



## Study Of *Dacryodes Edulis* Pulp Oil On 3-Methylcholanthrene Induced Breast Cancer and Expression of Cyclooxygenase-2 And Peroxisome Proliferator Activated Receptor-Gamma in Wistar Rats

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### ABSTRACT

**Background:** Breast cancer is a major cause of death in women, and dietary fat has been implicated among the factors that influence its incidence. This study investigated the effect of *Dacryodes edulis* pulp oil on 3-methylcholanthrene (MCA) induced breast cancer and the expression of cyclooxygenase-2 (COX-2) and peroxisome proliferator activated receptor gamma (PPAR- $\gamma$ ) in female Wistar rats. **Methods:** The pulp oil was extracted with n-hexane using Soxhlet apparatus and characterized by gas chromatography. **Results:** A total of 48 Wistar rats (4weeks old) were used and they were divided into three groups. Group A animals were fed with diet containing 10% *Dacryodes edulis* pulp oil for 14 days before MCA (250mg/kg) was intraperitoneally administered. Afterwards, they were fed with pulp oil for twelve weeks before sacrifice. Group B animals received MCA (250mg/kg) to induce breast cancer and fed with diet containing no *Dacryodes edulis* pulp oil. Group C animals were fed with diet containing 10% *Dacryodes edulis* pulp oil only. COX-2 and PPAR- $\gamma$  were significantly ( $P < 0.05$ ) less and more expressed on pulp oil fed animals respectively. Tumour latency period was longer on animals fed with seed oil compare with those not fed with diet containing pulp oil. **Conclusion:** Results from gas chromatography analysis indicated that the omega-3 polyunsaturated fatty acids were incorporated into the mammary gland cell membrane which opposed the progression of carcinogenesis animals treated with *Dacryodes edulis* pulp oil, this indicates that *Dacryodes edulis* pulp oil is able to oppose breast carcinogenesis induced by 3-methylcholanthrene.

#### Keywords:

*Dacryodes edulis*,  
3-methylcholanthrene,  
Cyclooxygenase-2,  
Carcinogenesis,  
Pulp oil.

### INTRODUCTION

*Dacryodes edulis* is a dieicious shade-loving specie of non-flooded forests in the humid tropical zone. It

Malaysia, Liberia and Sierra Leone<sup>1</sup>. It is known as ‘elemi’ in Yoruba, ‘Ube’ in Igbo and "Atili" in Hausa. It belongs to the family ‘Burseraceae’ whose members are characterized by an ovary of 2-5 cells, prominent as in ducts in the bark, wood and intrastaminal disk<sup>2</sup>. It was shown to possess antimicrobial, antioxidant, cardiovascular,

