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RESEARCH ARTICLE

ANTI OXIDATIVE STATUS OF NAUCLEA LATIFOLIA LEAVES EXTRACT ON CIPROFLOXACIN INDUCED OXIDATIVE STRESS IN RATS

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ABSTRACT

The antioxidative potentials of Methanol extract of *Nauclea latifolia* leaves on ciprofloxacin induced oxidative stress and hepatotoxic damage in rats was studied. Forty mature albino rats of the Wistar strain weighing between 200g and 300g were used, divided into four groups of ten each. Ciprofloxacin (70mg/kg/day) for 14 days was administered; Plasma and liver homogenate antioxidants were measured. Vitamin C, vitamin E concentrations, Glutathione Peroxidase (GPX) and superoxide dismutase (SOD) activities were estimated by employing colorimetric method while malondialdehyde was by Standard curve method..Result showed that plasma and liver homogenate lipid peroxidation marker (malondialdehyde, MDA) increased significantly ($P < 0.005$) when compared with the controls. The activities of blood and liver homogenate of superoxide dismutase and glutathione peroxidase were also reduced significantly ($P < 0.005$) in rats treated with ciprofloxacin when compared with the controls. Furthermore, levels of plasma and liver homogenate of vitamin C and E also reduced significantly ($P < 0.005$) in rats treated with ciprofloxacin when compared with the controls. In reverse, the activities of blood and liver homogenate of enzymic antioxidants; superoxide dismutase (SOD) and glutathione peroxidase (GPX) and levels of plasma and liver homogenate of non enzymic antioxidants also increased significantly on treatment with methanol extract of N. *latifolia leaves*. The results were also dose dependent. It was concluded that ciprofloxacin can induce hepatotoxic and oxidative damage in rats and treatment with methanol extract of *Nauclea latifolia* leaves reversed the effects.

INTRODUCTION

Ciprofloxacin is a fluoroquinolones group of antibiotics containing 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (Zimpfer *et al.,* 2004). Ciprofloxacin stimulates reactive oxygen species (ROS) production in *Escherichia Coli*, *Enterococcus faecalis* and *Staphyllococcus aureus*, (Albesa *et al.,* 2004) and increased levels of ROS in ciprofloxacin sensitive microorganisms (Becerra and Albesa, 2002; Goswami *et al.,* 2006). Reported that superoxide anions (O^-_2) and hydrogen peroxide (H_2O_2) are involved in the antibacterial action of ciprofloxacin. Removal or neutralization of ROS is achieved with antioxidants, endogenous (e.g. catalase, glutathione, super-oxide dismutase) or exogenous (e.g. vitamins A, C, E, bioflavonoids, carotenoids). Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may cause diseases, damage or kill cells (Valko *et al.,* 2007). Oxidative stress is evaluated in terms of thiobarbituric acid reactive substances (TBARS) and total lipid hydroperoxides (LOOH) while reduced glutathione (GSH) and total thiols (T-SH) have been studied as markers of non-enzymatic antioxidant status. The levels of these parameters have been shown to be valuable as indicators or biomarkers of oxidative stress and disease progression in a number of pathophysiologies (Singh *et al.,* 2003; Singh *et al.,* 2005). Furthermore, (Nkafamiya, 2006), studies on *Nauclea latifolia* (fruits) showed that it possesses the following biochemicals Copper, iron, cobalt, calcium, magnesium, zinc, phosphorus, vitamins A, B_1,B_2 ,C, and E, with vitamin E, zinc and phosphorus in a higher concentration

