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Original Research Article

Assessment of Ethanol Stem Extract of *Dennettia Tripetala* on Lipid Profile And Antioxidant Status in Rats Administered Thermoxidized Palm Oil

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Abstract: This study assessed the effect of ethanol stem extract of *Dennettia tripetala* on lipid profile and antioxidant status in rats administered thermoxidized palm oil. Twenty (20) apparently healthy albino wistar rats of both sexes weighing between 150 – 180g were divided into four (4) equal groups of 5 rats each and used for the study which lasted for 21 days. Group 1 which served as positive control was given normal rat chow and water daily without extract. Group 2 which served as negative control received 1ml of thermoxidized palm oil daily in addition to normal rat chow. Group 3 received 1ml of thermoxidized palm oil + 50mg/kg of the extract daily. Group 4 received 1ml of thermoxidized palm oil + 100mg/kg of the extract daily. All administrations were done orally.Standard analytical methods were used to determine lipid parameters (Total cholesterol (TC), Triglyceride (TC), High Density Lipoprotein cholesterol (HDL-C), Low Density Lipoprotein cholesterol (LDL-C) and Very low density lipoprotein cholesterol (VLDL-C)), serum malondialdehyde (MDA) level, serum glutathione peroxidase (GPx) and serum catalase activity. The result showed a significant increase in the concentrations of all the lipid parameters in the thermoxidized palm oil group (group 2) compared to the control group with the concentrations of TC, TG, HDL-C, LDL-C and VLDL-C in group 2 being 294.50mg/kg ± 4.50, 158.00mg/kg ± 4.11, 54.00mg/kg ± 9.32, 208.25mg/kg ± 12.10 and 20.00mg/kg ± 0.8 respectively. It also showed a significant decrease in the concentrations of TG and LDL-L in the extract-treated groups 3 and 4, significant decrease in the concentration of TC in group 4 (241.60mg/kg ± 9.99), significant increase in the concentration of VLDL-C in groups 3 and 4 (31.60mg/kg ± 5.76 and 30.80mg/kg ± 4.85 respectively) and HDL-C in group 4 (63.00mg/kg ± 4.55) when compared to group 2. The result further indicated an increase in the concentration of malondialdehyde in group 2 (15.86 IU/L ± 9.45) compared to control group (2.58IU/L ± 0.19), a decrease in the concentration of Glutathione peroxidase(30.83IU/L ± 2.48) and Catalase (82.60IU/L ± 2.30) in group 2 compared to control and their increase in groups 3 and 4 compared to group 2 with the concentrations being 41.20IU/L ± 1.92 and 97.20IU/L ± 2.17 respectively in group 3 and 53.80IU/L ± 5.68 and 118.80IU/L ± 5.81 respectively in group 4. In conclusion, stem extracts of *Dennettia tripetala* has lipid lowering potentials, antiatherogenic effect and antioxidant properties which become more potent with increase in dose. It can therefore be properly harnessed and applied in the management and treatment of various chronic and degenerative disorders.

Keywords: ***DennettiaTripetala*, thermoxidized palm oil, wistar rat, lipid profile, antioxidants.**

INTRODUCTION

The use and acceptance of roots and herbs for health care delivery have continued to gain popularity over the years. Using plants as medicine is an ancient practice common to all societies. According to Ingale and Hivrale, (2010), plants have been the basis of many traditional medicines throughout the world for thousands of years.The World Health Organization reported in 2001 that 60% of the world's population depends on traditional medicine (WHO, 2001), Also, Abdel-Azim *et al*., (2011) and Zhang and Moller, (2000) revealed that 80% of the population in developing countries depends almost entirely on traditional medicines for their primary health care needs.

