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EFFECT OF METHANOL EXTRACT OF *NAPOLEONAE IMPERIALIS* ON FREE RADICAL SCAVENGERS AND LIPID PROFILE OF WISTAR ALBINO RATS

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ABSTRACT

The present study was aimed at investigating the effect of methanol extract of *Napoleonae imperialis* on free radical scavengers and lipid profile of wistar albino rats. Sixteen (16) rats of mean weight 130g were assigned into four groups of four rats each. Two groups served as positive and negative controls, while the rest are the test groups that received 250mg/kg and 500mg/kg of the plant extracts. The positive control received only feed and water, while the other groups were induced with $CHCl_3$ and $C6H14$ (60:40) for hepatotoxicity. The negative control was induced but not treatment, while the test groups were orally administered with 250 and 500mg/kg body weight of the plant extract for 10 days. The rats were sacrificed after 10 days and the free radical scavengers status: vitamin C, vitamin E, catalase, superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione (GSH), and the lipid profile status: total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and triacylglycerol (TAG) were analysed. The result from the comparison of the mean difference of the test groups and the control groups analysis showed a significant increase ($p < 0.05$) in the level of catalase and vitamin C and a non-significant difference ($p > 0.05$) in the level of

vitamin E, GSH, SOD, and MDA, while the comparison of the mean difference of lipid profile between the test groups and control groups showed a non- significance difference ($p > 0.05$) in the total cholesterol, HDL, LDL, and TAG. The increased and decreased levels of the antioxidant status confirmed the free radical scavenging activity of *Napoleona imperialis*, while the non-significant difference in the lipid profile status shows that the plant extract has no effect on the lipid profile of the rats.

KEYWORDS: methanol extract of *Napoleonae imperialis,* lipid profile.

INTRODUCTION

The role of free radicals in disease initiation cannot be overemphasized. Most free radicals such as hydroxyl radical (OH^-), the superoxide radical (O_2-), lipid peroxide radicals and hydrogen peroxide (H_2O_2) are being implicated in some disease conditions. These include: cancer, gastrointestinal inflammation, asthma, cataracts, cardiovascular disease, diabetes mellitus, liver disorder, periodontal disease, and other inflammatory processes. (Gulcin *et al.*, 2003). These reactive oxygen species (ROS) are generated as a result of normal biochemical metabolism in the body which is due to high level of exposure to xenobiotics (Cook and Samman, 1996). Pathological conditions result when the generation of ROS induced by stimuli in the organism exceeds the antioxidant capacity of the organism (Yuan *et al.,* 2005). The harmful effect of these reactive species in normal metabolic processes which leads to disease condition is a consequence of their interaction with some biological compounds within and outside the cells. Recently, many natural and synthetic free radical scavengers and antioxidants have been employed in protecting biomolecules against free radical mediated damages (Lacey *et al.,* 1996).

The antioxidant activity or the inhibition of the generation of free radicals is important in providing protection against hepatic damage. A number of plants have been shown to possess hepatoprotective property by improving antioxidant status. Thus the efficacy of the drug would be preventive and passive for defending against damages (Decker *et al*., 2005).

Consequently, in this study we monitored the free radical scavenging effect, and the lipid profile effect of *Napoleonae imperialis* in wistar albino rats with a view to understanding at least in part, the mode by which methanol extracts of *Napoleonae imperialis* mediate or exert their free radical scavenging activity and lipid profile effect in wistar albino rats.

BACKGROUND OF THE STUDY/ JUSTIFICATION.

The background of this study lies on the fact that most people use extracts of *Napoleonae imperialis* to treat wounds and hypertension. The hypotensive effect may be due to the stimulation of muscarinic receptors of the parasympathetic nerve by the compounds or their actions as an antagonist of adrenergic receptor, but it may act as Ca^2+ ion channel block (Amran *et al*., 2004). For this reason, it is also suspected that the plant may enhance the activities of free radical scavengers and reduce the lipid profile of wistar albino rats. The study is therefore set to confirm or disprove this suspicion.

