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AFRICAN WALNUT SEED OIL ENRICHED DIET REDUCES PROSTATE CANCER INCIDENCE IN WISTAR RATS

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ABSTRACT

African Walnut (*Tetracarpidium conophorum*- Mull. Arg) is a perennial climbing shrub which grows mainly in West Africa, especially in Nigeria. The seeds are commonly processed by boiling and consumed as a snack. This study investigated the possible inhibition of 3-methylcholanthrene (MC) induced prostatic carcinogenesis and expression level of ethoxyresorufin-O-de-ethylase (CYP1A1), benzoxyresorufin-O-dealkylase (CYP1B1) and pentoxyresorufin-O-dealkylase (CYP2B1) activities by African walnut seed oil extract in male Wistar rats. The seed oil was extracted with n-hexane using Soxhlet apparatus. 60 Wistar rats of an average weight of 40-45g were randomly divided into four major groups of 15 rats each. Group A and B animals were fed for 12weeks with diet containing 10% of African walnut seed oil. Group C and D were fed for 12weeks with diet containing no African walnut seed oil. Group B and C animals were administered with 3-methylcholanthrene (200mg/kg) intraperitoneally after 4weeks of feeding. Results showed that group B had a significant decrease in the expression of CYP1A1, CYP1B1 and CYP2B1 enzymes ($p<0.05$) compared to group C. Prolonged latency period, reduce tumor weight and size characterized animals in group B. Histological analysis of the liver revealed that the progression of carcinogenesis was more rapid in animals that were not pretreated with African walnut seed oil, which is indicative that African walnut seed oil enriched diet oppose prostate carcinogenesis induced by MC.

KEYWORDS: 3-methylcholanthrene, African walnut, Prostate gland, Cytochrome-P450.

INTRODUCTION

The tropical African walnut, known as *Tetracarpidium conophorum* or *Plukenetia conophora* which belongs to the family Euphorbiaceae (Oyekale *et al.*, 2015). The plant is popularly known as African walnut, black walnut and Nigerian walnut (Ekwe and Ihemeje, 2013; Nwaichi *et al*., 2017; Uhunmwangho *et al*., 2022). In Nigeria, among the Yoruba tribe, the walnut is known as *awusa* or *asala*, *ukpa*, or *oke okpokirinya* in Igbo and *gawudi bairi* in Hausa; and it is known as *okhue* or *okwe* among the Bini tribe of Edo State (Chijoke, et al., 2015). All parts of *T. conophorum* have been used ethnomedically, including the stem bark, leaves, seeds and roots (Kanu *et al*., 2015). The bark is used by local people as a mild laxative (Janick & Paul 2008). The seed kernel, when eaten raw, has a bitter taste like the kola nut and is considered to be a tonic and aphrodisiac (Aiyeloja and Bello, 2006). Customarily, drinking water immediately after eating the edible nut has a bitter taste principle, which might be due to the presence of some alkaloid-containing compounds in the plant. Nwauzoma and Dappa (2013) reported ethnobotanical uses of *T. conophorum* seed in the treatment of fibroids; the boiled seeds are also eaten to improve sperm count in men while the leaf juice is used to improve fertility in women and regulate menstrual flow. Ayoola *et al*. (2011) reported the use of *T. conophorum* in the treatment of stomach disorders and for controlling high blood pressure. The bark is brewed as a tea for use as a laxative and is chewed for toothache. The fruits are edible and used for various purposes, including masticatory, thrush, anti-helminth, syphilis and also as an antidote against snake bites (Obianime and Uche, 2010). The leaves and young shoots are occasionally eaten with cooked rice in some parts of West Africa (Ayoola *et al*., 2011; Ogundolie *et al*., 2017). The oil from the nut has been used in the formulation of wood varnish, stand oil, vulcanised oil for rubber and leather substitutes (Oyenuga, 1997). Brown dyes have been extracted from the husk and the leaf extracts were used to reduce hiccups (Hogue, 2000). The African walnut contains protein, vitamins, magnesium and is a good source of antioxidants (Kim and Lee, 2002; Uhunmwangho and Omoregie, 2017). It is also rich in polyunsaturated fatty acids such as α-linolenic acid and it contains mono-saturated fatty acids (Kanu *et al*., 2015). Olaniyi *et al.*

(2016) reported on the chloroform extract of the *T. conophorum* fruit and noted that a 400 mg/kg dose significantly inhibited inflammation when compared with diclofenac but 200 mg/kg of the extract was pro-inflammatory. However, the molecular mechanisms underlying the chemoprotective effects of African walnut seed oil remain largely unknown. In the present study, we report that African walnut seed oil inhibits MC-induced prostate carcinogenesis in rats by suppressing Cytochrome P450 cancer metabolizing enzymes activities.

