ANTI-INFLAMMATORY EFFECTS OFEnantia chloranthiaAND Nauclea latifolia ON CRUDE OIL -INDUCED OXIDATIVE STRESS IN ALBINO WISTAR RATS

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ABSTRACT

The study investigated the prophylactic effects of extracts of the stem bark of *E. chloranthia* and *N. latifolia* herbal mixture (*HM*) on crude oil (5ml/kg b.w)-induced oxidative stress in rats. Thirty rats were divided into 6 Groups: Group 1 (untreated control), Group 2 (crude oil control), Group 3 (Ibuprofen and crude oil), Groups 4, 5 and 6 (200, 300, and 400 ml/kg b.w extracts respectively and crude oil), every other day for 14 days (prophylactic treatment). Phytochemical tests reveals presence of flavonoids, saponins and tannins. Crude oil treatment resulted in significant inhibition ($p<0.05$) of peroxidase but activation of catalase, superoxide dismutase, malondialdehyde levels, aspartate aminotransferase, alanine aminotransferase and alanine phosphatase when compared with untreated control. Prophylactic treatment with *HM* on the other hand resulted in non-significant alterations of the enzymes assayed when compared to that of untreated control. However, Group 3was significantly ($p < 0.05$) less active when compared to some doses (300 and 400 ml/kg extract) of the *HM*. The results suggest that combinations in herbal potion may be beneficial in preventing inflammation since the stem bark extract of *HM*was able to protect the hepatocyte against oxidative stress induced by crude oil. The results also indicates that the plants have anti-inflammatory activities and supports their ethno medicinal claims.

Keywords*: Anti-inflammatory activities, Enantia chlorantha, Nauclea latifolia, oxidative stress, phytochemicals, prophylactic effects*

INTRODUCTION

Traditional medicine has been in use all over the globe and stems from the use of locally available plants. Tribal communities, mostly use it because of the availability of these plants and its cost effectiveness.

Inflammation is a major consequence of diseases, especially in the under developed world like Africa, especially Nigeria where people are exposed to various kinds of stressors like pathogens, damaged cells and irritants. This is because inflammations have been implicated in elevated levels of nitric oxide (NO) formation which may bring about some undesired deleterious effects and numerous diseases states (1). Inflammation is a mechanism of innate immunity, and is characterized by pain, heat, redness, swelling and loss of function. Although it is not listed among the World Health Organization (WHO) diseases per se, inflammation still is a major cause of death to the world populace since majority of these undeveloped communities lack access to orthodox medicine due to illiteracy, poverty and non-availability. As a result, the people depend on the use of traditional medicine (herbal mixtures/ decoctions) for their cure (2, 3). These mixtures, being combinations, are believed to combat ailments in multiple dimensions to relieve sufferers undergoing treatment, just as, orthodox medicine, employ combination therapy is the treatment of some ailments (3).

Inflammation, aging, coronary heart disease, neurodegenerative disorders, atherosclerosis, cancer, etc., are degenerative or pathological processes that may occur as a result of oxidative stress (4). Oxidative stress usually results when antioxidants defense is overwhelmed by either endogenous or exogenous oxidants or free radicals called

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reactive oxygen species (ROS) or active oxygen species. These are various forms of activated oxygen, which include free radicals such as superoxide ions (O^{2-}) and hydroxyl radicals (OH**.**), as well as non-free .radical species such as hydrogen peroxide (H_2O_2) [5]. Reaction oxygen species may be a result of normal endogenous sources such as aerobic respiration, macrophages, perioxisomes or exogenous sources of free radicals such as ionizing radiation, certain pollutants, including pesticides, crude oil (5).

Antioxidant defense system scavenges and minimizes free radicals formation (6). The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals. It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich food and incidence of human disease (5).

Plant based antioxidants are preferred over synthetic antioxidants because of safety concerns (7). Therefore, research regarding antioxidant potential from plant source is important.

