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DIETARY INTERVENTION OF DACRYODES EDULIS PULP OIL ON 3-METHYLCHOLANTHRENE INDUCED PROSTATE CARCINOGENESIS AND EXPRESSION OF COX-2 AND PPAR-Γ IN WISTAR RATS

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ABSTRACT

Prostate cancer is the major cause of cancer death in man, and one of the factors that influence its occurrence is diet. The present study investigated the dietary intervention of feeding *Dacryodes edulis* (African Pear) pulp oil (DEPO) on 3-methylcholanthrene (MCA) induced prostate cancer in Wistar rats and expression of cyclooxygenase-2 (COX-2) and peroxisome proliferator activated receptor gamma (PPAR-γ) in the prostate gland. The pulp oil was extracted with n-hexane using Soxhlet apparatus and characterized by gas chromatography. A total of 52 male Wistar rats (28 days old) were used. Group-A was fed with diet containing 15% DEPO for 14 days before single intraperitoneal administration with MCA (250mg/kg). Afterwards, the animals were fed with DEPO for twelve weeks before sacrifice. Group-B animals were administered MCA (250m/kg) to induce prostate cancer, but was not fed with the DEPO, and group-C animals were fed with 10% DEPO only. In the MCA treated group, tumor latency period was 7weeks on animals fed with DEPO compare with those not fed with the DEPO diet which was 3weeks. The expression of cyclooxygenase-2 was significantly ($P < 0.05$) less, and PPAR-ɣ more on DEPO treated animals. The gas chromatography results showed that polyunsaturated fatty acids such as linolenic acid, eicosadienoic acid and docosadienoic acid were incorporated into the membrane of the prostate gland. This study showed the validity of *Dacryodes edulis* pulp oil in the management and treatment of prostate carcinogenesis.

KEYWORDS: *3-methylcholanthrene, Prostate cancer, Dacryodes edulis, Cyclooxygenase-2.*

1. INTRODUCTION

The modulation of cancer by nutritional variables has been an area of interest. Dietary fat has received considerable attention as a possible risk factor in the aetiology of prostate cancer (Uhunmwangho and Omoregie, 2017). Both the quantity and quality of dietary fat influence the development of spontaneous as well as chemically-induced neoplasm in laboratory animals. Dietary fat is an essential nutrient and important source for the essential fatty acid (FA), α-linolenic acids, linoleic acids and eicosapentaenoic acid (EPA) which contributes appropriate growth, development and anti-proliferative diseases, especially cancers (Wiseman, 2008; Kaur, 2014).

Dacryodes edulis is an indigenous fruit tree in the humid low lands and plateau regions of West Africa and belongs to the *Burseraceae* family (Emebiri and Nwufo, 1990). In South-East Nigeria, the trees are grown around homesteads and flowering takes place from January to April. The major fruiting season is between May and October (Kengue and Nyagatchou, 1990). *Dacryodes edulis* is a tree cultivated widely for its edible and nutritious fruits. Generally, the fruit may be cooked in hot water, or roasted/baked in an oven at about 50ºC. The cooked fruit can be eaten with maize, plantain, cassava, cocoyam, bread, etc. (Kengue and Nyagatchou (1990).

The entire plant of *D. edulis* has pharmaceutical properties that are variously exploited by many African communities (Kengue, 2002). Oral treatment against leprosy and it is also gargled as mouth-wash for the treatment of tonsillitis. In the western parts of Cameroon, the bark is crushed and used in concoctions against dysenteries while in central Cameroon, the bark is used to treat toothache (Ajibesin *et al*., 2008). The leaves are boiled in combination with Lantana camara, Cymbopogon citratus and *Persea americana* yielding a steam bath taken to treat fever/headaches and malaria in Republic of Congo (Jiofack *et al*., 2010). The leaves are also crushed and the resultant juice used to treat skin diseases such as scabies, ring worm, rashes, while twigs from branches are sometimes used as chewing sticks (Igoli *et al.*, 2005; Okwu and Nnamdi, 2008). The leaves

and seed are used in Nigeria for animal feed (Ajibesin *et al*., 2008).

The resin from the bark is used in some communities in Nigeria as incense and is believed to send off evil spirits in Nigeria (Sofowora, 2008). Uhunmwangho and Omoregie (2017), determined the oil content in *Dacryodes edulis* and predicts the profitability of Dacryodes edulis as a potential source of oil. The fatty acid composition of *Dacryodes edulis* were majorly palmitic acids as the major saturated fatty acids in *D. edulis*. Stearic isomer, oleic acid, eicosadienoic acid and linolenic acid were the major unsaturated fatty acids in matured fruits. This means therefore, there is a need to ascertain the role of *Dacryodes edulis* pulp oil influencing range it exerts on lipid bilayers which influences the carcinogen- metabolizing enzymes by which it may exert anti-cancer effects. In this study, we investigated the effect of feeding *Dacryodes edulis* pulp oil on 3-methylcholanthrene induced prostatic cancer in male Wistar rats.

