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Effect of *Imbrasia truncata* oil and docosahexaenoic acid on cancer antigen CA15-3, biochemical and histopathological markers of female Wistar rats with DMBA-Induced Breast Cancer

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ABSTRACT

This study aimed at evaluating the effect of *Imbrasia truncata* oil on cancer antigen CA15-3, biochemical and histopathological markers of female Wistar rats with 7,12-Dimethylbenz[a]anthracene-Induced (DMBA) breast cancer in comparison to docosahexaenoic acid (DHA). Oil was extracted from *Imbrasia truncata* caterpillar and analyzed for its quality. For the animal bioassay, 56 female rats aged 6-8 weeks (55-60 g each) were used. Breast cancer was induced in 50 rats using a single dose (50 mg/kg BW) of DMBA dissolved in 1 mL olive oil. At week 19, rats with similar tumor sizes (18) and those of the same batch that were not induced for breast cancer (06) were randomized into four groups of six rats each: The negative control, the positive control, the test and the normal groups that received distilled water (250 mg/kg), DHA (125 mg/kg), *Imbrasia* oil (1000 mg/kg) and distilled water (250 mg/kg) respectively by gavaging every day for 28 days. Tumor size was measured after every 02 days. The rats were allowed to fast on the 28th day and were sacrificed on the 29th day. The blood was collected and centrifuged to obtain the serum on which the breast cancer antigen CA15-3 level, the lipid profile, serum enzymes activities [Aspartate aminotransaminase (AST), alanine aminotransaminase (ALT) and lactate dehydrogenase (LDH)] and creatinine level were measured. Organs were collected for histopathological studies. Results showed that the oil quality indices were within the accepted standard ranges. Docosahexaenoic acid and *Imbrasia truncata* oil led to the lowest tumor sizes and significantly increased the HDL cholesterol and reduced breast cancer antigen tumor marker CA15-3 level. They also significantly reduced the AST, ALT, LDH activities and creatinine level of the serum compared to the negative control group. The microstructure of organs showed that docosahexaenoic acid and *Imbrasia truncata* oil have a protective effect on them. These findings suggest that *Imbrasia truncata* oil and DHA delay tumor progression and exhibit biochemical regulatory effects that may help in the management of breast cancer.

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Introduction

The idea that functional food ingredients and nutraceuticals present in food when supplemented to the diet can help in a significant manner in the prevention and management of non-communicable diseases such as cancer has been reported (Chatterjee et al., 2010; Siddiqui et al., 2013; Alqualshy et al., 2022; Noguchi et al., 1997). Nutraceuticals from natural food products as well as some natural functional food ingredients are gradually becoming a promising addition to anti-cancer drugs that are known for their high cost and toxic effect (Vanderpuye et al., 2017). They are also coupled to treatments such as surgery, chemotherapy and radiotherapy. These conventional methods of cancer treatment are not accessible to everyone and in some part of the African continent; the facilities are not yet available. The consequence is the increase in cancer cases due to late diagnosis and no accessibility to the above mentioned treatments (Vanderpuye et al., 2017). It is well known that cancer is one of the major cause of death in the world, with about 19.3 million cases and 10 million deaths in 2020 (Sung et al., 2021). In this ratio, breast cancer is reported to have the majority (50-80%) (Mvondo et al., 2021). This cancer is also known as the most prevalent in women in developing countries. In Cameroon, it remains a serious public health problem for women, with about 4170 (20.1%) new cases and 2108 (16%) deaths annually (Sung et al., 2021).

As solution, researchers have been working towards investigating the preventive and curative effect of nutraceuticals, natural plant extracts and functional food ingredients against cancer cell lines or breast cancer induced using a carcinogen [7,12 dimethylbenz(α)anthracene-(DMBA)] in female rats. Many chemopreventive studies used the DMBA to induce breast cancer in female rats, because the model appears to be the best method to mimic the genesis and development of breast cancer in humans (Witty et al., 1995).

Amongst the nutraceuticals targeted, omega-3 fatty acids are the most solicited. Reports show that population that consume food rich in omega-3 fatty acids have lower incidence to several cancer types including breast, colon and prostate cancers compared to those that consume lower amount of omega-3 fatty acids rich food (Hardman, 2002). The omega-3 fatty acids that have been highly related to anti-cancer activities are those from fish oil. These include docosahexaenoic acid and eicosapentaenoic acid. Similarly, fish oils, especially those rich in the previously mentioned fatty acids have been proven to be effective in the management and prevention of certain cancer when supplemented to the diet. As examples, Chatterjee et al. (2010) demonstrated that the combination of fish oil (Maxepa) and 1α, 25-dihydroxyvitamin D3 has chemopreventive effect against breast cancer induced with DMBA in female rats. Manna et al. (2011) reported that the combined supplementation of fish oil and vanadium stops tumor development, cell proliferation and induces apoptosis in female rats with DMBA-induced breast cancer. The anti-cancer activity of docosahexaenoic acid plus curcumin against breast cancer induced with DMBA in rats was reported by Siddiqui et al. (2013). Alqualshy et al. (2022) showed that docosahexaenoic acid has chemopreventive effect in hamster with DMBA induced buccal pouch cancer. Noguchi et al. (1997) demonstrated the chemopreventive potential of eicosapentaenoic acid and docosahexaenoic acid at low doses in rat with DMBA-induced mammary cancer. Though several studies have been done on the use of fish oil or the omega-3 fatty acids from it against DMBA-induced breast cancer, there is a need to explore other animal sources of omega-3 (rich in α-linolenic acid instead) that are edible and test their activity against cancer. Oil from edible insects from Cameroon represents a potential functional food ingredient that can help against this disease.

Imbrasia truncata oil, extracted from edible caterpillars commonly consumed in Central and Southern Africa, has gained attention as a potential source of bioactive lipids. It is traditionally valued for its nutritional density and sustainability, but more recently, its fatty acid profile has been scientifically examined. Womeni et al. (2009) analyzed the lipid content of this oil and found that it contains 38.01% α-linolenic acid (an omega-3 PUFA). The oil also includes appreciable levels of oleic acid (11.27%), linoleic acid (9.84%). Notably, the high content of omega-3 fatty acids in this oil is comparable to that found in marine sources, suggesting potential for anti-inflammatory and antioxidant action. Emerging evidence supports the immune-modulatory and anti-inflammatory effects of insect-derived oils rich in essential fatty acids. Studies have shown that such oils can attenuate inflammation, reduce oxidative stress, and regulate immune pathways features that are highly relevant in the context of cancer prevention (Nguimbous et al., 2021; Kolobe et al., 2023). However, despite its traditional use and favorable composition, *Imbrasia truncata* oil has not been systematically studied for its preventive or therapeutic potential in breast cancer models. The objective of this study was to evaluate the effect of some variety of *Imbrasia truncata* oil on cancer antigen CA15-3, biochemical and histopathological markers of female Wistar rats with DMBA-Induced breast cancer in comparison to docosahexaenoic acid.

