



Effect of Consumption of *Sphenostylis stenocarpa* Seed-formulated Diet on Oxidative Stress Biomarkers of Dexamethasone-treated Pregnant Rats

Funmilola Comfort Oladele ^{a*}, Olayinka Anthony Awoyinka ^a,
Joy Oluwadamilola Ofeimu ^b and Precious Moyinoluwa Adekanmbi ^b

^a Department of Medical Biochemistry, Ekiti State University, Ado-Ekiti, Nigeria.

^b Department of Science Laboratory Technology, Ekiti State University, Ado-Ekiti, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The present study tends to examine the effect of consumption of *Sphenostylis stenocarpa*-formulated diet on oxidative stress biomarkers of dexamethasone-treated pregnant rats.

Methodology: *Sphenostylis stenocarpa* was obtained locally from a market in Ado Ekiti. They were ground into powder and used to make feed for laboratory animals. Fifteen pregnant female rats were divided into three groups of five. Animals in group A were only fed standard animal feed. This served as the control group. Those in group B were exposed to *Sphenostylis stenocarpa*-formulated diet + 0.3 mg/kg body weight of dexamethasone, while those in group C were exposed to *Sphenostylis stenocarpa*-formulated diet. At the end of the eight days treatment, animals were sacrificed and blood sample, liver and kidney were collected.

Results: The results revealed that treatment of animals with dexamethasone significantly increased ($P < 0.05$) the activities of SOD and CAT and the concentration of MDA but decreased the concentration of GSH in plasma, liver homogenate and kidney homogenate respectively when compared with those in animals in the control group as well as those fed with *S. stenocarpa*-formulated diet only. The result further showed that feeding of animals with *S. stenocarpa*-

formulated diet only had no significant effect on oxidative stress biomarkers investigated when compared with those in the control group.

Conclusion: It can be concluded that exposure of animals to dexamethasone induced oxidative stress in animals while *S. stenocarpa*-formulated diet possesses the potential to alleviate the effect of oxidative stress generation.

Keywords: Dexamethasone; oxidative stress biomarkers; *Sphenostylis stenocarpa*-formulated diet.

1. INTRODUCTION

Antioxidants are substances that shield the body from the harmful molecules known as free radicals [1]. Antioxidants aid in the prevention of oxidation, which can cause cell damage and may contribute to ageing. They may boost immune function and reduce the risk of infection, cardiovascular disease, and cancer. Antioxidants can be found in foods as vitamins, minerals, and other compounds [2]. Antioxidants are substances that protect the body from the potentially harmful molecules known as free radicals [1]. Antioxidants help to prevent oxidation, which can damage cells and contribute to ageing. They may strengthen the immune system and lower the risk of infection, cardiovascular disease, and cancer. Antioxidants are found in foods in the form of vitamins, minerals, and other compounds [3].

Through the production of reactive oxygen species, oxidative stress induces the formation of free radicals such as superoxide anions (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radical (OH⁻), nitric oxide (NO), and organic hydroperoxide (ROOH) (ROS) [1]. Because they have a single pair of electrons in their outermost shells, these ROS are extremely unstable atoms. They have been implicated in processes such as mutagenesis, carcinogenesis, ageing, and so on, which are mediated by their inherent ability to cause cellular DNA damage [4]. Clearly, several human activities generate free radicals both endogenously and exogenously, resulting in debilitating pathological diseases. These pathological states are caused by an imbalance in the induction of oxidative stress and the biological system's ability to neutralise the free radicals released as a result of stress induction [5]. Of interest is the fact that the biological system has been packaged to nullify the destructive effect of these radicals generated through the inherent enzymatic antioxidants such as catalase, superoxide dismutase, and glutathione reductase [6]. However, there are synthetic antioxidants that are taken into the body to enhance the activities of the enzymatic

ones-tert butyl hydroxyl toluene, butylated hydroxyl anisole, butylated hydroxyl toluene, butylated hydroxyl anisole, butylated hydroxyl anisole, butylated hydroxyl anisole, butylated hydroxyl anisole, butylated hydroxyl anisole [3]. This has led to the resurgence of the search for herbal antioxidants that will be compatible with the biological system as well as boost the capacity of enzymatic ones with the aim of averting the health-related problems accredited to synthetics [2].