2. MATERIAL AND METHODS

2.1 The study location

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

2.2 Reagents/Chemicals

All reagents used were of analytical grade. Methanol (Sigma Chemicals Co, London), Chloroform (Sigma Chemicals Co., London), Benzene (BDH Chemicals Ltd., Eng.), NaCl (BDH Chemicals Ltd., Eng.),

2.3 Plant material

Fresh *Dacryodes edulis* fruits were obtained from farms in Ondo Town, Nigeria. The fruits were authenticated by a Taxonomist of the Botany Department, University of Medical Sciences, Ondo, Nigeria. At each harvest, 40 fruits were collected randomly. The collected fruits were and 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 oil extraction.

2.4 Extraction of oil from african walnut

The Soxhlet extraction method was employed according to AOAC (1996) as reported Uhunmwangho and Omoregie (2017).

2.5 Feeding the animals with diet containing walnut seed oil

The experimental diet comprised of *Dacryodes edulis* oil, (15.0%), of the feeds. Overall, 52 Wistar male rats were used, of which 4 was sacrificed to record zero-day observations. The remaining Animals were randomly divided into three major groups of 16 animals each. Group 1 animals were fed for 12weeks with diet containing *Dacryodes edulis* oil (15%) and the animals injected with 3-methylcholanthrene (150mg/kg body weight) through intraperitoneal injection after 4weeks of feeding. Group 2 were fed for 12weeks with diet containing *Dacryodes edulis* oil (15%) only. Group 3 animals were fed for `12weeks with diet containing no *Dacryodes edulis* oil, and will be given 3-methylcholanthrene (250mg/kg body weight) through intraperitoneal injection after 4weeks of feeding. The animals were palpated weekly to determine the time of appearance of tumors and body weight.

At necropsy, mammary glands were exposed and tumors were excised. Tumor incidence, volume and weight were determined. Animals from each group were sacrificed at and the serum and tissues collected for enzymes and biochemical analysis. Portions of mammary tissues from no tumor bearing and tumor tissue were preserved in RNA later for gene expression studies.

Fatty acid determination: Fatty Acids were determined according to the method of Manni and Caron, (1999) as described by Uhunmwangho and Omoregie (2017).

2.6 Cyclooxygenase-2 (COX-2) and PPAR-γ gene expression:

were placed in triazole (a molecular grid RNA isolating reagent) for COX-2 and PPAR--γ gene expression described by Rani and Kansal (2011).

2.7 Statistical analysis:

The values were expressed as mean ± SE. Kruskal-wallis one-way analysis of variance (ANOVA) was used for the feed intake, body weight, tumor weight, tumor volume and COX-2 and PPAR-γ gene expression using Systat 7.0 software (Spss Inc., Chicago, USA).

3. RESULTS

Table I.0 summarizes the data on incidence, latency period and weight and volume of tumours in prostate gland. The incidence of tumours on pulp oil diet (30.9%) was significantly (P<0.05) lower than on diet without Dacryodes edulis pulp oil (87.4%). The tumour latency period was 6weeks in animals fed with pulp oil compared to 4weeks in animals fed without pulp oil.

Table I.0 Effect of feeding *Dacryodes edulis* pulp oil on prostate carcinogenesis in MCA administered rats.

	Animals fed with pulp oil only	Animals fed with pulp oil + MCA	Animals fed with MCA only
Tumor latency period	No symptoms	6weeks	4weeks
Tumor incidence	Nil	30.9%	87.4%
Tumor weight (g)/ tumor bearing rat	Nil	3.7 ± 1.45	9.4 ± 2.26
Tumor volume (mm3)/ tumor bearing rat	Nil	2988 ± 2.16	7342 ± 1.48

Values are mean ± SE; *P<0.05 compared to Dacryodes edulis pulp oil group.

Major fatty acids composition (%) in prostate gland cells of animals fed with and without pulp oil diet

The major unsaturated FAs were *Dacryodes edulis* pulp oil fed rats' prostate gland cells were GLA, docosatrienoic acid, eicosadienoic acid, linoleic acids are shown in figure 1.

