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Effects of Hydro-Alcohol Extract of Englerina Drummondii Balleex Polhil & Wiens Leaves and Msg on Oxidative Stress Indicators (SOD, MDA) and Catalase, Total Cholesterol and Protein in Ovaria Tissues of Female Rats

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***Abstract*— Englerina drummondii Balle ex Polhill & Wiens commonly known as mistletoe is widely known and use for the treatment of various illments by the traditionalists in Africa. The aim of this study is to evaluate the effect of hydroalcohol extract of Englerina drummondii Balle ex Polhill & Wiens on biochemical parameters such as superoxide dismutase (SOD), malon dialdehyde (MDA), catalase, cholesterol and total protein following administration of mono sodium glutamate in female wistar rats. The results revealed that Superoxide dismutase (SOD) is significally increased in groups 5 and 9 when compared with control. control group and when the MSG 800mg/kg only group is compared with group 8, shows a significance decreased in SOD and when compared with group 9, it shows a significance increased in SOD. Catalase (CAT) shows a significance decreased in groups 2, 4, 5, 8, and 9 when compared with control group and shows significance increased in group 7 and when the MSG 800mg/kg only group is compared with groups 1, 6, and 7, it shows a significance increased in CAT and groups 4, 8, and 9 shows a significance decreased in CAT. Malon dialdehyde: When normal control group is compared with groups 2, 3, 4, 6, 7, 8, and 9 shows a significance increased in MDA and group 5 shows a significance decreased in MDA. Also, when MSG 800mg only group is compared with groups 1, 3, 4, 5, 6, 7, 8, and 9 shows a significance decreased in MDA.**

Total protein: When normal control group is compared with group 9 there is a significance increased in total protein and when MSG 800mg only group is compared with group 7 there is a decreased in total protein. Total cholesterol: When normal control group is compared with groups 4, 5, 7, and 9 shows a significance increased in total cholesterol and when MSG 800mg only group is compared with group 4 shows a significance increased in total cholesterol.

***Index Terms*— Englerina drummondii Balle ex Polhill & Wiens, Oxidative Stress, Indicators, Catalase, Total Cholesterol**

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I. INTRODUCTION

Englerina drummondii Balle ex Polhill & Wiens commonly known as mistletoe is widely known and use for the treatment of various illments by the traditionalists in Africa.
The plant Englerina drummomdii Balle ex Polhill & Wiens is commonly called mistletoe that belongs to a large family called loranthacae. Englerina drummomdii Balle ex Polhill & Wiens is a parasitice plants that bear green leaves and fruits. It is commonly called Atabe by the people of Khana in Ogoniland, Niger Delta, Nigeria (Gbaranor et al.2021). Several plants have been used across the globe by traditionalists for the treatment of various diseases without proper documentation especially in Africa (Gbaranor et al.2021).

Phytomedicine involves the use of various plant's parts such as leaves, stems, seeds, fruits, barks and roots to treat certain disease at home. Different plants show different phytochemical constituent while some shows similar phytochemical constituents (Gbaranor et al.2021).
There is presence of stress at all level in every human society which could come as social, physical and psychological stress (Sharma et al, 2013). Life-style parameters like "psychological stress, cigarette smoking, alcohol consumption, environmental and occupational exposures affect reproductive health of a female" according to Sharma et al (2013). Oxidative stress results from an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products (Gabriele Pizzino, et al, 2017). Reactive oxygen species (ROS) production, therefore causing the imbalance that leads

