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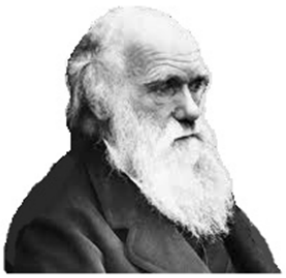

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Effect of *Tetracarpidium conophorum* nut extracts on body and organ weights of monosodium glutamate obesity-induced in albino wistar rats

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ABSTRACT

This study aimed to assess the effect of *Tetracarpidium conophorum* nut extracts on body weight and organ/tissue weight in monosodium glutamate obesity- induced in Wistar rats. With the global obesity epidemic lacking an effective cure, this investigation holds significance. Twenty-five Wistar rats (15 males and 10 females) were utilized, housed in well-ventilated cages at a 3:2 female-to-male ratio Monosodium glutamate was induced in pups using intraperitoneal monosodium glutamate injections from postnatal days 2 through 10. Normal controls received saline, and all experimental animals were raised on standard rat chow until reaching a weight of ≥150g. The study encompassed five groups, each consisting of 7 animals. Groups IV, V, and VI were treated with *Tetracarpidium conophorum* nut extracts: ethanol whole extract (EWE), ethyl acetate extract (EAE), and ethanol residue (ER), respectively. Group III, the standard control was given 5.14g/kg of Orlistat reconstituted in normal saline. Experimental animals of groups I and II served as the normal and obese controls, respectively. After a six-week treatment period, the animals were euthanized for organ harvesting. Results indicated varying weight changes among treatment groups compared to controls. The EWE-treated group displayed a notable decrease in weight (-1.40 ± 8.42) compared to the obese control (8.29 ± 8.29). Similarly, EAE-treated animals exhibited weight reduction (-6.90 ± 12.29), as did the ER-treated group (-0.10 ± 12.22). Evidently, EWE treatment induced the most substantial weight loss. *Tetracarpidium*

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conophorum nut extracts demonstrated potential in alleviating obesity-related weight gain in the rat model.

***Keywords*:** Tetracarpidium conophorum, Obesity, Nutt extract, Monosodium glutamatet, Orlistat

1. INTRODUCTION

As the world population increases, so is the prevalence of the Obesity epidemic. Obesity is characterized by excess body fat or adipose tissue distribution in the body and It is most often defined by the body mass index (BMI). Body mass index (BMI), calculated as weight in kilograms divided by height in meters squared (kg/m2) is easily obtained and widely used but is only an indirect measure of fatness because it cannot differentiate between lean body mass and adipose tissue [1]. Once a BMI estimate is obtained for an individual, it can be sorted into categories and the four commonly used categories include: “underweight,” “normal weight,” “overweight,” and “Obese,” although these categories differ between race and ethnicity groups [2]. Among race and ethnicity groups, Obesity is most prevalent in non-Hispanic Black adults, than Mexican-Americans, compared to non-Hispanic White Americans and the Asian adults generally have the lowest rates of Obesity [2]. The current cut-off points of each category of the BMI as determined by the World Health Organization (WHO) is: Severely Underweight: < 16 kg/m^2, Underweight: 16.0 - 18.4 kg/m^2, Normal weight: 18.5 - 24.9 kg/m^2, Overweight: 25.0 - 29.9 kg/m^2, Moderately Obese: 30.0 - 34.9 kg/m^2, Severely Obese: 35.0 - 39.9 kg/m^2 and Morbidly Obese: greater than or equal to 40.0 kg/m^2 [3]. Obesity is associated with many adverse health effects, including but not limited to increased risks of premature mortality, diabetes, heart disease, cancer, stroke, respiratory disease, lipid abnormalities, and pregnancy complications [4]. Hence the urgency to combat this menace.

