, College of Health Science and Technology, Nnamdi Azikiwe University, Nnewi Campus. The rats were fed with the commercially available rat feed. The rats were fed with standard rat-pelleted diet (Vital feed Growers mesh) manufactured by Grand Cereals Ltd (a subsidiary of UAC Nigeria PLC, Plateau state). The Rats had access to feed and tap water *ad-libitum.* The animals were randomly assigned into four (4) groups of eight (8) rats per cage via randomized blinding technique,[18] The groups were designated as groups I-IV. Group I served as the control group and received distilled water only while Groups II, Group III and Group IV served as the test groups and received 200, 400 and 600mg/kg body weight of the extract respectively. The extract administration was done in morning hours through oral gavage and lasted for 14 days. All procedures used in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the Care and Use of animals.[20] The Research and Ethical Committee of the Faculty of Basic Medical Sciences of Nnamdi Azikiwe University approved the study.

2.6 Blood glucose estimation

At the end of extract administration, the fasting blood glucose level was determined using glucometer kit (One-touch ultra) after over-night fasting for 7-11 hours. The tail was punctured and blood from the tail was dropped on the strip which has been inserted into the glucometer

to obtain blood glucose concentration in mg/dL for each rat in various groups.

2.7 Food and Water Consumption

The amount of food and water consumed were monitored daily as known amount of food and water was given to animals in each cage. After 24 hours, the remaining food and water was taken from the cage and measured. To find out the amount of food and water consumed, the leftover was deducted from the total amount. Food intake was recorded as grams of food consumed/day while water intake was recorded as milliliters of water consumed/day.

2.8 Body Weight

The body weight of each rat in each group was monitored daily as an index of the physical status of the animals over the period of study and the difference between initial and final body weight was regarded as the weight loss.

2.9 Statistical Analysis

All data were tabulated and statistically analyzed using SPSS (version 21) software package. Results were expressed as Mean ± SEM. One-way analysis of variance (ANOVA) followed by Bonferroni's Post Hoc test used for data comparison. Results were regarded significant at $P < 0.05$.

3.0 RESULTS

Table 1: Result of acute oral toxicity study of *Tetracarpidium conophorum*.

Phase	Groups	Dose	Mortality	Observation
I	1	10	0/3	The animals were calm
	2	100	0/3	The animals were calm
	3	1000	0/3	The animals were calm
II	1	1600	0/1	The animal was calm
	2	2900	0/1	The animal was calm
	3	5000	1/1	The animal was weak, twisted its neck, tail and died within 48hours

$LD_{50} = \sqrt{a \times b}$
A= maximum dose with 0% mortality = 2900 mg/kg
B= minimum dosed with 100% mortality= 5000 mg/kg
$LD_{50} = \sqrt{2900 \times 5000} = 14500000$
$= \sqrt{14500000} = 3807.88$ mg/kg
The LD_{50} of ethanolic seed extract of walnut (*Tetracarpidium conophorum*) in rats is therefore 3807.88 mg/kg

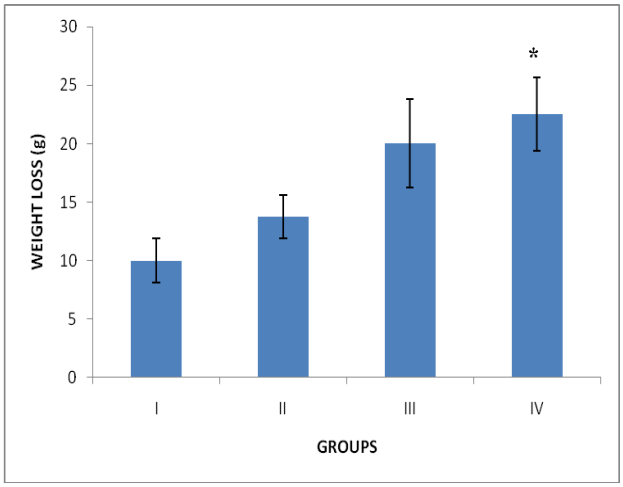

Figure 1: Effect of Ethanolic Seed Extract of *Tetracarpidium conophorum* on Body Weight Loss.

