EVALUATION OF THE ACUTE TOXICITY AND *IN VIVO* ANTIOXIDANT PROPERTIES OF *Tetracarpidium conophorum* (MULL. ARG) HUTCH & DALZIEL SEED AQUEOUS EXTRACT IN SWISS MICE

BY

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LSC1806998
SR/1773/RPR/21/23

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FACULTY OF LIFE SCIENCES
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BENIN CITY

DECEMBER, 2022

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE OF THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN. UNIVERSITY OF BENIN, BENIN CITY, EDO STATE

DECEMBER, 2022

CERTIFICATION

I certify that this project work was carried out by Ziongates AMEDU in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin-city, Nigeria.

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Dr. J. O. Erhabor **Date**

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DEDICATION

This project work is dedicated to God Almighty and my adored parent.

ACKNOWLEDGEMENTS

I cant cease being grateful to GOD ALMIGHTY for a he has done in the course of this program.

A special appreciation and acknowledging goes to my supervisor and mentor Dr. J. O. Erhabor for his fatherly assistance and mentorship guide towards the progress of this work. Thank you sir

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ABSTRACT

Herbs are useful in the management of diseases due to the reduced level of toxicity and antioxidant capacity as reported from folklore uses. Toxins harmful effect have been proven to be dose-dependent in relation to drug effects. *Tetracarpidium conophorum* belongs to the family Euphorbiaceae, with several therapeutic benefits (anti-diarrhea, constipation, antimicrobial, pain). This study investigated the acute toxicity of *T. conophorum* seeds aqueous extract using standard procedure. Graded doses (25, 50 and 100 mg/kg) of *T. conophorum* seeds aqueous extract, the standard drug (2 mg/kg diazepam orally) and the negative control were administered at a single dose to evaluate the antioxidant activities using standard protocol. The result of the acute toxicity revealed no abnormal physical signs and recorded no mortality across the doses. The aqueous extract of *T. conophorum* seed at 25 and 50 mg/kg showed a significant decrease in the level of Malondialdehyde compared to the untreated control (distilled water) ($p<0.05$). The results obtained from catalase, superoxide dismutase and glutathione peroxidase assays showed a significant increase in their scavenging capacity against free radicals. In conclusion, no adverse effect was observed with no mortality. This study scientifically validate the *in-vivo* scavenging property and acute untowards effect of *T. conophorum*.

CHAPTER ONE

INTRODUCTION

Plants have been used for human and animal health care from generation to generation. The therapeutic constituents of plants used by herbal practitioners may result from one or more of the compounds in the plant (Idu *et al*., 2007). It has been established that plants are potential medicine used to treat the different types of diseases. Traditional practitioner acclaims herbal materials are being projected to scientific investigations. Herbal plants are of great help to man, whether for providing food and shelter or as remedies for illness or cures for diseases (Ramakrishnan and Sivaranjani, 2013). Some natural products used scientific support to back up their different biological properties (Idu *et al*., 2007). The major advantage of using plant acquired-medicine is that they are harmless compared to synthetic drugs and offer better and affordable treatment. Nevertheless, it should also be distinguished that most medicinal plants are nontoxic for ingestion in raw forms. Conversely, systematic evidence and clinical test are essential to exhibit pharmacologically potent secondary metabolites effective in plant materials to establish the folklore benefits (Sasidharan *et al*., 2011). Scientific investigate proof, validity, reports and clinical trials exhibited sympathetic pharmacokinetics, safety, efficacy, drug interactions and bioavailability of therapeutic agents (Debbiea *et al*., 2012). Medicinal plants constituents have been used to derive chemical-substances for drugs production to management severa diseases.

Owing to effective or utility of plant medicine, reports widely established medicinal plants encloses as secondary metabolites for various protective functions with different medicinal effects, such as; antidiarrheal, antimicrobial, antioxidant, analgesic, antihypertensive, anti-inflammatory and anti-nociceptive (Idu *et al*., 2007; Meena and Pitchai, 2011). In Nigeria, plants are used in alternative medicine as a remedy, usually prepared in extracts or use as an

infusion for the management and treatment of different illnesses or diseases such as; gastrointestinal, respiratory, skin, urinary, and liver disorders many more. Medicinal plants are imperative in drug development (Raskin *et al*., 2002; Burke *et al*., 2017). Currently, it is estimated that approximately 80% of the population in developing countries depend on alternative medicine derived from plants and animals as primary sources of health care. The demand and recognition of herbal medicines are rapidly increasing. Approximately 500 medicinal plants used are stated in primordial literature, and about 800 of these plants are implicated in the aboriginal system of medicine. Herbal drugs derived from plant materials involve the application of whole plant parts or fractions of plant parts to care for ailments or diseases (Zhao and Yang, 2018). Herbs are considered harmless since they are derived from natural sources (Abhishek *et al*., 2006). They are useful with less or no toxic side effects in allopathic medicines; this has rapidly increased the number of herbal practitioners (Weber and Killen, 2015). However, some plants have shown some toxicity levels and have been withdrawn due to their harmful effects. Others are combined with or modified with herbs to reduce the adverse effects of toxic plants (Mutheeswarana *et al*., 2017). In previous decades, herbal medicines have been consumed by individuals without a prescription. Using plant as medicine has prompted the discovery of pharmacological actions against diseases (Zhao and Yang, 2018).

Toxicology is the science of toxins (Orisakwe *et al*., 2003). For human understanding of several agents capable of triggering damage to organisms , proper toxicology definition explains "the study of the adverse effects of chemicals or physical agents on living organisms". Adverse/side effects can take various forms, alternating from abrupt death to elusive variations not realized till several months or years later (Saidu *et al*., 2007). They occur at diverse concentrations in the body, like the organ, a form of cell, or definite biochemical. Understanding how poisonous mediator’s impairment to the body has

developed laterally with therapeutic information. It is recognized that numerous apparent structures or body roles change the actual effect from formerly unrecognized variations in explicit biochemical of the body. Toxicology studies the adverse effects in chemical or physical mediators on living entities (Oluwole *et al*., 2015). Toxic materials can be organ toxins or systemic poison (Saidu *et al*., 2010). Systemic toxicant affects the whole body or numerous organs somewhat than explicit spot. For instance, potassium cyanide possesses systemic toxin which affects practically all organ and cell in living entities by intrusive ability of the cell's to exploit oxygen. Toxin can as well affect certain organs or tissues without producing impair substance to our body. These selected sites, identified as mark tissues or organs. The toxicity of a substance depends on the following form and innate chemical activity dosage, especially dose-time relationship exposure route species, age, sex, ability to be absorbed, metabolism, distribution within the body, excretion, presence of other chemicals (Ajibade *et al*., 2012). The method in which constituent may be reflective to influence toxicity especially for metal elements.

Immoderate generation of intracellular reactive oxygen species (ROS) is a precursor of oxidative stress which subsequently catalyzes metabolic deficiency and cellular death through biochemical and physiological lesions (Ishola *et al*., 2014). The identification of antioxidants from natural products has become a matter of great interest in recent studies for their noteworthy role in nullifying the destructive effects of ROS (Chen *et al*., 2003; Ishola *et al*., 2014). Certain plants have been reported to possess enzymatic antioxidants, including catalase and superoxide dismutase, and non-enzymatic antioxidants, including vitamins C and E (Vijayameena *et al*., 2013). This caused a reduction in lipid peroxidation induced by cold immobilization stress in the brain and liver of rats, indicating the adaptogenic potential of this plant (Padma *et al*., 1997; 2001). The stem bark extract (200 mg/kg) also showed

protective effects against oxidative stress induced by carbon tetrachloride in rats. It significantly increased the oxidant levels and serum enzyme activities to near normal.

1.1 *Tetracarpidium conophorum* (Mull. Arg) Hutch & Dalziel Syn.

Tetracarpidium conophorum is commonly described as African Walnut and a perennial climbing shrub (GRIN, 2010). *T. conophorum* is native to tropical western and central Africa, dated from Togo to Congo throughout Sierra Leone, and is abundant in Nigeria, the Republic of Congo, Cameroon and the Democratic Republic of Congo (Wikipedia, 2016). In Nigeria, *T conophorum* is commonly found in Lagos, Akpabuyo, Kogi, Ajawa –Ogbomoso, Uyo, Akamkpa and Ibadan (Ayoola *et al*., 2011; Burkill, 1985). The seeds are edible usually as refreshments and snacks. It is known in English as walnut, in southern Nigeria as “ukpa” [Igbo], in western Nigeria as “awusa” or “asala” [Yoruba] and is known as “kaso” or ngak” in the littoral and western Cameroun (Dalziel, 1937). It is known in northern Nigeria as“gawudi bairi” (Hausa).

