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# INVESTIGATIVE STUDIES ON THE EFFECTS OF ETHANOL ROOT EXTRACT OF *DENNETTIA TRIPETALA* ON LIVER ENZYMES, PROTEIN AND BILIRUBIN LEVELS IN WISTAR RATS ADMINISTERED THERMOXIDIZED PALM OIL

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## ABSTRACT
This study investigated the effect of ethanol root extract of *Dennettia tripetala* on liver enzymes, protein and bilirubin level in wistar rats administered thermoxidized palm oil. Twenty wistar rats divided into 4 groups of 5 rats each were used for the study. Group 1 served as the normal control group, group II received 1ml of thermoxidized palm oil, group III animals were treated with 1ml of thermoxidized palm oil and 50mg/kg of ethanol root extract of *Dennettia tripetala* while group IV received 1ml of thermoxidized palm oil and 100mg/kg body weight of the root extract. Animals were fasted for 24 hours and sacrificed after 21 days of treatment. Aspartate amino-transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, globulin, total, conjugated and unconjugated bilirubin were determined using standard analytical methods. Treatment of the animals with 50mg/kg and 100mg/kg of root extract led to a significant ($p<0.05$) decrease in the levels of AST, ALT, ALP, total conjugated and unconjugated bilirubin when compared with negative control (Group II animals). Also animals that received 50mg/kg and 100mg/kg of extract showed a statistically significant increase in the levels of total protein, albumin and globulin when compared with the negative control. These effects were most pronounced in animals that received 100mg/kg body weight of rat. From the results, it can be deduced that thermoxidized palm oil causes derangement of liver function parameters while the root extract of *Dennettia tripetala* is hepato-protective.

**KEYWORDS:** Albumin, thermoxidized palm oil, bilirubin, liver enzymes, *Dennettia tripetala.*

## INTRODUCTION
Palm oil which is reddish in colour due to its high content of β-carotene is obtained from a tropical plant (oil palm) found in warm climate of Africa, Asia, Brazil at altitudes below 500 meters above sea level.[1,2] Its scientific name is Elaesis guineensis because it originates from the gulf of Guinea. Palm oil remains the most commonly consumed oil in Nigeria because its cheap, affordable and readily available.

Besides being used as a common cooking oil, ingredient and in commercial food industries because of the high stability of the refined product when used in frying, palm oil is also used in the manufacture of soap and other personal care products.[2,3] Fresh palm oil contains about 50% unsaturated and 50% saturated fatty acids which include oleic acid, Iinoleic acid, palmitic acid as well as carotenoid (α and β carotene) as well as vitamin A and E which are potent antioxidants.[4,5,6] However, the polyunsaturated component of paml oil can undergo thermal oxidation during frying, with the formation of cytotoxic and injurious products which are deleterious to cells, tissues and organs. Such products include but not limited to ketones, peroxides, aldehydes, ozonides.[7,8] Thermoxidized palm oil if consumed for a long time has been shown by many researchers to cause nucleic acid and micronutrient deficiency with concomitant deactivation of key metabolic enzymes, fatty liver disease, thrombosis, growth retardation as well as essential fatty acid deficiency and abnormal haematological indices.[5,9,10]

*Dennettia tripetala*, commonly called pepperfruit and variously known as mmimi, Ako, ata Igbere and Nkarika in different regions of Nigeria belongs to the family Annonaceae.[11,12] It is widely distributed in Southern Nigeria, Western Cameron and Coted'ivore where it is consumed for its spicey taste and used to welcome visitors.[11,12] *D.tripetala* is used in ethnomedicine for management of several ailments as antidiabetic, anticolvusant, anxiolytic, antimicrobial, anticancer and ant-inflammatory agent.[13,14,15,16,17] It is also used for

other conditions such as diarrhea, toothache fever and cough.[11] The use of *Dennettia tripetala* in folklore medicine is attributable to the presence of phytochemicals like tannins, steroids, flavonoids, glycosides, saponins, alkaloids etc in the plant extract.[18] Also proximate analysis of various parts of *D. tripetala* by various researchers has shown the plant to be rich in carbohydrates protein, fibre, ash, lipids. It also has a high content of vitamins A and C.[19]

