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Effects Of Aqueous And Methanolic Leaf Extracts Of *Lonchocarpus Cyanencens* Leaf On Oxidative Status In Normal Albino Wistar Rats

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Abstract

Lonchocarpus cyanescens, a shrub tree is one of the herbal plants used traditionally to improve human health in Nigeria. It is used in the treatment of Diabetes mellitus in the western part of the country. The aim of this study was to determine the biochemical effects of aqueous and methanol extracts of *Lonchocarpus cyanescens* leaf on oxidative status in normal Wistar albino rats. A total of twelve rats were divided into three groups of four rats each: control, aqueous and methanol extract groups. Rats in the two observation groups were administered 200 mg/kg body weight, of aqueous or method extract of the medicinal plant leaf. After 12 weeks of treatment, the rats were euthanized and blood samples were collected for analysis. The oxidative stress parameters measured included activities of catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) as well as concentrations of reduced glutathione (GSH) and malondialdehyde (MDA). The results showed that aqueous and methanol extracts of *L. cyanescens* leaves did not significantly alter the activities and concentrations of the measured indices of oxidative stress in rats' plasma when compared with the control group ($p > 0.05$). These results indicate that extracts of *L. cyanescens* can maintain the oxidative status of normal rats.

Keywords: Antioxidant enzymes, Catalase, Glutathione, Lonchocarpus cyanescens, Oxidative stress

Introduction

Extracts from plants have been used for centuries in the treatment and management of diseases (Abu *et al.,* 2015; Okpoghono *et al.,* 2018; Onyesom and Okoh, 2006). The medicinal benefits of plant extracts have been attributed to the presence of bioactive constituents known as phytochemicals (Abu *et al.,* 2017 and 2020; George *et al.,* 2012, 2015). However, some phytochemicals have been reported to be toxic to humans due to their ability to cause cellular damage via oxidative stress (Adebiyi and Abata, 2013). It has become necessary, therefore, to strike a balance between the effectiveness of medicinal plants and their relative safety (of what importance is a medicinal plant if its safety is not guaranteed).

Commonly called West African wild indigo, *Lonchocarpus cyanescens* is a medicinal plant used locally for the treatment of various diseases. The shrub is found in fringe, deciduous and savannah forests, growing up to a height of 5 m and 50 cm thickness (Ogungbaro, 2010). The bark and leaves are used for the treatment of bone pain, yaws and diabetes mellitus (Umoh and Nwafor, 2013). Decoctions of the root and leaves are used to treat venereal diseases and stomach aches (Moronkola and Oladosu, 2013). The plant leaves have been demonstrated to contain tannins, flavonoids, saponins, cardiac glycosides, steroids and reducing sugars (Iyoha and Onoagbe, 2016). Similarly, an acute toxicity assessment of the plant showed that it is practically non-toxic (Iyoha and Onoagbe, 2016). However, there are no scientific data to prove its effect on oxidative status in rats, hence this study was undertaken to determine the biochemical effects of aqueous and methanol extracts of *L. cyanenscens* leaves on oxidative status in normal Wistar albino rats.

Materials And Methods

Chemicals

All chemicals used in this study were of analytical grade, commercially sourced from Sigma-Aldrich Ltd (UK).

Medicinal plant

Fresh leaves of *Lonchocarpus cyanescens* were bought from a reliable source in Kwara State, Nigeria. The plant was identified in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. A herbarium specimen with voucher number UBHf0291 was deposited at the herbarium of the University of Benin.

Preparation of plant extracts

The plant leaves were washed and air-dried at room temperature for a period of three weeks, before it was pulverized into powder. The powdered leaves were then macerated in either distilled water (l00 g/1000 mL) or absolute methanol (l00 g/500 mL) and stirred periodically for 72 h. Filtration was done with the aid of a cheesecloth to remove debris and then through a Whatman No. 1 filter paper to obtain aqueous or methanol extract which was freeze-dried.

Experimental animals

Male albino rats weighing between 100 and 150 g were housed in wooden cages in the Animal House of Biochemistry Department, University of Benin, Benin City. The rats were allowed to acclimatize to the environment for a period of two weeks before the start of the experiment. They were placed on commercial feed (growers' pellet) and drank water *ad libitum.*

Experimental design

The rats were divided into three groups of four rats each: control, aqueous and methanol extract groups. Rats in the two observation groups were administered 200 mg/kg body weight aqueous and methanolic extracts of the medicinal plant leaves. This dose was determined from dose response study of both aqueous and methanolic leaf extracts conducted for a period of two weeks.