Sphenostylis stenocarpa (African yam bean) is a perennial member of the family leguminosae [7]. It is an important grain legume crop of rain-field agriculture in the tropics and subtropics. Compared with other grain legumes, African yam bean ranks only sixth in area and production, but it is used in more diverse ways than others [8]. The extracts or components of African yam bean are commonly used all over the world for the treatment of diabetes, dysentery, hepatitis and measles, as a febrifuge to stabilize the menstrual period. As a traditional Chinese medicine, the leaves of African yam bean have been widely used to arrest blood, relieve pain and kill worms [9]. Nowadays, African yam bean leaves are used for the treatment of wounds, aphtha, bedsores and malaria, as well as diet-induced hypercholesterolemia, etc. Protective effects of extracts from African yam bean leaf against hypoxic-ischemic brain damage and alcohol-induced liver damage have also been reported [10]. Chemical constituent investigations have indicated that African yam bean leaves are rich in flavonoids and stilbene, which are considered responsible for the beneficial efficacies of African yam bean leaves on human health [3].

A study hepatoprotective activity of African yam bean was carried out on sodium fluoride treated Swiss albino (BALB/C) mice. Treatment with extract of African yam bean exhibited significant anti-oxidant and hepatoprotective activity [2]. The present study tends to examine the effect of consumption of *Sphenostylis stenocarpa*-formulated diet on oxidative stress biomarkers of dexamethasone-treated pregnant rats.



Fig. 1. African yam bean [11]

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Materials

Dried *Sphenostylis stenocarpa* seeds were locally sourced from open bushes within Ado Ekiti, Nigeria. They were authenticated by the Chief botanist of the Department of Plant Science, Ekiti State University, Ado-Ekiti and deposited in the University's Herbarium with Voucher number UHAE-1010065. They were carefully selected to remove the perceived bad seeds. The seeds were sun-dried and milled into powder using an electric blender.

2.2 Experimental Design

Twenty one Albino rats (6 males and 15 females) were obtained from the Animal House, Faculty of Basic Medical Sciences, College of Medicine, Ekiti State University, Ado-Ekiti. They were grouped into three of 2 males and 5 females in each group using plastic cages with steel wire lids to copulate, since the experiment requires the female Albino rats to be pregnant. They were kept at room temperature with adequate access to rat chow and water throughout the experimental period. After a week of copulation, all the female Albino rats were confirmed pregnant by the animal house technician. The male rats were removed from their cages and the female pregnant rats were treated as follows: animals in group A were exposed to standard animal feed only. This served as the control group. Those in group B were exposed to *S. stenocarpa*-formulated diet + 0.3 mg/kg body weight of dexamethasone, while those in group C were exposed to *S. stenocarpa*-formulated diet only. All administrations were via oral route. At the end of the eight days treatment, animals were sacrificed and blood sample was collected into EDTA bottles and centrifuged. Plasma was separated and preserved at 4 °C for further analysis. Liver and kidney were also harvested

from the rats and were homogenized in phosphate water using a mechanical homogenizer and the homogenates were centrifuged for 5 minutes. The supernatant were collected and were used to carry out the biochemical assays.

2.3 Determination of Oxidative Stress Biomarkers

Determination of malondialdehyde (MDA), Reduced Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD) were carried out according to the methods previously described by Airaodion et al. [12].

2.4 Statistical Analysis

Results are expressed as mean \pm standard deviation. The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Turkey's test. All analyses were done using Graph Pad Prism Software Version 6.00 and p values < 0.05 were considered statistically significant.

3. RESULTS

Analysis of this study revealed that treatment of animals with dexamethasone significantly increased ($P < 0.05$) the activities of SOD and CAT and the concentration of MDA but decreased the concentration of GSH in plasma (Table 1), liver homogenate (Table 2) and kidney homogenate (Table 3) respectively when compared with those in animals in the control group as well as those fed with *S. stenocarpa*-formulated diet only. The result further showed that feeding of animals with *S. stenocarpa*-formulated diet only had no significant effect on oxidative stress biomarkers investigated when compared with those in the control group.