Plate 1: *Tetracarpidium conophorum* in its natural habitat (a) tree and (b) seed nuts

1.2 Ethnomedicinal Uses of Tetracarpidium conophorum

A study has suggested that consumption of walnut increases fat oxidation and reduces carbohydrate oxidation without affecting total consumption, implying that walnut consumption may Phytochemical, and Proximate Compositions of *T. Conophorum* (African Walnut) Seeds improve the use of body lipids in overweight adults. Local people use the bark as a mild laxative (Janick and Paul, 2008). The seed kernel is considered a tonic and aphrodisiac (Aiyeloja and Bello, 2006). *T. conophorum* has been used ethnomedically using the various parts, such as the leaves, seeds stem bark and roots. The nut is an excellent source of protein and provides high food energy value (Nwaoguikpe *et al*., 2012; Ojobor *et al*., 2015). Walnuts decrease endothelial dysfunction associated with high-fat diets (Anderson *et al*., 2001). Walnuts aid in treating Rheumatism, kidney pain, gout, cold, and substantial menstrual bleeding as a blood cleanser and worm expellant (Ekhuosuehi, 2008). It is used in managing asthma and is mainly prescribed between bouts of acute asthma. The young leaves and shoots are edible vegetables. It is used for the elderly as a constipation cure (Wikipedia, 2009).

The leaf is valuable in the management of cancers in the neck region. They are prepared as a tea for the gastro-intestinal system, diarrhoea and inflammation of the gums, mouth and throat. The root is especially good in treating piles. The bark is used in tea as a laxative and chewed for toothache. The oil from the nut has been used in the formulation of wood varnish, stand oil, and vulcanized oil for rubber and leather substitutes (Babalola, 2011). It helps to prevent and control high blood pressure (Ekhuosuehi, 2008). Walnut shell is well-suited with some materials and works filler in dynamite. The shells are used as fuel in co-generation power plants and oil-well drilling, the shell is ideal for the gritty, rough agents in soap, cosmetics and dental cleaners (Ekhuosuehi, 2008). Usually, drinking water directly after eating edible nut tastes bitter, this might be due to some alkaloid-containing compounds in

them. Nwauzoma and Dappa (2013) reported ethnobotanical uses of *T. conophorum* seed in managing fibroids; boiled seeds are also consumed to increase sperm count, thereby improving male fertility and for female aid in the regulation of menstrual flow. Ayoola *et al.* (2011) reported the use of *T. conophorum* in the treatment of stomach illnesses with a regulatory high blood pressure. They are said to tonify the kidneys, strengthen the back and knees and moisten the intestines. They are believed to stop asthma and are prescribed to be taken between bouts of asthma but not for acute asthma. They are used by the elderly to cure constipation and flatulence (Ayoola *et al*., 2011; Ogundolie *et al*., 2017). The fruits are edible and used in various ways, such as masticatory, thrush, anti-helminth, syphilis and as antidote against snake bites (Obianime and Uche, 2010). The leaves and young shoots are infrequently eaten in cooked rice in certain parts of West Africa. Brown dyes have been extracted from the husk and the leaf extracts were used to reduce hiccups (Hogue, 2000). The bark is brewed as a tea for use as a laxative and is chewed for toothache.

1.3 BOTANICAL DESCRIPTION OF *Tetracarpidium conophorum*

The African walnut is usually cultivated in the hot and humid zones of tropical Africa around gardens and backyards, mainly for subsistence consumption (Oyekale *et al*., 2015). The African walnut usually flowers between November and early January and fruits between February and September with peak production in July (Oyekale *et al*., 2015). It is abundant in Nigeria's cocoa-producing states and the southern part of Nigeria (Nwaichi *et al*., 2017; Udedi *et al*., 2014). The plant climbs up to the tops to benefit from full sunlight and it may bind trees together such that if one of the trees dies, it is held in position until it decays (Bailey 2006). The seed takes 4–6 months to mature (Akpuaka and Nwankwor 2000). The tree grows on moist, deep, fertile, well-drained loam soils and silt clay soils (Cogliastro *et al*., 1997). It grows in coves, bottomlands, abandoned agricultural fields and rich woodlands (Chijoke *et al*., 2015).

It is a monoecious plant that has separate male and female flowers on the same plant (Janick and Paul, 2008). The male flowers are in a narrow raceme-like panicle that is as long as the leaves, with one or two female flowers near the base. The flowers are arranged alternately on the axis of the raceme inflorescence. The style is stout and quadrangular with four spreading stigmas. There are many stamens, about 40 in number (Janick and Paul, 2008). The plant is a small tropical flowering plant, a woody perennial climber or climbing shrub of about 6 m – 18 m long on the attainment of the reproductive phase; its stem can be up to 16 cm in girth and dark grey when old, but is green and glabrous when young (Nwachoko and Jack, 2015). The root is fasciculate and the leaf ranges between 10 cm long and 5 cm broad, while the petiole may be up to 5 cm long (Ekwe and Ihemeje, 2013; Janick and Paul, 2008).

Furthermore, the leaf is simple, crenate and ovate with a serrated margin. They are rounded at the base with alternate leaf arrangement and abruptly acuminate (Ekwe and Ihemeje 2013; Janick and Paul, 2008). Usually, the walnut tree twines around other trees for support, especially the cocoa tree and kola nut tree (Oyekale *et al.,* 2015). The immature fruits are usually green in colour but turn dark brown to black as they reach maturity (Oluwole and Okusanya, 1993). The plants have swollen, fleshy, sparsely branched stems and are sometimes candle-broid in appearance; the fruit is a capsule 6 cm – 10 cm long by 3 cm – 11 cm wide containing sub-globular seeds (Janick and Paul, 2008). They are found in the forest zones as climbers and the seed is surrounded by a thick, hard testa, while the seeds are round and dark brown at maturity. The plants are found in primary and secondary forests (Okujagu *et al*., 2005).

1.4 Pharmacological properties of *Tetracarpidium conophorum*

1.4.1 Oxidative stress-induced brain damage

It was suggested that the inhibitory activities could be linked to the presence of phenolic constituents in *T. conophorum*. Akomolafe *et al*. (2017a) established the modulatory effects of *T. conophorum* aqueous leaf extracts on oxidative stress-induced brain damage and critical enzymes associated with brain cell dysfunction. The highest inhibitory effect was obtained in the penis at a concentration of 0.50 mg/mL, while the extract inhibited arginase activity in a dose-dependent manner and the IC_{50} (130.96 µg/mL) revealed higher inhibitory activity in the penile tissue than the testicular tissue homogenate (179.02 µg/mL).

1.4.2 Antioxidant activities

The evaluated antioxidant activity of *T. conophorum* nut extract described by Udedi *et al*. (2014) . Amaral *et al*. (2004) and Periera *et al*. (2007) reported that polyphenolic compounds identified in walnut leaf extract included 3-galactoside, lactoside, 3-pentoside, 3-arabinoside, quercetin, *p*-coumaric-acid and 3- and 5-caffeoylquinic acids, which could be responsible for its antioxidant activity. Amaeze *et al*. (2011) reported the *in vitro* antioxidant activity of *T. conophorum* leaf extract. They revealed that dried leaves have more antioxidant activity than fresh leaves. Still, the methanol extract possesses a high amount of plant bioflavonoids responsible for many plants families antioxidant activity.

1.4.3 Anti-lipidemic activities

The investigated the effects of cooked walnuts on blood lipids, lipoprotein and glucose among adult Nigerians decribed by Analike *et al*. (2017). Ezealisiji *et al*. (2016) reported on the anti-cholesterol activity of the ethyl acetate and n-hexane extracts of the *T. conophorum*

seed and showed that a 2.00 mg/kg dose of both extracts decreased low-density lipoprotein (LDL) and increased high-density lipoprotein (HDL) cholesterol when compared with atorvastatin (a standard cholesterol-lowering agent), which could be attributed to the oleic acid and α-linolenic acid. There was a significant reduction in plasma cholesterol, triglycerides, LDL-C and the LDL-C/HDL-C ratio of the subjects compared with their baseline values. Nwaichi *et al*. (2017) also reported on the nutraceutical potential of *T. conophorum* and *Buchholzia coriacea* in diet-induced hyperlipidaemia. The hyperlipidaemic rats were subsequently treated with normal feed supplemented at 500 mg/kg and 1000 mg/kg of *T. conophorum* and *B. coriacea* for two weeks. In comparison to test control animals, there was a reduction in weight gain, total cholesterol (TC), triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), plasma contents of LDL, very low-density lipoprotein (VLDL), non-HDL and atherogenic indices in a dose-dependent fashion. Clarisse *et al*. (2017) assessed some effects of the consumption of defatted flours of *Ricinodendron heudelotii* and *T. conophorum* on some biological and biochemical parameters in adult male rats.