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compared to the others. Phytochemical components of *Nauclea latifolia* are saponin, tannin, alkaloid and glycoside (El-Mahmood *et al.,* 2008). The roots of *Nauclea latifolia* showed the presence of sugars, saponins and flavonoids (Nworgu *et al.,* 2008). The phytochemical screening of *N.latifolia* leaves methanolic extract revealed the presence of alkaloids, tannins and terpenoids (highly present); saponnins, glycosides and flavonoids (moderately present) while anthraquinones (absent), while aqueous extracts, revealed flavonoids (highly present), saponnins, tannins, and terpenoids were moderately present and glycosides absent (Maikai, 2008). *Nauclea latifolia* is of the plant family Rubiaceae. It is called egbesi in Yoruba, uburu inu, mvunilu, ubulu inu or mbitinu in Igbo and marga in Hausa. It is a small evergreen tree or straggling shrub with leaves rounded ovate, apex shortly acuminate, rounded or cuneated base and stipule ovate. It is native to tropical Africa. Parts of the plant used for treatment of diseases include; leaves, roots, stem and fruits (Duke, 2008). The use of plants,' plant parts as sources of medicine to treat and prevent diseases predates history (Erdememeier *et al.,* 1996; Lino *et al.,* 2006). *Nauclea latifolia* is a plant and is used by traditional healers to cure so many diseases, arrest pre-term labour (Nworgu *et al.,* 2010). The leaves, barks and roots of *Nauclea latifolia* were discovered to have antibacterial actions against gram positive and gram negative bacteria e.g.p*seudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus* and *Shigella dysenteriae* (El-Mahmood *et al.,* 2008), and hypertensive action [10].The plant is also used as chewing sticks (Asubiojo *et al* 1982). *N.latifolia* is active against *plasmodium falciparium* (Traore-Keita *et al.,* 2000), *Bacilius subtilis*,and *Escherichia Coli* (Omar *et al.,* 1998). Klebsiella pneumonia (Tona *et al.,* 1999). *Salmonella enteriditis* and *Pseudomonas aeruginosa* (Hussein *et al.,* 1991) are sensitive to it. Aqueous leaves extracts of *Nauclea latifolia* at 200mg/kg body weight administered on Alloxan induced diabetic rats significantly lowered glucose levels ($P<0.05$) of the diabetic rats by 45% within four hours of administration (Gidado *et al.,* 2005). The bark of *Nauclea latifolia* is used in the treatment of wounds, coughs and gonorrhoea in Nigeria (Madubunyi *et al.,* 1995), while the fruits extract was shown to be active against Human Immuno-Deficiency Virus (Hussein *et al.,* 1999). However N. *Latifolia* has been extensively employed in the prevention and management of diseases without the practitioners having understanding of the mechanism of its action. Hence this research.

Justification

Many deaths in our localities have been attributed to adverse drug reactions (ciprofloxacin inclusive) and hypersensitivity as a result of indiscriminate use of antibiotics and herbal remedies. There is paucity of information on the potentials of N. *Latifolia* to reverse or improve adverse drug reactions especially due to ciprofloxacin administration.

MATERIALS AND METHODS

Plant materials

Plant Materials and Chemicals: Fresh and apparently uninfected leaves of *N. latifolia* were collected from plants growing within Owerri, Imo State. The botanical identification of the plant leaf was done at the Department of Plant Biology and Biotechnology, Imo State University, Owerri, where voucher samples are kept for reference.

Preparation of the Extract

Preparation of the Extract Fresh leaves of *N. latifolia* were dried in carbolite moisture extraction drying oven (Grant instruments, Cambridge, England) at $45^0C – 50^0C$ for 3 hours. Grinding was done using Thomas contact mill (pye Unicam, Cambridge, England). The ground materials were sieved through a 1mm sieve. Two hundred grammes (200 g) of fine power were dissolved in about 1000 ml of methanol and allowed to stand overnight. The extract was filtered. The filtrate was evaporated by hot air oven (Grant instrument, Cambridge, England) treatment at $45^0C – 50^0C$ for 72hrs. A 46g yield was noted and the appropriate concentrations were made based on the experimental design using distilled water (Nwanjo, 2006).

Preparation of Stock Solution of the Extract

On each day of the experiment, ten grammes of the plant extract was weighed and dissolved in one hundred millilitres (instead of mills) of distilled water to obtain a 100g/ml stock solution instead of 100mg/ml

DrugsandSolvents

Ciprofloxacin and Normal saline 0.9% infusion w/v {DANA Pharmaceuticals, Ibadan, Nigeria}, methanol {Sigma-Aldrich, Inc, USA}.

Laboratory Animals

Wistar rats weighing between 200 and 300 g bred in the Animal House of College of Medicine and Health Sciences, Imo State University, Owerri were used in this study. They were housed in stainless steel cages and kept in a room where a 12 h light/dark cycle was maintained. They were allowed free access to water and feed diet (product of Pfizer Nigeria Ltd) *ad libitum* throughout the period of the experiment.

Experimental design

Eighty male and female Wister rats were used in this study. The rats were randomized and divided into four groups of twenty animals each.

