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Effects of Methanol Leaf Extract of *Dryopteris filix-mas* on Catalase Activity and Malondialdehyde Levels in the Brain of Adult Male Wistar Rats

Awhin, P.E.[1], Ajoh, A.I.[*1], Erutere, B.[2] and Dennis-Eboh, U.[1]
Department of Medical Biochemistry, Delta State University, Abraka[1], Department of Nursing Science, Faculty of Health Sciences, Nnamdi Azikiwe Federal University, Awka, Anambra state, Nigeria[2]
Author for correspondence*: +234-816-832-4595/ajohalfred99@gmail.com.
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Abstract
This study was carried out to determine the effect of methanol leaf extract of *Dryopteris filix-mas* on catalase (an antioxidant) activity and malondialdehyde (an oxidative stress marker) levels in brain of wistar rats. Methanol extract of *Dryopteris filix*-mas was prepared by Soxhlet extraction. Twenty (20) adults male wistar rats were used for this study. The animals were grouped into four groups; 1 (normal control); 2 (administered 100mg/kg of *Dryopteris filix* methanol leaf extract) and 3 (administered 250mg/kg of *Dryopteris filix* methanol leaf extract); 4 (Administered 500mg/kg of *Dryopteris filix* methanol leaf extract). The rats were so treated and after 21 days, were sacrificed. The brain of the rats was harvested, and used to prepare brain tissue homogenates for the biochemical assay of catalase and MDA using standard procedures. Results obtained show that the varying doses of the plant extract caused a dose dependent increase in catalase activity when compared to the control group. However, the increases were found to be statistically insignificant ($p>0.05$). Administration of 100mg/kg of the plant extract slightly decreased MDA level, while 250mg/kg and 500mg/kg slightly increased MDA levels in brain of the experimental animals after the 21days treatment period when compared to the control group. However, these observed changes in MDA levels were found to be statistically insignificant ($p>0.05$). The results of this study shows that the different doses of the plant extract did not cause significant oxidative damage to the brain of the experimental animals.

Keywords: *Dryopteris filix-mas*, Oxidative stress, Antioxidant, Brain

Introduction

The brain is an organ of nervous tissue that commands task-evoked responses, senses, movement, emotions, language, communication, thinking, and memory (Maldonado and Alsayouri, 2021). The brain is the most complex organ in a vertebrate's body (Pelvig *et al*., 2008). The brain is highly susceptible to oxidative stress and increased oxidative stress has been implicated in several diseases affecting the brain.

The brain is especially sensitive to oxidative damage because of its high and specific metabolic activity. High consumption of oxygen, almost exclusive oxidative phosphorylation, no reserves of energy, high concentrations of lipids prone to peroxidation, and high levels of iron, all acting as a pro-oxidant (Saeed *et al*., 2007). Neuronal cells are, therefore, highly susceptible to metabolic/ischemic damage and associated oxidative stress. Lipid peroxidation is the main mechanism of oxidative damage by ROS (Shirley *et al*., 2014). Reactive species are unstable and react quickly with surrounding molecules. Oxidative stress is, therefore, a very rapid pathology, and it is hard to predict the type of damage.

The plant, *Dryopteris filix-mas* (commonly known as male fern) is usually found in moist environments, streams, open grounds, and brick walls (Uwumarongie, 2016). It is one of the medicinal plants employed in traditional medicine for the treatment and management of

various disorders (Duke, 2001; Beyrouthy *et al*., 2008; Tagarelli *et al*., 2010; Uwumarongie *et al*., 2016). *Dryopteris filix-mas* leaf extract has been found to possess potent antioxidant and cytotoxic activities (Ali *et al*., 2012), insecticidal activity (Shukla and Tiwari, 2011), as well as antimicrobial activities (Soare *et al*., 2012).

This present study was designed to investigate the effect of *Dryopteris filix-mas* on oxidative stress/antioxidant status in the brain by determining levels of Malondialdehyde (a marker of oxidative stress) and activity of Catalase (an antioxidant enzyme) in the brain of experimental rats.

Materials and Methods

Plant materials

Fresh whole plants of *D. filix-mas* were harvested from an uncultivated farmland in Abraka, Ethiope East Local Government Area of Delta State, Nigeria. The authentication of the plants was carried out by a Botanist at Botany Department, Delta State University, Abraka, Nigeria. The voucher number was 1013584.

Animals

20 Adults male Wistar rats (100-188 g) were used for this study. The Wistar rats were kept in plastic cages under controlled condition of 12hrs light / 12hrs dark cycle and allowed access to standard rat feed and water *ad libitum*. The maintenance of the animals was in accordance with approved guidelines by the Animal Ethics Committee, Delta State University, Abraka, Nigeria (ethical approval number - REC/FBMS/DELSU/2021/139).

Extract preparation

The leaves were plucked, washed, air-dried and blended to produce a fine powder (200 g) which was macerated in 1000 mL of methanol and allowed to stand for 48 hours before filtering. Thereafter, the extract was concentrated to form a gel-like liquid using cylindrical burner at 40°C. The percentage yielded (4.13%) was recorded. The percentage yielded was resuspended in normal saline for administration. The dose (g/kg) was converted to volume for easy administration as follows: V (ml) = D (g/kg) x B (kg)/ C (g/ml).

Where: D = Dose studied (g/kg body weight); B = Body weight (Kg); C = Concentration of the extract (g/ml); V = Volume of extract (ml) to be administered.