OBJECTIVE OF THE STUDY

 - To evaluate the effect of methanol extract of *Napoleonae imperiali*s on free radical scavengers or antioxidants in wistar albino rats
- To evaluate the effect of methanol extract of *Napoleonae impeialis* on the lipid profile in wistar albino

MATERIALS AND METHODS
PLANT MATERIAL

Fresh leaves of the plant *Napoleona imperialis* were obtained from a local farm in Umuariaga village, Umudike, Abia State, Nigeria, and identified by Dr. Garuba Omosun of the Plant Science and Biotechnology department, Michael Okpara Uiversity of Agriculture, Umudike.

PREPARATION OF PLANT EXTRACTS

The collected leaves were washed and dried and blended into powder using the grinder. The powdered plant material (100g) was extracted with methanol (95%v/v). The extract was air dried to evaporate the solvent.

ANIMALS

Healthy female Wistar albino rats having 130 grams mean weight were used for the study. All animals were housed in an animal house under normal room conditions and acclimatized for two (2) weeks. Commercial pellet diet (Vital growers mash by Grand Cereals and Oil Mills, Nigeria) and water were given to the animals *ad libitum*.

INDUCTION OF HEPATOTOXICITY

All the rats used for this study were initially subjected to hepatocyte damage using hexane and chloroform in the ratio of 4:6 at 2.5ml per rat orally except the normal control group.

EXPERIMENTAL DESIGN

Sixteen (16) female albino rats were used in the study of mean weight 130g. The Animals were grouped into four, gp1, gp2, gp3 and g4.The first two groups being the test groups and the last two are the control groups with four rats per group. Test Groups were given 250, 500mg/kg body weight respectively of methanolic leaf extract of *Napoleona imperialis* for 10 days via gastric intubation. Group three represented the normal control that did not receive the extract, but food and water only. Group four represented the negative control which was induced with hepatotoxin that is chloroform (60) and hexane (40) and treated without the extract. The animals were subsequently anaesthetized and blood samples were collected for catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD), and reduced gluthathione (GSH) assays.

DETERMINATION OF ANTIOXIDANT MARKERS
DETERMINATION OF SUPEROXIDE DISMUTASE (SOD)

xanthine-xanthine oxidase   system was used to generate a superoxide flux, and nitroblue tetrazolium (NBT) was used as an indicator of superoxide production. SOD activity was then measured by the degree of inhibition of the reaction unit of enzyme provides 50% inhibition of NBT reduction. Results are expressed as U/ml.

$$\% \text{ inhibition} = \frac{Ao - As}{Ao} \text{ x } 100$$

Where, A_0 is the absorbance of the control, and A_s is the absorbance of the tested sample.

DETERMINATION OF CATALASE ACTIVITY

Catalase (CAT) activity was measured using the method . Serum (10 µL) was added to test tubes containing 2.80 ml of 50Mm potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1ml of fresh Hydrogen Peroxide (H_2O_2) and the decomposition rate of H_2O_2 was measured at 240 nm for 5minutes on a spectrophotometer. A molar extinction coefficient of 0.041 mM^{-1}-cm^{-1} was used to calculate Catalase activity.

MALONDIALDEHYDE ESTIMATION

The degree of lipid peroxidation was assayed by estimating the thiobarbituric acid reactive substances (TBARS) using spectrophotometric method as described by earlier workers. The supernatant (50 µl) was deproteinized by adding 1ml of 14% trichloroacetic acid and 1ml of 0.6% thiobarbituric acid. The mixture was heated in a water bath for 30 min to complete the reaction, and then cooled on ice for 5 minutes. After centrifugation at 2000 g for 10 mins, the

absorbance of coloured product (TBARS) was measured at 535 nm with a UV spectrophotometer. Total protein content of the homogenate was determined spectrophotometrically using the Biurette method. The concentration of TBARS was calculated using the molar extinction coefficient of malondialdehyde (1.56×10^5 mol/L/cm) using the formularI, $A = \Sigma CL$, where A = absorbance, Σ = molar coefficient, C = concentration and L = path length. All TBARS concentration was expressed in μmol/g tissue protein.