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antidrapanocytary activities and also show no signs of toxicity<sup>3</sup>. It was also reported that *Dacryodes edulis* possess anti-proliferative, pro-aprotic effects against tumor cells, thus, being capable of breaking down multi-cellular tumors, regulate molecules, in apoptosis, signal transduction and cell cycle progression, hence, its anti-cancer activity<sup>4</sup> *edulis* is a versatile plant in African ethnomedicine as its various parts are employed to treat several diseases. The bark of the plant has long been used to cicatrize wound in Gabon<sup>2,4</sup>. In Democratic Republic of Congo, the plant is employed for the treatment of leprosy, tonsillitis and dysentery, anaemia, spitting blood, pains and stiffness and skin diseases<sup>5</sup>. In Congo Brazzaville, the leaves are boiled with those of *Lanata camara*, *Cymbopogon citretus* in water to form a decoction for treating malaria<sup>6</sup>. The bark resin is used in Nigeria to treat parasitic-skin diseases and jiggers and when applied in lotions and creams, the resin smoothens and protects the skin. The aroma of the resin is liberated through burning, which is believed to ward-off evil spirit in Nigeria<sup>7</sup>. The leaves are often crushed and the juice released to treat generalized skin diseases such as scabies, ringworm, rash and wound, while the stems are employed as chewing sticks for oral hygiene<sup>4</sup>. The essential oil of the plant showed potent antibacterial effect against *staphylococcus aureus*, *Bacillus aurens*, *Escherichia coli* and *proteus minibillis*<sup>8-10</sup>. *Dacryodes edulis* has been reported to decrease the HDL cholesterol level in serum of rats<sup>11</sup>. The genus *Dacryodes* consist of about 40 species<sup>8,9</sup>. Two varieties are recognized; *Var. edulis* and *Var. parricarpa*, whose conical fruits are smaller with the pulp. *Var. edulis* exhibit venticillate branching, while branching is slender and opposite in *Var. parricarpa* (Ajibesin, 2011). Breast cancer starts when cells in the breast begin to grow out of control<sup>13</sup>. These cells usually form a tumor that can often be seen on an x-ray or felt as a lump. The tumor is malignant (cancer) if the cells can grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body. Breast cancer occurs almost entirely in women, but men can get breast cancer too<sup>13</sup>. Around 5% of breast cancers and up to 25% of familial breast cancer cases are caused by a BRCA1 or BRCA2 mutation<sup>14</sup>. A woman carrying a BRCA1 mutation has a 65-95% lifetime risk of breast cancer, and more than 90% of hereditary breast and ovarian cancers are thought to be due to a mutation in BRCA1 or

BRCA2<sup>14</sup>. Everyone has BRCA1 and BRCA2 genes. Some people have an inherited mutation in one or both of these genes that increases the risk of breast cancer. BRCA1/2 mutations can be passed on from either parent and can affect the risk of cancers in both women and men. Breast cancer women who have a BRCA1 or BRCA2 gene mutation have an increased risk of breast cancer. Estimates of risk are different for BRCA1 carriers and BRCA2 carriers. BRCA1/2 mutations are thought to explain a large portion of hereditary breast cancers<sup>14</sup>. A person who has a BRCA1/2 mutation is sometimes called a BRCA1/2 carrier. The identification of the BRCA1 and BRCA2 genes will have vast significance in furthering our understanding of breast pathogenesis. These two genes are tumor suppressor genes shown to be involved not only in breast cancer but also in ovarian and prostate cancers. BRCA1 and BRCA2 are responsible for 80-90% of all familial breast cancer<sup>14</sup>. 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 shall investigate the effect of feeding *Dacryodes edulis* pulp oil on 3-methylcholanthrene induced mammary cancer in female Wistar rats.

## MATERIAL AND METHODS

### 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. Methanol (Sigma Chemicals Co, London), Chloroform (Sigma Chemicals Co., London), 3-methylcholanthrene, Sulphuric Acid Aldrich Chemical Company, USA.

### Plant material (Sample collection)

Fresh *Dacryodes edulis* fruits were obtained from farms in Laje town near Ondo city, Ondo State, Nigeria. The fruits were authenticated by a Taxonomist of the Botany Department, University

of Medical Sciences, Ondo. The collected fruits were cleaned with a moist soft cotton wool and then the seeds carefully separated from the fruits and dried at 65<sup>0</sup> C 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<sup>0</sup> C for extraction.

#### Extraction of oil from African Walnut

The Soxhlet extraction method was be employed<sup>15,16</sup>.

#### Ethical approval

Ethical approval was granted by the University of Medical Sciences, Ondo, Ethics committee for the use of animals.

