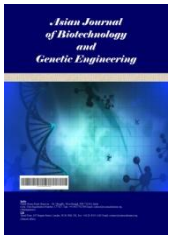

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Evaluating the Assault Caused by *Nauclea latifolia* on the Biochemical Parameters of Wistar Rats Following Testicular Tissue Damage

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Authors' contributions

This work was carried out in collaboration among all authors. Author EIB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JIN and KAO managed the analyses of the study. Author GDE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: The use of herbal products is very common among rural dwellers, although these products posses prophylactic, therapeutic and curative capacities. Unguarded doses most times often result in cellular and tissue damage in the body. This study was undertaken to evaluate the assault caused by *Nauclea latifolia* on lactate dehydrogenase, superoxide dismutase and DNA damage assay in Wistar rats following testicular tissue damage.
Methods: Forty (40) male Wistar rats divided into eight (8) groups were used for this investigation (n=5). The animals were administered with 3mg of alcohol/kg b.w alone, 5 mg of testosterone/kg b.w, 500 mg of ethanolic leaf extract of *Nauclea latifolia*/kg b.w, 1000 mg of ethanolic leaf extract of *Nauclea latifolia*/kg b.w, 1500 mg of ethanolic leaf extract of *Nauclea latifolia*/kg b.w, 3 mg of alcohol/kg b.w and 500 mg of of ethanolic leaf extract of *Nauclea latifolia*/kg b.w, 5mg of testosterone and 500 mg of ethanolic leaf extract of *Nauclea latifolia*/kg b.w and the control received

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15 % tween 80 (10 ml of tween 80/kg b.w) respectively. The experiment lasted for 21 days.
Results: Ethanolic leaf extract of *Nauclea latifolia* at doses of 500, 1000 and 1500 mg/kg b.w of rats significantly increased DNA damage and lactate dehydrogenase.
Conclusion: Increased in these biochemical parameters signify increased tissue damage.

Keywords: Nauclea latifolia; lactate dehydrogenase; superoxide dismutase; DNA damage assay; tissue damage; alcohol.

1. INTRODUCTION

Despite many achievements in human healthcare in the twentieth century, many of the world's population in developing countries lack regular access to affordable essential drugs. For these people, modern medicine is never likely to be a realistic treatment option. In contrast, traditional medicine is widely available and affordable, even in remote areas [1]. Traditional medicine has a wider acceptability among the people of developing countries than modern medicine due partly to inaccessibility of modern medicine. The major contributing factor is the fact that traditional medicine blends readily on the socio-cultural life of the people. Furthermore, traditional medicine remains popular because the practitioners have wisely formed an important economic contract to the mutual benefit of their practice and the population they serve [2]. Apart from the advantages of traditional medicine, many problems must be tackled to maximize the potential of traditional medicine as a source of healthcare [3]. Perhaps one of the greatest arguments against traditional medicine today is lack of scientific proof for its efficacy. There is no thorough scientific investigation on most of the claims made by the traditional medicine practitioners [4]. In 1964, the Organization of African Unity set up the Scientific and Technical Research Commission to initiate research on the proof of efficacy of medicinal plants. This initiative has greatly enhanced the development of medicinal plant research [5]. Over the years, traditional medicine has been used to treat various degrees and forms of ailment with significant positive therapeutic outcome [6]. One of such plants is *Nauclea latifolia* which has been widely used for prophylactic or curative purposes. Studies show that the root of *Nauclea latifolia* has antibacterial activity against gram positive and gram negative bacteria and antifungal activity [7]. The root of *Nauclea latifolia* is most effective against *Corynebacterium diphytheriae, Streptobacillus spp, Streptococcus spp, Neisseria spp, Pseudomonas aeruginosa* and *Salmonella spp* [8]. It is pertinent to note that although these herbal products possess therapeutic, prophylactic and curative capacities, the dosages and standardization must be taken into full consideration in order to minimize the risk of causing harm to the cells, tissues and organs of the body. This research was undertaken to evaluate the assault caused by the administration of ethanolic leaf of *Nauclea latifolia* on the biochemical parameters of Wistar rats following testicular tissue damage.