Group 1: (Control), received only normal saline 0.9% infusion w/v 5ml/kg body weight once daily.
Group 2: Received 70 mg/kg body weight of Ciprofloxacin, once daily.
Group 3: Received 70 mg/kg body weight of Ciprofloxacin and 1200mg/kg body weight of methanol extract of *N.latifolia* once daily.
Group 4: Received 70 mg/kg body weight of Ciprofloxacin and 2000mg/kg body weight of methanol extract of *N.latifolia* once daily.

Blood Sample Collection

After 14 days of treatment, Twenty four hours after the last doses were administered, the animals were weighed and sacrificed. Blood was collected by cardiac puncture after

fasting for 16 h in two different tubes i.e. one with EDTA anticoagulant for plasma separation and another without anticoagulant to separate serum for various biochemical estimations (Nwanjo *et al.,* 2007). The blood without anticoagulant were allowed to stand for about 30minutes to clot, and further centrifuged at 3500 rpm for 5 minutes using Wisperfuge model 1384 centrifuge (Samson, Holland). Serum was separated from clot with Pasteur pipette into sterile serum sample tubes for the measurement of biochemical parameters. The liver from both control and test animals were dissected out, and cleared off blood. They were immediately transferred to ice-cold containers containing 0.9% NaCl, washed there and homogenized in 0.1 N Tris-Hcl buffer (pH 7.4), and used for the estimation of malondialdehyde (MDA), glutathione peroxidase (GPX), and superoxide dismutase (SOD).

Acute toxicity tests

The acute toxicity of the extract was tested using 30 mice divided into 5 groups of 6 mice each, with each group receiving graded dose (1500-3000 mg/kg body weight, intraperitoneally) of the methanol extract of *N. latifolia* as described by (Ghosh, 1984). After administration of the extract the rats were observed for toxic effects after 24 h treatment. The toxicological effects were observed in terms of mortality expressed as LD50. The number of animals dying during a period was noted. The LD50 of the extract was estimated from the graph of percentage (%) mortality (converted to probit) against log-dose of the extract, probit 5 being 50% (Litch field *et al.,* 1959).

Laboratory method and procedure

All the reagents were commercially purchased and the manufacturer's standard operating procedure (SOP) were strictly followed.

(a)Estimation of non-enzymic antioxidants

i. **Methodology for Vitamin C (ascorbic acid) Estimation**

Principle: The ascorbic acid is converted to hydroascorbic acid by cupric sulphate solution and this couples with 2,4 dinitrophenyl hydrazine in the presence of thiourea as a mild reducing agent, sulphuric acid then converts the DNPH into a red coloured compound which is assayed spectrophotometrically.

Procedure: Plasma/ liver homogenate vitamin C (ascorbic acid) concentration was measured by (Omaye *et al.,* 1979) method. To 0.5 ml of plasma/ liver homogenate, 1.5 ml of 6% TCA was added and centrifuged at 3500g for 20 minutes using Wisperfuge model 1384 centrifuge (Samson Holland).To 5ml of supernatant, 0.5 ml of DNPH reagent (2% DNPH) and 4% thiourea in 9 N sulphuric acid) was added and incubated for 3 h at room temperature. After incubation 2.5 ml of 85% sulphuric acid was added and colour developed was read at 530 nm after 30 min.

ii. **Methodology for Vitamin E (α-tocopherol) Estimation**

Principle: The principle is based on the reduction of ferric to ferrous ion by vitamin E which then form a red complex with 2,2-α-dipyridyl. Vitamin E and carotenes were first extracted into xylene and the extinction read at 460nm to measure the carotenes. A correction is made for these after adding ferric chloride and reading at 520nm

Procedure: Plasma / liver homogenate vitamin E (α-tocopherol) was estimated by the method of Desai (1984). Vitamin E was extracted from plasma/liver homogenate by addition of 1.6 ml ethanol and 2.0 ml petroleum ether to 0.5 ml plasma/liver homogenate and centrifuged. The supernatant was separated and evaporated. To the residue, 0.2 ml of 0.2% 2,2-α-dipyridyl, 0.2 ml of 0.5% ferric chloride was added and kept in dark for 5 min, an intense red colour layer obtained on addition of 4 ml butanol was read at 520 nm.