Nauclea hatifolia is plant species which belongs to the Rubiacea family. It is commonly known as pin cushion tree which is native to tropical African and Asia. *N. hatifolia* is a shrub commonly seen in the South East of Nigeria. It is widely applied as herbal remedy in treatment of various illnesses (8). The medicinal values of this plant lies in their phytochemicals, which includes polyphenols, flavonoids, saponins, tannins and glycosides, important for antioxidant, anti-inflammatory and analgesic activities (9).

Different parts of *Enantia chlorantha* which belongs to the Annonaceae family, has been used folklorically to treat various ailments because they possess anti-inflammatory, anti-plasmodial, antibacterial and antifungal potentials (10).

The World Health Organization (11) recommendation of combination of drugs as a policy of malaria treatment, may be an impetus for the continued investigation of combinations of herbs with reported ethno-medicinal anti-protective potential usage as a promising approach for discovery of candidate drugs for amelioration of inflammation (3, 12).

Hence, the potentials of *E. chlorantha* and*N. latifolia*, as possible components of herbal mixtures, were evaluated in this study, using their aqueous stem barks extracts, oxidative stressed rats and the *in vivo* prophylactic models, with the aim of corroborating the *in vitro* anti-inflammatory potential and providing scientific evidence supporting their ethno-medicinal use.

MATERIALS AND METHODS

Plant materials

The stem barks of *E. chloranthia* and *N. latifolia* were purchased from herbal practitioners, identified and authenticated by a botanist in the Department of Plant Biology and Biotechnology, University of Benin, Nigeria.

Preparation and extraction of plant materials

The fresh stem barks were washed and air-dried at room temperature for a period of three weeks. They were pulverized using an electric grinder. Extraction was done using the solvent extraction method (maceration). A known amount in grams of powdered samples were soaked in a stopper container with 250 ml of ethanol for 72 h and rapidly stirred using glass rod every 6 h. After 72 h, filtration was carried out with Whatman filter paper No.1. The filtrates were concentrated using a rotary evaporator to dryness at reduced pressure. 50 mg of each extract was mixed together and labeled as Herbal Mixture (HM). The extracts were stored in a refrigerator at -4^{o}C until required for use.

Another quantity of the fresh stem barks were macerated with distilled water (250ml) for 48hours. The extract was filtered and concentrated to dryness with a rotary

evaporator in vacuo. 50 mg of each extract was mixed together and labeled as Herbal Mixture (HM). The dried powdery sample was stored at -4ºC.

Phytochemical analysis of plant extracts

The qualitative phytochemical test involves simple chemical test to detect the secondary metabolites using standard method of Trease and Evans (13). For each of the distilled water and ethanol extracts powder of HMqualitative phytochemical screening was determined for the presence of tannins, saponins, terpenoids, alkaloids, polyphenols and flavonoids.

Experimental Design

Acute oral toxicity test of crude oil and herbal mixture was carried out using Wistar albino rats. The crude oil and herbal mixture extract were administered using a feeding gavage attached a syringe. The rats were divided into four Groups with five rats per Group and treated orally with 2, 3, 5, and 10 ml/kg body weight of crude oil respectively (14). The rats were observed over a 24 hour period for nervousness, dullness, weight loss, in-coordination and/or death. Increased dullness and weight loss was concentration-dependent. Even though no death was recorded, the range of concentration used for the study was 5 ml/kg body weight. Acute toxicity test was also performed in healthy Wistar rats using increasing oral dose of the HM in water (100, 200, 400, 600 and 800 mg/kg body weight) in 20 ml/kg volume to different test Groups. Normal Group received water. The rats were allowed to feed *ad libitum,* kept under regular observation for 48 hour period for any mortality or behavioral changes (15).HM was safe up to a dose of 800 mg/kg body weight. The extract did not cause mortality in the rats during 48 hour period. However, observation indicates behavioral changes, locomotor ataxia, diarrhea and weight loss. Food and water intake had no significant difference among the Group studied.