DETERMINATION OF GLUTATHIONE

The colourimetric method of determing glutathione was used, 1ml portion of the sample was mixed with 7mls of distilled water (D.$H_2$0). The mixture was treated 2mls of 25% metaphosphoric acid solution. After a minute, it was centrifuged. The supernatant was used for the assay. To the extract (supernatant), in the test tube iml of 0.5m phosphate buffer solution was added followed by 1ml of 0.5m NaOH.

DETERMINATION OF VITAMIN C

The 2, 6- dichlorophenol indophenol colourimetric method was used.

A measure volume of the sample was mixed with 3mls of 5% metaphosphoric acid solution, mixed well and then centrifuged 2mls of the supernatant was mixed with 0.5mls 1.7% sodium citrate and its absorbance was measured.

DETERMINATION OF VITAMIN E

Pippete 0.5mls of the serum into a test tube, add 0.9mls of distilled water. Add 1mls of 0.27 ferric chloride and 1ml of acholic 0.5% a- dipyridyl solution and shake. Then add 5mls with distilled water and measure the absorbance at 520nm.

DETERMINATION OF LIPIDS

DETERMINATION OF HIGH DENSITY LIPOPROTEIN

High density lipoprotein (HDL) are one of the major classes of plasma lipoprotein. They are composed of a number of heterogenous particles including cholesterol which vary with respect to size and content of the lipid and apolipoprotein. HDL serve to remove the cholesterol from the pheripheral cells to the liver, where the cholesterol is converted to bile acids and excreted into the intestine.

An inverse relationship between HDL- cholesterol (HDL-C) levels in serum and the incidence of coronary heart disease (CHD) has been demonstrated in a number of epidemiological studies. The importance of HDL- c as a risk factor for CHD is now recognizes.

Accurate measurement of HDL- C is of vital importance when assessing patient risk from CHD. In this diagnostic test for a method of direct measurement of HDL-C without sample pre-treatment, is presented. Direct measurement gives improved accuracy and reproducibility when compared to precipitation methods.

Principle

Low density lipoprotein (LDL and VLDL) and chylomicrons fractions are precipitated quantitatively by the addition of phosphortungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, is determined.

TABLE 3.1 DETERMINATION OF HDL

Procedure

1.Precipitation
Pipette into centrifuge
Sample/ standard   Diluted precipitate ®
Mix and allow to sit for 10 minutes at room temperature
Then centrifuge for 10 minutes at 4,000rpm or   minutes at 12,000 rpm.

Separate off the clear supernatant within two hours and determine the cholesterol content by the CHOD PAP method. The supernatant may be stored up to five days at +2 to + 25°C

DETERMINATION OF LOW DENSITY LIPOPROTEIN (LDL)

Principle: LDL- cholesterol can be determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethylene-glycol monomethyl ether.

Procedure

1.Precipitation reaction: The precipitant (0.1ml(3 drops) and the sample (0.2ml) was mixed and allowed to stand for 15min approximately at room temperature (20-25^{0c}). It was

centrifuged at 2,000 x g/15min and the cholesterol concentration in the supernatant was determined.

2.Cholesterol assay: The concentration of the serum total cholesterol according to the QCACHOD-PAP method was determined.

LDL-cholesterol (mg/dl) = Total cholesterol (mg/dl) – 1.5 x supernatant cholesterol (mg/dl)

DETERMINATION OF TRIACYLGYLCEROL (TAG)

Triglycerides measurement is used in the diagnosis and treatment of diseases involving lipid metabolism and various endocrine disorders. e.g diabetes mellitus, nephrosis, and liver obstruction.

COLORIMETRIC METHOD

The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxidase, 4- aminophenazone and 4- chlorophenol under the catalytic influence of peroxidase.

Procedure

Using fresh ddH$_2$0 perform a new gain calibration in cuvette mode. Select TRI in the run. Test screen and carry out a water blank as instructed.