1.4.4 Male fertility-enhancing activities

Ikpeme *et al*. (2014) reported that *T. conophorum* seed extract increased the viability and sperm output of male albino rats and suggested that the seed should be included in the formulation of male fertility drugs. Results from a study conducted by Obianime and Uche (2010) on the effect of aqueous extract of *T. conophorum* seed on the hormonal parameters of male guinea pigs showed a significant dose– time-dependent increase ($p \leq 0.05$) in the level of testosterone; the highest increase was recorded after the seventh day of the treatment (3.40 ng/mL) when compared with standard drugs (Proviron). However, Akpan and Anietie (2014) had contrary findings on using an aqueous extract of *T. conophorum* seed nut as a

fertility enhancer in male albino Wistar rats. It was reported that 14.14 mg/kg and 21.21 mg/kg significantly decreased the percentage of sperm concentration but increased the follicle-stimulating (FSH) and luteinising hormones (LH), which implies that it stimulates biosynthesis and secretion of fertility hormones and also is clear evidence of toxic damage to the spermatozoa. However, it was concluded that there is a need for caution on excessive consumption of *T. conophorum* seed nuts among males with infertility problems.

1.4.5 Anti-diarrhea activity

Nwachoko and Jack (2015) understudied *T. conophorum* nut in the hot aqueous extract to protect rats against castor oil-induced diarrhoea; the inhibitory effect was attributed to some secondary metabolites and also justified ethnomedicinal use.

1.4.6 Anti-chelating activity

Olabinrin *et al*. (2010) investigated the *in-vitro* chelating capacity of aqueous extracts of *T. conophorum* nuts. The seed nut extract displayed a dose-dependent decreasing chelating effect at 2% w/v graded dose and had the highest chelating ability.

1.4.7 Anti-ulcer activities

The extract reduced the ulcer index, gastric volume, total and free acidity but increased the pH significantly ($p < 0.05$) compared to the control group. Ezealisiji *et al*. (2014a) evaluated the methanol extract of the *T. conophorum* nut using pyloric ligation-induced and ethanol-induced gastric ulceration methods. The result from a similar study carried out by Anosike *et al*. (2015) reported that the methanol extract of the seed nut showed significant anti-ulcer activity in the indomethacin-induced ulcer and there was significantly reduced ulceration ($p < 0.001$) when compared with the control group but it was not dose-dependent.

1.4.8 Anti-inflammatory activity

Olaniyi *et al*. (2016) reported the chloroform extract of *T. conophorum* fruit. It was observed that at 400 mg/kg dose, there was a significant inhibitory inflammation compared with diclofenac but 200 mg/kg of the extract was pro-inflammatory.

1.4.9 Anti-diabetic activities

Onwuli *et al*. (2014) and Ogunyinka *et al*. (2015) reported that the nuts have the potential to reduce hyperglycaemia; the authors also reported that the nuts increased the haemoglobin level and decreased urine output in the test group when compared with controls and could prevent diabetes associated with renal damage. Lepzem and Togun (2017) established the anti-diabetic and antioxidant effects of the methanolic extracts of the leaf and seed of *T. conophorum* on alloxan-induced diabetic Wistar rats. A study carried out by Ogbonna *et al.* (2013) indicated a significant reduction in blood glucose levels and suggested that the leaf and the root extracts of *T. conophorum* are more potent in lowering blood glucose in alloxan-induced diabetic rats when compared with oral hypoglycaemic agents.

1.4.10 Antimicrobial activities

Suara *et al*. (2016) also established an antibacterial assay of *P. conophora* methanol leaf extract and showed a concentration-dependent effect against *Bacillus subtilis* and *Proteus mirabilis* that could be formulated and used as a cream in the management of susceptible bacteria skin infection. Ogbolu and Alli (2012) suggested that the walnut had no *in vitro* antibacterial activity (leaf, stem bark, cooked or uncooked kernel) on Gram-positive and Gram-negative bacteria. These findings contradicted similar reports of Ajaiyeoba and Fadare (2006) that methanol extract and its fractions exhibited concentration-dependent antimicrobial properties, which were carried out in the same geographical areas. Bello *et al*.

(2013b) established the potential of walnut and onion bulb extracts as antimicrobial agents for fish. Akinwande (2015) reported antimicrobial activity of the leaves and isolated phytosterols (triterpenoids) – 3β, 22E-stigmata-5, 22-dien-3-ol and 3β-hydroxyolean-12-en-28-oic acid from petroleum ether fraction of *T. conophorum* leaves, which has high potential as an antimicrobial agent. It was recorded that 500 µg/mL of both extracts had the best minimum inhibitory concentration on the pathogens and could prevent the growth of microorganisms in fish feed production.

1.4.11 Antidepressant activity

Aladeokin and Umukoro (2011) evaluated the psychopharmacological properties of an aqueous extract of the *T. conophorum* nut in mice and oral administration at 50 mg/kg and 200 mg/kg produced a significant dose-related decrease in the duration of immobility in the forced swim test. The test doses showed no extend duration of sleeping time produced by thiopentone nor change the design of the stereotyped behaviour induced by the amphetamine. It was concluded that the nut extract demonstrated antidepressant-like activity.

1.4.12 Anti-malarial activity

Dada and Ogundolie (2016) assessed the *in-vivo* anti-plasmodial action of raw seed extract of *T. conophorum* in Swiss albino mice infected with *Plasmodium berghei*, revealing dose-dependent activity on chemo-suppression. Correspondingly, 600 mg/kg had a highest values of 47.22%, while chloroquine at 5 mg/kg produced 55.50% chemo-suppression.

1.4.13 Anticancer activity

Carvalho *et al*. (2010) reported that the methanol extract showed concentration-dependent growth inhibition towards human kidney and colon cancer cells. The results obtained

indicate that walnut tree has an excellent basis of actual natural antioxidants and chemopreventive agents. A-498 renal cancer cells all extracts exhibited similar growth inhibition activity (IC_{50}) values (between 0.226 mg/mL and 0.291 mg/mL), while for both 769-P renal and Caco-2 colon cancer cells, walnut leaf extract showed a higher anti-proliferative efficiency (IC50 values of 0.352 mg/mL and 0.229 mg/mL, respectively) than green husk or seed extracts.

1.4.14 Nutritional evaluation

Barber and Obinna-Echem (2016) assessed the nutritional composition, physical and sensory properties of wheat– African walnut cookies and recommended that the African walnut flour could be used successfully as a partial substitute for wheat flour at a range of 5% – 15%. Onawumi *et al*. (2013) carried out proximate analysis on the leaf *T. conophorum*, which contained moisture (29%), fat (5.63%), fibre (14.92%), protein (16.62%), ash (12.89%) and carbohydrate (20.94%). Suara *et al.* (2016) evaluated the nutraceutical properties of the methanol extract of *P. conophora* leaves and reported some vital mineral elements; the proximate analysis revealed 6.86% moisture content, 11.78% protein, 8.57% total ash, 20.12% crude fibre, 1.56% total fat and 51.8% total carbohydrate. Uhunmwangho and Omoregie (2017) evaluated the nutrition and anti-nutrition as well as mineral content of walnut seed oil at different stages of fruit maturation. In addition, Akpoghelie *et al*. (2016) evaluated the nutritional content of walnut seed (*P. conophora*) and revealed that the raw seed contains ash (3.18%), moisture (39.27%), crude fibre (8.40%), fat (5.19%), protein (20.74%) and carbohydrate (23.22%), while the mineral content revealed K (4029.14 mg/kg), Na (3480.00 mg/kg), Ca (3014.28 mg/kg), Mg (726.11 mg/kg), Fe (68.00 mg/kg), Zn (24.01 mg/kg), Mn (19.00 mg/kg) and Cu (14.00 mg/kg). This study revealed the nutritional profile of the fruit-nut as a good source of plant protein, carbohydrate and fat, with a reduction in the level of some anti-nutrients in matured fruits. Findings from a study carried out by Isong *et al*.

(2013) on conophor nut oil suggests that it is a non-drying oil suitable for paint and soap making as well as other industrial purposes.