A total of thirty (30) Wistar albino rats of both sexes, (weighing 170 to 200 g) were divided into six Groups with each Group containing five rats. The rats were housed in steel cages and allowed to acclimatize. Oxidative stress was induced by gavaging the rat 5 ml/kg of crude oil, using a 3.5 cm feeding tube attached to a syringe.

Group 1 which served as control, received rat pellets only (i.e. without treatment). Group 2 (rat pellets + 5ml/kg crude oil), Group 3 or the reference Group (rat pellets + 2ml/kgIbuprofen+ 5ml/kg crude oil after 7 and 14 days), Group 4 (rat pellets + 200ml/kg *HM* + 5ml/kg crude oil after 7 and 14 days), Group 5 (rat standard pellets + 300ml/kg *HM* + 5ml/kg crude oil after 7 and 14 days) and Group 6 (rat pellets + 400ml/kg *HM* + 5ml/kg crude oil after 7 and 14 days). Water was provided *ad libitium* to all Groups. The experiment lasted for fourteen days and rats sacrificed on the eighth and fifteenth day respectively, and the liver recovered for analyses.

Biochemical Analyses

The serum prepared from the blood was used to determine alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The liver function markers in the serum were measured using Randox test kit as specified by the manufacturers, Randox Laboratory Ltd (Antrim, UK, BT29 4QY). While the supernatant from liver homogenate was used to determine oxidative stress markers.

The total proteins were determined using the method of Lowry (1951). Determination of superoxide dismutase (SOD) activity was according to the method described by Misra and Fridovich (1972) and was expressed as units/mg tissue weight. One unit of enzyme was defined as the amount of the enzyme required for 50% inhibition of oxidation of epinephrine to adrenochrome in one minute.

Determination of catalase (CAT) specific activity was according to method of Sinha (1971) and was expressed as units/g wet tissue. Dichromate in acetic acid is reduced to

chromic acetate when heated in the presence of H_2O_2 with the formation of perchromic acid as an unstable intermediate. The amount of perchromic acid formed was taken as an activity unit.

Peroxidase (POX) activity was determined using the method of Nyman (1959) and was expressed as units/mg protein. The activity unit of the enzyme was defined as amount of purpurogallin formed in the oxidation of pyrogallol to purpurogallin by peroxidase at 20^0C. Malondialdehyde (MDA) levels was determined by reaction with thiobarbituric acid (TBA) and used as a lipid peroxidation marker (Gutteridge and Wilkins, 1982).

Statistical analysis

All values were expressed as mean ± standard error of mean (SEM) of three replicate experiments.

Statistical evaluations of all data were done using one-way analysis of variance (ANOVA) to test for differences in Groups, while Duncan's multiple comparisons test was used to determine significant differences between means. Instat-Graphpad software, San Diego, California, USA, was used for these analyses. A $p < 0.05$ was considered statistically significant.

RESULTS

Tables 1 and 2 shows the results of the phytochemical screening of the aqueous and ethanol stem bark extracts of both plants. The results revealed the presence of tannins, saponins and flavonoids, as the major phytochemical components (Tables 1 and 2) while the alkaloids, terpenoids and polyphenol contents were low. The phytochemicals were found in relatively equal proportions on both the ethanol and water extracts.

Table 3 shows the effects of crude oil on body weight and serum total protein in rats which had prophylactic treatment with Ibuprofen and *HM* before orally administering 5ml/kg crude oil.Significant ($p<0.05$) decrease in weight was observed in Group 2 rats (i.e. rats given 5ml/kg crude oil only), in the first week and second week of the study (Table 3). Rats in Groups 3 recorded no significant difference in their body weight when compared with control (Table 3). Rats in Groups 4, 5 and 6 recorded a non-significant ($p>0.05$) increase in body weight which corresponded to nearly control values in a dose dependent manner during the period of treatment when compared with control values.