(b) **Estimation of enzymic antioxidants**

i. **Methodology for Glutathione Peroxidase Estimation**

The kit was purchased from Randox Diagnostic LTD,Cat. No. RS 504.

Principle: Glutathione Peroxidase (GPX) catalyses the oxidation of Glutathione (GSH) by cumene hydroperoxide. In the presence of Glutathione Reductase (GR) and NADPH the oxidised Glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$.The decrease in absorbance at 340nm is measured (Paglia and Valentine, 1967).

$$2GSH + Rk\ OOH \xrightarrow{GPX} ROH + GSSG + H_2O$$
$$GSSG + NADPH + H^+ \xrightarrow{GR} NADP^+ + 2GSH$$

Procedure: 0.05ml of heparinized whole blood/plasma was diluted with 2ml of diluting agent. 20µl of the mixture was transferred into a test tube containing 1000µl of R1 (glutathione reductase + buffer) and 40 µl of R2 (Cumene). The solution was mixed and aspirated Initial absorbance of the sample and reagent blank was read after one minute .Read again after 1 and 2 minutes.Reagent blank value was then subtracted from that of the sample. Glutathione peroxidase concentration was calculated using the formula: U/l of Haemolysate =8412 x ▲A 340nm/minute.

ii **Methodology for Superoxide dismutase (SOD) Estimation**

The kit was purchased from Randox Diagnostic LTD, Cat. No. SD 125.

Principle: The role of superoxide dismutase (SOD) is to accelerate the dismutation of the toxic superoxide radical (O_2), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenol)-3(nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye (Wooliams *et al.,* 1983). The superoxide peroxide activity is then measured by the degree of inhibition of this reaction.One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay.

Procedure: 0.5ml of heparinized or EDTA whole blood was centrifuged for 10minutes at 3000rpm and then the plasma was

aspirated off the blood. Erythrocytes/liver homogenate were washed four times with 3ml of 0.9% NaCl solution by centrifuging for 10 minutes at 3000rpm after each wash. The washed centrifuged erythrocytes/liver homogenate were made up to 2.0ml with cold redistilled water, mixed and left to stand at +4^{o}C for 15 minutes. The lysate was diluted with 0.01 mol/l Phosphate buffer pH 7.0 so that the % inhibition falls between 30 % and 60%. Then, three test tubes, labelled standard (S1), standards (S2-S6), and samples were set up, each containing 1000µl of mixed substrates (R1) and 30µl of Ransod sample diluent (S1), standards (S2-S6) and diluted sample were transferred to each test tubes respectively. The mixture was shook and 150µl of xanthine oxidase (R2) was added to all the test tubes.The mixture was shaken and the cuvette was inserted into the RX Monza cell Holder and read. The assay was then calibrated using the standard .The following dilutions were made of the standard CAL (or S6) to produce a standard curve.

Standard	Volume of standard solution	Sample Dilute
S6	undiluted standard	-
S5	5ml of S6	5ml
S4	5ml of S5	5ml
S3	5ml of S4	5ml
S2	5ml of S3	6ml

S1 =Sample diluent 0.01mol phosphate buffer pH 7.0

(C) Lipid Peroxidation Marker

MALONDIALDEHYDE (MDA)

Principle: TEP reacts with hydrochloric acid in the required proportion to release malondialdehyde which then reacts with thiobarbituric acid (TBA) to produce thiobarbituric acid reactive material that absorbs maximally at 532nm.

Methodology for Lipid Peroxidation Standard Curve Using

Tetraethoxypropane

Procedure: A linear standard curve was prepared by dissolving 24.6mg tetraethoxypropane, in 100ml of deionised water to give a stock solution. Working standards were prepared by diluting the stock solution 1:25,1:50,1:75,1:100, 1:150,1:200,1:250,1:400,1:500,1:800,1:1000, with 0.01N HCL (which releases malondialdehyde almost instantly). Eleven tubes were set up and 0.5ml of each dilution were added accordingly. A quantity, 2.5ml of 0.05M H_2SO_4 and 3ml of TBA (0.67)% were added. The set up was carefully incubated for one hour in boiling water. After the incubation, the tubes were cooled under a running tap water and 4ml of butan-1-ol was added. The tube content was mixed with vortex mixer. This was then centrifuged for 30minutes at low speed, using whisperfuge centrifuge model 1684 and the layer on top containing the TBA-reactive substances was carefully collected using syringe and its absorbance read at 532nm against a blank on a spectrophotometer Pye-Unican sp 500. The standard curve of the O.D against MDA concentration was plotted see appendix ii.