to cell and tissue damage (oxidative stress). Various antioxidants have been discovered and tend to have beneficial effect against oxidative stress, and these antioxidants are vitamin E, flavonoids, and polyphenols (Gabriele Pizzino, et al, 2017). Superoxide radicals, hydrogen peroxide (H2 O2) hydroxyl radicals (•OH), and singlet oxygen (1O2) are commonly known reactive oxygen species (ROS) and they are produced as metabolic by-products by biological systems (Sato et al, 2013; Navarro-Yepes et al 2014). Increased in ROS production induce harmful effects on important cellular structures such as proteins, lipids, and nucleic acids (Wu et al, 2013). Study have shown that oxidative stress can be implicated, with various degrees of development and progression of several diseases (i.e., cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular diseases) (Taniyama and Griendling, 2003). Oxidative stress occurs due to an imbalance between free radical formation and antioxidants in the cells. Increase levels of hydroxyl radical and peroxynitrite may induce lipid peroxidation, thereby, causing harm to the cell membranes and lipoproteins and this results in malondialdehyde (MDA) and conjugated diene compound production, which are known to be cytotoxic as well as mutagenic. Lipid peroxidation spreads easily affecting several lipidic molecules [25]. (Gabriele Pizzino, et al, 2017; B. Frei, 1997). Studies have shown that oxidative stress may be implicated in causing delayed sexual maturation and puberty onset (Samuel et al, 2011; Interdonato et al, 2015).

Monosodium Glutamate (MSG) is widely used across the globe as food flavour's enhancer. Monosodium glutamate (MSG) is known to induced oxidative stress by causing lipid peroxidation, decrease the level of glutathione and also caused the rise of the activities of glutathiones- transferase, catalase and superoxide dismutase (Onyema et al. 2006).

II. Materials and Method

Forty-nine (49) female wistar rats were used for the study and hey were obtained from Animal House, Department of Pharmacology, Faculty of Basic Clinical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria. The were acclimatized for a period of two weeks before commencing the laboratory experiment, and it was carried as specified in the standard guide for the care and use of laboratory animals.

Plant collection and Preparation

The plants were collected from a forest in Ogoniland, Rivers State, Nigeria and a sample of it was taken to Department of Plant Science and Bio-technology, University of Port Harcourt where it was identified and the Herbarium Number given: UPH/V/1468. The leaves of *Englerina drummondii Balle ex Polhill & Wiens* were air-dried and extracted by cold maceration in alcohol. The extract was slowly evaporated to dryness in vacuum at 45°C using a rotary evaporator as described by (Eno *et al*, 2001).

Experimental procedure

The animals were randomly selected into 9 groups with Groups 1 to 5 having five (5) rats per group and groups 6 to 9 has six (6) rats per group.

Group 1: Normal control. (5ml/kg) of distill water was administered for 28 days

Group 2: Given only mono sodium glutamate (MSG) (800mg/kg) alone for 28 days

Group 3: Given mistletoe extract only (100mg/kg) for 28 days

Group 4: Given mistletoe extract only (200mg/kg) for 28days

Group 5: Given mistletoe extract only (400mg/kg) for 28 days

Group 6: Given mistletoe extract (100mgkg-1) + MSG (800mg/kg) for 28 days

Group 7: Given mistletoe extract (200mgkg-1) + MSG (800mg/kg) for 28 days

Group 8: Given mistletoe extract (400mg/kg) + MSG (800mg/kg) for 28 days

Group 9: Given letrozole (0.6mg/kg) + MSG (800mg/kg) for 28 days

Sample Collection

Administration of *Englerina drummondii Balle ex Polhill & Wiens* leaves extract was done for 28 days and on the 29 days, animals were sacrificed after been anesthesized with chloroform. Ovaries were harvested and thereafter, fixed or stored in Bruin fluid

Homogenization of Samples`

Homogenization of the right ovaries was done and used to determine Oxidative stress markers, cholesterol, and total protein.

Statistical Analysis

Statistical analysis was performed and presented as mean ± SEM and statistical analysis were done using ANOVA. Statistical Package for the Social Sciences (SPSS package version 23) was used .and p less than 0.05 was significant.

III. RESULTS

The study groups are significant at points a = $p < 0.05$ when normal control is compared with other groups and also notable at points b = $p < 0.05$ when compare to MSG group only (Table 1).