Serum total protein recorded no significant difference in the Group 3 to 6 rats but this cannot be said of the Group 2 rats which recorded significant decrease ($p<0.05$) in serum total protein when compared with control and the prophylactic Groups.

Exposure to crude oil induced significant ($p<0.05$) reduction in the activity of peroxidase (POX) but activated significant ($p<0.05$) increase in catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) (Figures 1, 2, 3 and 4). But the prophylactic Groups maintained relatively a near control value in a dose dependent manner (Figures 1, 2, 3 and 4).
Fig. 4 shows the results of the anti-inflammatory effects of orally administered *HM* on crude oil-induced oxidative stress in rats. *HM* showed statistically significant ($p<0.05$) dose-dependent anti-inflammatory activity. Herbal mixture (*HM*) showed remarkable and comparable anti-inflammatory effects at 300 and 400 ml/kg doses to the Ibuprofen Group. While *HM* Group revealed approximately between 94 - 98% inhibitions, Ibuprofen Group revealed approximately 92 - 98% of inhibition of oxidative stress.

The results of the liver marker enzymes are shown in Figure 5. The results recorded elevated activity of ALP, AST and ALT in crude oil exposed rats when compared with control (Fig. 5).

Table 1: Phyto-chemistry of stem barks of secondary metabolites from *E. chloranthia*

Phytochemicals		Tannins	Saponins	Flavonoids	Alkaloids	Terpenoids	Polyphenols
Solvents	Ethanol	++	++	++	+	+	+
	Water	++	++	++	+	+	+

(++) = Present in moderate concentrations; **(+)** = Present in low concentrations

Table 2: Phytochemistry of stem barks of secondary metabolites from *N. latifolia*

Phytochemicals		Tannins	Saponins	Flavonoids	Terpenoids	Alkaloids	Polyphenols
Solvents	Ethanol	++	++	++	++	+	+
	Water	++	++	++	+	+	+

(++) = Present in moderate concentrations; **(+)** = Present in low concentrations

Table 3: Effect of crude oil and prophylactic treatment with Ibuprofen and *HM*on body weight and total proteins in rats

Group /Assay	**Group 1 Control**	**Group 2 Crude oil**	**Group 3 Reference**	**Group 4 200ml/kg**	**Group 5 300ml/kg**	**Group 6 400ml/kg**
			DAY 7			
Body Weight	182.43±4.2^{a}	164.3±1.74^{b}	175.5±4.0^{a}	188.60±3.7^{c}	177.10±2.50^{a}	172.81±2.65^{a}
Protein	89.41±1.12^{a}	50.16±1.18^{b}	92.05±1.50^{a}	79.07±1.87^{a}	82.10±1.00^{a}	95.36±2.32^{d}
			DAY 14			
Body Weight	191.26±4.5^{a}	170.6±2.01^{b}	197.5±3.50^{a}	196.19±4.4^{a}	188.34±2.8^{c}	166.74±3.0^{b}
Protein	88.98±0.36^{a}	62.45±0.24^{b}	90.67±0.64^{a}	80.10±0.38^{a}	81.76±0.59^{a}	94.50±0.79^{d}

Results are expressed as means ± SEM of five determinations. Body weight is expressed as g. Protein is expressed as g/L. Means of the same row carrying different notations are statistically different at $p<0.05$ using Instat graphpad.