2. MATERIALS AND METHODS

2.1 Acquisition of Animals

Forty (40) adult male Wistar rats weighing between 170 and 260 g were used for the research. They were obtained from the Animal House of the Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria. They were kept in clean cages and allowed to acclimatize for 14 days under standard laboratory conditions. The animals were fed with commercial rat pellet (Vital Feeds, Grand Cereals Limited, Jos, Nigeria) and allowed access to water *ad libitum*. All procedures involving the care and use of experimental animals conformed strictly to the guidelines of American Physiological Society, 2002 [9].

2.2 Extract Preparation

The leaves of *Nauclea latifolia* were obtained from a local farm in Nsit Ibom Local Government Area of Akwa Ibom State. The plant was identified by a Botanist in the Department of Pharmacognosy, Faculty of Pharmacy, University of Uyo and authenticated in the Department of Botany, University of Uyo with a herbarium number UUH 031/15. The fresh leaves of *Nauclea latifolia* were dried for 14 days. The dried leaves were powdered and macerated in 70% ethanol and kept for 72 hours at room temperature. The macerated powder were filtered and the filtrate concentrated in water bath at 45^{o}C to dryness. Extract was weighed and stored in the refrigerator until when required for use.

2.3 Experimental Protocol

Forty (40) male Wistar rats were used for this investigation. They were randomly divided into 8 groups of 5 animals each which is shown below. The experiment lasted for 21 days.

Group 1: received 15 % tween 80 (10 ml of tween 80/kg body weight of rats).
Group 2: received 3mg of alcohol/kg body weight of rats alone.
Group 3: received 5 mg of testosterone/kg body weight of rats.
Group 4: received 500 mg of ethanolic leaf extract of *Nauclea latifolia*/kg body weight of rats.
Group 5: received 1000 mg of ethanolic leaf extract of *Nauclea latifolia*/kg body weight of rats.
Group 6: received 1500 mg of ethanolic leaf extract of *Nauclea latifolia*/kg body weight of rats.
Group 7: received 3 mg of alcohol/kg body weight of rats and 500 mg of of ethanolic leaf extract of *Nauclea latifolia*/kg body weight of rats.
Group 8: received 5 mg of testosterone /kg body weight of rats and 500 mg of ethanolic leaf extract of *Nauclea latifolia* /kg body weight of rats.

After the last day schedule, the animals were euthanized using chloroform inhalation method. Blood sample was taken via cardiac puncture and drawn into 10ml syringe and emptied into EDTA and plain sterile plastic bottles and taken to the laboratory for biochemical assay. The following biochemical parameters were measured; Lactate dehydrogenase, Superoxide dismutase and DNA damage assay (8-Hydroxy-Desoxyguanosine-8-OHdG).

2.4 Determination of Lactate Dehydrogenase, Superoxide Dismutase and Dna Damage Assay (8-Hydroxy-Desoxyguanosine-8-OHdG)

This was done according to the method of Ekaluo et al*.,* 2010.

2.5 Data Analysis

Data were analysed using one- way Analysis of variance (ANOVA) followed by student- Newman Keuls post hoc test at significant rate of P< 0.05.