1.5 Preliminary phytochemical screening

The phytochemical constituents present in the seeds are also present in the leaves.Nwaoguikpe *et al*. (2012) established the phytochemical and biochemical composition of varieties of walnut (boiled and mashed wet nuts and dried powdered nuts). Saponins (8.37, 5.03 mg/kg) were the highest constituent of the mashed wet nuts and the dried powdered nuts, respectively. The secondary metabolites revealed high alkaloid content (2.670 mg/kg) and low tannin content (0.56 mg/kg). Ayoola *et al*. (2013) reported on a comparative analysis of the phytochemical and nutrient composition of the leaves and seeds of *T. conophorum* and noted that the seeds have more nutritional and elemental composition than the leaves. Chijoke *et al*. (2015) reported the seed nut contains alkaloids (2.29 mg/100 g), glycoside (2.19 mg/100 g), saponins (8.07 mg/100 g), flavonoids (0.02 mg/100 g), tannins (0.89 mg/100 g), reducing sugars (4.10 mg/100 g) and soluble carbohydrate (1.06 mg/100 g). The seed nut also revealed high moisture content (31.40%), ash (6.01%), fibre (8.66%), protein (28.85%), carbohydrate (21.30%) and high energy value (234.57 kcal). The mineral and vitamin constituents of the seed were also documented by Nnorom *et al*. (2013). Udedi *et al*. (2013, 2014) reported comparative proximate analyses of raw and cooked walnut and noted that the nut is an excellent food material with potential in combating food insecurity in rural communities.

1.6 Toxicological studies

Bello *et al.* (2014) assessed the haematological and biochemical changes in African catfish fed a diet supplemented with *T. conophorum* leaf and onion bulb and reported no traces of

infections such as anaemia during the fish culture, suggesting that the extract of the walnut leaf and onion bulb could be helpful in stimulating immune responses. In a study carried out by Oladiji *et al*. (2010) on the toxicity of a *T. conophorum* nut oil–based diet in rats, there was a reduction in the activity of ALP, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in the liver and heart of the animals fed the nut oil–based diet. Akomolafe *et al*. (2017d) reported that the aqueous extract of *T. conophorum* leaves in rats did not reveal any pathological changes even at 2000 mg/kg. Agbaje *et al*. (2016) studied the acute and sub-chronic toxicity studies of the aqueous extract of the fresh nuts of *P. conophorum* and reported there was no mortality at a 2 g/kg dosage. However, ALT and AST were significantly reduced ($p < 0.05$) at 500 mg/kg and 750 mg/kg when compared with the control group and the haematological assessment was significant in all the treatment doses. It was concluded that the extract could be hepatoprotective and possibly serve as an immunostimulant.

1.6.1 History of Toxicity

The studies begins with Paracelsus (1493–1541), who determined specific chemicals responsible for the observed toxicity of plants and animals (Parasuraman, 2011). He demonstrated the harmless and beneficial effects of toxins and proved dose-response relationships for the effects of drugs. Paracelsus, a physician, alchemist, and astrologer, is widely regarded as the father of toxicology. His following statement is often quoted: “All substances are poisons; there is none which is not a poison (Oluwole *et al*., 2015). The right dose differentiates a poison and a remedy. Mathieu Orfila a Spanish physician in 1787–1853, determined the relationship between poisons and their biological properties and demonstrated specific organ damage caused by toxins. Orfila is the father of modern toxicology (Saidu *et al*., 2007). Toxicological screening methods and toxicological research

on individual substances developed in the mid-1900s, and environmental toxicological studies developed in the mid-20th century. The use of animals in toxicity studies began in 1920, when J. W. Trevan proposed using the 50% lethal dose (LD_{50}) test to determine the lethal dose of individual chemicals (Saganuwan, 2017). After the introduction of LD_{50}, a FDA scientist John Draize developed a method for testing eye and skin irritation using rabbits, and this method was widely accepted for testing the effects of chemicals and pharmaceuticals on the eye and skin. Later, the US National Cancer Institute (NCI) developed a test to identify carcinogenic chemicals through the daily dosing of rats and mice for 2 years. In the early 1960s, thousands of babies were born with debilitating congenital disabilities caused by thalidomide. After this, all the regulatory agencies concentrated on determining the toxicity profiles of all pharmaceutical substances available for regular patient use and made mandatory the submission of toxicity profiles of investigational new drugs (IND) (Saidu *et al*., 2010). In the late 1980s, the Organisation for Economic Co-operation and Development (OECD) and the International Conference on Harmonization (ICH) brought out the guidelines for toxicity testing of pharmaceutical substances (Agrawal and Paridhavi, 2007). Before conducting any toxicological testing in animals or collecting tissue/cell lines from animals, the study should be approved by the Institute Animal Ethics Committee (IAEC), or the protocol should satisfy the guidelines of the local governing body. The guidelines for conducting experiments and regulatory requirements vary from region to region.

1.6.2 Types of Toxicity Studies

1. Acute Toxicity Studies
2. Sub-acute Toxicity Studies
3. Chronic Toxicity Studies

1.6.3 Acute Toxicity Studies

Different types of toxicity studies are carried out to evaluate toxic effects of therapeutic agents or potential toxicants, which could threaten the lives of humans and animals (Saganuwan, 2017). The traditional methods of determining toxic effects of chemicals and drugs include acute toxicity study which is carried out to determine the immediate or short span toxicity effect of a toxicant (1-2 weeks). This is a short-term assessment and evaluation of potential hazard test substance or consequences of a single dose of a test substance. Acute toxicity testing may be used in risk assessments of chemicals for humans and non-target environmental organisms. Acute toxicity study is better described as LD_{50}, defined as the dose that kills 50% of animals (Saidu *et al*., 2010). LD_{50} is used for the estimation of the toxicity of the chemical agents. Acute toxicity provides guidelines on the dose to be used in more prolonged studies and provides the basis for other testing programs.. In acute toxicity studies, rodents are mostly used because they are economical, readily available, and easy to handle. This test is carried out in each animal species as the same route intended to be used in treatment (Agrawal and Paridhavi, 2007). Acute toxicity is important as it helps identify the specific organ of toxicity and helps workers map out safety measures and guidelines in the development and testing of test substances (Saidu *et al*., 2007).

1.6.4 Free Radicals

A free radical is any chemical species capable of independently possessing one or more unpaired electrons in an atomic orbital (Halliwell, 1997). Free radicals are constantly produced during metabolism (Lobo *et al.,* 2010). Cells use oxygen to generate energy in the mitochondria by-products are produced in the process. These by-products are mostly reactive oxygen species (ROS) and reactive nitrogen species (RNS) that result from cellular redox processes. Free radicals have a particular affinity for lipids, proteins, carbohydrates and nucleic acids (Velavan, 2011).

ROS/RNS can be generated (i) during UV light irradiation, by X-rays and gamma rays (ii) during metal catalyzed reactions (iii) by neutrophils, eosinophils and macrophages during inflammatory cell activation (iv) as by-products of mitochondrial catalyzed, electron transport reactions, (v) by cytochrome P450 metabolism and the enzyme xanthine oxidase, which catalyzes the reaction of hypoxanthine to xanthine and xanthine to uric acid (Cadenas *et al.,* 2000; Valko *et al.,* 2004). ROS can be detrimental and valuable in biological systems depending on the environment and concentration (Lopaczynski *et al.,* 2011). ROS's useful effects include the physiological roles in cellular responses to noxia such as defence against infectious agents, the function of several cellular signaling systems and gene expression. In contrast, at high concentrations, ROS can facilitate the impairment to cell structures, including lipids and membranes, proteins and nucleic acids; this damage is often referred as "oxidative stress" (Poli *et al.,* 2004).

1.7 Reactive Oxygen Species

Reactive oxygen species can be classified into oxygen-centred radicals and oxygen-centred non-radicals. Oxygen-centred radicals include superoxide anion (O_2^-), hydroxyl radical (OH), alkoxyl radical (RO), and peroxyl radical (ROO). Oxygen-centred non-radicals are hydrogen peroxide (H_2O_2), singlet oxygen (1O2), hypochlorous acid, and ozone. Other reactive species are nitrogen species such as nitric oxide ($NO\cdot$), nitric dioxide ($NO_2\cdot$), and peroxynitrite (OONO). (Halliwell *et al.,* 1995; Simon *et al.,* 2000).

