Biochemical Assessment of *Picralima nitida* Seeds on Oxidative Stress Parameters of Albino Rats

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**Authors’ contributions**

This work was carried out in collaboration between all authors. Author LAN designed the study, wrote the protocol and interpreted the data. Author AAE anchored the field study, gathered the initial data and performed preliminary data analysis. Authors ACU and UA managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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**ABSTRACT**

The effects of different percentage concentrations (5%, 10%, 20% and 30%) of ground seeds of *Picralima nitida* on the concentrations of ascorbic acid (AA), glutathione (GSH) and the activities of superoxide dismutase (SOD), catalase (CAT) and lactate dehydrogenase (LDH) of albino rats after 28 days were investigated. Albino rats fed without ground seeds of *Picralima nitida* served as the control. The concentrations of AA, GSH and the activities of SOD, CAT and LDH from groups of albino rats that were fed with different concentrations of ground seeds of *Picralima nitida* were determined/assayed using standard methods. Results showed that there were no significant (p<0.05) difference in the concentrations of glutathione of rats fed with 5% feed formulated with ground seeds of *Picralima nitida* when compared to those of the control. However, there were significant (p<0.05) differences in the concentrations of glutathione of rats fed with 5% feed-formulated with ground seeds of *Picralima nitida* when compared to those of the control. Also, there was a significant (p<0.05) reduction observed in the glutathione concentration of rats fed with 10%, 20% and 30% feed formulated with ground seeds *Picralima nitida*. The values obtained showed that, as the percentage concentration of ground seeds *Picralima nitida* in the formulated feed increased, the concentrations of ascorbic acid and glutathione decreased in contrast to the activities of SOD,

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CAT and LDH indicating that these changes are concentration-dependent. The high concentrations of the ground seeds of *Picralima nitida* in the formulated feed might have caused the observed changes in these oxidative stress parameters in the serum of experimental albino rats.

Keywords: *Picralima nitida*; seed; oxidative stress; albino rats.

1. INTRODUCTION

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous animals. There are claims by herbalists in recent times that certain ailments which have defiled Western medicine can be cured with local herbs.

Chemical compounds in plants mediate their effects on the animals through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects [1,2].

*Picralima nitida* is the only species of the genus Picralima. It is related to Hunteria and Pleiocarpa. It belongs to the hunterieae tribe of the Apocynaceae family, and is commonly called Osi-Igwe in Ibo and Abere in Yoruba. In other parts of West Africa, the plant is called Gbe-Fon dangne in Benin Republic, Adangme in Ghana, Abure ebissi in Ivory Coast and Susu balunyi in Sierra Leone [3].

It is widely distributed in high deciduous forest of West-Central Africa from Ivory Coast to West Cameroons, and extending across the Congo basin and Uganda [3-5].

*Picralima nitida* is an under storey tree which reaches up to 4-35 m in height, with dense crown. Its trunk is about 5-60 m in diameter; cylindrical, the wood is pale yellow, hard, elastic, fine-grained and taking a high polish. *P. nitida* bears white flowers (about 3 cm long) with ovoid fruits which at maturity are yellowish in colour. The leaves are broad (3-10 cm) and oblong (6-20 cm long) with tough tiny lateral nerves of about 14 to 24 pairs [3]. *P. nitida* has widely varied applications in West Africa folk medicine. Various parts of the plant; the leaves, seeds, stem, bark and roots are used by herbalists for the treatment of fever, hypertension, jaundice, gastro-intestinal disorders and for malaria [6,7].

