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Biochemical effect of aqueous extract *of Dialium Guineense* stem bark on oxidative status of normal wistar rats

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Abstract
The present study was undertaken to investigate the biochemical effects of aqueous stem bark extract of *D. guineense* on activities of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glucose 6-phosphate dehydrogenase (G6PDH)), and malondialdehyde (MDA) and total protein concentrations in normal Wistar rats. Ten adult male Wistar rats weighing 150 to 180 g were randomly assigned to two groups of five rats each: control group and observation group. The observation group received 1000 mg/kg body weight, BWT, of aqueous extract orally for twenty-eight days, and assays were performed on weekly basis. Results of phytochemical analyses of the plant stem bark revealed the presence of tannins (3.76 ± 0.31 %), alkaloids (1.22 ± 0.06 %), phenols (1.40 ± 0.03 %), flavonoids (0.92 ± 0.03 %), saponins (0.18 ± 0.02 %), glycosides (0.04 ± 0.01 %) and steroids (0.04 ± 0.01 %). There was no significant difference in the concentration of total protein between the two groups ($p > 0.05$). The concentration of MDA was significantly lower in observation group than in control group ($p < 0.05$). However, the activities of G6PDH, SOD, CAT and GPX were significantly higher in observation group than in control group ($p < 0.05$). The extract potentiated the activities of G6PDH, SOD, catalase and GPX.

Keywords: *Dialium Guineense*, oxidative damage, antioxidant enzymes, total protein, phytochemical

Introduction
Dialium guineense (velvet tamarind) belongs to the *Leguminosae* family. It has small, typically grape-sized edible fruits with brown hard inedible shells and grows in dense forests in Africa along the southern edge of the Sahel. The seed somewhat resembles watermelon seed *(Citrullus lanatus)*. The seeds are shiny, coated with a thin layer of starch. The pulp is edible and may be eaten raw or soaked in water and consumed as a beverage. Various parts of the plant have medicinal properties and are used to treat different diseases (Arogba *et al.*, 2006) [2].

The pathogenesis of a number of diseases is linked to free radical-induced oxidative damage. Increased lipid peroxidation and decreased antioxidant protection generate epoxides that spontaneously react with nucleophilic centers in cells and thus covalently bind to DNA, RNA, and protein (Yin *et al*., 1995; Rikans *et al*., 1997) [25, 19]. Such a reaction leads to cytotoxicity, allergy, mutagenicity, and/or carcinogenicity, depending on the properties of the epoxide in question. In addition, oxidative event plays an important role in the mechanism of action of ether lipids, and ability to oxidize may contribute to cellular drug sensitivity (Wagner *et al*., 1998) [24].

The enzymatic and non-enzymatic antioxidant defenses include SOD, GPx, catalase, ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), β-carotene, and vitamin A, which can be evaluated using easy photometric assays (Beaudeux *et al*., 1996; Hall *et al*., 1998; Stahl *et al.*, 1998) [3, 10, 23]. For the survival of organisms and maintenance of their health, there is usually a balance between the activities and intracellular levels of these antioxidants (Aleryani *et al.*, 1998; Brouwer *et al*., 1998; Grazioli *et al*., 1998) [1, 4, 8]. This study was undertaken to investigate the Effects of aqueous stem bark extract of *Dialium guineense* on activities of antioxidant enzymes, and MDA and total protein concentrations in normal Wistar rats.

Materials and Methods
Plant
Dialium guineense leaves were obtained from a forest in Benin City and identified at the Plant Biology and Biotechnology Department of the University of Benin, Benin City, Edo State Nigeria. After which the stem barks were obtained.

Experimental rats
Ten adult male Wistar rats weighing 150 to 180 g were randomly assigned to two groups of five rats each: control group and observation group. The observation group received 1000 mg/kg bwt of aqueous extract orally for twenty-eight days. All the rats were allowed free access to food and water.

Phytochemical analysis
This was performed using standard methods (Sofowora, 1993) [21].

Preparation of plant extract
The stem barks were shade-dried, pulverized and sieved. Extraction was by maceration over a 72 h period. Portions of the powdered stem bark (100 g) were covered up in 1000 ml of distilled water and the mixture was stirred intermittently. The aqueous extract was filtered with a mushlin cloth and freeze-dried by lyophilization.

Biochemical analysis

Plasma was analyzed for various biochemical parameters such as catalase activity (Cohen *et al.*, 1970) [6], SOD activity (Misra and Fridovich, 1972) [16], levels of MDA (Guttridge and Wilkins 1982), total protein concentration (Henry *et al.*, 1957) [11], G6PDH activity (Ells and Kirkman, 1961) [7] and GPx activity (Rotruct *et al*., 1973).

Statistical Analysis

Data are expressed as means ± SEM, and the statistical analysis was performed using SPSS (16.0). Groups were compared using Duncan Multiple Test Range and values of $p < 0.05$ were considered statistically significant.