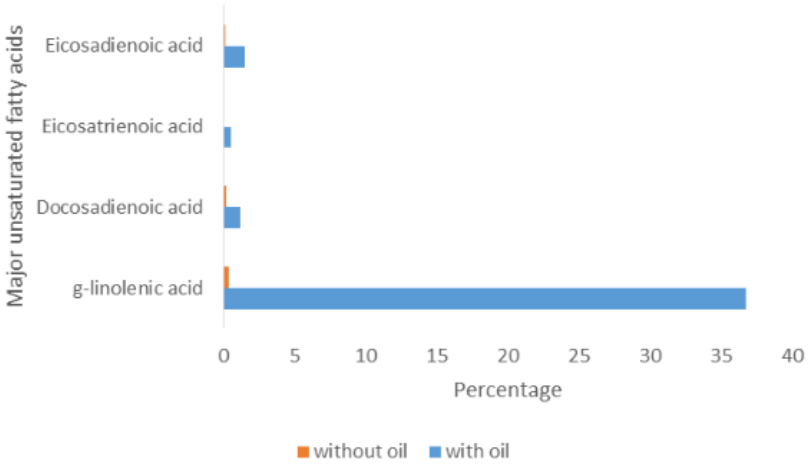

Figure 1: Major fatty acids composition (%) in prostate gland cells of animals fed With and Without pulp oil diet.

Expression of COX-2 and PPAR-γ: The effect of dietary fat on expression of COX-2 and PPAR-γ was investigated in normal prostate gland, *Dacryodes edulis* treated animal prostate gland and tumor tissue tumor bearing rats.

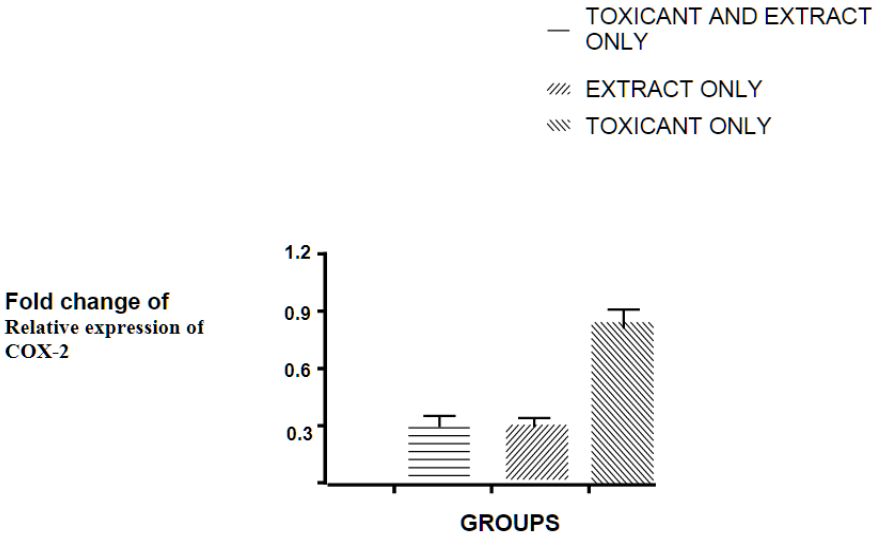

Fig. 2.0: Effect of feeding dacryodes edulis pulp oil on expression of COX-2 in prostate tissue of control and 3-MCA treated rats.

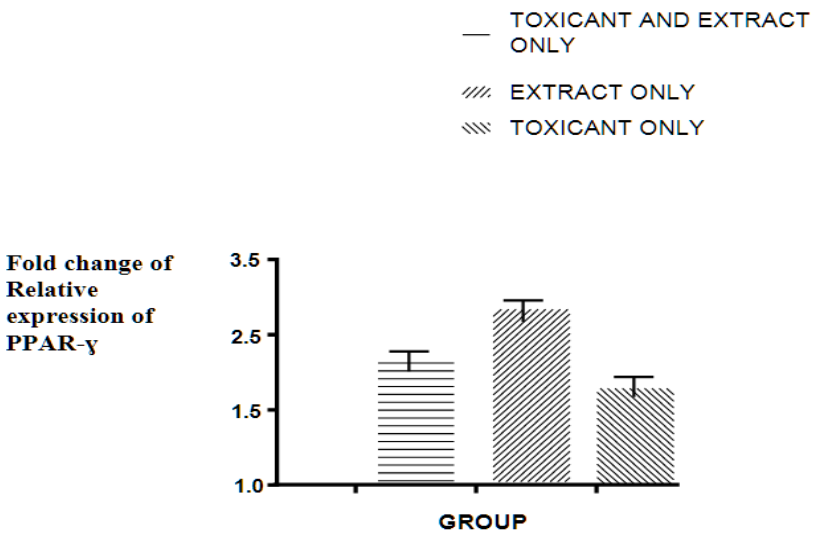

Fig. 3.0 Effect of feeding Dacryodes edulis pulp oil on expression of PPAR-γ in prostate tissue of control and 3-MCA treated rats.

4. DISCUSSION

The prevention of lifestyle-related diseases, such as prostate cancer is of worldwide interest. Fewer tumour incidence, smaller tumour size and greater tumour latency period on animals fed with *Dacryodes edulis* pulp oil was suggestive of protection conferred by *Dacryodes edulis* pulp oil in prostate gland carcinogenesis.