Materials and methods

Materials

7, 12-dimethylbenz (a) anthracene (DMBA) and docosahexaenoic acid (powder form) were purchased from sigma-Aldrich (Starnberg, Germany). All the other solvents and chemicals used were of analytical grade.

1,200 g of *Imbrasia truncata* caterpillar (Figure 1) was obtained from a farmer in East region, Cameroon in August 2024.

56 female albino Wistar rats aged 6-8 weeks old weighing 55-60 g were purchased from a farmer in Yaoundé, Center Region of Cameroon.

Figure 1. Caterpillars (*Imbrasia*) (Francis Hannawey, 2010)

Methods

Extraction and characterization of Oil

The extraction of the *Imbrasia truncata* oil was done using the mixture methanol-chloroform-water (2:2:1) (Bligh and dyer, 1959). About 100 g of *Imbrasia truncata* flour was weighed and introduced in a blender and 200 ml of methanol was added. The mixture blended for 2 min and 100 ml of chloroform was added. After blending for 2 min, another 100 ml of chloroform and 100 ml of distilled water were added. After proper mixing for 2 min, the solution was filtered using a muselin cloth and the cake was discarded. After that, the filtrate was again filtered using the whatman paper n° 1. The obtained filtrate was introduced into a funnel. The phase containing the mixture oil and chloroform was collected and evaporated using a rotatory evaporator at 50 ^{0}C under vacuum to eliminate the solvent. The extracted oil was transferred dark glass container and kept in the freezer at -18 °C for further use.

The oil sample was characterized by determining its quality indexes and by Fourier-Transformed Infrared Spectroscopy.

The quality indexes analyzed were: the peroxide value, which was determined using the standard spectrophotometric method of IDF 74A: 1991 (IDF, 1991); the p-anisidine and the acid values which were determined using the procedure in AOCS Official Method Cd 18-90 and Cd 1-15 (AOCS, 2003). The iodine value was measured by the AOCS method Cd 1-25 (AOCS, 2003).

The Fourier-transformed infrared spectroscopy of the oil sample was obtained using the method described by Liang et al. (2013). Shimadzu IRPrestige-21 equipment coupled with the DLATGS detector was used. Beam splitter was Potassium bromide and the controller IRsolution software. 20 µL of oil was released between two KBr disks, to create a thin film in the 4000-500 cm^{-1} IR region. The analysis was done in duplicate against the blank which was made up of clean empty KBr disks.

Animal assays

Ethical Clearance

All animals were cared for and used in agreement with international standard guidelines for animal use. An ethical clearance for animal handling and care was obtained from the University of Buea Institutional Animal Care and Use Committee with the reference number UB- IACUC N^0 02/2025.

Preparation of Animal Feed

A standard diet composed of corn-meal (36.7%), wheat meal (36.6%), bone meal (14.5%), crushed fish (4.8%), palm kernel cake (7.3%), iodide salt (0.3%) and vitamin (0.01%) (Zingue et al., 2024).

Animal Grouping and Feeding

For this study, 56 female albino Wistar rats aged 6-8 weeks (55-60 g) were used. The rats were allowed to acclimatize under a 12 h light-dark cycle at room temperature for 7 days in cages containing sawdust, with ad libitum access to food and water prior to the start of the experiment. Out of the 56 rats, 50 were anesthetized (using 10 mg/Kg diazepam and 50 mg/Kg ketamine intraperitoneally) and cancer induced in them by injecting each of them with a single dose (50 mg/kg BW) of DMBA dissolved in 1 mL olive oil via subcutaneous intra-mammary route as reported by Zingue et al. (2024). The six other rats that were not induced served as normal group. To avoid infection, all DMBA-induced rats received 89 mg/kg of amoxicillin by gavage for 10 days while the normal group received distilled water (Zingue et al., 2024). The rats were palpated after every 2 days to check if the tumor is developing. Breast tumor started appearing and being palpable around the 19th week. Rats with palpable tumors of similar sizes (18) were randomized into three groups of six rats each plus the normal group (06) for a total of 24 rats. Betadine and penicillin ointment were used to clean and treat the rats in case of any wounds twice daily. The randomized rats (Table 1) were used for the continuation of the study. The positive control group was given docosahexaenoic acid (125 mg/kg) (El-Mesery et al., 2009) while the test group was given *Imbrasia* oil (1000 mg/kg) by gavaging every day for 28 days (Djikeng et al., 2025). The tumor size was measured using a caliper after every 2 days to check for tumor size reduction and its volume (length, width, and height) calculated using the formula below Mvondo et al. (2009):

$$\frac{\pi}{6} \times length \times width \times height$$

On the 28th day the rats were allowed to fast overnight and they were sacrificed on the 29th day.

Animal sacrifice and sample collection

The animals were sacrificed on the 29th day after they were allowed to fast overnight. Before the sacrifice, they were anesthetized using Ketamine 60 mg/kg and Diazepam 10 mg/kg body weight. Blood was collected by cardiac puncture using a 5 mL syringe. The blood was introduced in tubes without EDTA and centrifuged at 3500 rpm for 15 min to obtain the serum. The serum was stored at -20˚C for the analysis of tumor marker and biochemical parameters.

Analysis of biochemical parameters

The lipid profile (triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol levels), serum enzymes activities (aspartate transferase, alanine transferase, lactate dehydrogenase) and creatinine level were measured following the procedure given on the commercial kits.

Analysis of serum breast cancer tumor marker

The measurement of cancer antigen 15-3 concentration in rat serum was performed using a sandwich enzyme-linked immunosorbent assay (ELISA) as described in the Biovantion Human CA 15-3 ELISA kit protocol, Lot number 202503.

Histopathology of organs

Histopathological evaluation was conducted to examine the microscopic architecture and potential pathological alterations of sampled organ tissues. Small sections of each organ were collected and processed to enable detailed cellular evaluation under a microscope. The analyzed samples included breast tumor, liver, pancreas, kidneys, brain, and heart tissues. Analyses were done according to the method described by Cardiff et al. (2014). The method involved fixing the specimen, trimming, pre-embedding (Dehydration, Clearing, and Paraffin Infiltration), embedding, sectioning, staining and mounting. Following staining and mounting, all tissue sections were examined under a light microscope connected to a computer, at appropriate magnifications of ×100 and ×200 to assess morphological features. The evaluation focused on cellular architecture and pathological changes, including inflammation characterized by leukocyte infiltration, fibrosis, and tumor characteristics. Images were collected, edited and interpreted.

Table 1. Animal Group Distribution and Characteristics

Groups	Characteristics
Group 1 (normal)	Normal rats receiving distilled water at a dose of 250 mg/Kg
Group 2 (negative control)	Rats with cancer receiving distilled water at a dose of 250 mg/Kg
Group 3 (positive control)	Rats with cancer receiving Docosahexaenoic acid at a dose of (125 mg/kg BW)
Group 4 (test group)	Rats with cancer receiving oral administration of 1000 mg/kg of Imbrasia oil daily for 28 days by gavaging

Statistical Analysis

Analysis was done in triplicate for oil characterization meanwhile for analysis regarding animals six assays were performed (n=6). The data were subjected to one-way analysis of variance (ANOVA) using Statgraphics Centurion version XVI in order to evaluate the statistical significance of the data. Pair comparison was done. A probability value at $p<0.05$ was considered to be statistically significant.