Blood sample preparation

After 12 weeks of treatment, the rats were euthanized and blood samples were collected in lithium heparin containers and centrifuged at 3000 rpm for 15 min to obtain plasma which was used for biochemical analyses.

Biochemical analyses

The oxidative stress parameters measured included activities of catalase (Cohen *et al.*, 1970), SOD (Misra and Fridovich, 1972), GPx (Nyman, 1959) as well as concentrations of GSH and MDA (Buege and Aust, 1978).

Statistical analysis

The results were analyzed using one- way analysis of variance (ANOVA). A post hoc multiple comparison test was utilized to determine the level of significance between treatment groups and control. Statistical significance was considered at $p < 0.05$. The statistical software used was SPSS version 21.

Results

Effects of aqueous and methanol extracts of *L. cyanescens* leaf on rat oxidative status

The results showed that aqueous and methanol extracts of *Lonchocarpus cyanescens* leaves did not significantly alter the activities and concentrations of the measured indices of oxidative stress in rat plasma when compared with the control group ($p > 0.05$) (Figures 1 to 5).

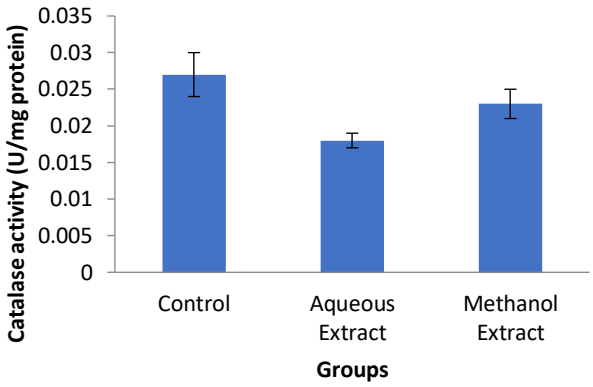

Figure 1: Activity of catalase in the plasma of normal rats
Values are expressed as mean±SEM (n=4).

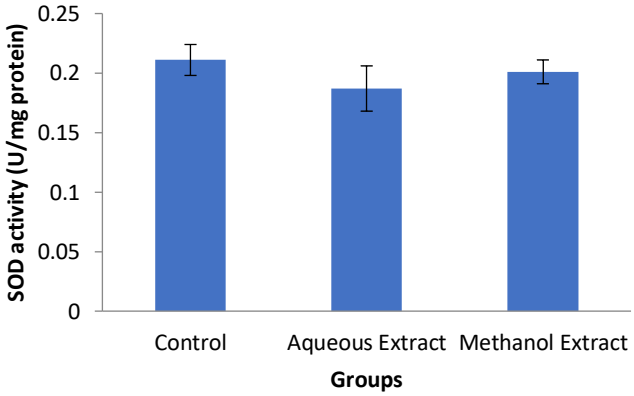

Figure 2: Activity of SOD in the plasma of normal rats .
Values are expressed as mean ± SEM (n = 4).

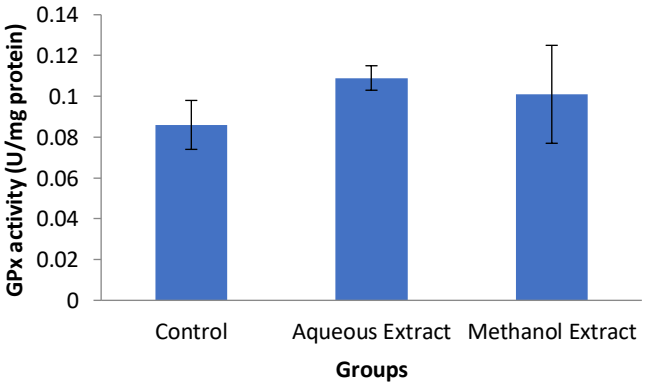

Figure 3: Activity of GPx in the plasma of normal rats.

Values are expressed as mean ± SEM (n = 4).