Preparations from different parts of the plant are employed as crude drug or crude herbal extract as remedy for various kinds of human disease. The seeds are widely used in West Africa especially in Nigeria, Cote d’ivoire and Ghana as antipyretic, aphrodisiac, for the treatment of malaria, pneumonia and other chest-conditions [7-9]. In Gabon the seeds are applied externally for the treatment of abscesses. The fruit is used in West Africa for the treatment of gastrointestinal disorder and dysmenorrhoea [10,11]. The leaves are used as a vermifuge and the leaf- sap is dripped into the ears for otitis [12]. The bark is used as laxatives and purgative, anthelmintic, treatment of venereal diseases, as febrifuges and also to treat hernia [13]. In Ivory Coast, a decoction of the bark is drunk in draught for jaundice and yellow fever [14]. The root is used as vermifuge, aphrodisiac, for fevers, malaria, pneumonia and gastrointestinal disorder [7,15,16].

All plants produce chemical compounds as part of their normal metabolic activities. The medicinal importance of *P. nitida* is based on its phytochemicals components. The plant is known to contain several phytochemicals such as alkaloids, tannins, polyphenols and steriods noted for their medicinal importance [17].

Despite the widespread abundance and numerous traditional uses of *Picralima nitida* seed in the treatment of various diseases, no study has been done on the effects of this herb on oxidative stress parameters to the best of our knowledge. Therefore, the aim of this study was to assess the effects of *Picralima nitida* seeds on oxidative stress parameters of albino rats.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Preparation

The pods of *Picralima nitida* were procured from Ekeonuwa market, Owerri, Imo State, Nigeria and were identified by a plant taxonomist at the
Department of Forestry and Wildlife, School of Agriculture, Federal University of Technology, Owerri. The seeds were removed from the pods, air-dried for two weeks and ground into a fine powder using a sterilized grinding machine.

![Image of Picralima nitida seeds](image1)

**Fig. 1. The seeds of Picralima nitida**

### 2.2 Experimental Animals

Twenty five (25) male albino rats of the Wistar strain weighing between 156 to 186 grams were purchased from the animal house of the Zoology Department, University of Nigeria Nsukka, Nigeria. The rats were transported to the animal house of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria. The rats were housed in partitioned wire-meshed cages under standard laboratory condition of humidity, temperature (25± ²C) and light (12 hr light/dark cycles). They were treated humanely as encapsulated in National Institutes of Health guidelines [18]. They were supplied with feed and water *ad libitum*

The rats were divided into five groups of five (5) rats in each cage according to their relative body weights. The animals were allowed to acclimatize to the environment for one (1) week on a regular feed and water *ad libitum*. After acclimatization, each group was fed with the feed formulated with 5%, 10%, and 20% w/w of ground *P. nitida* seed, except the control group which received 100% feed. During this period, observations were made on the animals' appetite and general wellbeing.

### 2.3 Animal Grouping and Feed Administration

The five (5) different experimental groups received designated concentrations of the feed mixed with ground *P. nitida* seed thus: Group 1 received 100% feed (Control), Group 2 received 95% feed with 5% ground *P. nitida* seed, Group 3 received 90% feed with 10% ground *P. nitida* seed, Group 4 received 80% feed with 20% ground *P. nitida* seed while Group 5 received 70% feed and 30% ground *P. nitida* seed respectively.

### 2.4 Collection of Blood Samples

The rats were anesthetized by exposure to dichloromethane vapor in covered transparent plastic container. Incisions were then made into their thoracic regions and were terminally bled by cardiac puncture using 5 mL hypodermic syringes and needles. The blood sample was collected and introduced into sterile sample bottles using 5 mL hypodermic syringes and needles. The blood samples were allowed to clot and centrifuged at 3000 rpm for 10mins. The serum was separated using micropipettes and used for the determination/assay of the various parameters.