MATERIAL AND METHODS

Ethical permission

Ethical permission was taken from UniMed Research Ethics Committee for the use of experimental rats for this studies.

The Study Location

Bioactive lipids in cancer and toxicology research laboratory, Department of Biochemistry, University of Medical Sciences, Ondo City, Ondo State, Nigeria.

Reagents/Chemicals

All reagents used were of analytical grade. Standard buffer tablets (BDH Chemicals Ltd., Eng.), 3-methylcholanthrene, Benzoxyresorufin, ethoxyresorufin, methoxyresorufin, pentoxyresorufin, resorufin sodium salt, were procured from Sigma-Aldrich (St. Louis, MO, USA).

Plant material (Sample collection)

Fresh *Tetracarpidium conophorum* fruits were obtained from farms in Ondo Town, Ondo State, Nigeria. The fruits were authenticated by a Taxonomist of the Botany Department, University of Medical Sciences, Ondo, Nigeria. The collected fruits were cleaned with a moist soft cotton wool and then the seeds carefully separated from the fruits and dried at 65^0C for 4 hrs. in an oven, crushed with a laboratory mortar and pestle and were kept in a well labeled air tight screw-capped bottles at 4^0C for extraction.

Extraction of oil from African Walnut

The Soxhlet extraction method according to AOAC (1996) as reported by Uhunmwangho *et al*. (2022).

Feeding the animals with diet containing walnut seed oil

Male Wistar rats (40-46g body weight) were obtained from the University of Medical Science animal unit, and were housed in metal cages in a well-ventilated room and allowed access to water and *ad libitum*. The experimental diet comprised of African walnut seed oil (10.0%) among other ingredients. 60 Wistar female rats were used. The animals were randomly divided into four major groups of 15 animals each. Group A and B animals were fed for 12weeks with diet containing 10% of African walnut seed oil. Group C and D were fed for 12weeks with diet containing no African walnut seed oil. Group B and C animals were administered with MC (200mg/kg body weight) intraperitoneally after 4weeks of feeding. The animals were palpated weekly to determine the time of appearance of tumors and body weight. Animals from each group were sacrificed at 12week, and tissues collected for enzymes and biochemical analysis. Prostate glands were exposed and tumors were excised. Tumor incidence, volume and weight were determined. The livers were preserved in RNA later for gene expression studies.

Preparation of liver microsomes: The preparatory of liver microsomes were as reported by Rani and Kansa, (2011).

Preparation of tissue homogenates: For enzyme activities, the tissue homogenates (10%) were prepared in 0.25 M sucrose in potassium phosphate buffer (0.01 M, *p*H 7.4) and centrifuged (12,000 x *g*) for 20 min. The microsomal and cytosolic protein content was determined by the method of Lowry *et al.,* (1951)

Enzyme assays: Microsomal ethoxyresorufin-O-de-ethylase (CYP1A1), benzoxyresorufin-O-dealkylase (CYP1B1) and pentoxyresorufin-O-dealkylase (CYP2B1) activities were assayed according to the procedure described by Burke *et al.,* (1985) and modified by Teel and Huynh, (1998).

Statistical analysis: The values were expressed as mean ± SE. Kruskal-wallis one-way analysis of variance (ANOVA) was used for the feed intake, tumor weight, tumor volume and cancer metabolizing enzymes gene expression using Systat 7.0 software (Spss Inc., Chicago, USA). A difference with $P<0.05$ was considered statistically significant.

RESULTS

Table I.0 Effect of feeding Male Wistar rats with or without Diet Containing African walnut seed oil.