Superoxide dismutase: Normal control group when compared with groups treated with extract 400mg/kg and letrozole 0.6 + MSG 800mg/kg, it shows notable rise in SOD and when MSG 800mg only group is compared with group treated with extract 400mg/kg + MSG 800mg/kg, it shows notable decreased in SOD and when compare to group treated with letrozole 0.6 + MSG 800mg/kg, it shows notable increased in SOD (Table 1).

Catalase: When normal control group is compare to groups MSG 800mg/kg only, extract 200mg/kg, 400mg/kg, extract 400mg/kg + MSG 800mg/kg, and letrozole 0.6mg/kg + MSG 800mg/kg it shows notable decreased in CAT and group treated with extract 200mg/kg + MSG 800mg/kg it shows notable increased in CAT and when MSG 800mg only group is compared with following groups: control, extract 100mg/kg + MSG 800mg/kg, and extract 200mg/kg + MSG 800mg/kg, it shows notable rise in CAT and groups treated with extract 200mg/kg, extract 400mg/kg + MSG 800mg/kg and letrozole 0.6mg/kg + MSG 800mg/kg, it shows a significance decreased in CAT (Table 1).

Malon dialdehyde: When normal control group is compared with groups treated with MSG 800mg/kg only, 100mg/kg, extract 200mg/kg, 100mg/kg + MSG 800mg/kg, 200mg/kg + MSG 800mg/kg, extract 400mg/kg + MSG 800mg/kg, and letrozole 0.6mg/kg + MSG 800mg/kg, shows a significance increased in MDA and when treated with extract 400mg/kg, it shows a significance decreased in MDA. Also, when MSG 800mg only group is compared with control and groups treated with extract 100mg/kg, extract 200mg/kg, extract 400mg/kg, extract 100mg/kg + MSG 800mg/kg, extract 200mg/kg + MSG 800mg/kg, extract 400mg/kg + MSG 800mg/kg, and letrozole 0.6mg/kg + MSG 800mg/kg, it shows a significance decreased in MDA (Table 1).

Total protein: When normal control group is compared with the treated group letrozole 0.6mg/kg + MSG 800mg/kg, there is a significance increased in total protein and when MSG 800mg only group is compared with the treated group extract 200mg/kg + MSG 800mg/kg, there is a decreased in total protein.

Total cholesterol: When normal control group is compared with the following treated groups extract 200mg/kg, extract 400mg/kg, extract 200mg/kg + MSG 800mg/kg, and letrozole 0.6mg/kg + MSG 800mg/kg it shows a significance increased in total cholesterol and when MSG 800mg only group is compared with the treated group extract 200mg/kg it shows a significance increased in total cholesterol (Table 1).

Table 1: Effect of EnglerinadrummondiiBalle ex Polhill & Wiens on OS markers, Total protein, and Total cholesterol following MSG administration in reproductive parameters in rats

GROUPS	SOD (U/mi) Mean±SEM	CAT (U/ml) Mean±SEM	MDA (µmol/L) Mean±SEM	TP (g/L) Mean±SEM	TC (mmol/L) Mean±SEM
Normal control	16.48±0.70	134.36±9.04	26.46 ±0.84	45.32±1.33	3.95 ±0.55
MSG 800mg/kg	22.16 ± 1.78	[a]112.05 ± 2.28	[a]87.58 ± 1.69	46.65 ± 0 .92	4.19 ± 0 .06
Extract 100mg/kg	20.56 ± 0 .68	123.90 ± 3.34	[ab]60.57 ± 0 .36	45.94 ± 1.00	4.34 ± 0.20
Extract 200mk/kg	20.93 ± 2.96	[ab]78.17 ± 5.33	[ab]53.54 ± 1.05	44.77 ± .69	[ab]4.71 ± 0 .29
Extract 400mg/kg	[a]25.64 ± 0 .30	[a]110.73 ± 14.87	[ab]1.05 ± 1.42	45.61 ± 2.44	[a]4.55 ± 0 .22
MSG + Extract 100mg/kg	21.07 ± 3.06	[b]113.54 ± 6.47	[ab]65.71 ±1.22	46.12 ± 1.26	4.19 ± 0 .11
MSG + 200mg/kg	16.42 ± 3.35	[ab]176.32 ± 9.09	[ab]50.47 ± 0 .29	[b]42.38 ± 2.22	[a]4.54 ± 0 .09
MSG + Extract 400mg/kg	[b]15.98 ± 0 .68	[ab]110.73 ± 14.87	[ab]42.55 ± 0 .02	46.21 ± 3.09	4.32 ± 0 .09
MSG + Letrozole 0.6mg/kg	[ab]22.94 ± 2.49	[ab]39.63 ± 5.03	[ab]31.54 ± .62	[a]51.15 ± 1.53	[a]4.54 ± 0 .03