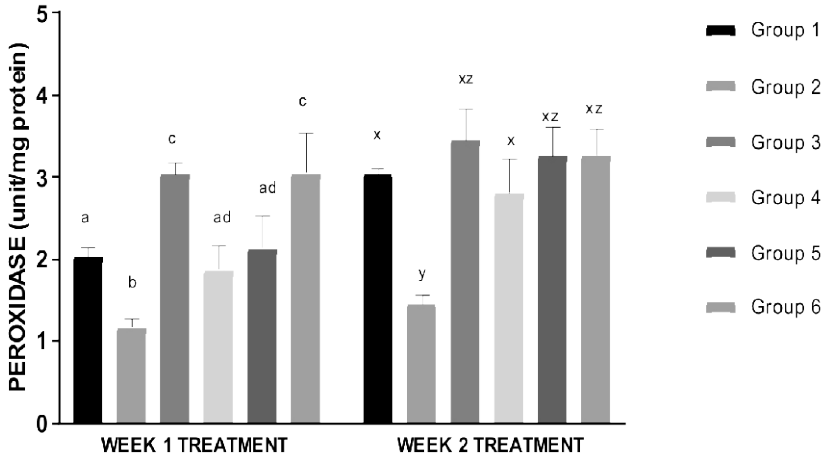

Fig. 1: Peroxidase activities in liver of rats exposed to crude oil and the prophylactic roles of Ibuprofen and herbal mixture (HM). Each bar represents the mean ± SEM. POX = Peroxidase activity is expressed as unit/mg protein. Bars with different letters are significantly different at ($p < 0.05$) using Instat graphpad.

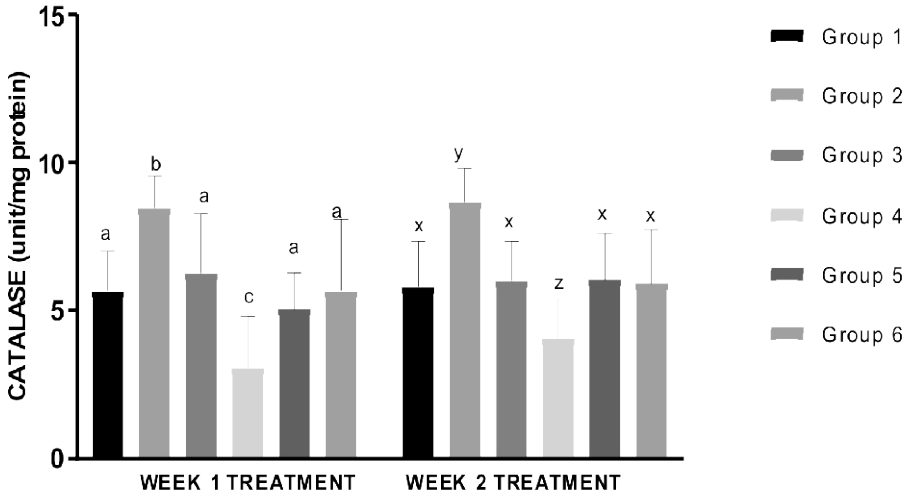

Fig. 2: Catalase activities in liver of rats exposed to crude oil and the prophylactic roles of Ibuprofen and herbal mixture (HM). The results are mean ± SEM of 5 determinants in each group. Catalase activity is expressed as unit/g wet tissueBars with different letters aresignificant different at ($p < 0.05$) using Instat Graphpad.