#### Feeding the animals with diet containing *Dacryodes edulis* oil

Female Wistar rats (28 days old) were obtained from the animal house of the University of Medical Science, Ondo, and were housed in metal cages in a well-ventilated room and they were allowed access to water and ad libitum. The experimental diet comprised among others *Dacryodes edulis* pulp oil (10.0%). Overall, 48 female Wistar rats were used. The animals were randomly divided into three major groups of 16 animals each. Group 1 animals were fed for 12 weeks with diet containing *Dacryodes edulis* pulp oil (10%) and the animals administered 3-methylcholanthrene (250 mg/kg body weight) intraperitoneally after 4 weeks of feeding. Group 2 were fed for 12 weeks with diet containing *Dacryodes edulis* pulp oil (10%) only. Group 3 animals were fed for 12 weeks with diet containing no *Dacryodes edulis* pulp oil, and were administered 3-methylcholanthrene (250 mg/kg body weight) intraperitoneally after weeks 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 the end of 12 weeks, and the serum and tissues collected for enzymes and biochemical analysis. Portions of mammary tissue from no tumor bearing and tumor tissue were preserved in RNA later for gene expression studies.

Another portion of tumor tissue was fixed in formalin (10%) for histopathological studies.

*Fatty Acid Determination:* Fatty Acids were determined according to the method of Manni and Caron<sup>17</sup>.

*COX-2 and PPAR-γ gene expression:* The liver samples were placed in triazole (a molecular grid RNA isolating reagent). The samples were homogenized and chloroform was added for homogenate gradient separation. This was followed by centrifugation at 15,000 rpm for 15 minutes. After centrifugation, the upper phase (clear supernatant containing RNA) was aspirated into a new sterile eppendorf tube of 1.5 ml. The clear supernatant was precipitated by adding isopropanol. This was followed by centrifugation at 15,000 rpm for 5 minutes. RNA pellet was air dried for 15 minutes and resuspended in nuclease free water (30 microliters). RNA samples were quantified and absorbance was checked using a spectrophotometer. RNA samples were optimized using PCR machine for 1 hour at 42<sup>0</sup> C. The samples were amplified and gel electrophoresis was carried out at 70 volts, 500 milli amperes for 10 minutes, the samples were placed in UV documentary for viewing the expression bands.

*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). Statistical analysis of tumor incidence was done by Chi-square test using Systat 7.0 software. A difference with P<0.05 was considered statistically significant.

## RESULTS

Table 1. Summarizes the data on incidence, latency period and weight and volume of tumors in mammary gland. The incidence of tumors on pulp oil diet (35.9%) was significantly (P<0.05) lower than on diet without *Dacryodes edulis* pulp oil (87.4%). The tumor latency period was 7 weeks in animals fed with pulp oil compared to 4 weeks in animals fed without pulp oil. The average size of tumor was generally larger in animals that was not fed with *Dacryodes edulis* pulp oil than in animals

fed with diet containing *Dacryodes edulis* pulp oil. Similarly, average tumor volume was significantly ( $P < 0.05$ ) less in animals fed with diet containing *Dacryodes edulis* pulp oil than animals fed with diet containing no pulp oil.

**Table 1** Effect of feeding *Dacryodes edulis* pulp oil on mammary 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	7 weeks	4 weeks
Tumor incidence	nil	35.9%	87.4%
Tumor weight (g)/ tumor bearing rat	nil	3.2 ± 1.45	9.4 ± 2.26
Tumor volume (mm <sup>3</sup> )/ tumor bearing rat	nil	2461 ± 2.16	7342 ± 1.48

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

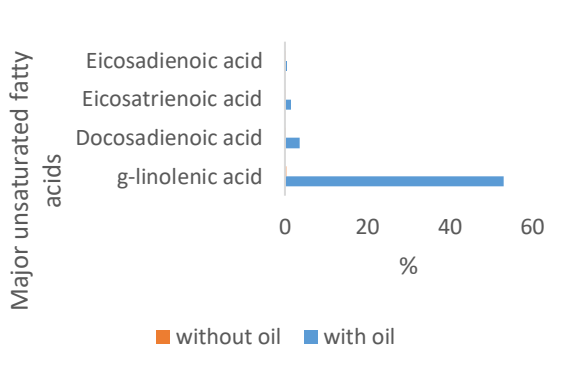


Figure 1: Major fatty acids composition (%) in mammary tissue of animals fed with and without pulp oil diet.