Prostatic Tumor	**Animals fed with *T. conophorum* only (Group A)**	**Animals fed with *T. conophorum* seed oil + MC (Group B)**	**Animals fed with MC only (Group C)**	**Animal fed with Normal rat pellets (Group D)**
Latency period		8weeks	4weeks	
Incidence	Symptoms not observed in these animals	33.1%	78.9%	Symptoms not observed in these animals
Weight (g)		3.1 ± 1.33	8.8 ± 2.3	

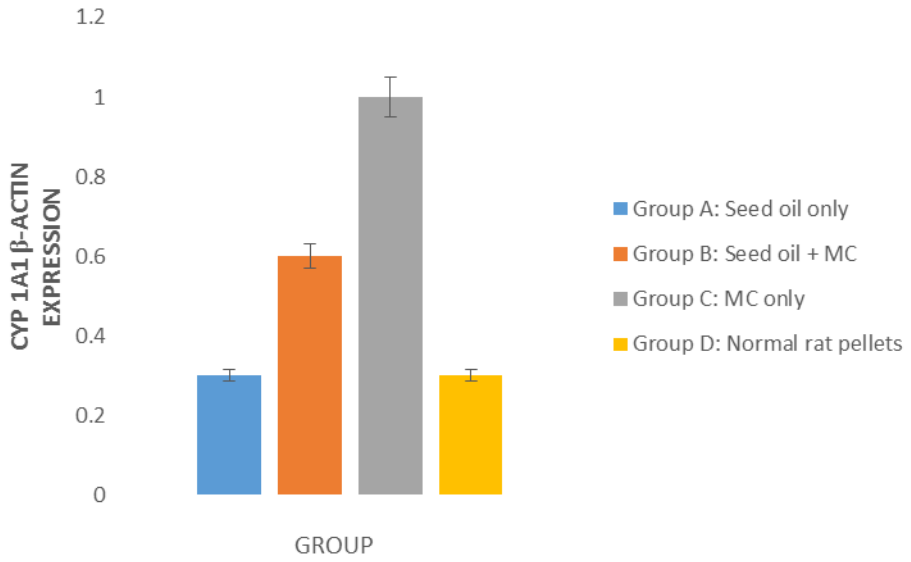

Figure 1.0 The effect of African walnut seed oil on expression of CYP1A1 in male Wistar rats. Values are mean ±SD ($p < 0.05$).

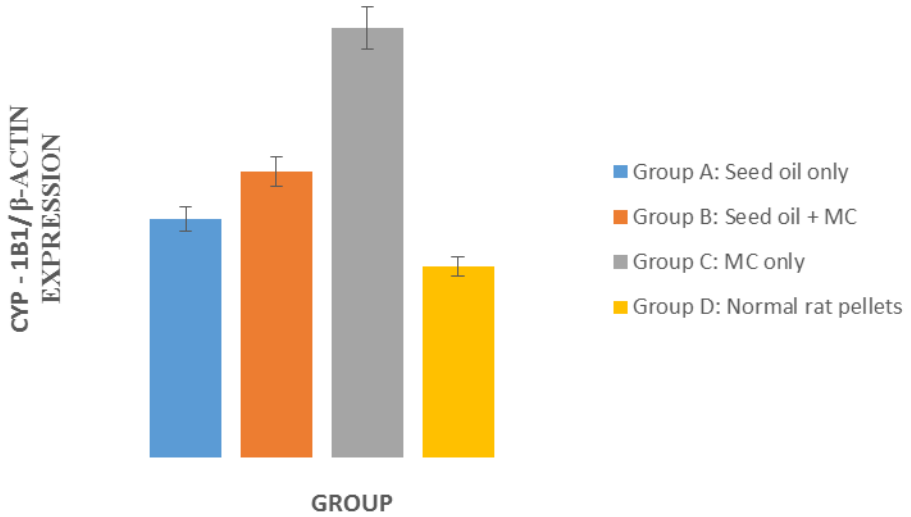

Figure 2.0 The effect African walnut seed oil on expression of CYP1B1 in male Wistar rats. Values are mean ±SD ($p < 0.05$).