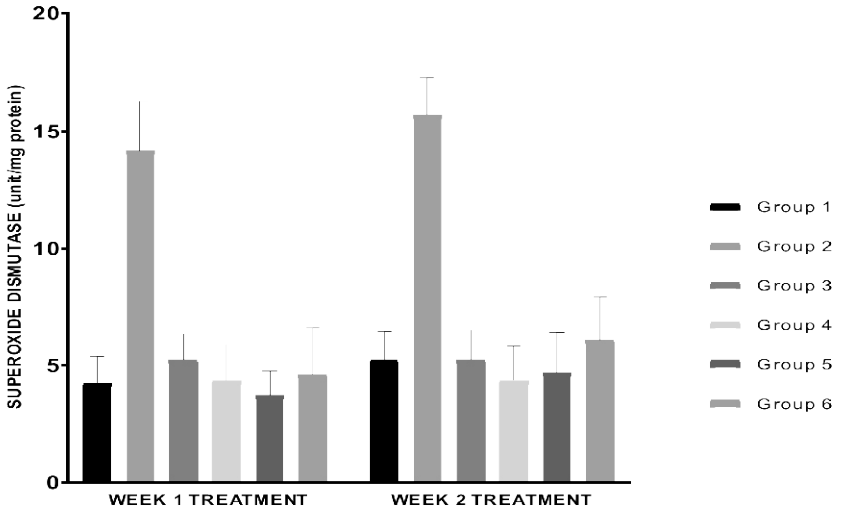

Fig. 3: Superoxide Dismutase activities in liver of rats exposed to crude oil and the prophylactic roles of Ibuprofen and herbal mixture (HM). The results are mean ± SEM of 5 determinants in each group. Superoxid dismutase activity is expressed as unit/mg protein. Significant difference was mentained at ($P < 0.05$) using Instat Graphpad.

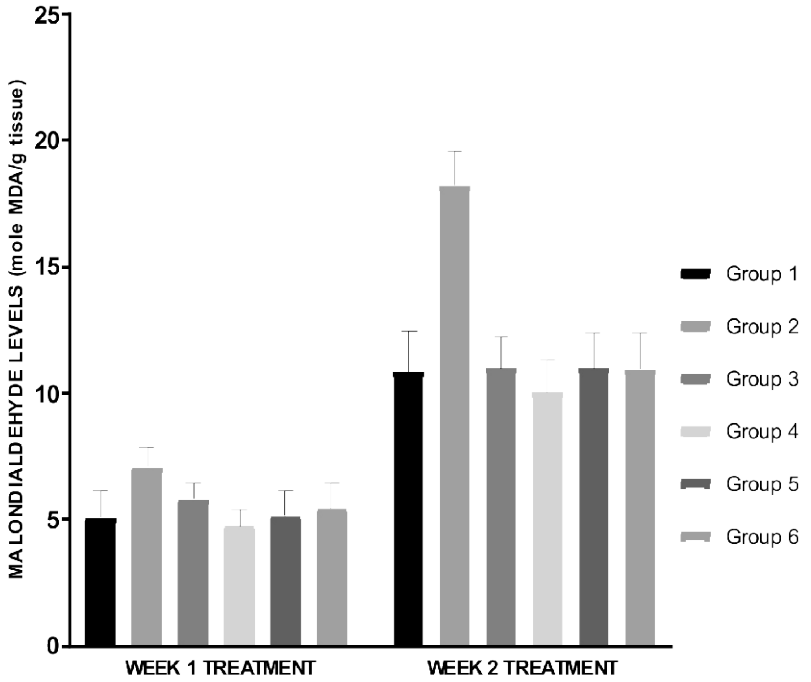

Fig. 4: Malondialdehyde levels in liver of rats exposed to crude oil and the prophylactic roles of Ibuprofen and hert mixture (HM). The results are mean ± SEM of 5 determinants in each group. Malondialdehyde level is presented i mole MDA/g tissue. Significant difference was mentained at ($P < 0.05$) using Instat Graphpad.