The major unsaturated FAs in mammary cells of animals fed diet containing *Dacryodes edulis* pulp oil were gamma linoleic acid, docosatrienoic acid, eicosadienoic acid, linoleic acid. The concentration of the FAs from the *Dacryodes edulis* pulp oil fed rats were significantly different ( $p < 0.05$ ) from animals not fed with *Dacryodes edulis* pulp oil.

The effect of dietary fat on expression of COX-2 and PPAR- $\gamma$  was investigated in normal mammary gland, *Dacryodes edulis* treated animal mammary gland and tumor bearing rats tissue. The COX-2 was not expressed in normal mammary

tissue but its expression was induced in response to MCA treatment.

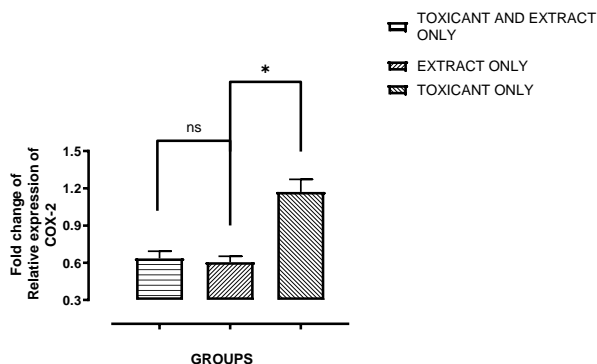


Figure 2. Expression of Cyclooxygenase-2 in the in the mammary tissues. Values are mean ± SEM. (\* =  $P < 0.05$ ).

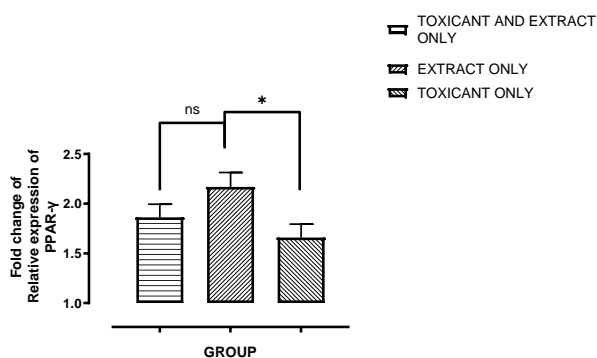


Figure 3. Expression of Peroxisome Proliferator activated receptor gamma in mammary tissue. Values are mean ± SEM. (\* =  $P < 0.05$ ).

In MCA treated rats, the expression of COX-2 was greater in tumor bearing than in no tumor bearing and *Dacryodes edulis* treated rats' tissues. Furthermore, the expression of COX-2 was greater in tumor tissues than in other tissues studied. In carcinogen treated rats wherein no tumor appeared, the expression of PPAR- $\gamma$  in *Dacryodes edulis* only fed rats was almost of the same magnitude as observed in treated counterpart's groups.

## DISCUSSION

Fewer tumour incidence, smaller tumour size and greater tumour latency period on animals administered with MCA pulp oil were suggestive of protection conferred by *Dacryodes edulis* pulp oil in

mammary gland carcinogenesis. The role of *Dacryodes edulis* pulp oil in mammary carcinogenesis may be explained by their ability to modulate pathway of prostaglandin synthesis. Mammary carcinogenesis is triggered by inappropriate induction and upregulation of COX-2. It was reported<sup>18</sup> that the expression of normally silent COX-2 gene results in excess production of prostaglandin E2 and increase in local estrogen biosynthesis by aromatase.