Animal Grouping and Extract administration

The rats were caged into four groups with five (5) rats each, to allow free and easy movement and to avoid crowdedness. The rats were each identified with different colours of indelible marker. The animals were grouped as follows:

Group 1: Normal control (Given only standard feed and water).
Group 2: Experimental 1 (administered 100mg/ kg of *Dryopteris filix* methanolic leaf extract).
Group 3: Experimental 2 (administered 250mg/ kg of *Dryopteris filix* methanolic leaf extract)
Group 4: Experimental 3 (administered 500mg/ kg of *Dryopteris filix* methanolic leaf extract).

The administration of extracts was through the oral route using syringe once a day for a period of 21 days.

Animal Sacrifice and Specimen Collection

On the 21st day of the experiment, the rats were fasted overnight and sacrificed the following morning by cervical dislocation. After sacrifice, 0.5g of the brain was weighed and used in preparing the brain homogenate in 4.5ml of buffer (pH 7.4) solution. The homogenate was then centrifuged (Cent 80D, Serico, China) at 10000rpm for 10 minutes to obtain the supernatant which was then used for the biochemical analysis of catalase activity and malondialdehyde (MDA) levels in the brain.

Biochemical Analysis

Catalase activity of the sample was determined according to the method previously described by Kaplan *et al*. (1972), while sample malondialdehyde levels were estimated by the thiobarbituric acid (TBA) method previously described by Ohkawa *et al*. (1979).

Statistical analysis

The results obtained were expressed as Mean ± SD for n = 5 rats/group. The data were analyzed using one-way analysis of variance (ANOVA) and Tukey HSD post-hoc analysis. Results were considered statistically significant when $p < 0.05$.

Results

The results obtained from the investigation into the effect of *Dryopteris filix-mas* on Catalase activity and Malondialdehyde levels in brain of adult wistar rats are presented in Tables 1 below.

Groups	Catalase (Unit/L)	MDA (µmol/L)
1	22.97±1.54[a]	1.22±0.31[a]
2	23.43±5.52[a]	1.14±0.14[a]
3	26.13±6.27[a]	1.25±0.45[a]
4	31.64±6.68[a]	1.23±0.17[a]

Values are expressed as Mean ± SD for n = 5 rats per group. Values that bear the same superscript on a column do not differ significantly ($p \geq 0.05$) when analyzed using one way analysis of variance (ANOVA)

1 – Control group
2 – Experimental (Administered 100mg/kg of *Dryopteris filix-mas*)
3 – Experimental (Administered 250mg/kg of *Dryopteris filix-mas*)
4 – Experimental (Administered 500mg/kg of *Dryopteris filix-mas*)

The varying doses of the plant extract (100mg/kg, 250mg/kg and 500mg/kg) caused a dose dependent increase in catalase activity when compared to the control group. However, the increases were found to be statistically insignificant ($p \geq 0.05$). Administration of 100mg/kg of the plant extract slightly decreased MDA level, while 250mg/kg and 500mg/kg slightly increased MDA levels in brain of the experimental animals after the 21days treatment period when compared to the control group. These observed changes were found to be statistically insignificant ($p \geq 0.05$).

Discussion

The brain is known to be highly susceptible to oxidative stress due to its high rate of metabolic activity and its elevated vulnerability to ischemic damage (Saeed *et al*., 2007). With *Dryopteris filix-mas* being increasingly employed in the treatment of several disorders, it becomes imperative to determine the effect of *Dryopteris filix-mas* extract on the brain. The study evaluated the levels of MDA as well as the activity of Catalase in the brain of wistar rats following the administration of *D. filix-mas* extract for a period of 21 days. From the result of this study (Table 1), the various concentrations of *Dryopteris filix-mas* methanolic leaf extract did not produce significant changes in oxidative stress/antioxidant status in brain of wistar rats.

MDA level is a valuable marker for oxidative stress in tissues (Camkurt *et al*., 2016). Malondialdehyde is one of the final products of polyunsaturated fatty acids (PUFAs) peroxidation in cell. An increase in free radicals causes overproduction of MDA. Hence, malondialdehyde level is commonly used as oxidative stress marker. While there were slight increases in the MDA levels in the experimental rats, the noticed increases were not statistically significant at the various concentrations of the extracts. This suggests that the different doses of the plant extract did not cause significant oxidative damage to the brain of the experimental rats.

Improved antioxidant status helps to minimize the oxidative damage and this can delay or decrease the risk of developing many free radical induced diseases. Catalase is one of the antioxidant enzymes that help in mitigating the levels of free radicals in the system. Catalase protects the cells by detoxifying generated H_2O_2 and plays a pertinent role in acquiring tolerance to oxidative stress as an adaptive response (Usui *et al*., 2009). Catalase can cause the maintenance of O_2 concentration either for several rounds of chemical reaction or for direct interaction with the toxin (Speranza *et al*., 1993). Inhibition of the activity of Catalase has been associated with increased ROS and enhanced cytotoxicity, further indicating the important role of Catalase in the maintenance of oxidative balance (Terlecky *et al*., 2006). From this study, the extract did not produce significant changes in the activity of brain catalase. While there were slight increases in catalase activity following administration of the

extract, the noticed increases were not statistically significant. Ali *et al.* (2012) had earlier reported the antioxidant activity of methanolic extract of *Dryopteris filix-mas*.

Conclusion

The findings from this study revealed that treatment of experimental animals with methanolic leaf extracts of *Dryopteris filix-mas* did not produce significant oxidative stress in the brain of experimental animals. The plant therefore has low potential of toxicity to the brain when administered at the appropriate dose.

Conflict of Interest

The authors declare no conflicting interest

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