4. DISCUSSION

Superoxide dismutase (SOD) is a vital enzymatic antioxidant required to reduce oxidative stress [13]. In this study, the activity of SOD in animals exposed to dexamethasone was significantly increased when compared with those in the control group. This is in agreement with the findings of Oladele et al. [14] who exposed pregnant rats to dexamethasone. This is also consistent with the observations of Zhu et al. [15] and Tanko et al. [16] who respectively reported that dexamethasone increased the activity of SOD in animals. However, the mean values

reported by Zhu et al. [15] and Tanko et al. [16] are significantly higher than that observed in this study. This might have occurred owing to the fact that the animals exposed to dexamethasone in this study were also fed with *S. stenocarpa*-formulated diet. The dexamethasone-induced oxidative stress might have generated elevated reactive oxygen species (ROS) in the plasma, liver and kidney which SOD tend to combat thereby increasing its activity. Increased activity of SOD has been reported to decrease in the presence of antioxidants and phytochemicals [17,18]. Analysis of *S. stenocarpa* has revealed

that that the plant is rich in phytochemicals some of which are antioxidants [19]. These phytochemicals might have been responsible for the antioxidant potential of *S. stenocarpa* seed. Interestingly, no significant difference was observed in the activity of SOD in animals fed with *S. stenocarpa*-formulated diet only when compared with those in the control animals. This corresponds to the findings of Ojuederie et al. [20] who reported that exposure of animals to *S. stenocarpa*-formulated diet had no significant effect on the activity of SOD.

Table 1. Effect of *S. stenocarpa*-formulated diet on Plasma Oxidative Stress Parameters of Dexamethasone-Treated Pregnant Rats

Treatment Group	SOD (U/mg)	CAT (U/mg)	MDA (U/mg)	GSH (U/mg)
Control	57.18 ± 1.58 ^b	0.46 ± 0.06 ^b	0.20 ± 0.00 ^b	61.81 ± 1.21 ^a
Dexamethasone + <i>S. stenocarpa</i> -formulated diet	69.58 ± 1.8 ^a	0.67±0.01 ^a	0.37 ± 2.81 ^a	52.82 ±1.33 ^b
<i>S. stenocarpa</i> -formulated diet only	55.30 ± 0.66 ^b	0.42± 0.00 ^b	0.22 ± 0.00 ^b	63.21 ± 2.19 ^a

Results are presented as mean±standard deviation with n = 5. Values with different superscripts along the same column are significantly different at P<0.05.

Legend: SOD = Superoxide Dismutase, CAT = Catalase, MDA = Malondialdehyde, GSH = Glutathione

Table 2. Effect of *S. stenocarpa*-formulated diet on Liver-Homogenate Oxidative Stress Parameters of Dexamethasone-Treated Pregnant Rats

Treatment Group	SOD (U/mg)	CAT (U/mg)	MDA (U/mg)	GSH (U/mg)
Control	43.18 ± 1.04 ^b	0.51±0.00 ^b	0.35 ± 0.00 ^b	49.89 ± 2.10 ^a
Dexamethasone + <i>S. stenocarpa</i> -formulated diet	57.88 ± 1.71 ^a	0.63 ± 0.03 ^a	0.43 ± 0.00 ^a	35.86 ± 1.31 ^b
<i>S. stenocarpa</i> -formulated diet only	44.67 ± 1.86 ^b	0.49 ± 0.01 ^b	0.32 ± 0.00 ^b	51.07 ± 1.55 ^a

Results are presented as mean±standard deviation with n = 5. Values with different superscripts along the same column are significantly different at P<0.05.

Legend: SOD = Superoxide Dismutase, CAT = Catalase, MDA = Malondialdehyde, GSH = Glutathione

Table 3. Effect of *S. stenocarpa*-formulated diet on Kidney-Homogenate Oxidative Stress Parameters of Dexamethasone-Treated Pregnant Rats

Treatment Group	SOD (U/mg)	CAT (U/mg)	MDA (U/mg)	GSH (U/mg)
Control	55.12 ± 0.93 ^b	0.84 ± 0.00 ^b	0.22 ± 0.00 ^b	48.64 ± 2.62 ^a
Dexamethasone + <i>S. stenocarpa</i> -formulated diet	67.88 ± 0.70 ^a	0.97± 0.01 ^a	0.32 ± 0.00 ^a	36.81 ± 1.43 ^b
<i>S. stenocarpa</i> -formulated diet only	55.81 ± 2.16 ^b	0.82± 0.00 ^b	0.22 ± 0.00 ^b	46.23 ± 3.08 ^a

Results are presented as mean±standard deviation with n = 5. Values with different superscripts along the same column are significantly different at P<0.05.