TABLE 3.2 DETERMINATION OF TRIACYLGLYCEROL (TAG)
Procedure

Using fresh ddH$_2$0 perform a new gain calibration in cuvette mode. Select TRI in the run. Test and carry out a water blank as instructed.

Pippete into a cuvette			
	Reagent blank SO	Standard S1	Sample
DdH20	-	-	-
Standard	-	5ul	-
Sample	-	-	5ul
Reagent R1	500ul	500ul	0,05ml
Mix, incubate for 10 min at 20- 25^{0}C or 5min at 37^{0}C. Insert into the RX Monza flow cell holder and press read within 60mins.			

DETERMINATION OF CHOLESTEROL

Cholesterol measurements are used in the diagnosis and treatment of lipid lipoprotein metabolism disorder. Lipids play an important role in the body, they serve as hormones or hormones precursors, aid in digestion, provide energy, storage and metabolic fuels, acts as functional and structural components of biomolecules and form insulation to allow nerve conduction and prevent heat loss.

In clinical chemistry, over the last decade however, lipids have become associated with lipoprotein metabolism and atherosclerosis. The Abel Kendall method, reported by Abet et al (1952) involved extraction of cholesterol by organic solvents and subsequent alkaline hydrolysis of the cholesterol esters. The reaction is highly specific but the reagents involved are corrosive and the method cumbersome, rendering it impractical for routine laboratory test.

The use of cholesterol oxidase following specimen saponification as described by Richmond (1973) provided the first step towards a total enzymatic procedure. In 1974, Albin et al and Roeschlaw et al published the first fully enzymatic procedure for cholesterol determination replacing chemical saponification with enzymatic saponification.

ASSAY PRINCIPLE

Cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxidase and 4- aminoantipyrine in the presence of phenol and peroxidase.

TABLE 3.3 DETERMINATION OF CHOLESTEROL

Procedure

Using fresh ddH_2O perform a new gain calibration in cuvette mode. Select TRI in the run. Test and carry out a water blank as instructed.

Pippete into a cuvette			
	Reagent blank SO	Standard S1	Sample
ddH_2O	-	-	-
Standard	-	5ul	-
Sample	-	-	5ul
Reagent R1	500ul	500ul	0,05ml

Mix, incubate for 10 min at 20- 250C or 5min at 370C. Insert into the RX Monza flow cell holder and press read within 60mins.

STATISTICAL ANALYSIS

Statistical analysis was carried out on the data obtained using analysis of variance (ANOVA) at 0.05 (95%) confidence level.

RESULTS AND INTERPRETATION

TABLE 4.1: Comparison of the mean difference of the lipid profile (T.CHOL, HDL, LDL, and TAG) between the test group that received 250mg/kg of the plant extract and Group A (normal control) and Group B (negative control)

Samples	T.CHOL	HDL	LDL	TAG
Group A positive control	$5.29^a \pm 0.85$	$1.40^a \pm 0.32$	$3.15^a \pm 0.17$	$2.55^a \pm 0.13$
Test group 250ml/kg extract	$5.51^a \pm 0.67$	$1.50^a \pm 0.18$	$2.70^a \pm 0.41$	$2.40^{ab} \pm 0.36$
Group B negative control	$5.86^a \pm 0.07$	$1.24^a \pm 0.05$	$2.70^a \pm 0.64$	$2.11^b \pm 0.18$

The result showed that for total cholesterol (T.CHOL), there was no significant difference ($p > 0.05$) between the test group given 250mg/kg of the plant extract and Group A (normal control) and Group B (negative control).

For high density lipoprotein (HDL), the analysis showed that there was no significant difference ($p > 0.05$) between the test group given 250mg/kg of the plant extract and Group A (normal control) and Group B (negative control).