Although a healthy lifestyle is the foundation of Obesity treatment, lifestyle modification alone produces only modest weight loss that is difficult to sustain therefore, adjuvant pharmacotherapy combined with lifestyle treatment could be recommended in patients with a body mass index (BMI) of 30 kg/m^2 or greater and in those with a BMI of 27 kg/m^2 or greater with adiposity-related complications [5]. Orlistat, the anti-obesity drug was used in this study is a medication used in the management of Obesity and it acts by reversibly inhibiting gastric and pancreatic lipases which prevents the hydrolysis of triglycerides, and thus free fatty acids are not absorbed [6]. A study reported that Orlistat also causes a significant reduction in BMI, waist circumference, total cholesterol, and LDL levels [7].

The tropical African walnut, known as *Tetracarpidium conophorum* or *Plukenetia conophora* belongs to the family Euphorbiaceae and is popularly known as African walnut, black walnut and Nigerian walnut [8]. The plants have swollen, fleshy, sparsely branched stems and are sometimes candle broid in appearance and the fruit is a capsule 6 cm – 10 cm long by 3 cm – 11cm wide containing subglobular seeds [8]. Adewale et al [9] reported that certain chemical compounds such as saponins, tannins, oxalates, phytates, trypsin inhibitors and cyanogenic glycosides which are known as secondary metabolites can be found in the phytogenic plant. Evaluation of its antinutritional factors (mg/100g) revealed tannins (0.89), oxalate (1.28), phytic acid (3.105), trypsin inhibitors (1.84), saponin (985.0) and alkaloid (40.91) as being inherent to the edible nut [10]. Ethnomedicinally, decoctions of the nut has also been reported to contain phenolic antioxidants with various biological activities including

antipyretic, anti-inflammation, anti-diabetic, anti-cancer, anti-malaria, anti-diarrhoeal, and antihypertensive effects [11]. A recent study, showed that African walnuts (*Tetracarpidium conophorum*) extracts can prevent hepatic lipid accumulation through reciprocal actions on HMG-CoA reductase and paraoxonase in Obesity [12]. Further study highlighted that African Walnuts (*Tetracarpidium conophorum*) extracts, may attenuate ectopic fat accumulation and its associated pathogenesis [13]. Of the studies done on the effect of African Walnut (*Tetracarpidium conophorum*) extracts on Obesity, to our knowledge, there are no reports on the effects of *Tetracarpidium conophorum* nut extracts on body weights, tissue and organ weights of Albino wistar rats. Therfore this study investigated the effect of Ethanol Whole Extracts (EWE), Ethyl Acetate Extracts (EAE), and Ethanol Residue (ER) fractions of *Tetracarpidium conophorum* nuts on body, organ and tissue weights of MSG - induced Obese rats.The parameters that were measured include; weekly body weight and abominal circumference, and relative (kidney, heart, brain, liver and white adipose tissue) weights.

2. MATERIALS AND METHODS

2. 1. Study Location

This study was carried out from the animal house of Biochemistry department in the University of Calabar, Cross River State, Nigeria.

2. 2. Sample collection

Nigeria and other African nations are the primary home of the African walnut which was used in this study. Also known as the "king of nuts " , African Walnut grows in all states that produce cocoa, primarily in the southern region of Nigeria, and is known as Asala in the western portion of the nation and Ukpa among the Igbos. In this Study, the African Walnut was purchased from Okuni in Ikom Local Government of Cross River State, Nigeria and was conveyed to Calabar in black polythene bags. The shells were removed, and the seeds shade dried, the *T. conophorum* seeds were pulverized and weighed into the sample container [14].

The pulverized sample was weighed and then extracted in 80% ethanol, and finally ethyl acetate to obtained two fractions (the ethanol whole extract and the ethyl acetate extract) and the ethanol residue. The fractions were collected and concentrated in rotary evaporator at 50 °C to 10%, and then allowed in an oven (55 °C) for complete evaporation to yield the final fractions for each solvent.

2. 3. Materials Used

Measuring tape (0.1 cm), weighing balance, feeding tube (for administration of extracts), stainless steel plates, wooden cages, plastic water bottles, knife, mortar and pestle, blender, syringes (1 ml, 5 ml, 8 ml) and needles, handgloves, tissue paper and liquid soap, trash can, Parker, broom, face masks, Vital grower feed, wood dust, EDTA vial, plain Vial, dissecting sets, and cages.