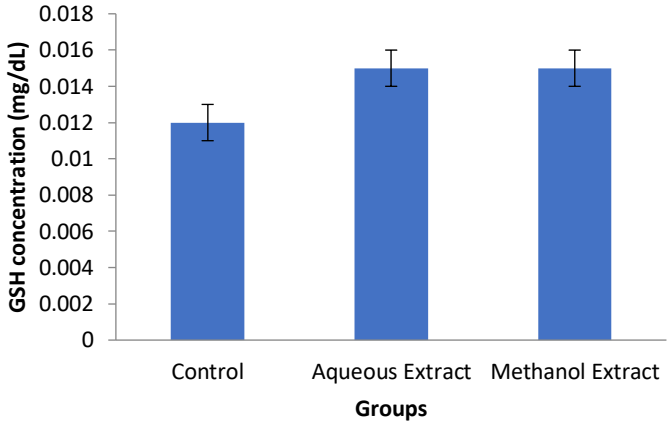

Figure 4: Concentration of GSH in the plasma of normal rats.
Values are expressed as mean ± SEM (n = 4).

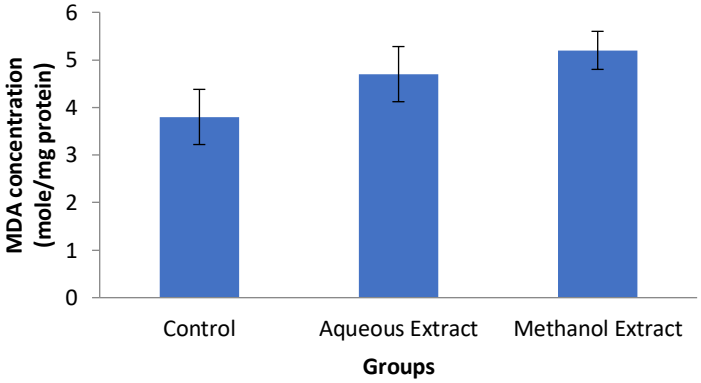

Figure 5: Concentration of MDA in the plasma of normal rats.
Values are expressed as mean ± SEM (n = 4).

Discussion

Oxidative stress occurs when there is an imbalance in the production and removal of free radicals within an organism. The majority of these radicals are reactive oxygen species (ROS) (Seis,1991). Excess ROS cause damage to DNA, lipids and cellular proteins (Poston *et al.,* 2011). Oxidative stress is characterized by increased lipid peroxidation and altered enzymatic and non-enzymatic antioxidant systems (Adewole and Caxton-Martins, 2006; Abu *et al.,* 2022a). Antioxidants are compounds that protect cells from peroxidation reactions caused by free radicals, thereby limiting cellular damage while maintaining cellular integrity. Antioxidant enzymes include catalase, SOD, GPx, and glutathione reductase (GR) while the non-enzymatic antioxidants include GSH, and vitamins C and E (Abu *et al.,* 2022b).

Superoxide dismutase (SOD) scavenges superoxide anion (O_2^-) into oxygen and hydrogen peroxide via a rapid dismutation reaction (Bannister, 1987; Zelko *et al.*, 2002). Catalase catalyzes the conversion of hydrogen peroxide to water and oxygen using either manganese or iron co-factor (Chelikani *et al.*, 2004). Glutathione peroxidase (GPx) catalyzes the reduction of organic peroxide. Reduced glutathione helps in scavenging free radicals. The aim of this study was to determine the biochemical effects of aqueous and methanol extracts of *L. cyanescens* leaves on oxidative status in normal Wistar albino rats. The administration of 200 mg/kg body weight of both extracts did not significantly ($p>0.05$) alter the enzyme and non-enzyme antioxidant as shown in figures 1 to 4 systems indicating that both extracts of the plant leaves have no deleterious effect on endogenous antioxidant levels in the normal rats as such cannot induce oxidative stress. Also the lipid peroxidation index (that is, MDA) was not significantly ($p>0.05$) increased (Fig. 5), indicating that the extracts did not affect cell membrane integrity. These results are in agreement with those reported by Omonkhua and Onoagbe (2012) where the roots of *Urena lobeta*, the bark of *Irvingia gabonensis* and the leaf of *Carica papaya* did not have any deleterious effect on oxidative status and lipid peroxidation in normal rats. In the study involving *Dialium guineense,* Abu and Onoagbe (2019) reported potentiation of the antioxidant defense systems of normal rats administered stem bark extract of the plant. *Lonchocarpus cyanescens* is one of the herb used in the treatment of various diseases.. From the results obtained in this study, the plant extracts (aqueous and methanolic) can be adjudged to be

safe and can be used in the treatment of the various diseases including Diabetes mellitus.

Conclusion

These results indicate that 200mg/kg of both extracts of *L. cyanescens* can maintain the oxidative status, cannot induce lipid peroxidation in normal rats and is therefore safe for the treatment of various ailments.

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