Results

Phytochemical composition of *Dialium guineense* stem bark

As shown in Table 1, tannins were present in the highest amount, while glycosides and steroids were present in the least amounts.

Table 1: Phytochemicals in the stem bark of *Dialium guineense*

Phytochemical	% Composition
Tannins	3.76 ± 0.31
Alkaloids	1.22 ± 0.06
Phenols	1.40 ± 0.03
Flavonoids	0.92 ± 0.03
Saponins	0.18 ± 0.02
Glycosides	0.04 ± 0.01
Steroids	0.04 ± 0.01

Data are percentage compositions of phytochemicals and are expressed as mean ± SEM (n = 3)

Activity of SOD in the two groups

The activity of SOD was significantly higher in the observation group than in control group, and increased time-dependently ($p < 0.05$; Figure 1).

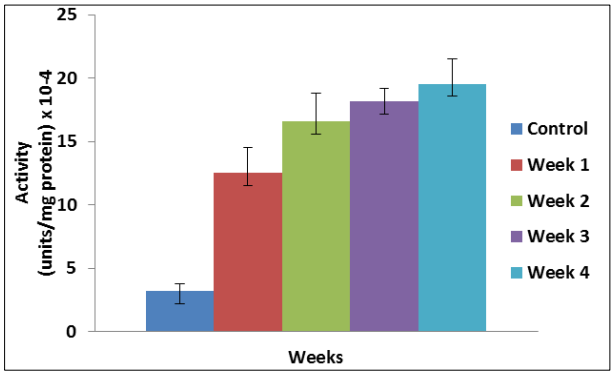

Fig 1: Effect of aqueous stem bark extract of *D. guineense* on SOD activity.

Data are SOD activities and are expressed as mean ± SEM (n = 5).

Catalase activity

The activity of catalase was significantly higher in observation group than in control group, and increased time-dependently ($p < 0.05$; Figure 2).

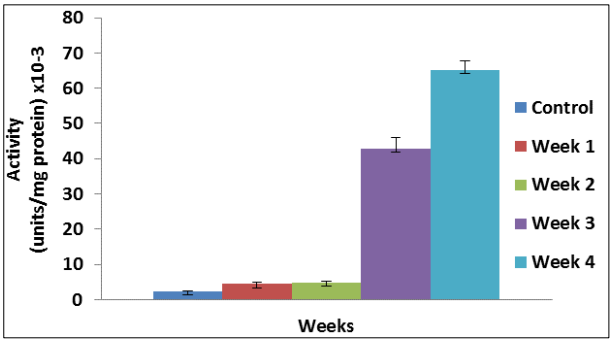

Fig 2: Effect of aqueous stem bark extract of *D. guineense* on the activity of catalase

Data are catalase activities and are expressed as mean ± SEM (n = 5).

Activity of GPX

The activity of GPX of rats treated with aqueous extract of *D. guineense* stem bark for four weeks (28 days) was significantly increased, relative to control group ($p < 0.05$; Figure 3).

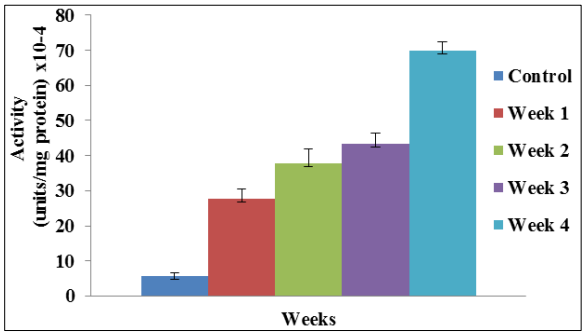

Fig 3: Effect of aqueous stem bark extract of *D. guineense* on the activity of GPx

Data are GPx activities and are expressed as mean ± SEM (n = 5).

Activity of G6PDH

As shown in Figure 4, the activity of G6PDH was significantly higher in the observation group than in control group, and was fairly constant throughout the period of treatment ($p < 0.05$).

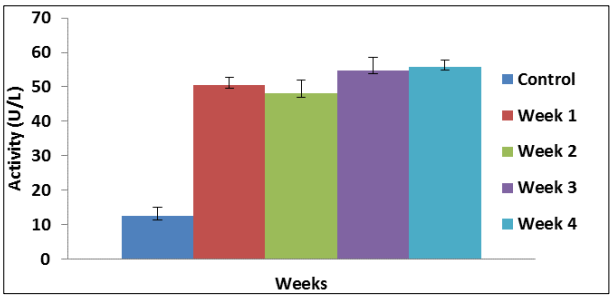

Fig 4: Effect of aqueous stem bark extract of *D. guineense* on the activity of G6PDH

Data are G6PDH activities and are expressed as mean ± SEM (n = 5).

Concentration of total protein

There was no significant difference in the concentration of total protein between the two groups ($p > 0.05$).