2. 4. Substances Administered

Monosodium glutamate, Orlistat, extracts of *Tetracarpidium conophorum*, water.

2. 5. Experimental animals

Twenty-five Wistar rats, 15 females and 10 males were obtained from the animal house of the Department of Biochemistry, University of Calabar, Cross River State, Nigeria. The animals were divided and co-habited in five well-ventilated cages in the ratio of 3:2 females to males respectively and fed with normal rat chow after three days of acclimatization. The pregnant animals were monitored closely for isolation when close to parturition. Pups produced by the co-habited animals were given monosodium glutamate (MSG), and each animal was injected a single dose of 4 mg/kg body weight of MSG reconstituted in normal saline, via intraperitoneal route once a day on postnatal days 2, 4, 6, 8 and 10 to produce the obese models.

The controls were similarly treated with normal saline. The pups were all together weaned immediately they started picking up food and that lasted for three weeks of postnatal life, while the animals were kept under close observation to ensure immediate isolation or separation once their secondary sex organ was beginning to get viable to avoid mating and pregnancy, the neonates were fed with rat chow and were allowed to grow weighing up to 140 and ≥150g.l

2. 6. Statistical Analysis

The results were expressed as means ± SD and tests of statistical significance were carried out using one-way analysis of variance (ANOVA), and post hoc test (LSD) using the Microsoft excel program. The acceptable level of significance was $P<0.05$ using a 2-tailed distribution and differences between and among the respective groups compared were considered significant at 95% probability level of confidence (i.e., $p< 0.05$).

2. 7. Experimental Design

The obesity-induced animals were randomly divided into five (5) experimental group according to their weights with each containing at least 7 animals per group. While the non-induced animals, formed the normal control group as shown in Table 1.

Table 1. Showing the different treatment groups in present study

S/N	GROUPS	NO OF ANIMALS	TREATMENT
1	NC	7	Normal saline
2	OBC	7	Normal saline
3	OBSC	7	Standard anti - Obesity drug (orlistat) (5.14 mg/kg body weight)
4	OB-EWE	7	Whole extract of *T. conophorum* (2 g/kg body weight).
5	OB-EAE	7	Ethyl acetate extract of *T. conophorum* (2 g/kg body weight).
6	OB-ER	7	Ethanol extract of *T. conophorum* (2 g/kg b.w.)

Key

NC: Normal control, OBC: Obese control, OBSC: Obese standard control, OB-EWE: Obese ethanol whole extract, OB-EAE: Obese- ethyl acetate extract, OB-ER: Obese ethanol residue.

The EWE and EAE came out in oily form and were administered directly in calculated doses of 2 g/kg body weight once a day to the animals in group IV and V respectively by oral gavages for 6 weeks. While, in group VI, the obese animals were treated with 2g/kg body weight of the ethanol residue reconstituted in normal saline. Also, the animals in group I and II were treated with equimolar volume of normal saline and 5.14 mg/kg body weight of orlistat (standard anti-obesity drug) respectively. The weight (g), nasal-anal length (cm) and waist circumference (cm) of the animals were taken fourth-nightly and then random blood sugar was taken every two weeks. After six weeks of treatment and overnight fasting of the animals, they were all euthanized using 2 µL of ketamine per gram body weight of animals. All the experimental animals were also measured before the sacrifice and the weight differences of the animals for the period 6 weeks were calculated by subtracting the baseline weight from the weight of the animal in each successive week while the relative organ/tissue weight in percentage were calculated as ratio of organ/tissue weight to final weight of animal before sacrifice multiplied by 100 (%) [28]. Thus,

$$\text{Weight gain (g)} = \text{New weight} - \text{baseline weight}$$

$$\text{Relative organ/tissue weight} = \frac{\text{Organ/tissue weight (g)}}{\text{Final weight of animal}} \times 100\%$$

The rats were “knocked-out” using ketamine. Immediately after cardiac puncture in each experimental animal, the heart, liver, kidneys were harvested with forceps and blotted with filter paper and weighed with electronic weighing balance. Also, the brain and the white adipose tissue (WAT) comprising of epididymal and perineal fats in male and female respectively were harvested and weighed.