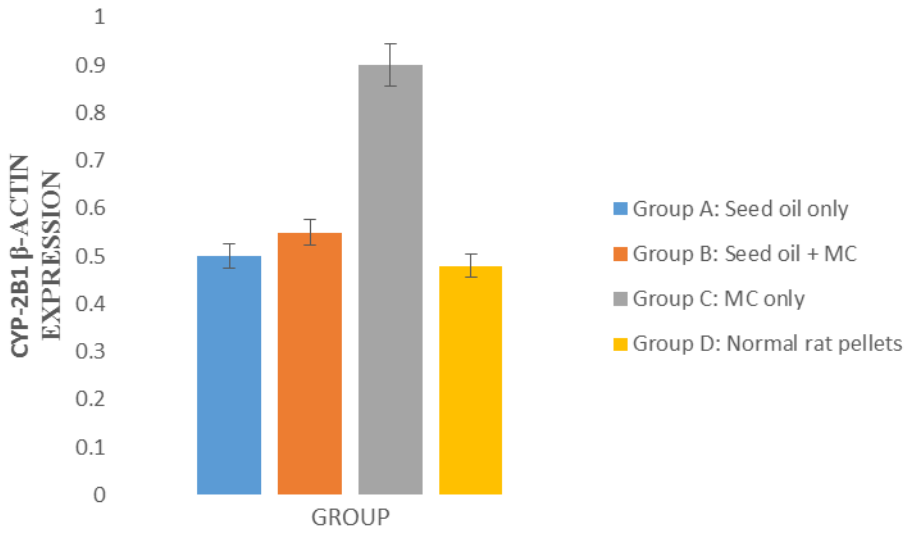

Figure 3.0 The effect African walnut seed oil on expression of CYP2B1 in male Wistar rats. Values are mean ±SD ($p < 0.05$).

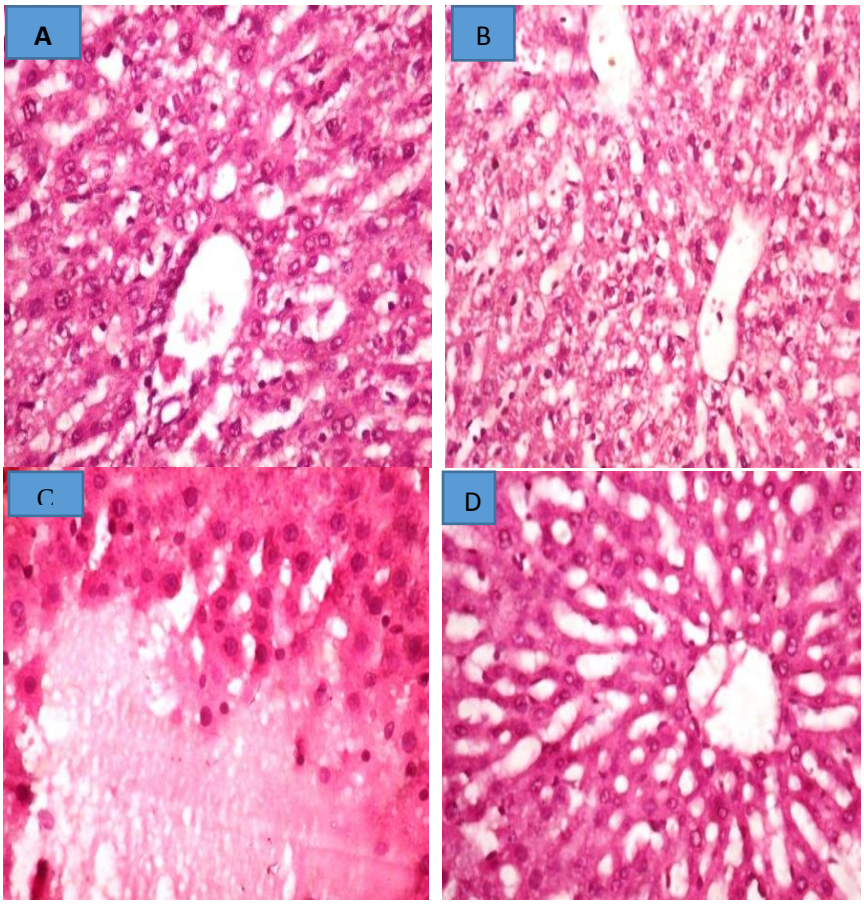

Fig 4.0 Effects of African walnut seed oil on MC-induced histological changes in the livers of rats. Sections were stained with hematoxylin and eosin ×400.