Many diseases of the liver are characterized by elevated levels of serum bilirubin as well as variations in the levels of liver biormarkers such as alanine aminotransferase (ALT), aspartate aminotransferase (ALT) and alkaline phosphatase.[3,12,20] Iseghohi *et al*,[20] reported the ameliorative effect of extract of *D. tripetala* on rats with hepato-renal toxicity induced by exposure to carbon tetrachloride. Since palm oil is commonly consumed and oxidized products are deleterious to hepatic tissue, the present study was therefore aimed to investigate the effect of *D. tripetala* on liver function status in rat exposed to thermoxidized palm oil.

## MATERIALS AND METHODS

### Preparation of root extract

The roots of *Dennettia tripetala* were obtained from the farmland of Imo State University, Imo State, Nigeria. They were identified and authenticated by Prof. F.N Mbagwu, of Plant Science Department, Imo State University Owerri. Roots were thoroughly washed, cut into small sizes and sun dried for about 4 weeks until a constant weight was obtained. The dry roots were chipped into smaller sizes and ground with a grinding machine to obtain a fine powder. 650g of the dry root powder was soaked in 2 litres of 95% ethanol. After 48 hours, the suspension was filtered using a whatman No1 filter paper and the filtrate concentrated using a Rotary evaporator at $40^0$C. The concentrate was allowed in water bath for complete dryness at $37^0$ C and then re-constituted to an appropriate concentration in distilled water prior to administration.

### Preparation of thermoxidized palm oil

The palm oil sample was prepared according to the method described by Oboh *et al*,[21] Fresh palm oil was purchased from Orie market in Ideato South, Imo State, Nigeria. The palm oil was oxidized by heating at a temperature of $160^0$ C in a stainless steel pot for 4 different intervals of 10, 15, 20 and 25 minutes and after each interval, the palm oil was allowed to cool for 10 hours before re-heating. The thermoxidized palm oil was kept in air tight amber bottles at room temperature prior to administration.

### Animals

A total of twenty (20) wistar rats weighing between 140-170g which were purchased from the department of Animal and environmental Biology, Imo State University were used for the study. Animals were allowed seven (7) days to acclimatize prior to commencement of experiment. Rats were kept under normal standard environmental conditions of humidity (35-60%), room temperature ($25\text{-}28^0$ C) and 12 hour light/ 12 hour dark cycle and were allowed free access to water and feed. Ethical principles of World Health Organization for good laboratory practice regulations of 1998 and United states guideline for experimental animal[22,23] were strictly adhered to throughout the experiment.

### Experimental Design

The animals were randomly divided into four groups with each group consisting of five wistar rats.

Group 1: Normal control rats received daily normal feed and water

Group II: Rats treated with 1 ml of thermoxidized palm oil

Group III: Rats treated with 1ml of themoxidized palm oil + 50mg/kg body weight of ethanol root extract of *Dennettia tripetala*.

Group IV: Animals treated with 1ml of thermoxidized palm oil + 100mg/kg body weight ethanol root extract of *Dennettia tripetala*.

The doses were administered orally to the rats for 21 days and animals had free access to feed and water *ad libitum* throughout the course of the experiment.

### Sample collection

At post 21 days of treatment, animals were fasted overnight, anaeesthetized with chloroform and sacrificed. Blood samples were collected by cardiac puncture using sterile syringe and needle and dispensed into heparinized sample bottles for clinical chemistry analysis and allowed for about 10 minutes to clot. Sample was then centrifuged at 100rpm for 5 minutes with Wisperfuge model 1384 centrifuge (Samson, Holland) and collected with a pasteur pipette into clean labeled sample bottle. Serum was used for analysis.