1.7.1 Superoxide anion

Superoxide anion (O_2^-) is a reduced form of molecular oxygen created by receiving one electron. Mitochondrial electron transport systems, in their early stage produce superoxide anion. During cellular metabolism, mitochondria generate energy using four electron chain reactions, reducing oxygen to water. Some of the electrons leaking from the chain reaction of

mitochondria react with oxygen and form superoxide anions (Harman, 2000). Superoxide anion plays a vital role in forming other reactive oxygen species, such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in biological systems (Stief, 2003). It can react with nitric oxide ($NO\cdot$) and form peroxynitrite ($ONOO^-$), which can generate toxic compounds such as hydroxyl radical and nitric dioxide:

1.7.2 Hydroxyl radical

Hydroxyl radical ($\cdot OH$) is the most reactive free radical in biological systems, generated by Fenton reaction in which free metal ions (copper or iron) catalyze breakdown of hydrogen peroxide (H_2O_2) or Haber-Weiss reaction involving superoxide ion with hydrogen peroxide. Hydroxyl radicals have short half of 10^{-9}s with the greatest 1-electron reduction potential of 2310 mV, and is mainly responsible for the cytotoxic effect in aerobic organism (Pastor *et al.,* 2000). Unlike superoxide anion which can be detoxified by superoxide dismutase, the hydroxyl radical cannot be eliminated by an enzymatic reaction (Reiter *et al.,* 1995).

1.7.3 Peroxynitrite

Reaction of nitric oxide ($NO\cdot$) and superoxide anion (O_2) can generate peroxynitrite ($OONO^-$).

$$O_2^{\cdot -} + \quad NO\cdot \longrightarrow OONO^-$$

Peroxynitrite is a cytotoxic species, causes tissue injury and oxidizes low density lipoprotein (LDL) (Halliwell *et al.,* 1997). Peroxynitrite appears to be an important tissue damaging species generated at the sites of inflammation (Papas *et al.,* 1999) and has been shown to be involved in various neurodegenerative disorders and several kidney diseases (Knight *et al.,* 1999). Peroxynitrite ($OONO^-$) can cause direct protein oxidation and DNA base oxidation and modification acting as a “hydroxyl radical-like” oxidant. The impact of peroxynitrite as an oxidant in biological system is due to its high diffusibility across cell membranes.

Nitrotyrosine, which can be formed from peroxynitrite-mediated reactions with amino acids, has been found in age-associated tissues (Knight *et al.,* 1999).

1.7.4 Peroxyl and Alkoxyl radicals

Peroxyl radicals (ROO^-) are formed by a direct reaction of oxygen with alkyl radicals ($R\cdot$), for example, the reaction between lipid radicals and oxygen. Decomposition of alkyl peroxides (ROOH) also results in peroxyl ($ROO\cdot$) and alkoxyl ($RO\cdot$) radicals. Hemolysis of peroxides produces peroxyl and alkoxyl radicals by UV irradiation and transition metal ions. Peroxyl and alkoxyl radicals are good oxidizing agents. They can abstract hydrogen from other molecules with lower standard reduction potential. This reaction is often observed in the propagation stage of lipid peroxidation. Alkyl radical formed from this reaction can react with oxygen to form another peroxyl radical, resulting in chain reaction. Some peroxyl radicals break down to liberate superoxide anions or can react with each other to generate singlet oxygen. Aromatic alkoxyl and peroxyl radicals are less reactive than respective open-chain radicals because of the delocalization of electrons in the ring (Eboh, 2014; Stief, 2003; Girotti *et al.,* 2000).

1.7.5 Hydrogen peroxide (H_2O_2)

Hydrogen peroxide can be generated through a dismutation reaction from superoxide anion by superoxide dismutase. Enzymes such as amino acid oxidase and xanthine oxidase also produce hydrogen peroxide from superoxide anion. Hydrogen peroxide is highly diffusible and crosses the plasma membrane easily (Eboh, 2014). Hydrogen peroxide is the least reactive molecule among reactive oxygen species and is stable under physiological pH and temperature in the absence of metal ions. Hydrogen peroxide is a weak oxidizing and reducing agent and is thus regarded as being poorly reactive. Hydrogen peroxide can generate the hydroxyl radical in the presence of metal ions and superoxide anion (Halliwell, 1997).

Hydrogen peroxide can produce singlet oxygen through reaction with superoxide anion or with HOCl or chloroamines in living systems. It can degrade certain heme proteins, such as hemoglobin, to release iron ions (Eboh, 2014).

1.7.6 Nitric Oxide

In biological tissues, nitric oxide (NO•) is generated by specific nitric oxide synthetases (eNOS, iNOS) metabolization of arginine to citrulline via a 5 electron oxidative mechanisms (Ghafourifar *et al.,* 2005). In normal physiological processes, nitric oxide ($NO^{\cdot}$) acts as an important oxidative biological signaling molecule in neurotransmission, blood pressure regulation, defense mechanisms, smooth muscle relaxation and immune regulation (Bergendi *et al.,* 1999). Oxidative burst inducing inflammatory processes releases superoxide anion and nitric oxide by immune system cells. Nitric oxide and the superoxide anion may react together under these conditions to produce significant amounts of highly reactive oxidative molecule (peroxynitrite anion (ONOO-). This potent oxidizing agent can cause DNA fragmentation and initiate lipid peroxidation (Beckman*et al.,* 1996).

1.7.7 Oxidative Stress

Oxidative stress (OS) is an expression of surplus reactive oxygen species (ROS) or oxidants against cell defence mechanism of depleted antioxidants. Physiologically, brain cells present optimally and attain their functional competencies before a regulated point of reactive oxygen species. During oxidative stress, a distinct increase in ROS levels, intracellular calcium and tyrosine kinase result in increased cyclic adenosine monophosphate (cAMP). Upregulated cAMP develops free radicals, a state generally known as stress-induced depression (Azantee *et al.,* 2016; Bakos *et al.,* 2011; Azantee *et al.,* 2016).

1.8 Aim of study

This study evaluated the acute toxicity profile and antioxidant capacity of *Tetracarpidium conophorum* seeds aqueous extract in Swiss mice

1.8.1 Objectives of study

The following are the objectives of this study:

1. To investigate the acute toxicity of *Tetracarpidium conophorum* in Swiss mice
2. To evaluate the *in-vivo* antioxidant capacity of *T. conophorum* in Swiss mice

CHAPTER TWO

MATERIALS AND METHODS

2.1 Plant Collection and Identification

The freash seeds of *Tetracarpidium conophorum* were obtained from Ugbojiobo market in Benin Cith, Edo State. It was identified and authenticated by Prof. MacDonald Idu of the Department of Plant Biology and Biotechnology, University of Benin, with voucher specimen number UBH.

2.2 Preparation of Samples

Fresh seeds of *T. conophorum* were rinsed in clean water, sliced into pierces and shade-dried for fourteen (14) days. It was further oven dried at 45 ^{O}C for 24 hours. The powder seeds sample was weighed (1550 grams) and extracted with distilled water (2500 mls) using the maceration technique for 72 hours with intermittent stirring and shaking. It was filtered and the filtrate was concentrated using crucibles on the water bath to concentrate into a semi-solid. The percentage yield was calculated using the formula below

$$\% \text{ yield of extract} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times \frac{100}{1}$$

2.3 Purchase and Handling of Animals

Twenty-five (25) Swiss mice weighing 30-35 g of either sex from the Animal House of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo state for the experimental study. They were randomly divided into 5 groups (n=3). The animals were acclimatized to laboratory condition for fourteen days (14) before the experiment and were allowed free access to a grower pellet diet and water *ad libitum*. Animals were fasted

overnight with free access to water before each experiment. The ethical guide for the use of animals guild was adhered to during this study.

2.4 Acute toxicity study

The determination of acute toxicity was done using the modified method of Lorke (1983) and OECD, (2001) for the use of Chemical substances in animals. Twelve (12) mice were randomly divided into four (4) groups of three (3) each. Extracts were administered in single dose of 25, 50 and 100 mg/kg) orally. The acute toxicological signs were observed for 4 to 24 hours and further observed for 14 days for mortality. Observations were focused on parameters such as piloerection, sensitivity to sound and touch, locomotion, aggressiveness, the appearance of faeces, salivation, urinating, convulsing, coma and death. Fifty (50) lethal dose (LD_{50}) was evaluated and classified according to the Globally Harmonized System (GHS) for the classification of chemicals (OECD, 1998). The LD_{50} was calculated based on the final results in the square root (of product) with the lowest fatal dose and highest non-fatal dose (geometric mean at repeated doses where 0 and 100 % are the survival rates recorded). The LD_{50} was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose with no mortality,

D_{100} = Lowest dose with mortality.