For low density lipoprotein (LDL), the result from the table above showed that there is no significant difference ($p > 0.05$) between the test group given 250mg/kg of the plant extract and Group A (normal control) and Group B (negative control)

For the triacylglycerol (TAG), the result from the table above showed that there is no significant difference ($p > 0.05$) between the test group given 250mg/kg of the plant extract and Group A (normal control) and Group B (negative control), but there is a significant difference ($p < 0.05$) between Group A (normal control) and Group B (negative control).

TABLE 4.2: Comparison of the mean difference of the lipid profile (T.CHOL, HDL, LDL, and TAG) between the test group that received 500mg/kg of plant extract with Group A (normal control), and Group B (negative control)

Samples	T.CHOL	HDL	LDL	TAG
Group A positive control	$5.29^a \pm 0.85$	$1.40^a \pm 0.32$	$3.15^a \pm 0.17$	$2.55^a \pm 0.13$
Group B negative control	$5.86^a \pm 0.07$	$1.24^a \pm 0.05$	$2.70^{ab} \pm 0.64$	$2.11^a \pm 0.18$
Test group with 500mg/kg extract	$4.55^a \pm 1.23$	$1.25^a \pm 0.13$	$2.40^b \pm 0.26$	$2.58^a \pm 0.19$

The result from the table above showed that, for total cholesterol (T.CHOL) there was no significant difference (p> 0.05) between the test group that received 500mg/kg of the plant extract and Group A (normal control) and Group B (negative control).

For high density lipoprotein (HDL), the result from the table above showed that there is no significant difference (p> 0.05) between the test group that received 500mg/kg of the plant extract and Group A (normal control) and Group B (negative control).

For low density lipoprotein (LDL), the result from the table above showed that there is a significant difference (p< 0.05) between the test group that received 500mg/kg of the plant extract and Group A (normal control), and no significant difference between the test group and Group B (negative control)

For the triacylglycerol (TAG) the result from the table above showed that there is no significant difference (p> 0.05) between the test group that received 500mg/kg of the plant extract and Group A (normal control) and Group B (negative control).

TABLE 4.3

Samples	T.CHOL	HDL	LDL	TAG
Group A positive control	$5.29^{ab}\pm0.85$	$1.40^{a}\pm0.32$	$3.15^{a}\pm0.17$	$2.55^{a}\pm0.13$
Group B negative control	$5.86^{a}\pm0.07$	$1.24^{a}\pm0.05$	$2.70^{ab}\pm0.64$	$2.11^{b}\pm0.18$
Test group 250ml/kg extract	$5.51^{ab}\pm0.67$	$1.50^{a}\pm0.18$	$2.70^{ab}\pm0.41$	$2.40^{ab}\pm0.36$
Test group with 500mg/kg extract	$4.55^{b}\pm1.23$	$1.25^{a}\pm0.13$	$2.40^{b}\pm0.26$	$2.58^{a}\pm0.19$

The table above shows a summary of the comparism of the mean difference of the lipid profile (T.CHOL, HDL, LDL, and TAG) of the test group that received 250 and 500mg/kg of the plant extract and Group A (normal control) and Group B (negative control).

TABLE 4.4: Comparison of mean difference of free radical scavengers (vit C, vit E, GSH, SOD, CATALASE, and MDA) between the test group that received 250mg/kg of the plant extract with Group A (normal control) and Group B (negative control).

Samples	VITAMIN C	VITAMIN E	GSH	SOD	CATALASE	MDA
Group A positive control	$1.72^{a}\pm0.69$	$1.12^{a}\pm0.24$	$3.30^{a}\pm1.88$	$0.99^{a}\pm0.11$	$4.56^{a}\pm1.59$	$1.92^{a}\pm0.55$
Test group 250ml/kg extract	$0.58^{b}\pm0.16$	$1.04^{a}\pm0.02$	$5.12^{a}\pm0.14$	$0.89^{a}\pm0.03$	$1.09^{b}\pm0.06$	$1.89^{a}\pm0.23$
Group B negative control	$1.30^{a}\pm0.11$	$1.12^{a}\pm0.08$	$5.01^{a}\pm0.24$	$0.88^{a}\pm0.07$	$3.08^{a}\pm0.61$	$2.07^{a}\pm0.56$

The result of the table showed that for vit C, there is a significant difference ($p < 0.05$) between the test group that received 250mg/kg of the plant extract with Group A (normal control) and Group B (negative control).