3. RESULTS

3.1 DNA Damage Assay

The mean DNA damage in mg/dl obtained in this study were: 0.31 ± 0.01, 1.67 ± 0.18, 0.38 ± 0.02, 1.60 ± 0.02, 1.71 ± 0.01 and 1.45 ± 0.02, 1.43 ± 0.03,1.40 ± 0.00, for the control, alcohol, testosterone, low dose (500 mg of ethanolic leaf extract of *N. latifolia*/kg b.w. of rats) medium dose (1000 mg of ethanolic leaf extract of *N. latifolia /*kg b.w. of rats) and high dose (1500 mg of ethanolic leaf extract of *N. latifolia/*kg b.w. of rats), alcohol with *Nauclea latifolia*, testosterone and *Nauclea latifolia* groups respectively (Table 1). The DNA damage that occurred in group 3 treated with testosterone alone was not significantly different from control but was significantly lower than alcohol and *Nauclea latifolia* groups ($p<0.05$). The *Nauclea latifolia* and alcohol groups caused significant increase in DNA damage compared to the control group ($p<0.05$) (Table 1). Comparison between the group where alcohol was co-administered with ethanolic leaf extract of *Nauclea latifolia* (1.43 ± 0.03 mg/dl) showed significant reduction in DNA damage compared to alcohol group (1.67 ± 0.18 mg/dl) ($p<0.05$) (Fig. 1). Comparison between the group where testosterone was co-administered with ethanolic leaf extract of *Nauclea latifolia* (1.40 ± 0.00 mg/dl) showed significant increase in DNA damage compared to testosterone group (0.38 ± 0.02 mg/dl) ($p<0.05$) (Fig. 1).

3.2 Lactate Dehydrogenase (LDH)

The mean LDH in mg/dl obtained in this study were: 0.66 ± 0.00, 0.78 ± 0.00, 0.88 ± 0.00, 0.04 ± 0.00, 1.35 ± 0.00 and 1.28 ± 0.00, 1.04± 0.00, 0.79±0.00 for the control, alcohol, testosterone, low dose (500 mg of ethanolic leaf extract of *N. latifolia/*kg b.w. of rats), medium dose (1000 mg of ethanolic leaf extract of *N. latifolia* /kg b.w of rats), high dose (1500 mg of ethanolic leaf extract of *N. latifolia* /kg b.w. of rats) , alcohol and *N. latifolia,* testosterone and *N. latifolia* groups respectively (Table 1). Treatment with 500 mg of ethanolic leaf extract of *Nauclea latifolia* /kg b.w. of rats significantly lowered LDH compared to control group and other groups while treatment with 1000 mg (medium dose) and 1500 mg (high dose) of ethanolic leaf extract of *N. latifolia* /kg b.w of rats significantly increased LDH compared to control group, alcohol group and testosterone group. The alcohol and testosterone groups caused significant elevation of LDH level

compared to control group ($p<0.05$). The value obtained in testosterone group was significantly higher than alcohol group ($p<0.05$) (Table 1). Co-administration of alcohol with ethanolic leaf extract of *Nauclea latifolia* (1.04 ± 0.00 mg/dl) increased the level of LDH compared to control group and also significantly elevated LDH level compared to alcohol group (0.78 ± 0.00 mg/dl) ($p<0.05$) (Fig. 2). Co-administration of testosterone with ethanolic leaf extract of *Nauclea latifolia* (0.79 ± 0.00 mg/dl) significantly lowered LDH level compared to testosterone group (0.88 ± 0.00 mg/dl) ($p<0.05$) (Fig. 2) and showed an increase in the level of LDH compared to control group.