### Biochemical analysis

Liver enzymes (aspartate amino transferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP) levels were estimated spectrophotometrically using standard laboratory kits (Randox Laboratory Ltd co. Antrim UK). Total protein, albumin, globulin, total and conjugated bilirubin levels were also spectrophotometrically determined using standard laboratory kits (Randox laboratory Ltd, Co. Antrim, UK). All the biochemical estimations were performed under total adherence of manufacturers guidelines. Unconjugated bilirubin was estimated thus: Unconjugated bilirubin = total bilirubin – conjugated bilirubin.

### Data Analysis

Data obtained from this study were presented as mean ± SD of four determinations. Statistical analysis was by one way analysis of variance using the SPSS 21.0 version. This was followed by students t- test of

significance. A p- value of < 0.05 was considered statistically significant.

## RESULTS

**Table 1: Liver indices of rats administered thermoxidized palm oil and ethanol root extract of *D.tripetala*.**

| Groups | AST (IU/L) | ALT (IU/L) | ALP (IU/L) |
|---|---|---|---|
| I | 41.40 ± 1.14[a] | 42.60 ± 1.51[a] | 87.40 ± 1.60[a] |
| II | 62.55 ± 5.47[b] | 68.60 ± 5.69[b] | 81.45 ± 4.56[a] |
| II | 55.60 ± 3.88[b] | 55.66 ± 8.76[c] | 74.60 ± 8.90[b] |
| IV | 48.50 ± 2.38[a] | 50.24 ± 3.55[a] | 69.22 ± 4.83[c] |

Values are mean ± SD of four determinations. Values bearing different superscripts (a – d) in the same column are significantly different (p<0.05). AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase.

**Table 2: Effects of Ethanol Root Extracts of *D. tripetala* on some biochemical parameters of wistar rats administered thermoxidized palm oil.**

| Group | Total protein (mg/dl) | Albumin (mg/dl) | Globulin (mg/dl) | Total bilirubin (mg/dl) | Conjugated bilirubin (mg/dl) | Unconjugated Bilirubin(mg/dl) |
|---|---|---|---|---|---|---|
| I | 98.60 ± 4.15[a] | 59.80 ± 5.35[a] | 38.80 ± 4.38[a] | 0.75 ± 0.07[a] | 0.55 ± 0.13[a] | 0.19 ± 0.05[a] |
| II | 72.60 ± 2.07[b] | 43.40 ± 2.70[b] | 29.20 ± 2.28[b] | 2.09 ± 0.45[b] | 1.37 ± 0.29[b] | 0.70 ± 0.03[b] |
| III | 83.40 ± 2.30[c] | 50.80 ± 1.09[a] | 31.20 ± 2.38[b] | 0.89 ± 0.09[a] | 0.65 ± 0.14[a] | 0.24 ± 0.06[a] |
| IV | 89.26 ± 2.34[a] | 55.20 ± 1.48[a] | 37.23 ± 1.78[a] | 0.60 ± 0.18[a] | 0.47 ± 0.13[a] | 0.13 ± 0.0.9[a] |

Values are mean ± SD of four determination. Values bearing different superscript letters (a -d) in the same column are significantly different (p <0.05).

Table 1 shows the effects of ethanol root extract of *Dennettia tripetala* on liver enzymes of rats administered thermoxidized palm oil. The result revealed a statistically significant increase (p<0.05) in the serum levels of AST and ALT in animals that received only thermoxidized palm oil when compared with the group I. Treatment of experimental animals with 50mg/kg body weight and 100mg/kg body weight of ethanol root extract of *D. tripetala* led to a significant decrease in the levels of AST, ALT and ALP when compared with the negative control (Group II animals). Effects of ethanol root extract of *D. tripetala* on biochemical parameters of wistar rats administered thermoxidized palm oil is shown on table 2. From the result, animals that received 1 ml of thermoxidized palm oil daily for 21 days showed a statistically significant decrease (p<0.05) in the levels of total protein, albumin and globulin when compared with the group I animals. Also, there was a statistically significant increase (P<0.05) in the levels of total, conjugated and unconjuagted bilirubin when compared with the normal control (group I rats). However, treatment of the experimental animals with *D. tripetala* root extract brought about a statistically significant increase in the level of total protein, albumin and globulin as well as a significant decrease (P>0.05) in the levels of total, conjugated and unconjugated bilirubin in group III and IV animals when compared with the negative control (group II). These changes are dose dependent with the greatest effects seen in animals that received 100mg/kg of the root extract.