### 2.5 Determination of Oxidative Stress Parameters

Ascorbic acid concentration was determined by the method of Reo and Kuether [19]. Reduced glutathione (GSH) concentration was determined by the method of [20]. The method is based on the formation of a relatively stable chromophoric product on reacting with a sulphur hydryl compound (GSH) with Ellman’s reagent. Catalase activity (CAT, E.C. 1. 11.1.1.) was assayed by measuring spectrophotometrically at 570 nm the rate of decomposition of hydrogen peroxide (H₂O₂) over a period of 30 minutes at (1 minutes interval) as described by [21]. The enzyme activity was expressed in terms of Katalase feihigkeit (Kat.f) as ks⁻¹ mg⁻¹ protein where K is the first order rate constant. Superoxide dismutase (SOD, E.C.1.15.1.1.) activity was assayed according to the methods of [22]. Lactate dehydrogenase (LDH, E.C. 1.1.1.27) activity was assayed following standard procedures as described in the assay kit by the manufacturer from Randox Laboratories Ltd, United Kingdom.

### 2.6 Statistical Analysis

Each reading was taken in triplicate. All data were expressed as mean ± standard deviation and analyzed for statistical significance by using one way Analysis of Variance (ANOVA). Values were considered significance at p≤ 0.05.
3. RESULTS AND DISCUSSION

Although most herbal medicines have a long history of traditional use, only their experimental validation at known doses may give a clearer idea about its safety and efficacy, in line with the objectives of World Health Organization Traditional Medicine Strategy.

A paradox in metabolism is that, while the vast majority of complex life on earth requires oxygen for its existence, oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species [23]. Consequently, organisms by containing a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipid [24].

Ascorbic acid is involved in many physiological functions in living organisms. In a variety of other functions, the role of ascorbic acid in cellular metabolism can be accounted for by its reducing properties to protect cellular components from oxidative damage. It acts as a scavenger of free radicals and harmful oxygen-derived species, such as the hydroxyl radical, hydrogen peroxides and singlet oxygen [25]. The concentration of ascorbic acid in the serum could therefore be used to assess the presence of free radicals capable of causing damages to cellular components.

The results obtained in this study as shown in Fig. 2 revealed that the concentration of ascorbic acid reduced in albino rats fed with different percentage concentrations of ground seeds of Picralima nitida as the concentrations of ground seeds of Picralima nitida increased when compared to those of the control.

The reduction in serum concentration of ascorbic acid observed in this study could indicate an increased utilization of the ascorbic acid to maintain normal reactive oxygen species homeostasis by using the ascorbic acid to mop up the reactive oxygen species generated following feeding with the ground seeds of Picralima nitida. Since the lowest concentration of ascorbic acid was obtained in the group fed with highest concentration of the grind seeds, it showed that oxidative stress was high in the group. It corroborates the clinical evidence for the unequivocal benefits of antioxidant supplementation to protect against excessive reactive oxygen species. Polidori et al. [26] reported that low level of serum ascorbic acid concentrations are known to occur in several conditions of increased oxidative stress.

The critical role of reduced glutathione in a variety of detoxification reactions against oxidizing species produced during the metabolism of xenobiotics and its involvement in the formation of conjugates with electrophilic metabolites is well established [26]. Indeed, many in-vivo and in-vitro studies have associated with a decrease in the intracellular level of this tripeptide with conditions of oxidative stress or the formation of glutathione-S-conjugates [26]. In fact, due to the rapid nature of the reduction of GSSG relative to its synthesis or secretion, the ratio of GSH to GSSG is a good indicator of oxidative stress within cells [27].

The results obtained in this study as shown in Fig. 3 showed that the concentrations of glutathione (GSH) in the test samples reduced as the concentration of the ground seeds of Picralima nitida increased when compared to the control. The reduction in the serum glutathione concentration of the albino rats fed with different concentrations of the ground seeds of Picralima nitida could be attributed to an increased utilization of reduced glutathione (GSH) to mop up the free radicals produced following feeding with the ground seeds of Picralima nitida.

Another possible reason for the decrease in serum glutathione concentrations of the test samples could be the presence of toxic components of the seeds. For this reason, the glutathione was used up by conjugating the toxic components in order to make them soluble for excretion.