The working solutions must be fresh daily. A 1:100 dilution of stock solution

Contains 15nmol (2.46ug) malondialdehyde per ml.

Statistical Analysis: All values were expressed as mean ± S.D. The statistical analysis was carried out using one-way analysis of variance (ANOVA) using SPSS version 20.0 was employed to express the significance of difference between results with p values set at 0.05

RESULTS

Table 1. Mean and Standard Deviation of Body Weights of Experimental and Control Rats Before and After Treatment with Methanol extract of *N. latifolia leaf*

Groups	Mean Weight Before(g) Treatment	Mean Weight After(g) Treatment	Mean Weight change(g)
Control (1)	155.00± 12.25	157.00±12.43	2.0±0.18*
Group (2)	168.33±9.83	164.67±10.39	-3.66±0.56
Group (3)	175.00±5.48	172.50±8.20	-2.50±2.72
Group (4)	180.00±20.98	185.00±21.10	5.0±0.12*

*Significantly different from group II and group III ($P < 0.05$).

Analysis of results

Effect of Methanol extract of *N. latifolia leaf* on body weight changes of Experimental and Control Rats Before and After Treatment

The results of body weight changes in control, ciprofloxacin treated rats and *N. latifolia* treated rats were shown in Table 1. There was a significant ($p < 0.05$) increase in body weight of rats in Groups I, III and IV when their respective weights before and after treatment were compared. Administration of methanol extract of *N. latifolia leaf* at a dose of 1200mg/kg body weight significantly ($p < 0.05$) increased body weight of rats in (Group III and Group IV). The results were found to be in a dose dependent manner. The body weight change of rats treated with 70mg/kg body weight ciprofloxacin (group II) was significantly less ($p < 0.05$) than the normal control which then returned to near normal in rats treated with ciprofloxacin 70mg/kg body weight and methanol extract of *N. latifolia leaf* at a dose of 1200mg/kg and 2000mg/kg body weight.

Effects of Methanol extract of *N. latifolia leaf* on non-enzymic antioxidant status

The levels of plasma and liver vitamins C and E were significantly depleted in rats treated with ciprofloxacin 70mg/kg body weight only (group II).Treatment with methanol extract of *N. latifolia leaf* at a dose of 1200mg/kg and 2000mg/kg body weight significantly increased the levels of these non enzymic antioxidants in rats treated with ciprofloxacin ($P < 0.05$) (Table 2).

Effects of Methanol extract of *N. latifolia leaf* on enzymic antioxidant status

A significant decrease ($P < 0.05$) in the activities of enzymic antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase of the blood and liver were noted in rats treated with ciprofloxacin 70mg/kg body weight only(group II),when compared with the normal control(group I) rats. Upon treatment with methanol extract of *N. latifolia leaf* at a dose of 1200mg/kg and 2000mg/kg body weight , the activities of both SOD and glutathione peroxidase were significantly reversed to near normal (Table 3).

Table 2. Mean values of plasma and liver homogenate non enzymic antioxidants in experimental and control rats

Treatment	Vitamin C (mg/dl)		Vitamin E (mg/dl)	
	Plasma	Liver Homogenate	Plasma	Liver Homogenate
Control(Group I)	1.31±0.20*	1.07±0.17*	1.50±0.15*	1.22±0.22*
Groupii(Ciprofloxacin70mg/kg)	0.63±0.17	0.55±0.15	0.60±0.18	0.63±0.20
Groupiii Ciprofloxacin70mg/kg+1200mg/kg *N.latifolia*	0.97±0.05**	1.03±0.15**	1.07±0.16**	0.95±0.09**
Groupiv Ciprofloxacin70mg/kg+2000mg/kg *N.latifolia*	1.05±0.19*	1.11±0.13*	1.33±0.23*	1.30±0.16*

*Significantly different from group II and group III ($p < 0.05$); **Significantly different from group II ($p < 0.05$)

Table 3. Mean values of blood and liver homogenate enzymic antioxidant in experimental and control rats