Results

Oil Quality Parameters

The peroxide, p-anisidine, acid, iodine and Thiobarbituric acid values were respectively 13.83 (meq O_2/kg), 12.21, 11.28 (mg KOH/g), 74.33 (g I_2/100g), and 6.60 (ppm), as presented in Table 2.

The Fourier transform infrared spectroscopy of *Imbrasia truncata* caterpillar oil sample is presented in Figure 2. No significant ($p>0.05$) peak was noted between 3250 and 3750 cm^{-1}. However, a peak with high intensity appeared at 3011, 2850 and 2920 cm^{-1}. A strong and abundant peak was also observed at 1750 cm^{-1} and a low one around 1650 cm^{-1}. At the level of fingerprint region, abundant peaks were recorded around 1500, 1160 and 721 cm^{-1}.

Average Tumor Size and Morphology

Figure 3 presents the average tumor size of Wistar rats with cancer throughout the experimental period. The negative control group showed significantly ($\rho<0.001$) higher tumor size compared to the other groups. It was followed by the group consuming *Imbrasia truncata* oil and the group taking Docosahexaenoic acid. No significant ($p>0.05$) difference was recorded in the tumor size of both the groups taking DHA and *Imbrasia truncata* oil.

Figure 4 presents the tumor morphology of Wistar rats. Negative control (DMBA Alone) presented the highest dimension (8.5 cm), followed by DMBA+*Imbrasia truncata* oil (4.5 cm). DMBA+DHA presented the smallest (4 cm) tumor size. The normal group had no tumor.

Lipid Profile

Table 3 presents the lipid profile of various groups after the experimental period. Groups receiving DHA and *Imbrasia truncata* oil presented significantly ($p<0.01$) higher HDL cholesterol levels compared to the other groups. The lowest HDL level was recorded with the negative control group. The normal group and the group taking *Imbrasia truncata* oil presented significantly ($p<0.001$) higher triglycerides levels. The lowest value was obtained with negative control group. For the total and LDL cholesterols, the group receiving *Imbrasia truncata* oil exhibited the highest ($p<0.001$) value compared to the other groups. It was followed by the negative control group.

Serum Enzymes Activities, Creatinine and CA15-3 Tumor Marker Levels

Table 4 presents the serum enzymes [Alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH)] activities, creatinine and breast cancer antigen CA15-3 levels of DMBA-induced breast cancer wistar rats. Generally, the negative control group disclosed significantly ($p<0.05$) higher creatinine level, LDH, AST and ALT activities compared to the other groups.

Table 2. Oil quality indices

Parameter	Value	(FAO/WHO, 2017)
Peroxide value (meq O_2/Kg)	13.83 ±1.75	≤15
p-Anisidine value	12.21 ±0.34	≤20 in fish oil
Acid value (mg KOH/g)	11.28 ±0.00	≤4
Iodine value (gI_2/100g)	74.33 ±2.47	/
Thiobarbituric acid value (ppm)	6.60 ±2.07	/

Table 3. Lipid profile

Groups	HDL cholesterol (mg/dL)	Triglycerides (mg/dL)	Total cholesterol (mg/dL)	LDL cholesterol (mg/dL)
Normal group	60.73±1.40^{a}	122.60±15.64^{a}	132.54±16.89^{ab}	47.28±4.12^{a}
Negative control (DMBA Alone)	57.75±2.1^{b}	85.50±5.90^{b}	142.06±16.72^{a}	67.20±b5.18^{b}
DMBA + Docosahexaenoic acid	86.29±0.00^{c}	99.40±2.33^{b}	129.76±1.68^{b}	23.58±0.56^{c}
DMBA + *Imbrasia* caterpillar oil	95.21±2.4^{d}	198.86±5.21^{c}	228.57±0.00^{c}	93.59±0.00^{d}

n=6. Values are presented as mean ±standard deviation $^{a-d}$ values of the same column with different super scripts are significantly different at $p<0.05$

Table 4. Serum enzymes, creatinine, and CA 15-3 levels of DMBA-Induced Breast cancer Wistar rats

Groups	Creatinine (µmol/L)	LDH (U/L)	AST (U/L)	ALT (U/L)	CA15-3
Normal group	0.85±0.03^{a}	157.85±15.23^{a}	28.81±2.55^{a}	19.64±2.98^{a}	13.07±0.65^{c}
Negative control (DMBA Alone)	1.60±0.34^{a}	475.31±11.24^{b}	129.78±16.22^{b}	50.63±0.00^{b}	34.91±0.98^{b}
DMBA + Docosahexaenoic acid	0.68±0.02^{b}	293.44±6.68^{c}	81.19±16.04^{c}	23.74±3.62^{a}	26.08±0.64^{a}
DMBA + *Imbrasia caterpillar* oil	0.75±0.03^{ab}	343.03±27.18^{d}	51.01±3.87^{d}	18.16±2.34^{a}	26.41±0.74^{a}

n=6. Values are presented as mean ±standard deviation $^{a-c}$ values of the same column with different super-scripts are significantly different at $p<0.05$.