No significant increase ($P > 0.05$) in weight loss was observed in test groups II (13.75 ± 1.83 g) and III (20.0 ± 3.78 g) when compared with the control group I (10.0 ± 1.89 g). However, a significant increase ($P < 0.05$) in weight loss was observed in test group IV (22.5 ± 3.13 g) when compared with the control group I (10.0 ± 1.89 g).

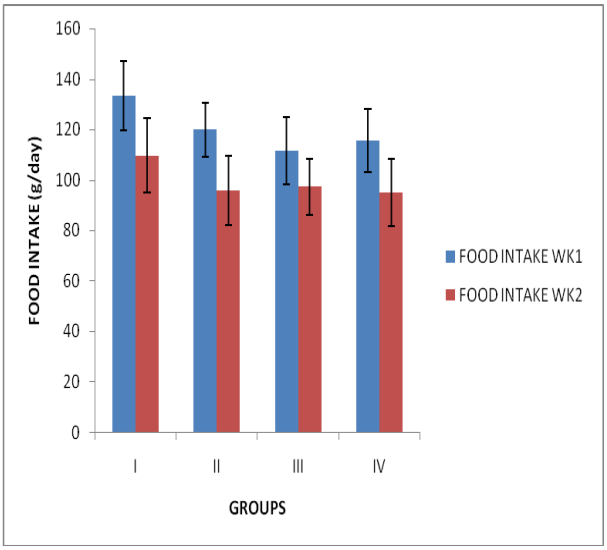

Figure 2: Effect of Ethanolic Seed Extract of *Tetracarpidium conophorum* on Food Intake.

No significant difference ($P > 0.05$) in food intake was observed in test groups II (119.98 ±10.6 g/day), III (111.61 ±13.17 g/day) and IV (115.57 ±12.64 g/day) when compared with the control group I (133.29 ± 13.8 g/day) in week one (1). Also, there was no significant difference ($P > 0.05$) in food intake observed in test groups II (95.89 ± 13.86 g/day), III (97.37 ± 11.07 g/day) and IV (95.06 ± 13.28 g/day) when compared with the control group I (109.69 ± 14.82 g/day) in week two (2).

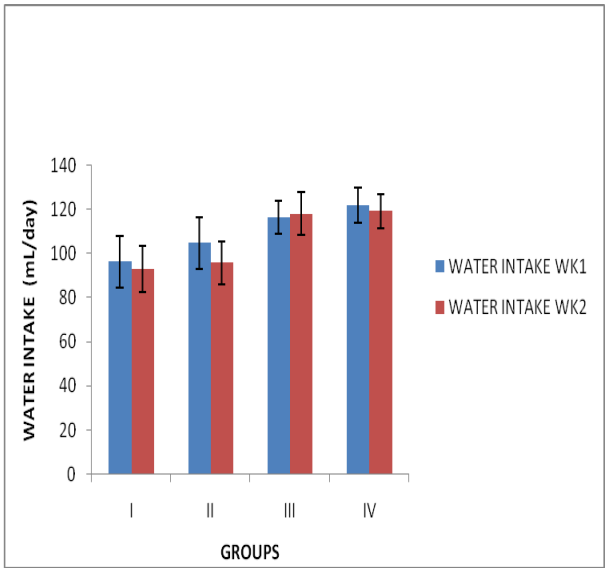

Figure 3: Effect of Ethanolic Seed Extract of *Tetracarpidium conophorum* on Water Intake.

No significant difference (P > 0.05) in water intake was observed in test groups II (104.85 ± 11.66 mL/day), III (116.43 ± 7.43 mL/day) and IV (122.0 ± 8.0 mL/day) when compared with the control group I (96.29 ± 11.93 mL/day) in week one (1). Also, there was no significant difference (P > 0.05) in water intake observed in test groups II (95.85 ± 9.81 mL/day), III (118.14 ± 9.86 mL/day) and IV (119.29 ± 7.61 mL/day) when compared with the control group I (93.14 ± 10.63 mL/day) in week two (2).