Treatment	SOD (U/ml)		GPX (U/ml)	
	Blood	Liver Homogenate	Blood	Liver Homogenate
Control (Group I)	7.75±0.74*	6.84±0.54*	16.43±1.32*	36.20±1.45*
Group II (Ciprofloxacin70mg/kg)	3.78±0.30	3.17±0.38	8.85±0.49	16.22±0.55
Groupiii Ciprofloxacin70mg/kg+1200mg/kg *N.latifolia*	4.66±0.30**	4.28±0.44**	13.77±0.64**	28.82±1.92**
Group IV Ciprofloxacin70mg/kg+2000mg/kg *N.latifolia*	5.60±0.64*	5.08±0.20*	15.39±0.59*	32.20±1.32*

*Significantly different from group II and group III ($p < 0.05$); **Significantly different from group II ($p < 0.05$)

Effects of Methanol extract of *N. latifolia leaf* on MDA

The levels of MDA in plasma and liver were significantly ($p < 0.05$) increased in rats treated with ciprofloxacin 70mg/kg body weight only (group II) compared to control rats. Treatment with methanol extract of *N. latifolia* leaf resulted in a significant decrease in the levels of lipid peroxidation products (MDA) in rats treated with ciprofloxacin (Table 4).

DISCUSSION

There was a slight gain in body weight of animals in Control and group IV ($p<0.05$) whereas the rats in group II and group III showed loss of body weight ($p<0.05$) when their respective body weights before and after treatment were compared. The observed loss of body weight of animals in group II and III could be attributed to the effect of induced oxidative stress by ciprofloxacin which was normalised by treatment with higher dose of methanol extract of *N.latifolia* leaf(table 1,group IV). (Goswami *et al.,* 2006) has reported that superoxide anions (O_2) and hydrogen peroxide (H_2O_2) are involved in the antibacterial action of ciprofloxacin. Thus increased levels of ROS could be responsible for the loss of weight in the rats (group II and group III). Treatment with methanol extract of *N. latifolia leaf* at a dose of 1200mg/kg and 2000mg/kg body weight increased the values of the antioxidants vitamin C, E, SOD and glutathione peroxidase when compared with the control and protected the cells (hepatocytes) by decreasing the production of free radical derivatives. The results were also dose dependent. It is observed in this research that *Nauclea latifolia* extract has significant effect on lipid peroxidation. This report is in contrast with the study of (Udem, and Madubunyi, 2008), that *Nauclea latifolia* extract has no significant effect on lipid peroxidation. Nkafamiya 2006, has shown that *Nauclea latifolia* contains the antioxidants Vitamin C and E. Probably the antioxidative effect of the *Nauclea latifolia* extract observed in this study is due to the presence of the antioxidants Vitamin C and E in *Nauclea latifolia.* Vitamin E protects the membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Herrera and Barbas, 2001; Traber *et al.,* 2007). This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidised α-tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol (Traber *et al.,* 2007). This is in line with findings showing that α-tocopherol, but not water-soluble antioxidants, efficiently protects glutathione peroxidase 4 (GPX4)-deficient cells from cell death (Seiler *et al.,* 2008). Treatment with methanol extract of *Nauclea latifolia* leaf significantly ($p<0.05$) decreased the levels of liver and plasma MDA concentrations to near normal (table 4) and increased the values of the antioxidants vitamin C, E, SOD and glutathione peroxidase when compared with the control and protected the cells (hepatocytes) through attenuation of lipid peroxidation by decreasing the production of free radical derivatives. The results were also dose dependent. It is observed in this research that *Nauclea latifolia* extract has significant effect on lipid peroxidation. This report is in contrast with the study of (Udem and Madubunyi, 2008), that *Nauclea latifolia* extract has no significant effect on lipid peroxidation. In conclusion, it is observed from this study that *Nauclea latifolia* has both lipid peroxidative and antioxidative potentials.

Conclusion

Methanol extract of *Nauclea latifolia* leaf exhibits strong antioxidant activities which enhanced the levels of antioxidant defense system.

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We appreciate the management of Reene Medical Diagnostic Centre, 12A Nwaziki Avenue, Awada, for granting us permission to run the assays in their research Centre.

Recommendation

I recommend further research on the effect of this *Nauclea latifolia* leaf extract on some specific hepatotoxin such as aflatoxin B1, aflatoxin G1 and aflatoxin G2.

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