## DISCUSSION

Various parts of *Dennettia tripetala*, be it leaves, fruits, stem or roots are used in ethnopharmacology in the management of diabetes, fever, convulsion and cancer.[20,24,25] The medicinal properties of *D. tripetala* has been attributable to the presence of phenolic compounds like saponins, flavonoids, tannins as well as vitamins such as C and E in the extract. Falade *et al*,[5]; Oboh *et al*,[21] reported the formation of volatile organic compounds, aldehydes and peroxides during oil thermoxidation. Peroxides are known to cause oxidative stress.

The liver which is the largest and heaviest internal organ of the body is essential for xenobiotic metabolism/ detoxification, synthesis of biomolecules, secretion of bile and bilirubin metabolism.[20,26,27] The levels of ALT, AST, ALP and bilirubin which are known markers of liver disease increase following cellular damage and necrosis of the liver and are hence used to assess hepatic function.[27,28] ALT is more specific to the liver as AST is also found in large quantity in the heart and muscles while ALP level is also elevated in bone, placental and GIT diseases.[29] In this study, treatment of animals with thermoxidized palm oil which is well known to be cytotoxic caused an increase in plasma levels of ALT, AST, ALP and bilirubin (total, conjugated and unconjugated). Our findings agree wish those of Falade *et al*,[5] where treatment of wistar rats with thermoxidized palm oil for 30 days led to a significant increase in the levels of AST, ALT, ALP, total bilirubin, triglycerides, low density lipoprotein as well as plasma/ liver MDA. This increase in serum level of these enzymes and bilirubin is most likely due to free radical damage to the membrane of the liver cells.[20,30,31] Some researchers have shown that thermoxidized oil and fats contain free radical and dihydroxy esters which are capable of causing injury to the cells.[31] Treatment of the rats exposed to thermoxidized palm oil with root extract of

*Dennettia tripetala* resulted in a dose dependent significant decrease in levels of serum AST, ALT, ALP total, conjugated and unconjugated bilirubin. Our findings corroborate those of Akpakpan *et al*,[12] and Iseghohi *et al*,[20] where administration of *D. tripetala* for 14 days prior to a single administration of cytotoxioc carbon tetrachloride significantly protected the liver from damage and prevented elevation of AST, ALT and ALP. This may be due to the high antioxidant content of *D.tripetala* which has been shown to contain antioxidants like vitamin A and C as well as phytochemicals such as flavonoids.[19]

Albumin is primarily synthesized in the liver cells while globulin is produced by the liver and to some extent by the immune system. Albumin represents a major synthetic protein and therefore serves as a serum marker of liver's ability to synthesize proteins.[29] Chronic damage to the cytoarchitecture of the liver results in decrease in the levels of serum proteins albumin and globulin.[5] Our results showed a significant decrease in the levels of total protein, albumin and globulin in experimental animals that were fed thernoxidized palm oil only. This shows that thermoxidized palm oil diets may alter protein synthesis in the liver. Falade *et al*,[5] reported similar results where there was a decrease in plasma and liver total proteins and albumin levels of groups fed with 20 minute-thermally oxidized palm oil. Ttreatment of the exposed rats to ethanol root extract of *Dennettia tripetala* restored the levels of total protein, albumin and globulin to normal (when compared to control) and this indicates that the extract enhanced the synthetic function of the liver. This is similar to the findings of Akpakpan *et al*,[12] where treatment of experimental rats with ethanol root extract of *D. tripetala* did not decrease the levels of total protein and albumin in such rats.

## CONCLUSION

In conclusion, thermoxidized palm oil is deleterious to the liver and cause an unhealthy change in the various biomarkers of liver function which can be restored by the root extract of *Dennettia tripetala*.

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