(A) Liver from rats fed with normal rat pallets (B) Liver from rats treated with diet containing African walnut seed oil plus MC (200mg/kg) (C) liver from the rat treated with MC (200 mg/kg) only (D) liver from a rat treated with African walnut seed oil.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

DISCUSSION

African walnut is currently not a staple crop in Africa as it still grows in the wild. Hence, it can be refer to as one of the neglected and underutilized sources of oil that is beginning to gain attention among scientists in Africa. The nutritional potentials of African walnut as reported by previous researchers (Oyekunle *et al*., 2013; Okon and Atai, 2014) have shown that it has high protein content which can serve as alternative source in the wake of animal protein scarcity and high cost in developing Nations. It is also reported to have good amino acid profile with essential amino acids present in appreciable amounts which are essential in the fight against protein-energy malnutrition (Akomolafe *et al*., 2015; Onwuli *et al*., 2014). African walnut – kernel, leaves, root and bark - is constantly mixed in decoctions used in treatment or management of different ailments including chronic diseases such as diabetes and cancer (Bhat and Karim, 2009; Onwuli *et al.,* 2014; Akomolafe *et al.,* 2015). This existing practice of treatment of diseases, using African walnut extracts in local herbal medications, underscores the need for adequate research to validate the claims and much more, re-package the possible products from African walnut for global commercial acceptability. Our present study data on incidence, latency period, weight and volume of tumors in Prostate gland are summarized in Table 1.0. The incidence and latency period of tumors on African walnut seed oil extract pretreated animals was 33.1% and 8weeks respectively, and was significantly (P<0.05) lower than animals administered MC only with 78.9% and 4weeks respectively. The average size and volume of tumor was generally larger in MC administered group than in Walnut seed oil extract diet containing groups. Hence, the feeding of animals which started during the pubescent period of prostate gland development might have resulted in the decreased tumor incidence and progression to malignancy. Studies have also indicated that diets enriched with high levels of ω-3, ω-6 and ω-9 fatty acids reduce tumor growth, metastasis and tumor leukocyte infiltration (Comba *et al.,* 2010). African walnut seed oil has been shown to contain ω-3, ω-6 and ω-9 fatty acids (Uhunmwangho and Omoregie, 2017). Also, gamma linolenic acid (GLA) and dihommo-gamma linolenic acid (DGLA), known to be biologically important (ω-6) long chain PUFAs, were found to be capable of exerting cytotoxic action on tumor cells (Wangs *et al.,* 2012). *In vitro*, GLA or DGLA are known to show selective tumoricidal action (Watkins *et al.,* 2005).

Cytochrome P450 (CYP450)-dependent monooxygenases are phase I enzymes that mostly activate pro-carcinogens. In Phase I reaction, substrates usually undergo reduction, oxidation or hydroxylation reactions yielding more polar metabolites (Guengerich, 2017). In most cases, phase I metabolism is followed by phase II conjugation reactions. During phase II, phase I metabolites are conjugated to a more polar molecule, a process that usually produces inactive and water soluble compounds which can easily be excreted by urine or bile (Turesky, 2004). Phase-II enzymes therefore can reduce the cellular exposure to carcinogens whereas Phase-I enzymes can increase it (Sheweita *et al.,* 2002). Chemical inducers of CYP450 such as 3-methylcholanthrene stimulate CYP450 activity by enhancing its transcription rates, thereby upregulating CYP450s (Swinney *et al*., 2006). According to Figure 1 - 3, African walnut seed oil downregulated the expression of CYP 1A1, 1B1 and 2B1 activities in group B compared to group C (p < 0.05). In group A and D, CYP450 enzymes induction and activities were very low compared to group C (p<0.05) the untreated toxicant group. This shows that African walnut seed oil downregulated the expression of Phase I enzymes by suppressing the activities of CYP450 stimulated by 3-methylcholanthrene. The histopathology of the liver shows that there was ameliorated effect of African walnut seed oil on 3-methylcholanthrene-induced prostate cancer in pre-treated rats as normal morphology of the liver and no pathological lesion were observed (Group B), while normal histology of the liver was observed in group A, B and D. Damage is seen in MC-induced untreated group C, as necrosis and steatosis were observed. Researches have showed that bioactive components of plants are essential for their beneficial roles (Amardeep *et al.,* 2019). The ameliorative effects exerted by African walnut seed oil could probably result from the various bioactive components present in the oil.

CONCLUSION

The nutritional approach to the management of prostate cancer is quite novel and promising. From our findings, the incorporation of African walnut seed oil into diets significantly down-regulates CYP450 enzyme activities.

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