Legend: SOD = Superoxide Dismutase, CAT = Catalase, MDA = Malondialdehyde, GSH = Glutathione

This study also revealed that there was no significant difference in catalase activity between control animals and those treated with *S. stenocarpa*-formulated diet alone. This is

consistent with the findings of Ojuederie et al. [20], who found that exposing animals to *S. stenocarpa*-formulated diet seed had no effect on catalase activity. The activity of catalase in

animals exposed to dexamethasone was significantly increased when compared with those in the control group. This is in agreement with the findings of Oladele et al. [14] who exposed pregnant rats to dexamethasone. Chen et al. [21] have previously reported that glucocorticoid increases the generation of oxidative stress. Dexamethasone is a known glucocorticoid; thus its action in elevating the activity of catalase in the plasma, liver and kidney is not surprising.

The result of this study showed that dexamethasone exposure significantly elevated the concentration malondialdehyde (MDA) in the plasma, liver and kidney indicating enhanced peroxidation and breakdown of the antioxidant defense mechanisms [22]. Decomposition products of lipid hydroperoxide such as malanaldehyde and 4-hydroxynonenal, can cause chaotic cross-linkage with proteins and nucleic acids, which plays an important role in the process of carcinogenesis [23]. Furthermore, extensive damage to tissues in a free radical mediated lipid peroxidation (LPO) results in membrane damage and subsequently decreases the membrane fluid content [24]. The result of this present study is line with the findings of Zhu et al. [15] and Tanko et al. [16] who respectively reported that dexamethasone increased the concentration of MDA in animals. However, the values reported for both studies are significantly higher than that observed in this study. This might have occurred owing to the fact that the animals exposed to dexamethasone in this study were also fed with *S. stenocarpa*-formulated diet simultaneously. The dexamethasone-induced oxidative stress might have led to increased decomposition of lipid hydroperoxide which subsequently increased the concentration of MDA.

Increased concentration of MDA has been reported to decrease in the presence of antioxidants and phytochemicals [17,18]. Analysis of *S. stenocarpa* has revealed that that the plant is rich in phytochemicals some of which are antioxidants [19]. These phytochemicals might have been responsible for the antioxidant potential of *S. stenocarpa* seed. The result of this present study further showed that no significant difference was observed in the concentration of MDA in animals fed with *S. stenocarpa*-formulated diet only when compared with those in the control animals. This corresponds to the findings of Ojuederie et al. [20] who reported that exposure of animals to *S. stenocarpa*-formulated

diet had no significant effect on the concentration of MDA.

Glutathione (GSH) is the most abundant soluble antioxidant and is found in all cell compartments [25]. In response to metabolic challenges, hepatic glutathione synthesis and antioxidant protection are critical for efficient detoxification processes [21]. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism [26]. Glutathione directly inhibits ROS like lipid peroxides and also plays an important role in xenobiotic metabolism [26]. Glutathione detoxifies hydrogen peroxide and lipid peroxide by donating an electron to hydrogen peroxide, reducing it to water and oxygen and protecting macromolecules from oxidation, such as lipids [27]. In this study, the decrease in the concentration of glutathione in animals treated with dexamethasone might not be unconnected with dexamethasone-induced oxidative stress. However, no significant difference was observed in the concentration of GSH in animals treated with *S. stenocarpa*-formulated diet only when compared with those in the control group. This is an indication that *S. stenocarpa*-formulated diet did not generate ROS and had no adverse effect on oxidative stress.

5. CONCLUSION

Exposure of animals to dexamethasone was observed to induce oxidative stress in animals while *S. stenocarpa*-formulated diet possesses the potential to alleviate the effect of oxidative stress generation.

DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

The use of animals for this study was approved by the Experimental Animal Research Ethics Committee of Ekiti State University, Ado-Ekiti

with ethical approval number
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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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