For vitamin E, there is no significant difference ($p > 0.05$) between the test group that received 250mg/kg of the plant extract with Group A (normal control) and Group B (negative control).

For glutathione (GSH), there is no significant difference ($p > 0.05$) between the test group that received 250mg/kg of the plant extract with Group A (normal control) and Group B (negative control).

For superoxide dismutase (SOD), there is no significant difference ($p > 0.05$) between the test group that received 250mg/kg of the plant extract with Group A (normal control) and Group B (negative control).

For catalase (CAT), there is significant difference ($p < 0.05$) between the test group that received 250mg/kg of the plant extract with Group A (normal control) and Group B (negative control).

For malondialdehyde (MDA), there is no significant difference ($p < 0.05$) between the test group that received 250mg/kg of the plant extract with Group A (normal control) and Group B (negative control).

TABLE 4.5: Comparison of the mean difference of free radical scavengers (vit C, vit E, GSH, SOD, CATALASE, and MDA) of the test group that received 500mg/kg of the plant extract with group A (normal control) and group B (negative control)

Samples	VITAMIN C	VITAMIN E	GSH	SOD	CATALASE	MDA
Group A positive control	$1.72^a \pm 0.69$	$1.12^a \pm 0.24$	$3.30^a \pm 1.88$	$0.99^a \pm 0.11$	$4.56^a \pm 1.59$	$1.92^a \pm 0.55$
Group B negative control	$1.30^{ab} \pm 0.11$	$1.12^a \pm 0.08$	$5.01^a \pm 0.24$	$0.88^a \pm 0.07$	$3.08^a \pm 0.61$	$2.07^a \pm 0.56$
Test group with 500mg/kg extract	$0.78^b \pm 0.23$	$1.05^a \pm 0.16$	$4.48^a \pm 0.24$	$0.90^a \pm 0.03$	$1.13^b \pm 0.07$	$1.69^a \pm 0.23$

The result from the table showed that for vitamin C, there is a significant difference ($p < 0.05$) between the test group that received 500mg/kg of the plant extract with Group A

(normal control), but no significant difference between the test group and Group B (negative control).

For vitamin E, the result from the table showed that there is no significant difference (p> 0.05) between the test group that received 500mg/kg of the plant extract with Group A (normal control) and Group B (negative control).

For glutathione (GSH), the result from the table showed that there is no significant difference (p> 0.05) between the test group that received 500mg/kg of the plant extract with Group A (normal control) and Group B (negative control).

For superoxide dismutase (SOD), the result from the table showed that there is no significant difference (p> 0.05) between the test group that received 500mg/kg of the plant extract with Group A (normal control) and Group B (negative control).

For catalase (CAT), the result from the table showed that there is a significant difference between (p> 0.05) between the test group that received 500mg/kg of the plant extract with Group A (normal control) and Group B (negative control)

For malondialdehyde (MDA), the result from the table showed that there is no significant difference (p> 0.05) between the test group that received 500mg/kg of the plant extract with Group A (normal control) and Group B (negative control).

TABLE 4.6

Samples	VITAMIN C	VITAMIN E	GSH	SOD	CATALASE	MDA
Group A positive control	$1.72^a \pm 0.69$	$1.12^a \pm 0.24$	$3.30^b \pm 1.88$	$0.99^a \pm 0.11$	$4.56^a \pm 1.59$	$1.92^a \pm 0.55$
Group B negative control	$1.30^{ab} \pm 0.11$	$1.12^a \pm 0.08$	$5.01^a \pm 0.24$	$0.88^a \pm 0.07$	$3.08^b \pm 0.61$	$2.07^a \pm 0.56$
Test group 250ml/kg extract	$0.58^c \pm 0.16$	$1.04^a \pm 0.02$	$5.12^a \pm 0.14$	$0.89^a \pm 0.03$	$1.09^c \pm 0.06$	$1.89^a \pm 0.23$
Test group with 500mg/kg extract	$0.78^{bc} \pm 0.23$	$1.05^a \pm 0.16$	$4.48^{ab} \pm 0.24$	$0.90^a \pm 0.03$	$1.13^c \pm 0.07$	$1.69^a \pm 0.23$