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Dennettia tripetala is of the family annonaceae, a well-known forest fruit and spicy indigenous medicinal plant found in the rain forest zone of Africa. It grows to a height of 12 – 15m and has a girth of 0.6m (Ejechi and Akpomedaye, 2005). *D. tripetala* has a bark that possesses a very strong characteristic scent, a wood that is white and soft, fruits which are edible with spicy taste and green in color when developing but turns red with ripening and leaves which are elliptical in shape with sizes of 3 – 6inches long and 1.5 – 2.5inches broad (Okwu and Morah, 2004). It is widely domesticated in the Southern, Eastern and Western parts of Nigeria and consumed by inhabitants of Western Cameroons, Ivory Coast, Southern and Eastern Nigeria (Ihejeme *et al*., 2013). *Dennettia tripetala* is popularly known as pepper fruit. In Nigeria, it is called "Mmimi" in Ibo, "Nkarika" in Efik and "Ata Igbere" in Yoruba (Isehohi, 2015).

Phytochemical screening of ethanol extract of *D. tripetala* revealed the presence of tannins, alkaloids, steroids, flavonoids, cardiac glycosides, saponins and terpenoids (Sparg, 2004). There is also presence of proteins, fiber, ash, lipids and moisture which increases with ripening (Ihejeme *et al*., 2013). Also found in *D. tripetala* are antioxidants such as lycopene, ascorbic acid, p-coumaryl alcohol, ethoxyquin and capsaicinoids (Luo *et al*., 2011) and essential oils like carvacrol, carvone, caryophyllene, limonene and thymol (Helander *et al.*, 1998; Kotamballi *et al*., 2013).

The medicinal and health benefits of *Dennettia tripetala* have been documented. *D. tripetala* was reported to have antimicrobial activity (Lampe, 2003; Yamasaki *et al*., 2011, Ribeiro *et al*., 2011; Cruz *et al*., 2010), anticancer activity (Mori *et al*., 2006; Luo *et al.*, 2011; Alonso-Castro *et al*., 2011; R'ezanka and Sigler 2008; Maoka *et al*., 2001), antinociceptive and anti-inflammatory activities (Ejechi *et al*., 1999; Oyemitan*et al*., 2008; Luo *et al*., 2011; Mueller *et al*., 2010); antiviral activity (Bourne *et al*., 1999); antihyperglycemic effect (Anaga and Asuzu, 2011); antifungal and insecticidal potentials (Oyemitan*et al*., 2008; Adedire and Akinkurolere, 2005) and antioxidant potentials (Oboh and Akindahunsi, 2011; Aderogba *et al*., 2011; Isabella *et al*., 2010; Park *et al.*, 2012). In some Nigerian communities, fruits of *D. tripetala* are used for the treatment of cough, toothache (Adebayo *et al.*, 2010) and also serve as an appetite stimulant, mouth wash and anti-pyretic (Gill, 1992; Enwere, 1998).

Lipid profile or lipid panel is a panel of blood test that serves as an initial screening tool for abnormalities in lipids such as cholesterol and triglycerides, and diagnostic tool for disease conditions like heart failure, hypertension, stroke (Merck Manual, 2012; Togun *et al*, 2007). The result of this test can identify certain genetic diseases and can determine approximate risks for cardiovascular diseases, acute pancreatitis, cerebrovascular diseases (Enechi *et al*, 2014; Milleu *et al*, 2011). A lipid profile usually includes the level of total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides and calculated low density lipoprotein (LDL) cholesterol which are effective tools in the diagnosis of dyslipidaemia and atherosclerosis (Ezekwesili *et al*, 2008; Onyeyoli *et al*, 1992)

Antioxidants are substances that when present at low concentrations compared with that of an oxidizable substrate significantly delays or inhibits the oxidation of that substrate (Halliwell and Gutteridge, 1995). They protect the cellular components of the body from oxidative stress and also constitute a defense system against free radicals and reactive oxygen species that arise from the body's biochemical reactions (Boxion *et al*., 2002). Free radicals are produced either from external sources or generated *in situ* as a result of normal cellular metabolism (Parke and Sapota, 1996). They are short-lived, highly reactive and utilized by the immune system for lyses of micro-organisms, but become toxic when produced in excess quantities (Senthil and Manoharan, 2004).