COX-2 is one of the two enzymes that catalyze the first step of prostaglandin synthesis from polyunsaturated fatty acid and in constract to the other cyclooxygenase (COX-1); it is not detected in normal tissues (Mestre *et al*., 1997). It has been suggested that COX-2 is a promising target for cancer treatment and prevention and many pharmacological studies suggests that COX-2 is a therapeutic target in cancer management (Subbaramaiah and Dunnenberg, 2003; Yao *et al*., 2003). Cyclooxgenase-2 gene expression is induced by a wide variety of stimuli in cells fighting inflammatory disorders and cancer, therefore, the level of COX-2 is elevated in various types of cancer tissues in comparison to non-malignant tissues, a study has shown that COX-2 may participate in cancer development through multiple mechanisms such as stimulation of growth, resistance to apoptosis and enhancement of angiogenesis (Liao *et al*., 2007). In the present study, COX-2 (fig.2) was undetectable in normal prostate tissue of group fed with *Dacryodes edulis* pulp oil only, and its expression induced by 3-MCA treatment was significantly higher in tumor tissue as compared to *Dacryodes edulis* pulp oil treated prostate tissue. This shows that gamma-Linolenic acid, docosahexaenoic acid and other polyunsaturated fatty acids present in the pulp oil extract suppressed COX-2 activity by reducing the proliferation of prostate cancer cells.

PPAR-Gamma belongs to the nuclear receptors (NRs), comprising a large family of high conserved transcription factors, which regulates many key processes in normal and neoplastic tissues and plays an important role in inflammation, glucose metabolism and cancer (Olefsky, 2001). Basic research studies have demonstrated that PPAR-Gamma ligands are efficient in the control of tumor progression through their effects on various cellular processes including anti-proliferation, apoptosis, anti-inflammation, metastasis and resistance to angiogenesis.

Figure 3, PPAR- γ activity in animals administered with *Dacryodes edulis* pulp oil only significantly increased, ($p > 0.05$), compared to the group of animals that were administered with MCA only. In the group of animals administered with MCA and treated with the seed oil, PPAR- γ protein activity was also higher compare to animals treated with MCA only ($p<0.05$), but there was significant difference between the PPAR- γ activity of animals administered with MCA but fed with the seed oil, and the animals administered MCA only. Vanden *et al.,* (2012) reported that PPAR- γ can be activated by lipid-rich walnut extract and as showed in the results, *Tetracarpidium conophorum* seed oil extract administered to the rats resident high amount of gamma-Linolenic acid, docosahexanoic acid and eicosatrienoic acid which are unsaturated fatty acid, hence the increase in PPAR- γ protein activity.

Emerging data studies suggests that omega-3 PUFAs, which are found in food, may slow growth of many tumors, including prostate cancer (Berquin *et al*., 2003). Animal studies have suggested that these poly-unsaturated fatty acids are involved in anti-inflammatory, proapoptotic, anti-proliferative and anti-angiogenic pathways making them perfect anti-tumor molecules (Williams *et al*., 2011). Omega-3 PUFA may also regulate other metabolic process, including β-oxidation, cellular signaling of membrane-bound proteins, eicosanoid synthesis, and direct activation of nuclear receptor and transcription factor, all of which may influence the development and progression to cancer.

The results from figure 1.0 shows that in the animals fed with *Dacryodes edulis* exhibited in their plasma membrane the presence of gamma linolenic acid, docosadienoic acid and eicosatrienoic acid but the animals not fed with the pulp oil have these polyunsaturated fatty acid in very low quantity but have oleic acid and arachidic acid being significantly higher. This supports the fact that omega-3 PUFAs resident in the *Dacryodes edulis* pulp oil are involved in anti-inflammatory, anti-proliferative and anti-angiogenic pathways which slows down the growth of cancer tumors and cancer progression, and supports the work of Chamberland and Moon (2015) that the omega-3 fatty acid suppresses cancer development.

These above lines of evidence from this study shows that *Dacryodes edulis* pulp oil contains the essential omega-3 PUFAs which helps in the management and control of prostate cancer, as animal fed with the pulp oil of *Dacryodes edulis* showed the presence of high percentage of gamma linolenic acid and docosahexanoic acid incorporates omega-3 PUFAs in the lipid bio-membrane of the animals and has influenced COX-2 and PPAR-gamma protein expression by inhibiting COX-2 expression and activating PPAR-gamma expression through the disruption of cell cycle progression and induction of apoptosis leading to the prevention of prostate carcinogenesis.

4.1 CONCLUSION

This study has proven that *Dacryodes edulis* pulp oil contains the vital polyunsaturated fatty acid which suppresses COX-2 and activates PPAR-γ thereby curbing prostate cancer and prolonging the latency period.

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Conflict of interests

None.

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