2.5 Administration of Extract and Standard drug.

To make a solution, T. conophorum seed aqueous extract was re-dissolved in distilled water. It was orally administered at graded doses of 25, 50 and 100 mg/kg of the extract. Standard drug (50 mg/kg ascorbic acid) were prepared in distilled water and was given orally to the mice. The following are the various groups for this study;

Group 1: Untreated control treated with 0.2 ml distilled water alone

Group 2: Reference drug administered 50 mg/kg ascorbic acid

Group 3- 25 mg/kg of *T. conophorum*

Group 4- 50 mg/kg of *T. conophorum*

Group 5- 100 mg/kg of *T. conophorum*

The animals were sacrificed and the brain isolated, homogenized (weighed 1 g of the brain and grind with 5 ml normal saline). It was centrifuged for 15 minutes at 10,000 ppm. The supernatants were collected and refrigerated at 4^{o}C until ready for further analysis.

2.6 Determination of antioxidant property of *T. conophorum* seed aqueous extract

2.6.1 Superoxide dismutase

Superoxide Dismutase (SOD) was carried out using the method described by Bagul *et al.* (2005) and Magili and Bwatanglang, (2018).

Principle

Auto-oxidation with heamatoxylin (an increases in the absorbance wavelength 560 nm) was blocked by SOD activity to assay at pH 7.8; fraction of SOD quantity within specific range. SOD effect in these samples was analyzed via reading heamatin amount present. The crucial principle to assay is revealed schematically via the following equation:

$O_2 + HTH_2$ SOD $H_2O_2 + HT$ (Abs560↑)

Procedure:

Aliquant mixture of plasma 0.20 ml diluted microsome enclosed using 2.5 mls solution of 0.05 M carbonate buffer. The reactions started via addition of 0.3ml of 0.3mM adrenaline. Reference combination with 2.5 mls solution of 0.05 M carbonate buffer, 0.3 ml solution of 0.3 mM Adrenaline and 0.20 ml distilled water. Absorbance was read after 30 secs. to 150 secs. using wavelength of 480 nm. Calculations augment absorbance/ minute = % inhibition

= 100 - Where As is increase absorbance of substrate and Ab is increase absorbance of blank 1 unit of SOD property is equivalent to the quantity of SOD involved to obtain 50% inhibition of oxidation via adrenaline to adenochrome in 1 minute. 5 .21 5 A A 100 xAAxs

2.6.2 Catalase activity

Catalase activity was assayed for using a standard protocol described by Bagul *et al.* (2005). Test principle: Catalase scavenging hydrogen peroxide, converted into molecular oxygen and water. The action of catalase in this sample was resolute following decreased rate of absorbance using wavelength of 240 nm and by monitoring the consumption of H_2O_2 substrate at 240 nm spectrophotometrically. 40 H_2O_2 Catalase H_2O +$_2O_2$

Procedure

Tissue homogenate (10 µls) (100-150 µg protein) was added to 2.8 mls solution of 50 mM potassium phosphate buffer at pH 7.0 in 3 mls cuvette. Reaction was instigated via addition of 0.1 ml solution freshly prepared 30 mM H_2O_2 with decomposed rate of H_2O_2 read at 240 nm wavelength for 300 seconds in spectrophotometer. Molar loss coefficient at 0.041 mM-1cm-1 utilized in calculating catalase effect in H_2O_2 mole reduced/min/mg/protein.

2.6.1 Malondialdehyde activity

Malondialdehyde assays was carried out with a described method of Bagul *et al.* (2005) and Magili and Bwatanglang, (2018). Test principle: MDA assay is majorly on reaction of MDA with thiobarbituric acid (TBA); forming MDA-TBA2 adduct that absorbs strongly at 532 nm.

Procedure

Following the 24 hours incubation, treated brain culture was centrifuged for 20 mins, and consequently isolation of the supernatants. 1300 µls of R1 was withdrawn from the microcentrifuge tube. 1 ml supernatant was diluted ten times in Tris HCl and 200 µls further dilute the supernatant in every 200 µls culture added of distilled water and vortexed. 300 µls

of R2 to all the test tube, then vortexed and place under incubation at 45°C for 40 min. following the incubation process, each tube were chilled in ice and centrifuged with a speed of 15000 g in 10 mins. at 4 °C. Every sample measured in spectrophotometer for 586 nm.

2.7.2 Gluthatione Peroxidase

Glutathione peroxidase activity was measured in terms of the first order rate constant for the decomposition of tetra-butyl hydroperoxide according to Bagul *et al.* (2005).

2.8 Statistical Analysis

Values were presented as Mean ± standard error to represent data in bar chart. The software package used was Graph pad prism 7 for data analysis.

CHAPTER THREE

RESULTS

3.1 Acute Toxicity Test

No mortality was observed at 5000 mg/kg highest dose after fourteen (14) days of the study.

No significant toxic sign was recorded within fourteen (14) days of administering the extract (Table 1).

Table 1. Acute toxicological effects of *Tetracarpidium conophorum* in mice

Parameters	**Treatment/ Doses mg/kg**			
	Dw (ml/kg)	**25**	**50**	**100**
Number of mortality	0	0	1	0
% mortality	0	0	33.33	0
Adverse effects	Nil	Nil	Nil	Nil

values were expressed as Mean ± SEM; DW----Distilled water. n=3 (total number of animals per group)

Table 2. Physical observable effects of *Tetracarpidium conophorum* in mice

Groups	Doses (mg)	Observational effect
Control	DW	0
TCSAE	25	0
TCSAE	50	0
TCSAE	100	0

DW----Distilled water. TCSAE--- *Tetracarpidium conophorum* seed aqueous extract

ADVERSE EFFECT: 1. Writhing, 2. Hyper-respiration, 3. Pilo-erection, 4. Vomiting, 5. Stooling blood, 6.Restlessness, 7. Jerking, 8. Salivation, 9. Lacrimation, 10. Hemorrhage**,** 11. Nausea, 12. Diarrhea, 13. Motor- movement, 14. Dizziness, 15. Drowsiness 16. Convulsion 17. Cough, 18 coma and 19. Death

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Hyper-respiration

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Table 3 and Figure 1 showed the effect of *Tetracarpidium conophorum* seed aqueous extract showed a significant decrease in Malondialdehyde across the treatment group except for 100 mg/kg, which had a slight increase when compared with the standard and untreated control.

Table 3. Effects of *Tetracarpidium conophorum* seed aqueous extract in Malondialdehyde *in-vivo* antioxidant assay.

Treatment	**Doses (mg)**	**Malondialdehyde ($^{X}10^{-3}$ mmole/ml)**
Control	DW	32.57 ± 0.75^{a}
Ascorbic acid	50	30.91 ± 0.23^{a}
TCSAE	25	28.51 ± 0.21^{b}
TCSAE	50	28.47 ± 0.03^{b}
TCSAE	100	35.01 ± 0.13^{a}

$p<0.05$. values were expressed as Mean ± SEM; values with the same alphabetical superscript are non-significant across the column. DW----Distilled water. TCSAE--- *Tetracarpidium conophorum* seed aqueous extract