Thermal oxidation of oil produces free radicals and lipid peroxidation which always lead to tissue damage and cell death, and which has been implicated in the pathogenesis of various chronic and degenerative disorders such as cancer, aging, alzeheimer's disease, diabetes, autoimmune disorders and cardiovascular diseases (Ames *et al*., 1993; Halliwell *et al*., 1994). Palm oil derived from the pulp of the fruit of *Elaeis guineensis* is used in most homes in Nigeria for cooking and frying. Often times, palm oil is heated overtime before use. Thermal oxidation of edible oils has become a common practice in most homes in Nigeria. Also very common is the tendency of people to repeatedly reuse thermally heated oil in frying and cooking with little or no knowledge of its adverse effects on health and general well-being. Eating more plant-based foods which are rich in phytochemicals according to Heather and Talcott (2009) prevent oxidative stress in the body. As much as works have been done to substantiate the antioxidant potentials of *Dennettia tripetala*, there is still paucity of information on the practical applications of the antioxidant activities of this plant to prevent potential hazardous effects of heated oils on the body system. This study therefore aimed at assessing the effect of ethanol stem extract of *Dennetiatripetala* on lipid profile and antioxidant status in rats administered thermoxidized palm oil.

MATERIAL AND METHODS

Identification and Collection of Plant materials

Fresh stem of *Dennettia tripetala* were obtained from different local farmlands in Ulakwo, Egbelu and Ngwoma in Owerri-North L.G.A of Imo State Nigeria. They were identified and authenticated in the department of Plant Science and Biotechnology, Imo State University, Owerri. The stem was cut to

pieces with knife (to facilitate drying), thoroughly washed to remove un-wanted debris and air-dried at room temperature for 7 days until constant weight was obtained. The dried stem was then pulverized to fine powder with the aid of a mechanical grinder and kept in labeled airtight containers under dry conditions until required for use.

Ethanol stem extraction of *Dennettia tripetala*

The ethanol stem extraction of *Dennettia tripetala* was done using the modified method of Abdulrahman *et al* (2004). Six hundred and fifty grams (650g) of grounded stem of *Dennettia tripetala* was macerated in 2 litres of 95% absolute ethanol for 24hrs. It was filtered with sterile filter paper and evaporated afterwards to dryness at 40°C in a vaccum using a rotary evaporator RE52. Approximate concentrations of the extract were constituted to the required doses for the treatment of the animals using normal saline.

Preparation of Thermoxidized Palm Oil

Fresh palm oil were purchased from three different major markets namely, Ekeukwu Owerri market, Relief market and Egbeada market all in Owerri, Imo state, Nigeria. The purchased palm oil were thoroughly mixed, thermoxidized by heating at temperature of 150^0C in a stainless steel pot for 20 minutes and allowed to cool for eight (8) hours. This process was repeated for four (4) times. The resultant thermoxidized palm oil was kept in air-tight amber bottles at room temperature and used for treating the animals.

Animals and Experimental Design

Twenty albino wistar rats of both sexes, weighing between 150 – 180g were randomly selected and used for the study. The animals were randomly divided into four groups of 5 rats each and were acclimatized for 7 days under standard environment at a temperature of 22 – 25^0C, 12hrs light and 12hrs dark cycle before commencement of administration. While being acclimatized, the rats were fed normal rat chow (Product of Vital Feed Nig. Ltd) and water *ad* libitum.*D. tripetala* extracts were orally administered to the rats using oral gastric tube inserted through their mouth. The extracts were administered for 21 days.