Superoxide dismutase, is an important enzymatic antioxidant defense system present in nearly all cells exposed to oxygen, counteracts damaging reaction of superoxide radical, thus protecting the cells from superoxide toxicity. Quantification of superoxide dismutase activity is therefore essential in order to fully characterize the oxidative stress status in a biological system.

The results obtained in this study as shown in Fig. 4 revealed that the superoxide dismutase activity increased in the test samples in a concentration-dependent manner when compared to the control. This corroborates the work of [28] on the hepato-protective potentials of Moringa oleifera leaf extract on alcohol-induced hepato-toxicity in Wistar rats. The increase in the serum superoxide dismutase
activity of the test samples of our study could be attributed to the presence of superoxide radicals generated following feeding with ground seeds of *Picralima nitida*.

**Fig. 2.** Serum ascorbic acid concentrations (mg/dl) of the rats in groups 1 – 5. Bars (mean ± standard deviation) with different superscript letter(s) are statistically significant (p<0.05)

**Fig. 3.** Serum glutathione concentration (µM) of the rats in groups 1–5. Bars (mean ± standard deviation) with different superscript letter(s) are statistically significant (p<0.05)
Catalase is an enzymatic antioxidant which has one of the highest turnover number of all enzymes, one catalase molecule can converts millions of molecules of hydrogen peroxide to oxygen and water [29]. Catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules [30]. Catalase can be used to protect cell from oxidative damage in the system by reactive oxygen species. The results obtained in this study as shown in Fig. 5 indicates that there was a significant (p< 0.05) increase in the activities of catalase of rats fed with 5%, 10%, 20% and 30% of Picralima nitida formulated feed respectively when compared to the control. This corroborates with the work of on the hepatoprotective potentials of Moringa oleifera leaf extract on the alcohol-induced hepato-toxicity in wister rat. The increase in serum of catalase activity following feeding of albino rats with mixture of ground seeds of Picralima nitida could be attributed to increase oxidative damage possibly caused by some components of the ground seeds.

Lactate dehydrogenase (LDH) is an enzyme found in animals, plant and prokaryotes. It is of medical significance because it is found extensively in body tissues, such as blood cells and heart muscles, because it is released during tissue damage, it is a marker of common injuries and disease. It is made up of five isoenzymes [31]. The activity of lactate dehydrogenase in the serum can be used to assay different tissue damage.

The results obtained in this study as shown in Fig. 5 indicates that there was increase in activity of lactate dehydrogenase of rats fed with 10%, 20% and 30% of Picraima nitida formulated feed when compared to those of the control, indicating the release of free radicals which subsequently might had caused oxidative damage in the rats.

The increase in serum lactate dehydrogenase activity following feeding of albino rats with feed formulated with ground seeds of Picralima nitida could be attributed to tissue damage possibly caused by some components of the ground seed.

![Graph showing serum superoxide dismutase activity (U/L) of the rats in groups 1–5](image)

**Fig. 4.** Serum superoxide dismutase activity (U/L) of the rats in groups 1–5. Bars (mean ± standard deviation) with different superscript letter(s) are statistically significant (p<0.05)
Fig. 5. Serum Catalase activity (U/l) of the animals in groups 1–5. Bars (mean ± standard deviation) with different superscript letter(s) are statistically significant (p<0.05)

Fig. 6. Serum lactate dehydrogenase activity (U/l) of the rats in groups 1–5. Bars (mean ± standard deviation) with different superscript letter(s) are statistically significant (p<0.05)
4. CONCLUSION

The study has shown that *Picralima nitida* seeds irrespective of its efficacy in the treatment of various ailments have adverse effects on some important oxidative stress parameters studied. We therefore conclude that herbal preparations with *Picralima nitida* seeds may not be completely safe in albino rats when repeatedly administered for 28 days at high doses.

ETHICAL APPROVAL

We, hereby declare that this study was approved by the ethics committee of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria and all experiments were in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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