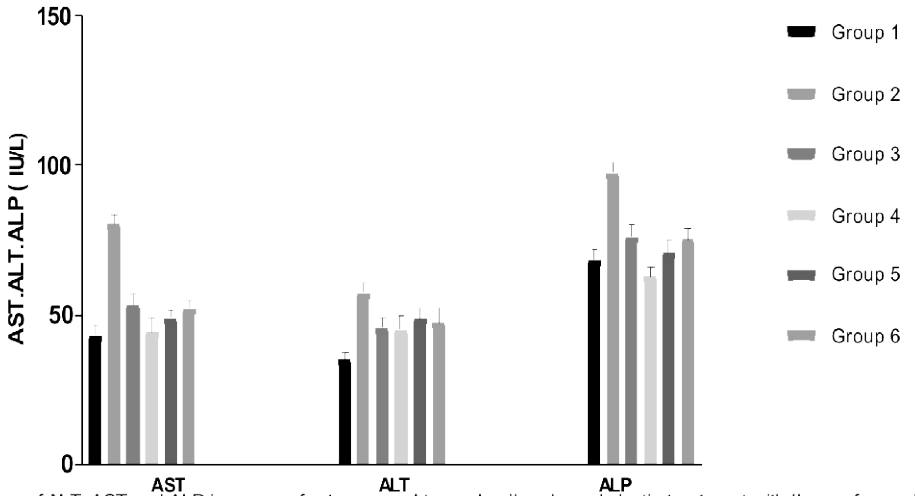

Fig. 5: Activities of ALT, AST and ALP in serum of rats exposed to crude oil and prophylactic treatment with Ibruprofen and herbal mixture (HM). Each bar represents the mean ± SEM. ALT = Alanine aminotransferase, AST=Aspartate aminotransferase, ALT=alkaline phosphatase, were expressed as IU/L. Bars with different letters are significantly different at ($p < 0.05$) using Instat Graphpad.

DISCUSSION

This study has revealed that the stem bark extract of *E. chloranthia* and *N. latifolia* possess useful and vital bioactive agents which can be used in the design and formulation of drugs. This result is in line with others similar studies carried out to access the phytochemical composition and biological activities of the plant constituents (16).

The detection of a host of secondary metabolites (flavonoid, saponin, alkaloids, tannins, terpenoids and polyphenols) in the stem bark of both plants *(E. chloranthia* and *N. latifolia)* in this study indicates that these plants possesses nutritional and medicinal properties as reported in similar studies by other researchers. Some of these properties includes antioxidant,vasodilatory, anti-carcinogenic, anti-inflammatory,

antibacterial (17), anti-allergic, antiviral, estrogenic and immune system stimulating effect, antibacterial antifungal and antimicrobial (18, 19).

As shown by the results, the induction of oxidative and possible inflammation due to crude oil stress was inhibited by the herbal mixture. This suggests a possible inhibition of cyclooxygenase synthesis as it applies to non-steroidal anti-inflammatory drugs such as ibuprofen, whose mechanism of action is the inhibition of the cyclooxygenase enzyme. Flavonoids and saponins are well known for their ability to inhibit pain perception as well as anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation (19, 20). This hypothesis is supported by our previous study, which showed that each plantpossesses antioxidant and anti-inflammatory activity due to the presence of flavonoid content (21, 22).

Researches have shown that a variety of diseases are a result of tissue damage and inflammation brought about by the release of ROS and excessive nitric oxide (NO) [19, 23]. Since phenols are potent inhibitors of NO synthase activity and NO production, the presence of polyphenols in this study may be responsible for the significant free radical scavenging abilities of the *HM*. This may be responsible for the prevention of inflammation in the crude oil-induced oxidative stress in the rats.

Any substance with good prophylactic activity, would when absorbed into the blood stream, be able to prevent inflammation in the organism. The *HM* was able to prevent oxidative stress (inflammation) that may have been induced by crude oil stress by suppressing MDA levels to values comparable to control values.

CONCLUSION

The study suggests the possible use of herbal mixture (*HM*) of the stem bark of *E. chloranthia* and *N. latifolia* as an alternative or supplementary remedy for the prevention/treatment of pain and inflammatory diseases. The study also suggests further investigation of the *HM* for possible development as new class of analgesic and anti-inflammatory drugs.

Although, the activities of the standard drug (ibuprofen) appear low/less active in comparison with some of the doses of the herbal mixture, they all show significant prophylactic effects in the rats. This can be used to justify their ethno-medicinal claims as anti-inflammatory. The study shows the importance of the use of herbal mixtures in the prevention of inflammation.

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AUTHOR CONTRIBUTIONS

Stella Oghomwen Olubodun (SOO): initiated the project, supervised the B.Sc. degree project of Oyinye Uche-Aboh, which is part of this publication. Ododo Augustine Osagie (OAO) assisted in the herbal combination experiments. SOO wrote the manuscript, while SOO and OAO processed for publication.

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