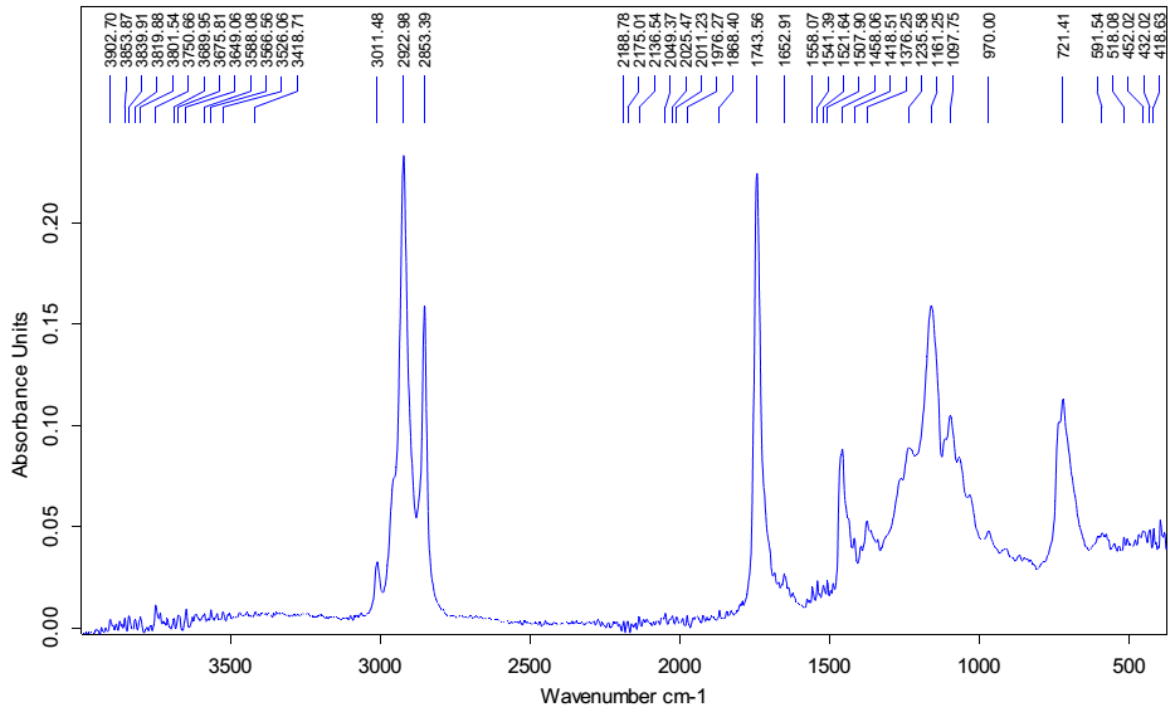


Figure 2. Fourier-Transformed Infrared spectrum of *Imbrasia* caterpillar oil

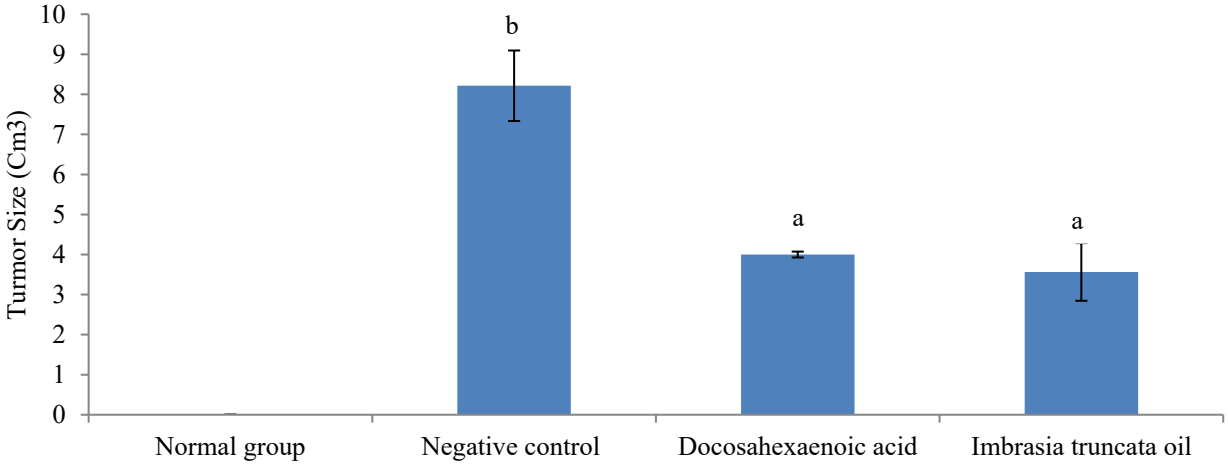


Figure 3. Tumor volume

n=6 values are presented as Mean±Standard deviation. a-b values of the same column with different superscripts are significantly different at $\rho<0.05$.

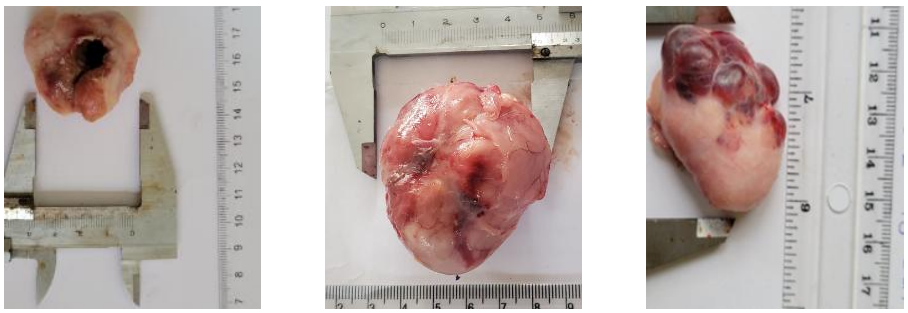
Histopathological Studies

The effect of *Imbrasia truncata* oil and docosaheaenoic acid on the micro-architechture of organs is shown in Figure 5.

Looking at the tumor, in the negative control group, mucin is observed within the ductal lumens, and the cytoplasm of the cells appeared highly granulated. Some ducts have interruptions in the basement membrane, along with papillary projections of malignant cells. The tumor displayed varying levels of differentiation, consistent with adenocarcinoma. The tissue section has characteristic features of adenocarcinoma, including tubular and acinar arrangements of neoplastic cells, marked pleomorphism, and a streaming growth pattern. The tumor cells are surrounded by dense fibrous connective tissue and infiltrated by mononuclear cells with small, round nuclei.

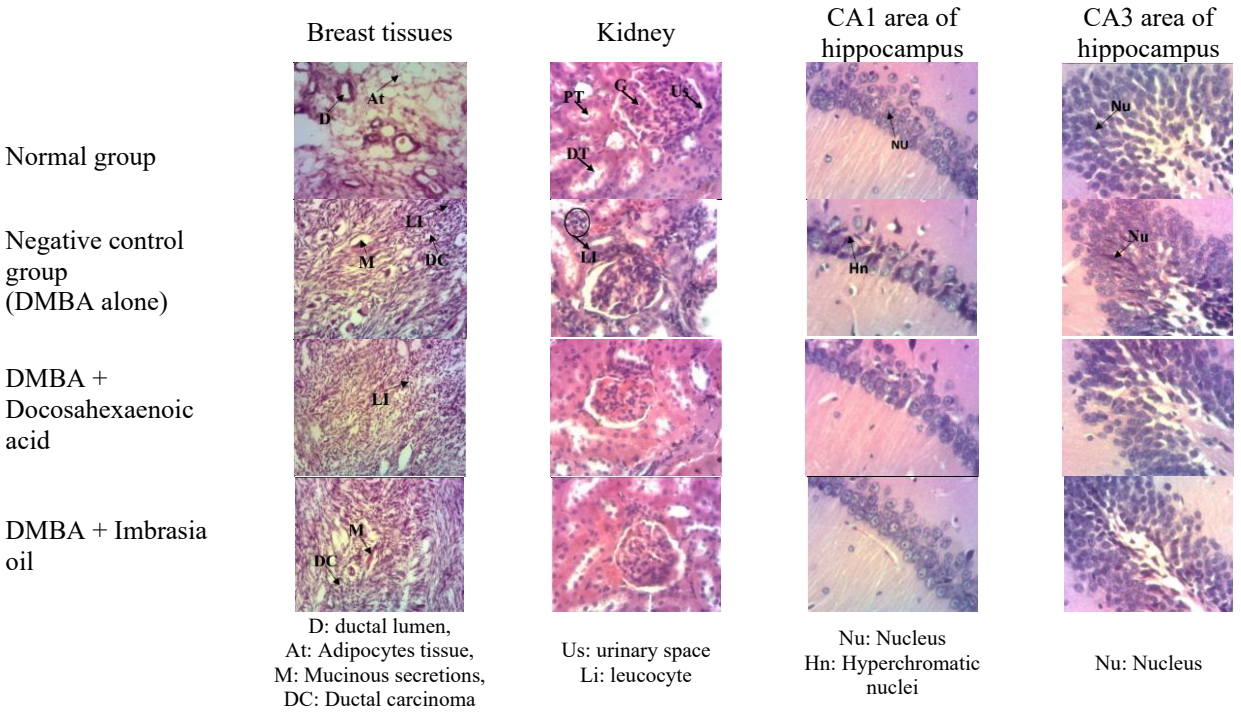
The structure of the kidney the normal group has a normal structure with visible urinary space, distal and proximal convoluted tubules, and a clearly defined glomerulus. In the negative control group, there are signs of kidney damage, including leukocyte infiltration, mesangial expansion, and glomeruli with sclerosis. However, administration of *Imbrasia truncata* oil and DHA helped protect the kidney structure and prevented these histopathological changes.