3.3 Superoxide Dismutase (SOD)

The mean SOD assay in µg/dl obtained in this study were: 1.07 ± 0.00, 0.65 ± 0.01, 1.65 ± 0.02, 1.24 ± 0.05, 1.20 ± 0.03 and 1.18 ± 0.01, 0.65±0.01, 1.35± 0.03 for the control, alcohol, testosterone, low dose (500 mg of ethanolic leaf extract of *N. latifolia* /kg b.w. of rats), medium dose (1000 mg of ethanolic leaf extract of *N. latifolia* /kg b.w. of rats), high dose (1500 mg of ethanolic leaf extract of *N. latifolia* /kg b.w. of rats), alcohol and *N. latifolia,* testosterone and *N. latifolia* groups respectively (Table 1). Ethanolic leaf extract of *Nauclea latifolia* at all treated doses significantly showed marked increased in SOD assay which was significantl
y higher than control and alcohol treated group ($p<0.05$). Alcohol treated group showed a significant reduction of SOD compared to testosterone. The values for the varied (low, medium and high) doses of ethanolic leaf extract of *N. latifolia* were however not significantly different from one another (Table 1). The SOD assay in testosterone group was significantly higher than the control group and alcohol group, ($p<0.05$) (Table 1). Comparison between alcohol treated group and group co-administered alcohol and ethanolic leaf extract of *N. latifolia* showed that the level of SOD was significantly higher in group with co-administration (1.01 ± 0.00 µg/dl) than the group with alcohol alone (0.65 ± 0.01 µg/dl) (Fig. 3). Co-administration of testosterone and ethanolic leaf extract of *N. latifolia* (1.35 ± 0.03 µg/dl) showed a significant increase in SOD compared to control group, alcohol group and *Nauclea latifolia* groups but significantly lowered SOD relative to group treated with testosterone alone (1.65 ± 0.02 µg/dl) (Fig. 3).

Table 1. DNA, LDH and SOD assay obtained following treatment with various substances for 21 days

Groups	DNA (mg/dl)	LDH (mg/dl)	SOD (mg/dl)
Control	0.31 ± 0.05	0.66 ± 0.00	1.07 ± 0.00
Alcohol	1.67 ± 0.18^{a}	0.78 ± 0.00^{a}	0.65 ± 0.08^{a}
Testosterone	0.38 ± 0.02^{b}	0.88 ± 0.00^{a,b}	1.65 ± 0.02^{a,b}
Low dose *N. latifolia*	1.60 ± 0.02^{a,c}	0.04 ± 0.00^{a,b,c}	1.24 ± 0.05^{a,b,c}
Medium dose *N. latifolia*	1.71±0.01^{a,c}	1.35±0.00^{c}	1.20 ± 0.03^{b,c}
High dose *N. latifolia*	1.45±0.02^{a,b,c}	1.28±0.00^{c}	1.18 ±0.01^{b,c,e}
Alcohol *and N. latifolia*	1.43± 0.03	1.04±0.00	0.65±0.01
Testosterone and N. latifolia	1.40±0.00	0.79±0.00	1.35±0.03

Values are mean± SEM, n = 5 rats per group, a = significantly different from control group (p<0.05), b = significantly different from alcohol group (p<0.05), c = significantly different from testosterone group (p<0.05), e = significantly different from medium dose of N. latifolia group (p<0.05)

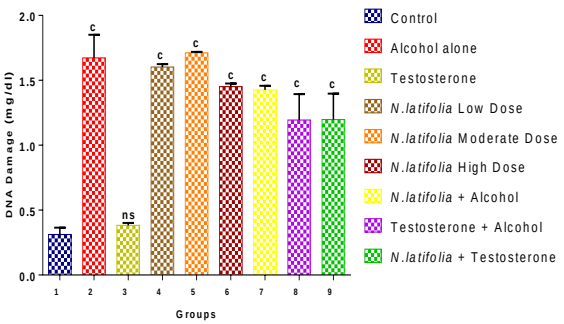

Fig. 1. DNA damage assay among the groups

*Values presented as mean ± SEM, *=p<0.05. c = Extremely significantly higher than control group and testosterone group, ns = Not significantly different from control group*