3. RESULTS AND DISCUSSION

3. 1. Effects of treatment on body Weight of experimental animals

The effects of the treatment on the weight of the experimental animals over a period of 6 weeks is shown in Figure 1. The results generally show variation in weight increment between the treatments groups relative to the control groups across the treatment period. The data obtained, it showed that, from the 3rd week the rate of weight increment significantly decreased between the test groups and the controls. In the 3rd week EWE (-1.40 ± 8.42), EAE (6.90 ± 12.29) and ER (0.10 ± 12.22) shows a decreased in weight gain relative to ObSC (10.21 ± 4.78) and ObC (8.29 ± 8.29) though the ObSC did not show any decrease in weight relative to ObC. The trend persisted into the 4th and 5th week where EWE experimental animals, EAE experimental animals and ER experimental animals showed a mark decreased in the rate of weight gained (4.43 ± 8.54, 12.60 ± 8.44), (5.05 ± 12.38, 10.60 ± 19.2) and (2.12 ± 11.03, 13.75 ± 3.72) respectively relative to ObSC (16.04 ± 9.05, 29.99 ± 13.86). The increase in weight gained was significant ($p < 0.05$) with EWE and ER in week 4 and 5 relative to the ObSC treated with Orlistat. While experimental animals in groups treated with EAE only showed significant decrease in weight gain in week 5 relative to ObSC. On the 6th week, the decrease in weight gain between the treated groups and the ObSC group was still recorded though it was insignificant.