At the level of the hippocampus, specifically in the CA1 and CA3 regions (Horns of Amun 1 and 3) and the dentate gyrus it can be observed that compared to the normal group, the inducer led to the appearance of hyperchromatic nuclei in the CA1 region. However, *Imbrasia truncata* oil and DHA administrations helped protect and maintain the integrity of neurons in this area by preventing the formation of hyperchromatic nuclei.



	DMBA + Docosahexaenoic acid (4 cm)	Negative control (DMBA Alone) (8.5 cm)	DMBA + Imbrasia truncata oil (4.5 cm)
No Tumor Normal group			

Figure 4. Tumor morphology



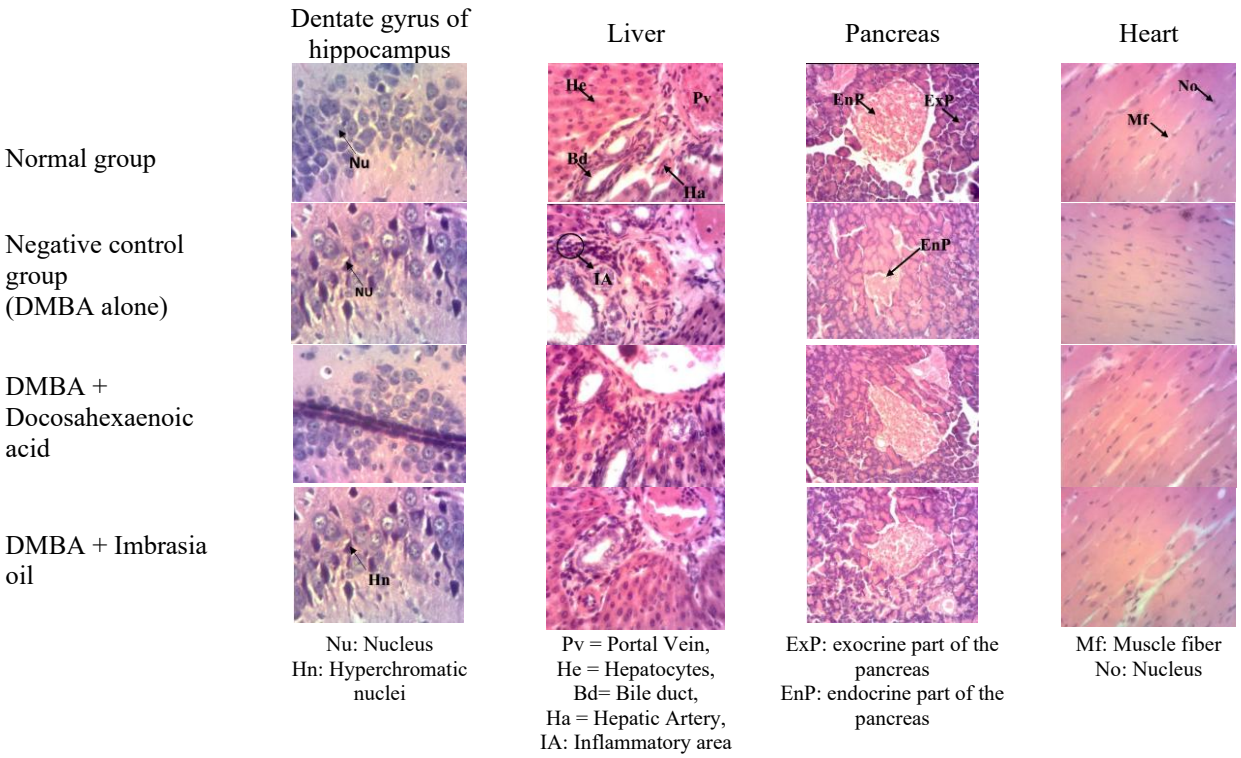


Figure 5. Effects of docosaheaenoic acid and Imbrasia oil microarchitechtures of organs

In the CA3 region, there is no noticeable differences between the *Imbrasia truncata* caterpillar-administered group, DHA administered group, the normal group, and the negative control. In the dentate gyrus, many hyperchromatic nuclei are seen in both the negative control and the group administered with *Imbrasia truncata* oil compared to the normal group and the group taking DHA. Neuronal degeneration was also seen in both the negative control and the group that received *Imbrasia truncata* oil but not in the one taking DHA.

Looking at the liver, the structure appears normal in the normal group with clearly defined hepatic parenchyma, including the portal vein, hepatic artery, bile canaliculi, and distinct hepatocytes. In the negative control group, leukocyte infiltration is observed in the portal area, indicating inflammation. However, in rats administered with *Imbrasia truncata* oil and DHA, this inflammatory area was completely absent, showing protective effects compared to the negative control.

The microphotograph of a normal pancreas shows a typical structure, with an endocrine part (EnP) made up of the islets of Langerhans, and an exocrine part (ExP) containing acinar cells. In the negative control group, the pancreatic islets appeared smaller than in the normal group, indicating reduced secretory activity. In both the positive control and the groups administered with *Imbrasia truncata* oil and the DHA, the pancreas showed slightly altered architecture, with islets displaying irregular outlines.

The heart of rats in the normal group shows long, tapered muscle fibers with many nuclei that are orderly and well-aligned at the level of the cardiac parenchyma. In the negative control group, the cardiomyocyte nuclei appear disorganized compared to the normal group.