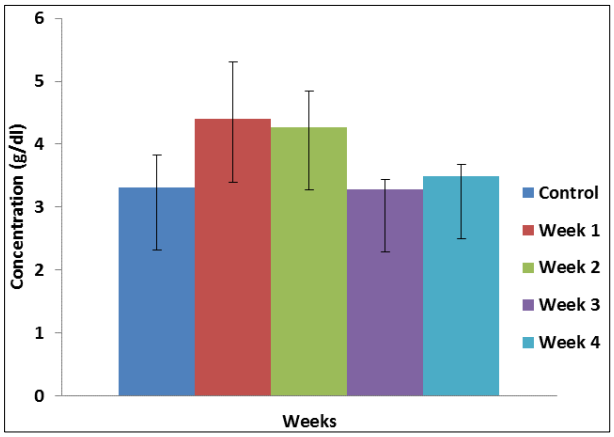

Fig 5: Effect of aqueous stem bark extract of *D. guineense* on the concentration of total protein

Data are concentrations of total protein and are expressed as mean ± SEM (n = 5).

Concentration of MDA

The concentration of MDA was significantly lower in observation group than in control group, and was reduced time-dependently ($p > 0.05$).

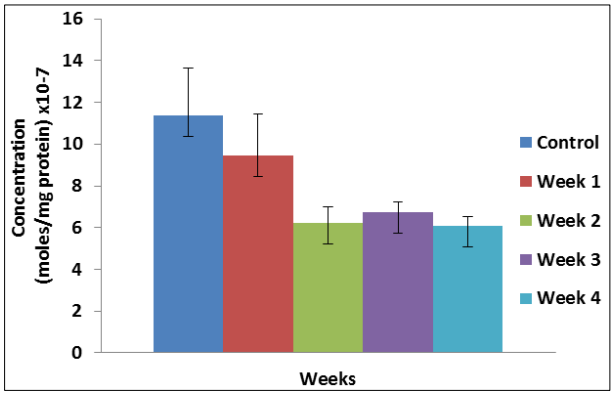

Fig 6: Effect of aqueous stem bark extract of *D. guineense* on the concentration of malondialdehyde

Data are concentrations of MDA and are expressed as mean ± SEM (n = 5).

Discussion

Aerobic organisms possess antioxidant defense systems that deal with reactive oxygen species (ROS) produced as a consequence of aerobic respiration and substrate oxidation. Small amounts of ROS, including hydroxyl radicals ($^{\bullet}OH$), superoxide anions ($O^{2\bullet-}$) and hydrogen peroxide (H_2O_2) are constantly generated in aerobic organisms in response to both external and internal stimuli (Hurst *et al*., 1997; Jornot *et al*., 1998; Mills *et al.*, 1998) [12, 13, 15].

Superoxide dismutase (SOD) detoxifies $O^{2\bullet-}$ which otherwise damage cell membrane and macromolecules (Okolie *et al.*, 1994) [17]. In animals, hydrogen peroxide is detoxified by catalase and GPx. Catalase protects cells from hydrogen peroxide generated within them. Even though catalase is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells. The increased sensitivity of transfected catalase-enriched cells to some drugs and oxidants is attributed to the ability of catalase to prevent drug-induced consumption of O_2 (Speranza, *et al*., 1993) [22]. Suppressed action of this enzyme results in enhanced sensitivity of cells to free radical- induced cellular damage (Caroline, *et al*., 2008) [5]. In the present study, G6PDH assay was employed as an indirect way of assessing the level of glutathione (reduced), since the reaction catalyzed by G6PDH in the erythrocyte membrane and cells of other sensitive tissues provides the coenzyme, NADPH which furnishes the hydride ion (or hydrogen) needed to keep or maintain glutathione in the reduced state where it is active as a free radical scavenger. Reduced glutathione (GSH) is a major non-protein thiol in living organism, which act against xenobiotics and neutralize ROS, and disturbances of its intracellular level in biological system has been reported to lead to serious consequences (Pastore, *et al*., 2003) [18]. Malondialdehyde (MDA), a commonly used biomarker of lipid peroxidation, is synthesized from the breakdown of lipid peroxyl radicals during oxidative stress. Measured level of MDA is considered a direct index of oxidative injuries associated with lipid peroxidation (Khan, *et al*., 2010) [14].

In this study, the activities of all the antioxidant enzymes measured were significantly higher in the observation group than in control group, and increased time-dependently. There was no significant difference in the concentrations of total protein between the two groups. The MDA concentrations was significantly lower in observation group than in control group, and was reduced time-dependently. It is possible that the extract potentiated the antioxidant system of the rats. The extract may be used as a potential crude drug for conditions that result from oxidative stress. The results of phytochemical analysis showed the extract had high level of tannins and low levels of glycosides and steroids. The observed enhanced antioxidant effect may be due to the presence of many important phytochemicals in the extract of this medicinal plant.

Conclusion

This study has provided a first time experimental evidence for the antioxidant properties of aqueous extract of *Dialium guineense* stem bark.

Funding

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