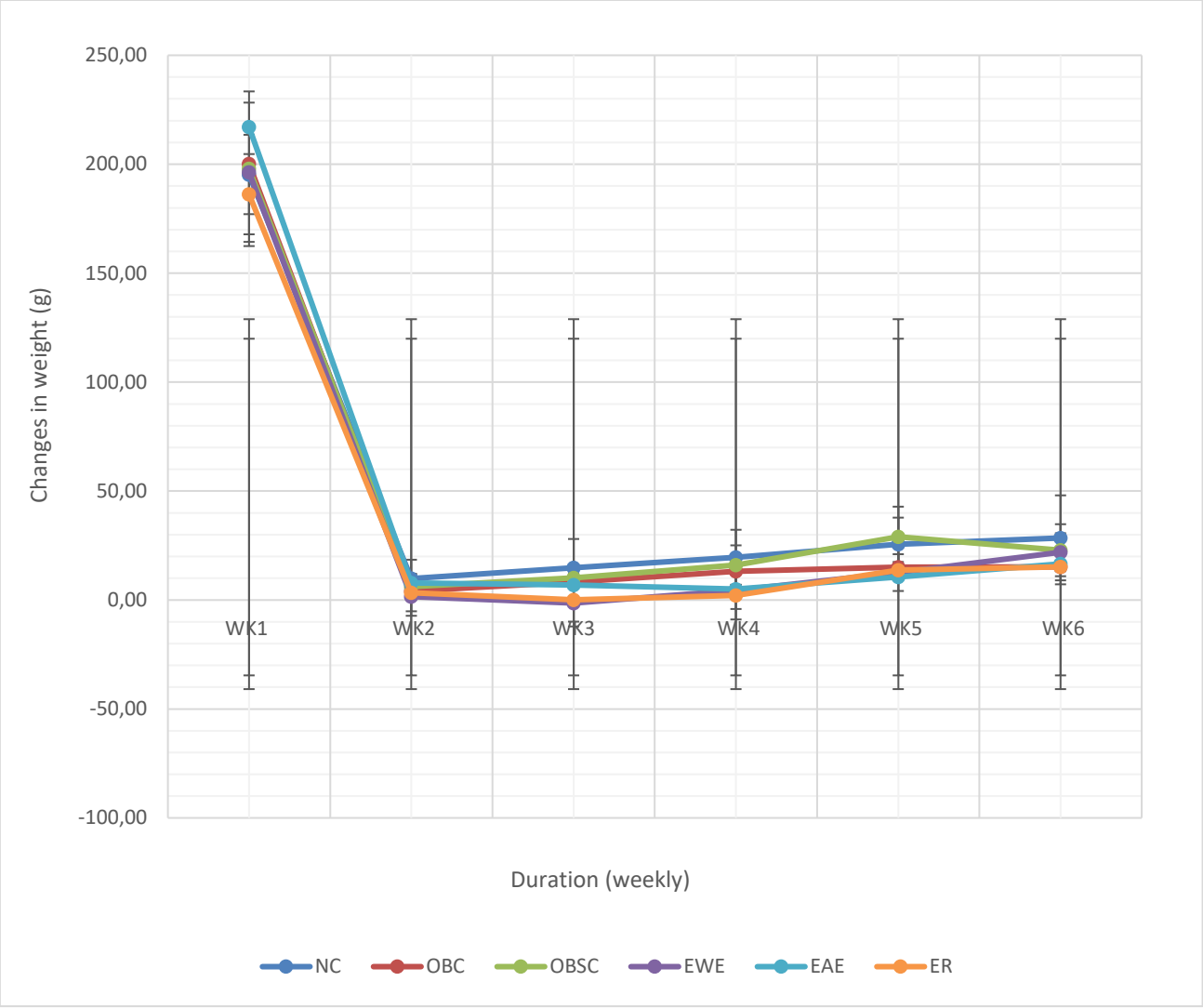

Figure 1. Curve showing weight changes of experimental animals Values are expressed as mean ± SD, n = 7 Significant ($p < 0.05$)

3. 2. Effects of the Treatment on the organ/tissue weight of the experimental animals

The treatment did not show any significant effect on the liver as shown in Figure 2 below as there was no significant ($p > 0.05$) decrease or increase in the liver weight of the test groups relative to the controls. Meanwhile, the treatment affected the relative heart weight of the EWE treated group. There was a significant ($p < 0.05$) increased in the relative heart weight of the group treated with EWE relative to the controls. While EAE and ER treated groups showed insignificant increase and decrease relative to ObSC and ObC respectively. While the ObC decrease insignificantly relative to NC, ObSC increases insignificantly ($p > 0.05$) relative to the ObC group. The EWE and EAE treated groups affected the relative weight of the kidney relative to the control. While EWE treated group shows a significant ($p < 0.05$) increase in relative weight of the kidney (0.75 ± 0.10) relative to the ObSC, EAE treated group shows ($p <$

0.05) decrease relative weight (0.35 ± .24) relative to the ObC group. The increase in kidney relative weight of the ER treated group was insignificant ($p > 0.05$) relative to the controls. The treatment regiments also elicited changes on the relative weight of the brain of the experimental animals. The change was more significant ($p < 0.05$) with group treated with EWE and ER fractions with the former showing significant increase of (2.09 ± 0.72) and the latter insignificantly increases to (0.74 ± 0.12) relative to the brain weight of ObSC (0.67±0.16). Whereas, the brain weight increases insignificantly in ObC relative to NC group, the EAE treated group similarly decreases relative to ObSC. The treatment increased white adipose tissues deposition. The results show that, NC, ObC, ObSC, EWE, EAE and ER were 0.69 ± 0.86, 1.73 ± 0.31, 2.52 ± 1.08, 2.09 ± 0.72, 2.27 ± 1.16 and 1.78 ± 1.30 respectively. While there was no significant ($p > 0.05$) difference between ObSC group relative to ObC which shows significant ($p < 0.05$) difference relative to NC, and the experimental groups shows significant ($p<0.05$) increased in relative weight of WAT when compared with the NC, but there was no significant difference between test groups (EWE, EAE and ER) relative to the control groups.