Discussion

Oil Quality Parameters

The quality parameters of *Imbrasia truncata* oil were evaluated using quality indexes and Fourier- Transformed Infrared Spectroscopy (FTIR). FTIR analysis revealed a low-intensity peak in the range 3250–3750 cm^{-1}, along with significantly higher-intensity peaks at 2922, 2853, and 1743 cm^{-1}. Additional peaks were observed in the fingerprint region, particularly around 1040, 1000, and 721 cm^{-1}, with low-intensity signals also recorded near 1650 and 3011 cm^{-1}. The broad, low-intensity peak between 3250–3750 cm^{-1} is indicative of hydroxyl (–OH) groups, commonly associated with alcohols, water and hydro-peroxides. Similar peaks were reported in FTIR spectra of groundnut, sesame, and palm oils (Mirghani et al., 2003). The peak near 3011 cm^{-1} corresponds to cis double bond (C=C–H) stretching, while the peaks at 2922 and 2853 cm^{-1} reflect the asymmetric and symmetric stretching vibrations of methylene ($–CH_2$) groups, commonly observed in edible oils like African walnut and groundnut oils (Djikeng et al., 2024). The strong absorption at 1743 cm^{-1} can be attributed to ester carbonyl (C=O) groups of triglycerides. The weak peak near 1040 cm^{-1} may represent the C=O stretching of free fatty acids, as noted in comparative FTIR analyses of edible oils (Kalaimani et al., 2022). In the fingerprint region, the peaks at approximately 1000 and 721 cm^{-1} can be attributed to isolated trans double bonds (C=C–H) and CH_2 rocking vibrations, respectively. These findings align with spectral observations reported in African walnut, palm, and groundnut oils (Douky et al., 2025).

In terms of quality indexes, peroxide value (PV), acid value (AV), thiobarbituric acid (TBA) value, and iodine value, were assessed. The peroxide value (PV) of *Imbrasia truncata* caterpillar oil was determined to be 13.83 meq O_2/kg, which lies within the Codex Alimentarius safety threshold of ≤15 meq O_2/kg for crude fats and oils (Codex STAN, 1999). This value may suggest that the oil has retained an acceptable degree of oxidative stability at the time of analysis, with limited formation of hydro-peroxides, the primary products of lipid peroxidation. As PV reflects the initial stages of oxidation, its measurement is crucial for evaluating early degradation, particularly in oils with moderate to high levels of unsaturation (Flores, et al., 2021).

The p-anisidine value (p-AV) provides insight into the extent of secondary oxidation, particularly the accumulation of aldehydic compounds such as 2-alkenals and 2,4-dienals. The recorded p-AV of 12.21 in the oil samples falls well within the Codex limit of ≤20 for fish oil (WHO/FAO, 2009), suggesting the formation of secondary oxidation products but at low concentrations

The acid value (AV), assesses the extent of triglyceride hydrolysis into free fatty acids (FFAs), and is a widely accepted marker of both hydrolytic rancidity and overall oil degradation. The *Imbrasia truncata* oil sample showed an acid value of 11.28 ± 0.00 mg KOH/g, which exceeds the Codex limit of ≤4 mg KOH/g for crude oils (Codex STAN, 1999). This indicates a degree of triglyceride breakdown, potentially resulting from enzymatic action or moisture exposure during processing or storage (Sharma and Jain, 2015). Though such levels are elevated, they are not uncommon in unrefined oils and do not inherently signify inferior quality. Nonetheless, the observation underscores the critical role of appropriate handling and storage conditions in minimizing hydrolytic deterioration.

The iodine value (IV) is a well-established parameter used to quantify the degree of unsaturation in fats and oils. A higher IV reflects a greater presence of double bonds, indicating a higher content of unsaturated fatty acids, which are nutritionally beneficial but also more prone to oxidation (Pulassery et al., 2022). In this study, *Imbrasia truncata* caterpillar oil exhibited an iodine value of 74.33 g I_2/100g, indicating a moderate level of unsaturation. This suggests a nutritionally favorable fatty acid profile with a balanced mix of saturated and unsaturated fats. Among insect oils, *Imbrasia truncata* oil is similar to mopane worm oil, which showed iodine values around 70–85 g I_2/100g (Bukkens, 1997; Rumpold and Schlüter, 2013). In contrast, oils from *Hermetia illucens* (black soldier fly) tend to be more saturated, with iodine values between 40 and 60 g I_2/100g (Spranghers et al., 2017). Thus, the unsaturation level of *Imbrasia truncata* oil suggests both functional stability and potential health benefits, placing it among the more versatile edible insect oils (Tzompa-Sosa et al., 2014).

The thiobarbituric acid (TBA) value, commonly expressed as thiobarbituric acid reactive substances (TBARS), reflects the accumulation of secondary lipid oxidation products, particularly malondialdehyde (MDA), a breakdown product of polyunsaturated fatty acids. TBA measurements are widely used to assess the extent of

rancidity, especially in oils subjected to thermal treatment or prolonged storage (Custodio-Mendoza et al., 2019).

In the present study, the *Imbrasia truncata* oil exhibited a TBA value of 6.60 ppm. Although no universally accepted threshold exists for TBA in edible oils, this value falls within the range reported in other studies on insect and fish oils that have undergone some degree of oxidative change. For example, Tzompa-Sosa et al. (2014) reported TBA values between 4 and 7 ppm in insect oils subjected to solvent extraction and thermal stress, while values in certain fish oils have been shown to exceed 5 ppm during early stages of oxidation (Frankel, 2005).

Effect of DHA and Imbrasia truncata oil on cancer antigen CA15-3, biochemical and microstructure of organs

Tumor size and morphology are important signs of how far cancer has progressed. As expected, the normal control group had no tumors because they were not exposed to any cancer-causing agents. On the other hand, the negative control group developed large and aggressive tumors, showing how serious the disease was without treatment. The positive control group (docosahexaenoic acid) had the smallest tumors, which supports previous findings about its strong ability to slow cancer growth. The group that received *Imbrasia truncata* caterpillar oil also showed smaller tumors than the negative control group, though the effect was more moderate. DHA helps fight cancer by reducing inflammation, encouraging cancer cell death, and slowing down the growth of new cancer cells (Zhang et al., 2024; Xue et al., 2014). The moderate effect seen with *Imbrasia truncata* oil may be due to its healthy fat content, especially omega-3 fatty acids like alpha-linolenic acid (ALA), along with antioxidants that help lower inflammation and cell damage. A similar protective effect has been seen with *Ricinodendron heudelotii* ("Njansang") oil, which is also rich in omega-3 fatty acids and has been shown to reduce tumors and oxidative stress in lab studies (Zingue et al., 2024). These results support the therapeutic potential of omega-3-enriched oils including DHA and *Imbrasia truncata* oil as complementary dietary agents in breast cancer management. By mitigating tumor growth and improving the biochemical environment, such functional oils could contribute to improved prognosis when integrated into broader cancer care strategies.