Table 4.6 above, shows a summary of the comparison of the mean difference of free radical scavengers (vit C, vit E, GSH, SOD, CATALASE, MDA) between the test group that received 250mg/kg and 500mg/kg of the plant extract with Group A (normal control) and Group B (negative control).

DISCUSSION

Antioxidant enzymes (made in the body) and antioxidant nutrients (found in foods) can scavenge and deactivate this reactive free radicals turning them to harmless particles (Chu *et al.*, 2002). Improving body antioxidant status is a way to fight against degenerative diseases. This could be achieved by higher consumption of vegetables and fruits (Oboh and Rocha, 2007). The positive effect attributable to antioxidant is due to the presence of carotenoids, flavonoids, lycopene, phenolics, vitamin C and B-carotene (Zhang and Hanazu, 2004). The effective of the antioxidants usually increases with their concentration (Deck et al., 2005).

The effect of the methanol extract of Napoleonae imperialis on some parameters were examined, for the test group that received 250mg/kg and 500mg/kg of the plant extract in comparison with the mean difference of the normal and negative control, there was no significant difference ($p > 0.05$) in the level Vitamin E, Glutathione, Superoxide dismutase, and malondialdehye, signifying that the level of these free radical scavengers did not increase much in the test group in comparison with the normal and negative control.

There was no significant difference in the levels of SOD across the treated groups, and the control groups, which is the only enzyme that employs the superoxide anions as a substrate and produces the hydrogen peroxide as a metabolite, this is more toxic than O2 radical and has to be disposed by catalase.

Also, there was no significant level in the levels of MDA with the normal and negative control groups. MDA is the end product of lipid peroxidation and measures free radical generation. Thus, validating the scavenging property of the extract at high concentration against free radicals generate

Glutathione (GSH) is a tripeptide found in most cells and reacts with the free radicals to protect cells against hydroxyl radical, singlet oxygen and superoxide radical (Schulz *et al.,* 2000). The activity of GSH reduced in hexane (40) and chloroform (60) control is an indication of decreased liver functions (Ahmed *et al.,* 2005). Its level increased significantly in the treated groups to a near normal value; this shows the ability of the leaf extract in increasing the competency of liver in detoxification of xenobiotics, as GSH is a major detoxifier in the liver. This shows that the leaf extract possesses antioxidant properties that help to stabilize the integrity of cell membrane and also prevent hepatic insult mediated free radicals.

There was no significant difference in the level of vitamin E in both the test group and the control groups involved indicating that the plant extract was unable to raise the level of vitamin E in the rats.

There was a significant difference (p> 0.05) in the level of catalase and vitamin C in the test groups and the controls groups indicating a significant increase in levels of catalase and vitamin C in all the treated groups, indicating the aqueous extract scavenges the hydrogen peroxide, which is generated by SOD.

The effect of the methanol extract of *Napoleonae imperialis* on the lipid profile of the rats in comparison with the mean difference treated with 250mg/kg and 500mg/kg of the plant extract and the control groups showed no significant difference (p> 0.05)   indicating that the plant extract was unable to lower the lipid profile of the rats.

The present investigation showed that the methanol extract of *Napoleona imperialis has* antioxidant properties by scavenging free radicals, decreasing lipid peroxidation and increasing the endogenous blood antioxidant enzymes levels.The result of the study, shows that *Napoleona imperialis* has a significant difference (p< 0.05) in the activity of Catalase and vitamin C. The plant should therefore be employed as sources of natural antioxidant boosters and for the treatment of oxidative stress disorders in which free radicals are implicated.

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