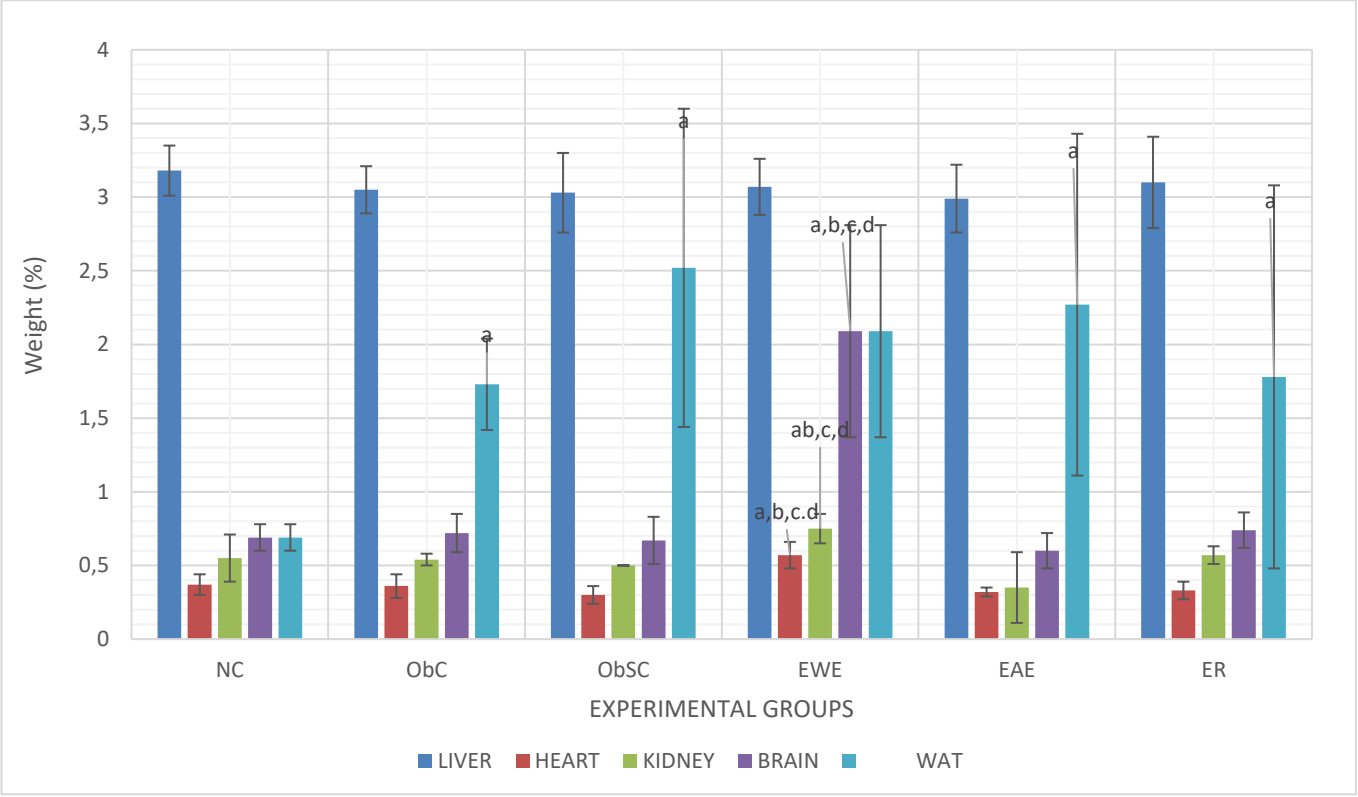

Figure 2. Comparison of relative organ/tissue weight of the different experimental groups. Values are expressed as mean ± SD, n = 7. a = $p < 0.05$ vs NC, b = $p < 0.05$ vs ObC, c = $P < 0.05$ vs ObSC, d = $p < 0.05$ vs another test group.

Data obtained in the course of this study points that Tetracarpidium conophorum nut extracts reduced weight gain in the body of the experimental groups relative to the standard drug treated group, notwithstanding there was general increase in all th body weights of

experimental groups from the baseline weight. This agrees with the epidemiological study showing a neutral or even negative association between nut intake and biological mass index - BMI [16]. It was reported by [17] that in a walnut supplemented diet study, the fasting blood levels of test groups were significantly ($p < 0.05$) lower relative to normal control (NC) for 3,7 and 10th day of the test. It was also observed that he body weight and haemoglobin concentration of the test animals also significantly ($p < 0.05$) increase, while urine decreased compared to the controls.