The lipid profile is a critical metabolic marker reflecting systemic homeostasis and serving as an indirect indicator of both cardiovascular and oncological disease states. In cancer models, including DMBA-induced breast carcinogenesis, dyslipidemia is frequently observed due to disruptions in lipid metabolism associated with tumor proliferation, membrane biosynthesis, and systemic inflammation (Santos and Schulze, 2012). In this study, the groups taking *Imbrasia truncata* oil and docosahexaenoic acid exhibited significant increase in HDL cholesterol levels compared to other groups, with the lowest levels recorded in the negative control. This is notable, as higher HDL levels are generally associated with anti-inflammatory effects and reduced cancer risk. HDL is known to inhibit tumor-promoting cytokines, support cholesterol efflux from immune and cancer cells, and suppress oxidative stress mechanisms that help restrain tumor progression and modulate the tumor microenvironment (Barter et al., 2004; Yin et al., 2019). The elevated HDL in groups taking *Imbrasia truncata* oil and DHA may thus reflect a protective metabolic shift, supporting their potential as a modulator of cancer-associated lipid imbalance. On the other hand, the *Imbrasia truncata* oil group showed elevated triglycerides, total cholesterol and LDL cholesterol levels, which are often linked to poor cancer prognosis. Elevated cholesterol can promote tumor development by increasing cholesterol availability for cancer cell membranes and steroid hormone synthesis, while high triglycerides are associated with increased lipogenesis and tumor aggressiveness (Ferro et al., 2020). The rise in these lipids could be influenced by the specific fatty acid profile of *Imbrasia truncata* oil, which contains both saturated (e.g., palmitic and stearic acids) and unsaturated fatty acids (e.g., oleic and linoleic acids) in unique proportions (Rumpold et al., 2013). The levels of LDL, total cholesterol and triglycerides in the group taking DHA were relatively lower compared to that of the group taking *Imbrasia truncata* oil, showing a potential protective effect. It was shown that DHA has the ability to reduce the cholesterol level within the lipid rafts of breast cancer cells membranes which are capital for cell movement, invasion and the whole cancer growth (Fabian et al., 2015).

CA15-3 is a well-established biomarker used to monitor tumor burden and treatment response in breast cancer, with elevated levels typically reflecting ongoing tumor growth and potential metastasis. In this study, the negative control group (DMBA induced rats with no treatment) exhibited significantly increased CA15-3 levels, confirming successful induction of tumor genesis. This result aligns with findings by Anugerawat et al. (2024), who reported that elevated CA15-3 is commonly associated with breast cancer progression and systemic inflammatory responses. The rise in CA15-3 observed here supports its diagnostic relevance and reflects the biological impact of DMBA-induced malignancy. Animals which received *Imbrasia truncata* oil and DHA demonstrated a notable reduction in serum CA15-3, suggesting decreased tumor activity or delayed progression. These results indicate the protective action of DHA and *Imbrasia truncata* oil tested against breast cancer growth and development. Similar results were reported by Zingue et al. (2024) with Njansang oil in menopause-like conditions female wistar rats with DMBA-induced breast cancer. Although direct investigations on *Imbrasia truncata* oil's modulation of CA15-3 are currently limited, the observed results may be attributed to the oil's rich composition of bioactive compounds, especially omega-3 fatty acids. *Imbrasia epimethea* oil, along with other edible insect oils, is known to contain significant quantities of unsaturated fatty acids (notably oleic and linoleic acids), tocopherols, phytosterols, and other antioxidant micronutrients (Mba et al., 2019; Lautenschläger et al., 2017). These components have been widely associated with anticancer, anti-inflammatory, and lipid-modulatory activities (Jinadasa et al., 2022; Idris, 2025; Tzompa-Sosa et al., 2020). Studies have further highlighted that edible insect oils, including those from caterpillars, are excellent sources of α-tocopherol and omega-3 fatty acids, which contribute to reduced oxidative stress, immune modulation, and potential suppression of tumor markers (Paul et al., 2017; Nyangena et al., 2023).

Serum transaminases, namely alanine aminotransferase (ALT) and aspartate aminotransferase (AST), along with lactate dehydrogenase (LDH), are widely used in both experimental and clinical settings as indicators of hepatocellular integrity and systemic tissue injury. ALT is considered liver-specific, making it a more reliable marker for hepatocellular necrosis, while AST and LDH are found in multiple tissues, and their elevation often reflects broader cellular damage (Chinnappan et al., 2023). In this study, the negative control group, which received 7,12-dimethylbenz[a] anthracene (DMBA) without therapeutic intervention, exhibited significantly elevated ALT, AST, and LDH activities compared to all other groups. Research done by Olawale et al., (2021) aligns closely with our observations. Their study demonstrated that DMBA administration in Wistar rats caused significant hepatic enzyme elevations particularly ALT and AST indicative of liver injury, while co-treatment with antioxidant-rich agents helped mitigate this damage. This supports the view that hepatocellular enzyme elevations seen in DMBA models are reproducible and modifiable through dietary or pharmacologic interventions, validating the hepatotoxic effect of DMBA and the potential for therapeutic attenuation. In our study, rats receiving *Imbrasia truncata* oil and DHA showed significantly reduced serum ALT, AST, and LDH activities**,** compared to the negative control. These reductions suggest that both agents may confer a protective effect on hepatocytes**,** potentially through antioxidant activity or modulation of liver detoxification pathways. Notably, the hepato-protective trends observed here are consistent with those reported by Olayaki et al., (2018)**,** who found that omega-3 fatty acids attenuated oxidative liver injury in rats exposed to chemical toxins. The shared outcome of enzyme normalization across these studies suggests that lipids rich in unsaturated fatty acids and antioxidants such as *Imbrasia truncata* oil may be effective in blunting the hepatotoxic cascade induced by DMBA**.**

Serum creatinine levels remain a cornerstone biomarker for evaluating renal function due to its exclusive clearance via glomerular filtration. Elevated serum creatinine typically indicates nephron impairment, decreased glomerular filtration rate (GFR), or systemic oxidative stress affecting renal tissues (Lin, 2023; Selby, 2019). In the current study, rats exposed to DMBA without therapeutic intervention (negative control) showed significantly elevated serum creatinine levels, indicating impaired kidney function. This finding is consistent with the nephrotoxic effects of DMBA reported by Olawale et al., (2021), who observed that DMBA exposure leads to oxidative stress that damages renal microvasculature and tubular structures. These structural disruptions impair glomerular filtration, causing the accumulation of nitrogenous waste products like creatinine. The elevated creatinine levels in our study therefore reflect similar renal injury mechanisms, reinforcing the established link between DMBA-induced oxidative stress and kidney dysfunction. By contrast, rats which received *Imbrasia truncata* oil and rats which received DHA led to notably reduced serum creatinine levels, indicating a potential reno-protective effect. Although direct studies on insect oils in DMBA-induced nephrotoxicity models remain limited, supportive evidence exists regarding the general organ protective and antioxidant roles of insect lipids. For instance, Rumpold et al., (2013) emphasized that edible insect oils, including those from *Lepidoptera* larvae, are rich in essential fatty acids, sterols, and antioxidant compounds factors that may contribute to cellular protection in high-stress environments such as chemically induced carcinogenesis. Similarly, studies on insect-derived dietary supplements have demonstrated systemic antioxidant activity and modulation of inflammation two key pathways implicated in preserving renal function under toxic stress. Finke (2002) reported that larvae oils contain appreciable quantities of oleic and linoleic acids, which may help maintain membrane integrity and reduce oxidative injury to renal tissues. In this light, the activity recorded with *Imbrasia truncata* oil can be attributed to the presence of α-linolenic acid (Womeni et al., 2009) an omega-3 fatty acid which has the ability to be converted into DHA in-vivo and exert a protective role (Domenichiello et al., 2014).