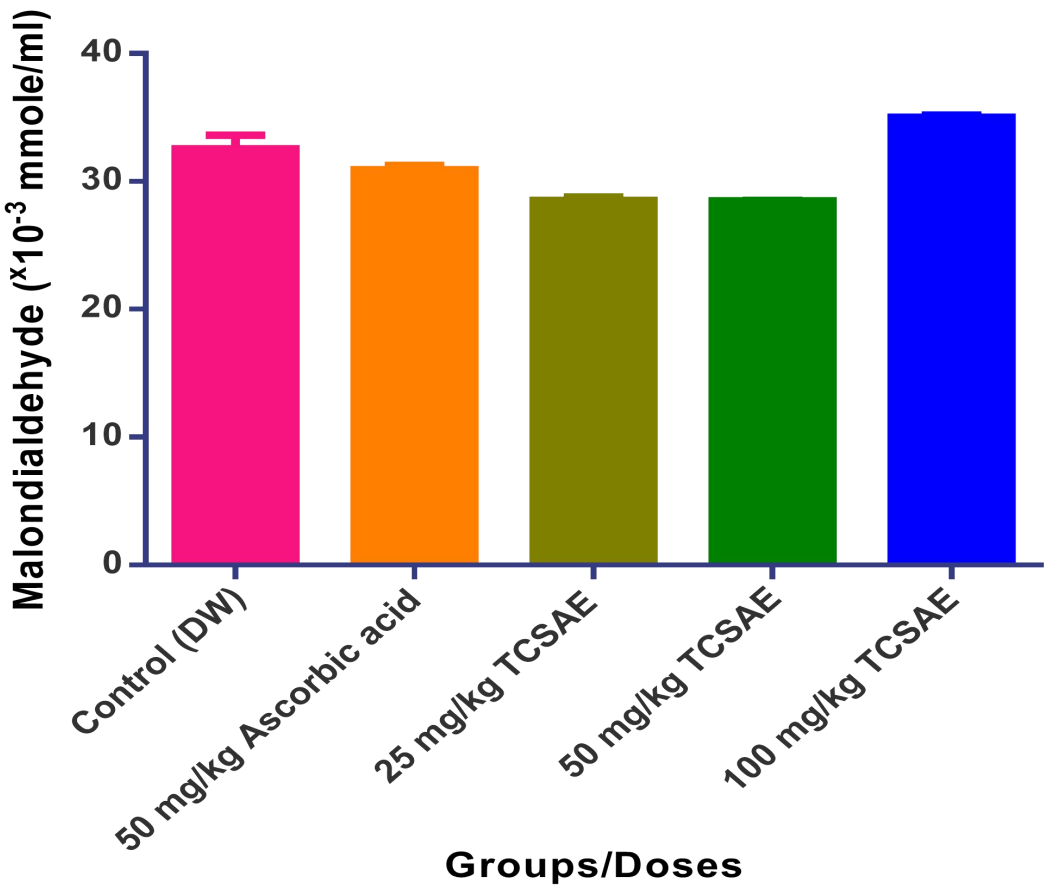

Figure 1. Effects of *Tetracarpidium conophorum* seed aqueous extract in Malondialdehyde *in-vivo* antioxidant assay. *p<0.05. values were expressed as Mean* ± SEM; values are non-significant. DW--

--Distilled water. TCSAE--- *Tetracarpidium conophorum* seed aqueous extract

Table 4 and Figure 2 revealed the effect of *T. conophorum* seed aqueous extract with a significant increase in Superoxide dismutase across the treatment group except for 100 mg/kg which showed a slight decrease when compared with the control groups.

Table 4. Effects of *Tetracarpidium conophorum* seed aqueous extract in superoxide dismutase *in-vivo* antioxidant assay.

Treatment	**Doses (mg)**	**Superoxide dismutase (U/ml)**
Control	DW	6.93±0.02[a]
Ascorbic acid	50	6.64±0.09[a]
TCSAE	25	8.66±0.05[b]
TCSAE	50	8.64±0.05[b]
TCSAE	100	5.68±0.04[a]

p<0.05. values were expressed as Mean ± SEM; values with the same alphabetical superscript are non-significant across the column. DW----Distilled water. TCSAE--- *Tetracarpidium conophorum* seed aqueous extract

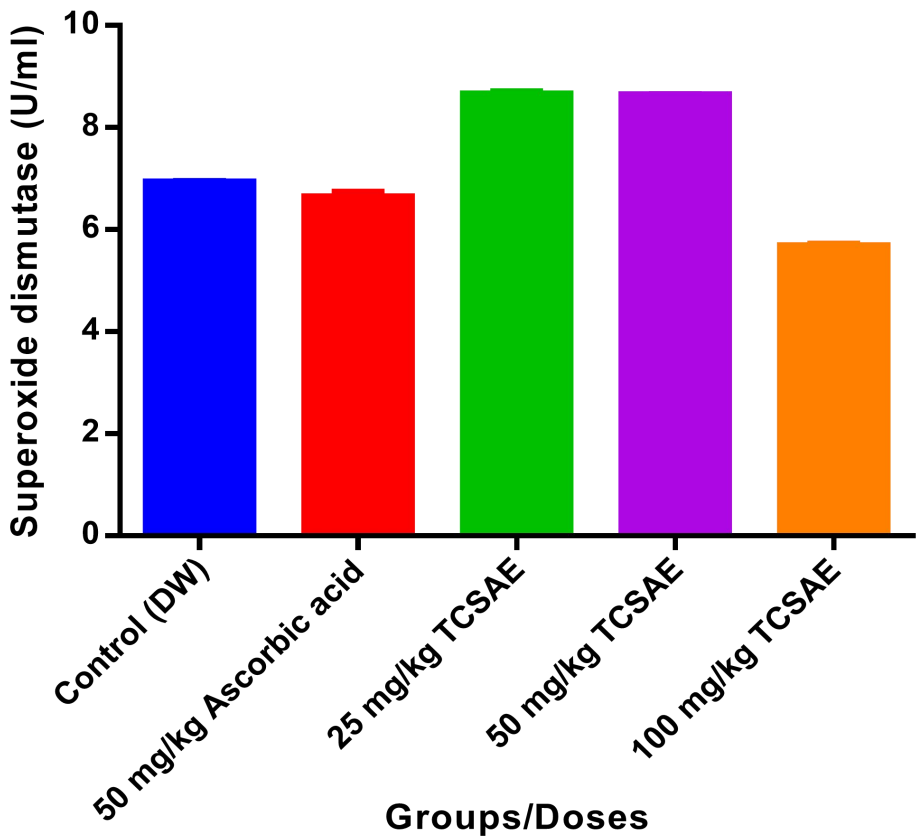

Figure 2. Effects of *Tetracarpidium conophorum* seed aqueous extract in superoxide dismutase *in-vivo* antioxidant assay. *$p<0.05$. values were expressed as Mean* ± SEM; values are non-significant. DW----Distilled water. TCSAE--- *Tetracarpidium conophorum* seed aqueous extract.

Graded doses of *T. conophorum* seed aqueous extract elicited a significant increase in the level of catalase except for 100 mg/kg, which showed a slight significant decrease when compared with the untreated control, as shown in Table 5 and Figure 3

Table 5. Effects of *Tetracarpidium conophorum* seed aqueous extract in catalase *in-vivo* antioxidant assay.

Treatment	**Doses (mg)**	**Catalase (mole/g)**
Control	DW	220.10±1.54[a]
Ascorbic acid	50	229.00±1.46[a]
TCSAE	25	238.80±2.56[b]
TCSAE	50	237.50±0.95[b]
TCSAE	100	212.80±1.39[a]

p<0.05. values were expressed as Mean ± SEM; values with same alphabetical superscript are non-significant across the column. DW----Distilled water. TCSAE--- *Tetracarpidium conophorum* seed aqueous extract.

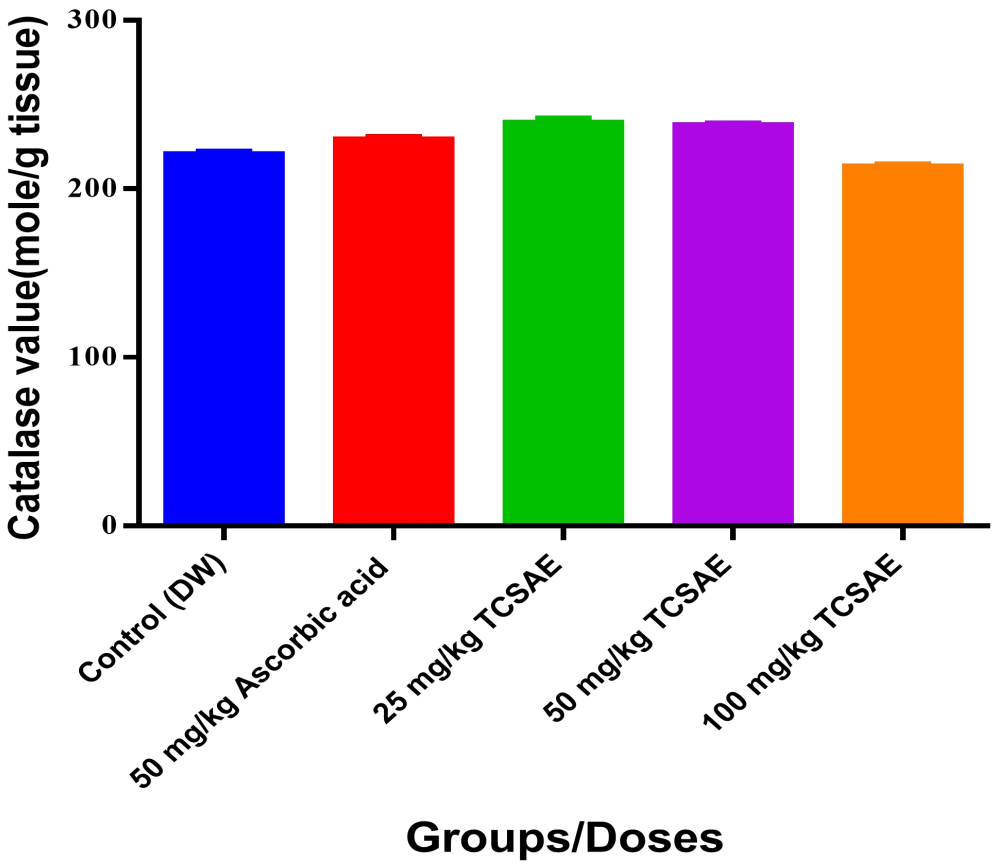

Figure 3. Effects of *Tetracarpidium conophorum* seed aqueous extract in catalase *in-vivo* antioxidant assay. *p<0.05. values were expressed as Mean* ± SEM. DW----Distilled water. TCSAE---*Tetracarpidium conophorum* seed aqueous extract

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T. conophorum seed aqueous extract at graded doses had no significant difference in glutathione peroxidase except for 100 mg/kg, which showed a slight decrease in glutathione peroxidase when compared with the control groups (Table 6 and Figure 4).