This results agrees with the findings of [18] where the authors likewise reported that African Walnuts increased the hemoglobin level and decreased urine output in the experimental group when contrasted with controls and could forestall diabetes related with renal damage. According to the result obtained in this present study, the reduction in weight gain was observed to be in the order was ethyl acetate (EAE) treated group, ethanol residue (ER) and ethanol whole extract (EWE). This decrease in weight may be connected with the rich supply of the proteins in the African Walnut (T. conophorum) where a study found the amino acids contained in the African Walnut to be a total of seven essential amino acids identified and the major essential amino acid was Lycine (20.38 mg/100g) while the lowest was Isoleucine (5.24 mg/100g) but the order depending on the contents of the essential amino acids in T.conophorum seeds was Lycine (20.38) > Threonine (18.58) > Methionine (16.21) > Phenylalanine (13.47) > Valine (13.30) > Leucine (6.60) > Isoleucine (5.24) [19]. The bioavailability of these amino acids can equally enhance protein synthesis and muscle mass as well as adipogenesity in consumers. Notably, EWE exhibited remarkable efficacy, suggesting its possible use as a pharmacological aid against obesity.

These findings underscore the need for further research into the bioactive compounds within these extracts, their mechanisms of action, and long-term safety. Comparisonly many pharmacological treatments are available to combat obesity. With the weight loss medication Tirzepatide—in the RCT, participants who received the lowest (5mg) dose had an average 11.9% reduction in body weight, specifically with a significant reduction in fat mass compared to lean mass [20]. But before settling on a path for treatment, the actual causes of obesity hypothetically need to be investigated and addressed, although we may be able to identify effective treatments without knowing how or why they work [2]. A recent study suggests that BMI may not necessarily increase mortality independently of other risk factors in those with BMI of 25.0–29.9 and in older adults with BMI of 25.0–34.9 [21]. *Tetracarpidium conophorum* nut extracts may hold promise as a complementary approach to combating obesity, offering hope in the ongoing battle against this global health concern.

4. CONCLUSIONS

In conclusion, this study investigated the potential effects of *Tetracarpidium conophorum* nut extracts on body weight and organ/tissue weight in a monosodium glutamate-induced Obesity rat model. The findings suggest promising implications for combatting Obesity. The experimental groups treated with *Tetracarpidium conophorum* nut extracts demonstrated varying degrees of weight reduction compared to the Obese control group. Among the extracts, the ethanol whole extract (EWE) exhibited the most significant impact on weight reduction, showing a considerable decrease in body weight compared to the Obese control group. This substantial weight loss observed in the EWE-treated group indicates its potential as a viable and

effective therapeutic agent for addressing obesity. The ethyl acetate extract (EAE) and ethanol residue (ER) extract also showed weight-reducing effects, albeit to a lesser extent. The study's results suggest the potential of *Tetracarpidium conophorum* nut extracts as a pharmacological adjunct in combating obesity. While the underlying mechanisms behind these weight-reducing effects require further investigation, the current findings open avenues for future research into the bioactive components responsible for the observed outcomes. Overall, this study contributes to the growing body of evidence supporting the role of natural compounds in managing obesity, offering new insights into potential therapeutic strategies. *Tetracarpidium conophorum* nuts, particularly the ethanol whole extract, emerge as promising candidates for further exploration and development in the quest to address the global obesity epidemic. Further studies are warranted to elucidate the molecular mechanisms underlying these effects, evaluate long-term safety, and explore potential synergies with existing anti-obesity treatments.

Abbreviations

HMG - COA - 3-hydroxy-3-methylglutaryl-CoA
MSG - Monosodium glutamate
NC - Normal Control
OB-EAE -. Obese - Ethyl Acetate Extracts
OB-EWE- Obese - Ethanol Whole Extracts
OB-ER - Obese Ethanol Residue
OBC- Obese Control
OBSC - Obese Standard Control
WAT - White Adipose Tissue

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