Histological sections of the breast tissues from the negative control group revealed well-defined features of ductal adenocarcinoma, including mucin accumulation, papillary projections, basement membrane disruption, and extensive cellular pleomorphism. These findings are consistent with previous reports by Rakha et al., (2017) and Binnewies et al., (2018), who described aggressive tumor phenotypes following DMBA induction in rats. In contrast, the test group (rats taking *Imbrasia* oil) or positive control group (rats taking DHA) showed reduced tumor severity. Glandular structures were partially maintained, and cellular pleomorphism was significantly less pronounced. These improvements suggest that omega-3-rich oils, particularly DHA and *Imbrasia truncata* oil, may help slow tumor progression. This supports existing evidence that omega-3 fatty acids can inhibit cell proliferation, alter membrane lipid composition, and suppress tumor-promoting signaling pathways (Calder, 2020; Xue et al., 2014).

Kidney tissues from the normal control group showed healthy glomeruli and well-maintained tubules, indicating normal renal structure. In contrast, the negative control group displayed clear signs of kidney damage, such as glomerular sclerosis, tubular distortion, and infiltration of white blood cells evidence of nephrotoxicity. However, the test control group (rats that received *Imbrasia truncata* oil) or positive control group (rats which received DHA) showed significant preservation of kidney architecture. Both treatments helped maintain glomerular and tubular integrity. This protective effect is consistent with previous studies showing that omega-3 fatty acids reduce renal oxidative stress and inflammatory responses, thereby supporting overall kidney function (Mori et al., 2007; Khan et al., 2013; Dai et al., 2015). While DHA appeared to offer slightly stronger protection, the omega-3 content in *Imbrasia truncata* oil particularly alpha-linolenic acid (ALA) also contributed to notable nephron-protective effects.

In the negative control group, the CA1 region of the hippocampus exhibited marked neuronal necrosis, evidenced by hyper-chromatic and pyknotic nuclei. This is consistent with the known vulnerability of CA1 pyramidal neurons to oxidative and excito-toxic damage, attributed to their dense glutamate receptor distribution and limited

antioxidant capacity (Gao et al., 2015). In contrast, the CA3 sub-region showed relatively preserved morphology, aligning with previous reports that CA3 neurons possess stronger intrinsic defenses, including astrocytic calcium buffering and anti-apoptotic signaling (Sun et al., 2018).

Among the groups which received DHA and *Imbrasia truncata* oil, visible neuro-protection in the CA3 region was observed, with well-preserved neuronal structure. However, in the dentate gyrus (DG), degeneration persisted in the group taking *Imbrasia truncata* oil, suggesting incomplete neuro-protection. This regional discrepancy could reflect differential oxidative stress or insufficient activation of glial repair mechanisms (Cao et al., 2009; Heras-Sandoval et al., 2016).

Liver histology in the negative control group revealed peri-portal leukocyte infiltration, suggesting inflammatory liver damage likely driven by systemic oxidative stress following DMBA administration. In contrast, rats taking *Imbrasia truncata* oil and DHA exhibited preserved hepatic architecture and reduced inflammatory cell presence, indicating a clear hepato-protective effect. This protection may be mechanistically linked to the oil's high content of unsaturated fatty acids (notably linoleic and oleic acids), phyto-sterols, and tocopherols compounds known to upregulate hepatic antioxidant enzymes such as glutathione peroxidase and catalase, while downregulating pro-inflammatory mediators like NF-κB (Tung et al., 2017; Alagawany et al., 2020; El-Sayed et al., 2021).

Pancreatic tissues from the normal group showed well-structured acinar cells and intact islets of Langerhans. Exposure to DMBA caused disruption of the islets and breakdown of acinar cells, reflecting damage to both endocrine and exocrine functions. The test group (rats taking *Imbrasia truncata* oil) and positive control group (rats taking DHA) maintained healthier pancreatic tissue architecture. Omega-3 fatty acids contribute to preserving pancreatic health by reducing inflammatory cytokines and enhancing insulin signaling pathways. Although DHA is recognized for its higher bioactivity, alpha-linolenic acid (ALA) found in *Imbrasia truncata* caterpillar oil also provides protective effects on endocrine function, particularly under oxidative stress conditions (Chen et al., 2018).

Cardiac tissue from the normal control group showed well-aligned myocardial fibers with centrally positioned nuclei, reflecting healthy heart structure. In contrast, rats exposed to DMBA exhibited significant disruption of myocardial fibers and nuclear degeneration, indicating marked cardiotoxicity, especially in the negative control group (Gupta et al., 2020). Treatment with DHA resulted in near-complete restoration of heart tissue architecture, while *Imbrasia truncata* oil provided moderate structural recovery. Omega-3 fatty acids particularly DHA are well known for their cardioprotective properties, including reducing inflammation, enhancing mitochondrial efficiency, and stabilizing myocardial cell membranes (Kaur et al., 2018). The presence of alpha-linolenic acid (ALA) in *Imbrasia truncata* oil likely contributed to these effects through local anti-inflammatory activity. Similar benefits were reported in a study by Tchana et al., (2023), where omega-3-rich insect oils improved cardiac function and lipid profiles in experimental models.

Conclusions

Results revealed that *Imbrasia truncata* oil is stable with an average iodine value that shows it appreciable content of unsaturated fatty acids. Docosahexaenoic acid and *Imbrasia truncata* oil reduced tumor progression, improve HDL cholesterol level, and considerably reduce cancer antigen CA15-3 level. DHA and *Imbrasia truncata* oil reduce the AST, ALT, LDH activities and creatinine levels in the serum of rats with DMBA-induced breast cancer. They also have a protective effect against the potential damages caused on them by the progression of the cancer. In summary, this research supports *Imbrasia truncata* caterpillar oil as a promising omega 3 rich functional food with potential benefits in cancer prevention and management.

Declarations

Ethical Approval Certificate

All animals were cared for and used in agreement with international standard guideline for animal use. An ethical clearance for animal handling and care was obtained from the University of Buea Institutional Animal Care and Use Committee with the reference number UB- IACUC N^0 02/2025.

Author Contribution Statement

Epingi Dalmaris Orock-Naka: Conceptualization; Investigation; Writing - original draft; Writing - review & editing; Visualization; Validation; Methodology; Software; Formal analysis; Resources; Data curation. *Fabrice Tonfack Djikeng*: Conceptualization; Investigation; Writing - original draft; Writing - review & editing; Visualization; Validation; Methodology; Software; Formal analysis; Resources; Supervision; Data curation. *Achidi Aduni Ufuan***:** Conceptualization; Investigation; Writing - original draft; Writing - review & editing; Visualization; Validation; Methodology; Software; Formal analysis; Resources; Supervision; Data curation. *Bernard Tiencheu*: Conceptualization; Investigation; Writing - original draft; Writing - review & editing; Visualization; Validation; Methodology; Software; Formal analysis; Supervision; Resources; Data curation.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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