In this study, COX-2 was undetectable in normal mammary tissue, the 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 mammary tissue. Cyclooxygenase-2 is an inflammation associated enzyme involved in the pathogenesis of carcinogenesis. Inhibition of COX-2 and blockade of prostaglandin cascade may lead to the reduction of carcinogenesis<sup>19</sup> but over expression of COX-2 initiates and promotes carcinogenesis. Figure 2.0 shows there was no significant difference ( $p > 0.05$ ) between the group fed with *Dacryodes edulis* pulp oil only and the animals in the group administered with MCA but treated with *Dacryodes edulis* pulp oil. Hence, there was a significant difference ( $p < 0.05$ ) in the expression of COX-2 between the groups fed with *Dacryodes edulis* pulp oil and the group which was induced with 3-Methylcholanthrene but was not treated with *Dacryodes edulis* pulp oil which implies that the use of *Dacryodes edulis* pulp oil has a high activity against COX-2 in groups fed with *Dacryodes edulis* pulp oil. This proves that *Dacryodes edulis* pulp oil extract was effective in suppressing the expression of COX-2 in groups that were fed with the pulp oil. This result is in agreement with Chinery et al<sup>20</sup> who conducted an experiment on cyclooxygenase-2 expression to colorectal cancer cells. Peroxisome proliferators activated receptor- $\gamma$  (PPAR- $\gamma$ ), a key component in regulation of growth and progression of mammary cancer, is expressed in normal as well as in malignant mammary epithelial cells and its activation by ligands induces cellular differentiation<sup>20-22</sup>. The activation of PPAR- $\gamma$  induces proapoptotic caspase-3 protein in human liver cancer cell lines and reduces antiapoptotic Bcl-2 protein level in human colon cancer cell<sup>23</sup>. An inverse relationship between COX-2 and PPAR- $\gamma$  expression was observed in the present study and it

was associated with decreased mammary tumour incidence in *Dacryodes edulis* pulp oil fed rats compared to non-*Dacryodes edulis* pulp oil fed ones. In the genesis of breast cancer, evidences suggest that induction of COX-2 and downregulation of PPAR- $\gamma$  can be the key components<sup>24,25</sup>. Simultaneous targeting with COX-2 inhibitor (celecoxib) and PPAR- $\gamma$  agonist [N - (9-fluorenyl-methyloxycarbonyl)-L-leucine] has been reported to inhibit mammary gland carcinogenesis in rats<sup>24</sup>. Further, activation of PPAR- $\gamma$  by ciglitazone (PPAR- $\gamma$  ligand) decreases the COX-2 expression (Yang and Fruchth, 2001), and the inhibition of COX-2 induces PPAR- $\gamma$  expression<sup>26</sup>. Feeding GLA during pubescent mammary gland development period lowers the population and proliferating activity of the terminal end buds' cells<sup>27</sup>, which are the target sites for development of adenocarcinomas in response to carcinogenic stimulus. In this present study, the feeding of animals with *Dacryodes edulis* pulp oil started during the pubescent period of mammary gland development might have resulted in the decreased tumour incidence and progression to malignancy. The anticarcinogenic effect of GLA may be partly explained by its effect on the COX-2. Gamma linolenic acid affects the COX-2 at the level of mRNA as well as protein in cultured macrophage cell line<sup>27</sup>. It represses AP-1 mediated activation of COX-2 transcription in MCF-7 breast cancer cells<sup>27</sup>. The treatment of colon cancer cells with CLA inhibits cell proliferation; increases expression of PPAR- $\gamma$  and downregulates APC and c-myc proteins<sup>12,28</sup>. The higher tumour incidence and faster progression of MCA induced mammary carcinogenesis in rats fed on *Dacryodes edulis* pulp oil compared to animals not fed with *Dacryodes edulis* pulp oil could be due, partly, to high content of GLA, eicosadienoic acid, dihomotrienoic acid and linoleic acid in *Dacryodes edulis* pulp oil.

## CONCLUSION

The conclusion from this study is that *Dacryodes edulis* pulp oil opposed MCA induced mammary carcinogenesis and the effect is mediated through decreased expression of COX-2 and increased expression of PPAR- $\gamma$ .



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#### CONFLICTS OF INTEREST

No conflict of interest among the authors.

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