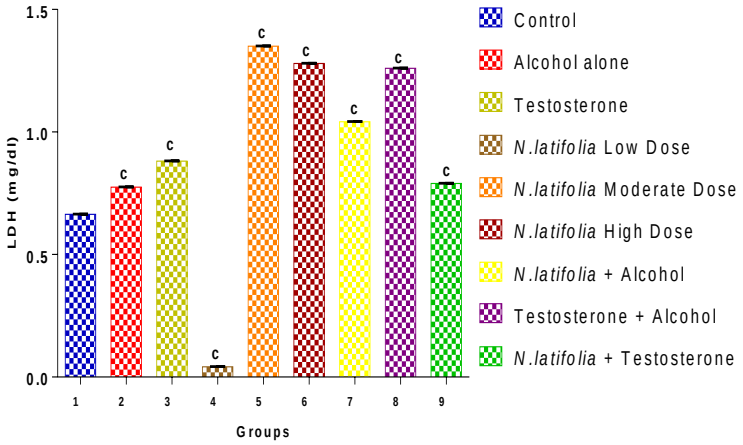

Fig. 2. Lactate dehydrogenase Assay among the groups

*Values presented as mean ± SEM, *=p<0.05. a = significantly higher than control group, b = significantly lower than control group, c = extremely significantly higher than control group.*

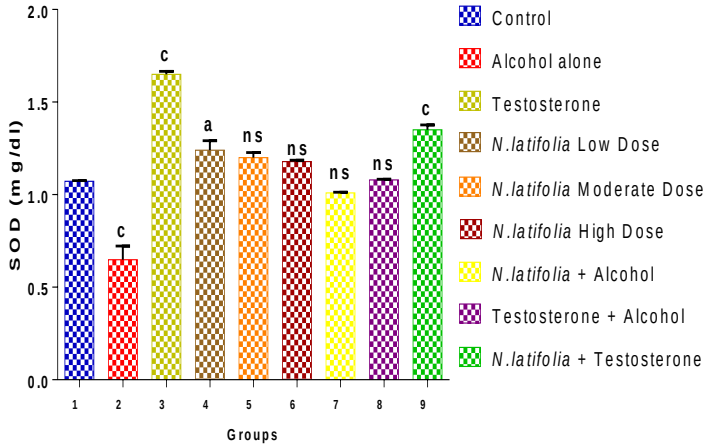

Fig. 3. Superoxide dismutase Assay among the groups

*Values presented as mean ± SEM, *=p<0.05. a = significantly lower than control group,c = significantly higher than control group. ns = not significantly different from control group*

4. DISCUSSION

Increased DNA damage has been implicated in loss of a nuclear protein known as Paired box (PAX) transactivation domain interacting protein (PTIP). This nuclear protein is involved in both DNA repair and histone methylation during spermatogenesis. The differentiation of matured sperm from male germ cells requires chromatin remodeling and compaction as well as DNA double stranded break repair of sister chromatids [10]. The loss of PTIP may lead to developmental arrest of spermatocytes, testicular atrophy and infertility. The result from this study indicates that ethanolic leaf extract of *Nauclea latifolia* at doses of 500, 1000 and 1500 mg /kg b.w. to adult male rats caused a significant increase in DNA damage. An increase in DNA damage also reflected in the group administered testosterone and ethanolic leaf extract of *Nauclea latifolia*. This shows that the hazardous effect of *Nauclea latifolia* ethanolic leaf extract on DNA was not ameliorated by testosterone. In mammals, the generation of spermatozoa is a continuous process that encompasses multiple molecular mechanism for generating mature haploid sperm, such mechanism include stem cell maintenance, cellular differentiation, DNA damage and repair during recombination, epigenetic remodeling of DNA, histone modification and chromatin