Table 6. Effects of *Tetracarpidium conophorum* seed aqueous extract in glutathione peroxidase *in-vivo* antioxidant assay.

Treatment	**Doses (mg)**	**Glutathione peroxidase (U/ml)**
Control	DW	124.70±0.39[a]
Ascorbic acid	50	122.00±0.41[a]
TCSAE	25	125.00±0.39[a]
TCSAE	50	125.30±0.03[a]
TCSAE	100	117.20±2.44[a]

p<0.05. values were expressed as Mean ± SEM; values with the same alphabetical superscript are non-significant across the column. DW----Distilled water. TCSAE--- *Tetracarpidium conophorum* seed aqueous extract

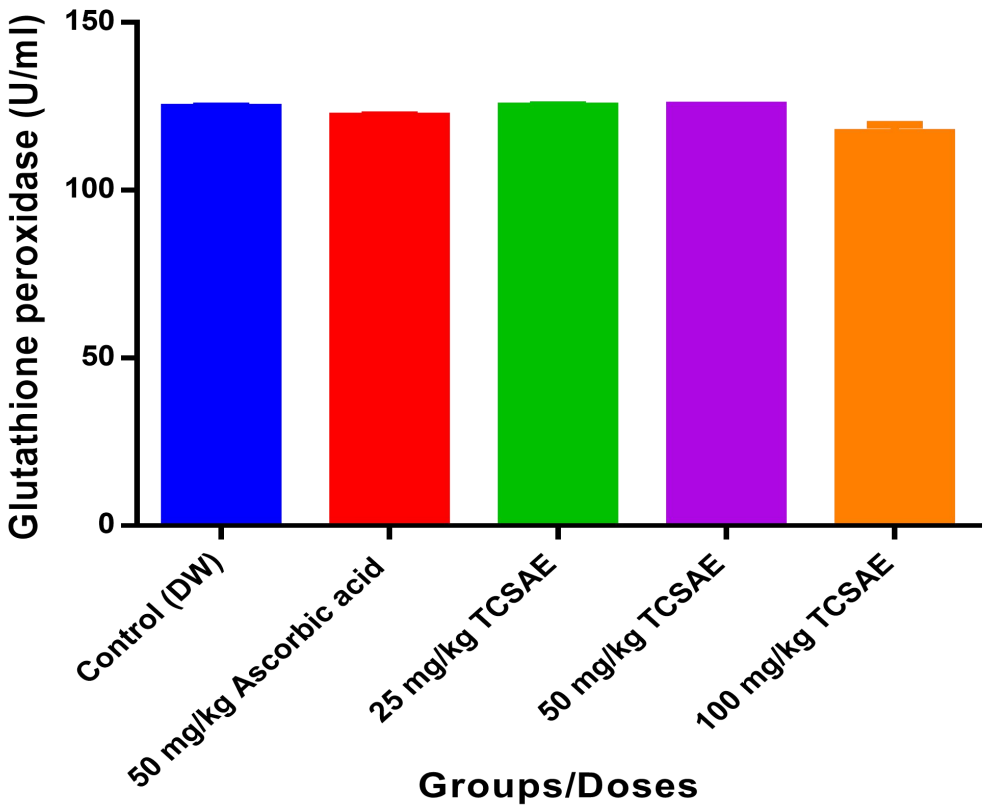

Figure 4: Effects of *Tetracarpidium conophorum* seed aqueous extract in glutathione peroxidase *in-vivo* antioxidant assay. *$p<0.05$. values were expressed as Mean* ± SEM; values are non-significant. DW----Distilled water. TCSAE--- *Tetracarpidium conophorum* seed aqueous extract

CHAPTER FOUR

DISCUSSION

Folk medicine in Africa involves the preparation of extracts across the various plant parts to manage diverse human disorders, including yaws, pains, swellings, stomach ulcers, *Diabetes mellitus*, and oxidative stress (Igbe, 2009). This present research study showed the absence of acute toxic effect of *Tetracarpidium conophorum* with no clinical toxicity signs like respiratory distress, change in hair advent, stooling, maternal mortality, salivation, coma and death evaluated. This showed from the acute toxicity study that the understudied doses had no trace of adverse effects when compared with the control. ly, Tajuddin et al. (2005) showed similar acute toxicity study on ethanolic extract of Myristica fragrans with absent mortality and adverse effects as shown in Table 1. The toxicity presents in non-essential and metal elements for human stimulating rise in kidney, blood pressure and brain damage, and nervous system disorder (Obiajunwa *et al*., 2002; Khan *et al*., 2011).

Félix-Silva *et al*. (2014) stated that most plant act via mechanisms dependent on antioxidant action. *In-vivo* antioxidant property of *Tetracarpidium conophorum* could be attributed to their capability to block the excessive release of neurotransmitters. *In-vivo* study exhibited by the report of Falodun and Irabor (2008) agreed with this present study, which serves as an indication of the plant seed extract acting via the inhibitory effect of dopamine imbalances with secretory eliciting its effect as antioxidant properties presumed to be responsible for the blocking effects displayed by some enzymes involved in the discharge of specific neurotransmitter metabolism, this was similarly shown in Longanga *et al*. (2000), and Félix-Silva *et al*. (2014) reports. Classical uses of bio-markers, including transaminases, have evaluated the redox state of the brain organs with antioxidant activity enzymes and measurements of macromolecule oxidation (Santosh *et al*., 2010). The present study inhibited oxidative damage on cellular membranes of the rain cells accessed by thiobarbituric acid

reactive substances (TBARS) assay. This assay quantified malondialdehyde (MDA), a product of lipid peroxidation (LPO). *Tetracarpidium conophorum* did not cause overproduction of free radicals of MDA levels significantly reduced in the treatment groups compared with untreated control (Figure 1). *T. conophorum* controlled the level of oxidants, probably due to the presence of natural antioxidant compounds such as certain phytochemicals. These compounds, previously reported, have been investigated extensively and there is evidence that they are not involved in the scavenging of radicals, as well as indirect, inactivate transcription factors that regulate the expression of genes encoding the brain for antioxidant enzymes (Barcelos *et al*., 2011). Tissue sulfhydryl groups act against reactive oxygen species (ROS), which are related to toxic brain damage triggered by some herbs. The principal non-protein sulphhydryl (NPSH), comprising 75%–90% of total intracellular NPSH reduced glutathione (GSH) (Moran *et al*., 2001). GSH plays a vital role in antioxidant defence because it possesses direct radical-scavenging properties and an essential component of glutathione peroxidase (GPx) systems, which eliminate different hydroperoxides (Dickinson and Forman, 2002).Toxic plants investigated in acute assays depleted the GSH content in the brain due to principally the pro-oxidant effects of extract at graded doses. To overcome this free-radical stress, GSH is being utilized as the first line of defence. This contradicted the present study proposed by Santosh *et al*. (2010), which showed the hepatotoxicity effect using *Pueraria tuberosa*. Concerning non-enzymatic defences, there are no changes in thiols content (NPSH), indeed because a synergistic antioxidant effect occurs between NPSH content and SBSB phytochemicals in the brain tissue (Figure 2). The CAT and SOD antioxidant activities were also evaluated in the brain tissue of animals treated with *Tetracarpidium conophorum* seed extracts. CAT, SOD and GPx, constitute the primary enzymatic defence, catalyzing the decomposition of ROS.

The acute toxicity effect in the treatment groups showed no behavioural toxicity across the understudied doses compared to the control. There was an increase in CAT and SOD activities derived from *T. conophorum* compared with the control (Figures 3 and 4) (Halliwell and Gutteridge, 2007; Bhor *et al*., 2004).

5.1 CONCLUSION

The conclusion, the study showed that the extract of *T. conophorum* showed no adverse effect or mortality. Hence the scavenging effect of the extract had a significant antioxidant activity, which concurred with the ethnomedicinal reports.

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