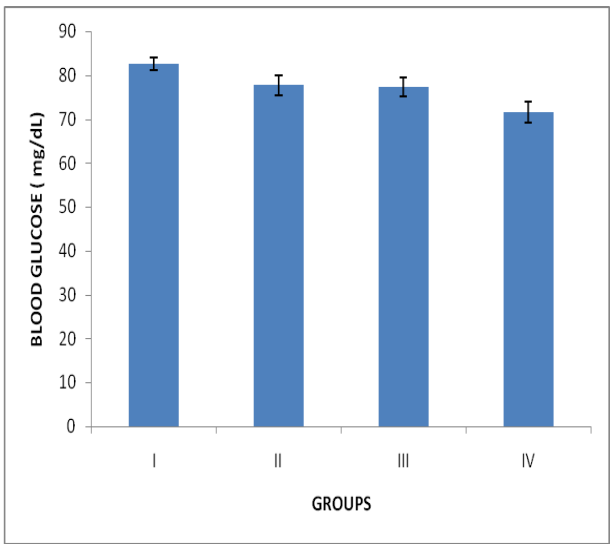

Figure 4: Effect of Ethanolic Seed Extract of *Tetracarpidium conophorum* on Blood glucose level.

No significant decrease (P > 0.05) in blood glucose level was observed in test groups II (77.75 ± 2.34 mg/dL) and III (77.25 ± 2.14 mg/dL) when compared with the control group I (82.63 ± 1.40 mg/dL). However, a significant decrease (P < 0.05) in blood glucose level was observed in test group IV (71.63 ± 2.34 mg/dL) when compared with the control group I (82.63 ± 1.40 mg/dL).

4.0 DISCUSSION

Natural products of plant origin have played an important role globally in ethno-medical studies showing their efficacy in anti-hyperglycemic and anti-obesity activities. However, *Tetracarpidium conophorum* is not an exception to such plants. The result of the present study revealed that the dose that can cause fifty percent (50%) mortality (LD_{50}) in rats is 3807.88 mg/kg. This indicates that the consumption of the extract should be done with caution since it has a toxic dose. It also revealed a significant decrease in body weight in group IV which received 400mg/kg. No significant difference in food and water intake in all the test groups when compared to control. The decrease in the body weight may result from the phytochemical composition of *Tetracarpidium conophorum* such as saponin which has an anti-obesity activity.[21] It may also be linked with the regulation of body weight by the central nervous system (CNS), which receives numerous neural impulses from the gastrointestinal mucosa and fat tissue to control food intake and energy expenditure.[22,23] The involved neurons modulate the hypothalamic–pituitary–adrenal axis. The gut peptides signaling to the hypothalamus mediate the appetite-stimulating effect through the activation of neurons containing neuropeptide Y (NPY) and agouti-related peptide (AgRP) or, on the contrary, an appetite-inhibitory effect via other neurons. NPY constitutes an important regulator: it increases food intake and reduces dietary fat oxidation.[24,25] The role of many peptides, such as NPY, AgRP, cholecystokinin (CCK), ghrelin and glucagon-like peptide 1 (GLP-1), has been taken into account. Leptin and insulin are also involved in hypothalamic appetite regulation, and they constitute potential therapeutic targets to treat obesity.[24] Leptin is an adipocyte-derived protein that acts as a regulator of energy homeostasis. This regulator acts centrally, inhibiting the synthesis of NPY.[26]

There was a significant decrease at (P<0.05) in blood glucose levels of the rats in group IV which were given 600 mg of the extract (high dose) when compared with control. The decrease in blood glucose observed is in line with the report of,[12] that investigated the antihyperglycemic effect of *T. conophorum* nuts. The mechanism of action of antihyperglycemic effect of the extract is not fully understood but may be attributed to the presence of some active phytochemicals in the plant such as (alkaloid, tannin and flavonoids) which play a role in glucose metabolism employing different mechanisms in maintaining blood glucose levels within a narrow range, such as increasing glucose uptake and also increasing insulin secretion[27]

Another possible reason for the non significant decrease in food intake and non significant increase in water intake as well as reduced body weight could be due to the ability of African walnut and other nutrient-rich nuts

to contribute to satiety, which can help control appetite and caloric intake.[5,6] This action may result to increased contact of the carbohydrate with receptors in small intestine and consequent enhanced release of insulin leading to decrease in blood glucose.

5.0 CONCLUSION

Conclusively, from this study it can be said that *Tetracarpidium conophorum* may have control effect on hyperglycemia and obesity hence its consumption by diabetic and overweight patient is highly recommended. However, this should be done with caution to avoid toxicity.

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Conflict of interest: None declared.
Ethical Approval: Approved by Institutional ethical approval.

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