Group 1 (Positive control Group) was fed with normal rat chow and water without extracts.
Group 2 (negative control group) received normal rat chow with 1ml of thermoxidized palm oil.
Group 3 received 1ml of thermoxidized palm oil + 50mg/kg of stem extract of *D. tripetala*
Group 4 received 1ml of thermoxidized palm oil + 100mg/kg of stem extract of *D. tripetala*

Analytical Procedure

Within 24hrs after the 21st day of administration, the rats were sacrificed under ethyl ether anaesthesia and have their cavities cut open to expose the heart. Blood samples of the rats were collected by cardiac puncture using a sterile syringe and allowed to clot for 1 hour. Serum was obtained by centrifuging at 1000rpm for 5 minutes using a Wisperfuge centrifuge (model 1384), collected with a Pasteur pipette into clean labeled sample bottle and was later used for biochemical analysis.

Biochemical Analysis

Total cholesterol (TC), triglyceride (TG) and High density lipoprotein cholesterol (HDL-C) were determined by enzymatic assay method using analytical kits from Randox laboratories Ltd. UK while Low density lipoprotein cholesterol (LDL-C)and very low density lipoprotein cholesterol (VLDL-C) were derived by calculation using the Friedewald formula: LDL = TC – HDL – VLDL and VLDL = Triglycerides/5.Determination of serum malondialdehyde, glutathione peroxidase and serum catalase activity were according to the modified level Draper and Hadley (1990), modified method of Atawobi (2011) and method of Ellman (1959) respectively.

Statistical Analysis

Data generated in this study was entered, cleaned and coded in excel sheets and was statistically analyzed using the SPSS/IBM version 21 software. Means and standard deviations of means were calculated for all parameters under investigation. Statistical differences between the experimental and control groups were determined using ANOVA and students t-test. Values were considered significant at $p<0.05$.

RESULT

The results are presented in tables.

Table 1: Effect of ethanol stem extract of *Dennettia tripetala* on lipid profile

GROUPS	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
1 (Control)	232.40 ± 1.67	71.60 ± 5.02	32.00 ± 2.34	105.80 ± 2.38	14.60 ± 1.51
2 thermoxidized palm oil	294.50 ± 4.50*	158.00 ± 4.11*	54.00 ± 9.32*	208.25 ± 12.10*	20.00 ± 0.81*
3 (oil+50mg/kgextract)	289.60 ± 9.06	144.00 ± 7.94**	58.00 ± 4.95	175.40 ± 8.90**	31.60 ± 5.78**
4 (oil+100mg/kg extract)	241.60 ± 9.99**	119.00 ± 8.63**	63.00 ± 4.55**	63.80 ± 7.93**	30.80 ± 4.85**

Values are presented as mean ± SD of four determinants. n = 5. Values in the same column with single asterisk (*) are significantly different from group 1 while those with double asterisks (**) are significantly different from group 2 ($P < 0.05$).

Table 2: Effect of ethanol stem extract of *Dennettia tripetala* on serum concentration of malondialdehyde (MDA) and glutathione peroxidase (GPx) and serum catalase (CAT) activity

Groups	MDA (IU/L)	GPx (IU/L)	Catalase (IU/L)
1 (Control)	2.58 ± 0.19	59.25 ± 1.71	126.00 ± 6.52
2 (thermoxidized palm oil)	15.86 ± 9.45*	30.83 ± 2.48*	82.60 ± 2.30*
3 (palm oil + 50mg/kg extract)	13.98 ± 0.88	41.20 ± 1.92**	97.20 ± 2.17**
4 (palm oil +100mg/kg extract)	7.90 ± 0.16**	53.80 ± 5.68**	118.80 ± 5.81**

Values are presented as mean ± SD of four determinants. n = 5. Values in the same column with single asterisk (*) are significantly different from group 1 while those with double asterisks (**) are significantly different from group 2 ($P < 0.05$)