compaction [11]. This complex process requires two rounds of meiosis to produce the haploid gametes from a diploid germ cell. Before a germ cell completes meiosis I, chromosome pairs undergo homologous recombination involving the formation of synaptonemal complexes in which double strands DNA breaks occur followed by repair. Failure in the repair of DNA breaks by nuclear protein due to loss or a decline in the level of these proteins will prevent the cell division of the haploid secondary spermatocytes which is important in the separation of individual chromatid strands to generate two spermatid cells. The differentiation of spermatids involving extensive chromatin compaction and replacement of histones produce mature spermatozoa. The nuclear protein (PTIP) is implicated in both the DNA damage response and in the epigenetic modification of gene expression states [12]. Increase in the level of lactate dehydrogenase has been linked to tissue and cellular damage. Results from this study revealed an increased in the level of lactate dehydrogenase in rats administered moderate dose (1000 mg of ethanolic leaf extract of *Nauclea latifolia*/kg b.w. of rats) and high dose (1500 mg of ethanolic leaf extract of *Nauclea latifolia*/kg b.w. of rats). This increased might have been as a result of systemic ischemia caused by *Nauclea latifolia* leaf extract, leading to a release of lactate dehydrogenase enzyme from various damaged tissues [13]. Total serum LDH principally could rise due to the release of red blood cell lactate dehydrogenase as a consequence of intravascular hemolysis. The rats administered with low dose (500 mg of ethanolic leaf extract of *Nauclea latifolia* /kg b.w. of rats) and the group where testosterone was co-administered with ethanolic leaf extract of *Nauclea latifolia* showed a significant decrease in the level of lactate dehydrogenase compared to the group where alcohol was co-administered with ethanolic leaf extract of *Nauclea latifolia*. This implies that testosterone may have abated the activity and production of lactate dehydrogenase by the ethanolic leaf extract of *Nauclea latifolia* at this level with a decrease in the level of damage to other tissues while the reverse was the case in the combined group of ethanolic leaf extract of *Nauclea latifolia* and alcohol. LDH catalyzes the conversion of lactate to pyruvate and vice versa, as it converts NADH to NAD^+. At high concentration of lactate, the enzyme exhibits feedback inhibition and the rate of conversion of pyruvate to lactate is decreased. Conversion of pyruvate to lactate is increased due to the associated regeneration of NAD^+. High $NADH/NAD^+$ ratio shift the lactate dehydrogenase equilibrium to lactate, so that less pyruvate is formed. Biochemical (immunoassay) findings of superoxide dimustase level in the rats administered with 500, 1000 and 1500 mg of ethanolic leaf extract of *Nauclea latifolia*/kg b.w. of rats when compared with the control group showed a significant increase. This result is an indication that superoxide dismutase served as a scavenger of free radicals as an anti-oxidant to mob up the free radicals responsible for oxidative stress in order to protect the cells from free radicals [14]. This increase in the level of superoxide dismutase in the varied groups of *Nauclea latifolia* might have been due to the presence of flavonoids in *Nauclea latifolia* leaf extract, which activates the antioxidant levels of superoxide dismutase against free radical. This implies that though *Nauclea latifolia* could serve as an antioxidant, its benefit in this aspect could not ameliorate, counter or overcome its detrimental effect on other biochemical parameters (DNA damage and lactate dehydrogenase). In the group where alcohol was co-administered with ethanolic leaf extract of *Nauclea latifolia*, the level of superoxide dismutase was significantly increased compared to the group which alcohol alone was administered to the rats. This implies that co-administration of alcohol and ethanolic leaf extract of *Nauclea latifolia*, could increase the level of antioxidants in the body. The group administered testosterone and ethanolic leaf extract of *Nauclea latifolia* showed a decrease in the level of superoxide dismutase compared to the group administered testosterone alone. This is an indication that *Nauclea latifolia* promotes oxidative stress by reducing the activity of superoxide dismutase when combined with testosterone.

5. CONCLUSION

Ethanolic leaf extract of *Nauclea latifolia* at doses of 500, 1000 and 1500 mg/kg b.w of rats significantly increased DNA damage and lactate dehydrogenase signifying increased tissue damage. Elevated superoxide dismutase level was recorded following the administration of ethanolic leaf extract of *Nauclea latifolia*. This increase may be due to its high flavonoid content. This implies that though *Nauclea latifolia* could serve as an antioxidant, its benefit in this aspect could not ameliorate, counter or overcome its detrimental effects on other

biochemical parameters (DNA damage and lactate dehydrogenase).

ETHICAL APPROVAL

Animal Ethical committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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