DISCUSSION

Assessment of lipid profile is an essential tool that helps in the diagnosis of cardiovascular diseases (Shai *et al*, 2004; Pischon *et al*, 2005; Walldius and Jungner, 2006) and to a lesser extent diagnosis of diabetes (Jiang *et al*, 2004; Bruno *et al*, 2006) and cancer (Santos and Schulze 2012; Muntoni *et al*, 2009). Lipid peroxidation which is free radical oxidation of polyunsaturated fatty acids such as linoleic acid or arachidonic acid is associated with thermal oxidation of edible oils and has been implicated in various diseases and pathogenic conditions (Negre-Salvayre *et al*., 2010) such as cardiovascular diseases (Glass and Witztum, 2001; Lee and Blair, 2001), neurodegeneration (Bradley *et al*., 2010) carcinogenesis (Marnett, 2000) and aging (Muller *et al*., 2007). A variety of free radical scavenging antioxidants are found in fruits, leaves and other plant parts and these antioxidants help in converting the ROS to less reactive species (Yadav *et al*., 2016) thereby constituting a defense system against free radicals arising from the body's biochemical reactions. This study was undertaken with the aim of assessing the effect of ethanol stem extract of *Dennettia tripetala* on lipid profile and antioxidant status in rats administered thermoxidized palm oil.

In the present study, comparison of the concentrations of the lipid parameters between group 2 rats given thermoxidized palm oil and the control group revealed a statistically significant increase in the concentrations of all the lipid parameters (TC, TG, HDL-C, LDL-C, VLDL-C) in group 2 (Table 1). When concentrations of the lipid parameters were compared between group 2 and groups 3 and 4 which were given varying doses of extract, the study showed a significant decrease in the concentration of TG and LDL-C in groups 3 and 4, significant decrease in the concentration of TC in group 4and a significant increase in the concentrations of VLDL-C in groups 3 and 4 and HDL-C in group 4 (Table 1). Phytochemical analysis of *Dennettia tripetala* revealed the presence of steroids, saponins, cardiac glycosides and flavonoids (Sparg, 2004). Studies have shown that these phytochemicals acting wholly or partly may be responsible for the lipid lowering action of some plant extracts (Gaamousi *et al*., 2010). The observed increase in the concentration of HDL-C with the administration of *D. tripetala* extract has some health benefits considering the antiatherogenic functions of HDL-C. HDL-C exerts its anti-atherogenic effects partly by counteracting LDL-C oxidation (Adaramoye *et al*., 2006). It also inhibits oxidation of LDL by transition metal ions and also prevents 12- lipoxygenase mediated formation by lipid hydroperoxide (Nofer *et al*., 2002).

On the concentrations of MDA, GPx and CAT, the present study showed a significant increase in the concentration of malondialdehydein group 2 rats when compared to the control group and a significant decrease in its concentration in group 4 compared to group 2 (Table 2). This result is indicative of the role of thermal oxidation of palm oil in promoting lipid peroxidation. Malondialdehyde which is the most abundant lipid peroxide is widely used as an indicator of lipid peroxidation (Kawase *et al*, 1989). There is significant decrease in the levels of glutathione peroxidase and catalase in group 2 when compared to the control. The decrease in the concentration of these antioxidants is believed to have resulted from the depletion of antioxidants while trying to scavenge free radicals resulting from thermal oxidation of palm oil and its associated lipid peroxidation. Antioxidant enzymes such as SOD, CAT and GPx play important roles in scavenging free radicals and preventing cell injury (Bergendi *et al*, 1999). Comparison of the levels of the antioxidants between group 2 and groups 3 and 4 revealed their significant increase in groups 3 and 4. This therefore indicates that stem extract of *Dennettia tripetala* has antioxidant property that increases with increase dose.

The findings of this study suggest that thermal oxidation of palm oil increases the level of free radicals, increases lipid peroxidation and increases depletion of antioxidants. It also suggests that *Dennettia tripetala* stem extract has lipid lowering potentials and anti-atherogenic effect. It is a pointer to the powerful antioxidant property of *Dennettia tripetala* which increases in potency with increase in dose.

In conclusion, stem extracts of *Dennettia tripetala* has lipid lowering potentials, anti-atherogenic effect and antioxidant properties which become more potent with increase in dose. It can therefore be properly harnessed and applied in the management and treatment of various chronic and degenerative disorders.

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