a = p lower than 0.05 when compare to normal control
b = p low than 0.05 when compare to MSG (800mg) only treated group
SOD = Superoxide dismutase; MDA = Malon dialdehyde; TP = Total protein
CAT = Catalase; TC = Total cholesterol; MSG = Mono sodium glutamate

IV. Discussion

The study shows that there was meaningfully increased in superoxide dismutase (SOD) in the groups treated with extract 400mg/kg and letrozole 0.6mg/kg + MSG 800mg/kg when compare to control group. Also, there is notable decrease in group treated with extract 400mg/kg + MSG 800mg/kg and significance increase in group treated with letrozole 0.6mg/kg + MSG 800mg/kg when compared with the MSG 800mg/kg only group. This increased in SOD in the groups treated with extract 400mg/kg and letrozole 0.6mg/kg may be due to increase in free radicals that may be generated by MSG administration. Also, increased SOD may mop up the free radicals to prevent oxidative stress.

Catalase was notably decreased in groups treated with MSG 800mg/kg, extract 200mg/kg, extract 400mg/kg, extract 400mg/kg + MSG 800mg/kg and letrozole 0.6mg/kg + MSG 800mg/kg when compared to control group. It was also notably decreased in groups treated with "extract 200mg/kg, extract 400mg/kg + MSG 800mg/kg" and letrozole 0.6mg/kg + MSG 800mg/kg and significantly increase in control group, extract 100mg/kg when compared with MSG 800mg/kg only group. However, there is significance increase in catalase in group treated with extract 200mg/kg + MSG 800mg/kg when compared with both control group and MSG 800mg/kg only group. The study shows that MSG may induce free radicals and oxidative damage as seen in the reduction of antioxidant markers and this may lead to decrease fertility rate in the female wistar rats. The study also revealed that when the extract is given in moderate dosage of 200mg/kg + MSG 800mg/kg, it increases the antioxidant (catalase) level which may restore normal body function.

Malon dialdehyde significantly increase in all the groups except the group treated with extract 400mg/kg where it decreases significantly when compared with control group. This shows that the extract may have increase the MDA, suggesting prevention of oxidative stress. However, MDA significantly decrease in all the groups when compare to MSG 800mg/kg only group and this could be that MSG have the ability to induce free radicals and oxidative damage and this may delay fertility.

Total protein significantly increases only in the group treated with letrozole 0.6mg/kg + MSG 800mg/kg when compared with the control group. "Increased crude protein (12.7 to 19.3%) had a negative influence on reproductive parameters" (Jordan and Swanson, 1979). Though increase in total protein may suggest increase in oxidative stress that could result to oxidative damage to the ovarian tissue. This resulted in protein production to save the cells from been destroy. It could also be that letrozole has no effect on total protein. Total protein neither increase or decrease in all other treated groups. Also, Total protein significantly decrease in only the group treated with 200mg/kg + MSG 800mg/kg when compared with MSG 800mg/kg only group. This decrease could be due to moderate dosage of the extract, resulting in reducing the effect of MSG on protein. Hence may enhance reproduction (fertility). All other groups treated with extract only significantly increase or decrease total protein.

Total cholesterol significantly increases in groups treated with extract 200mg/kg, extract 400mg/kg, extract 200mg/kg + MSG 800mg/kg and letrozole 0.6mg/kg + MSG 800mg/kg when compared to control group. TC also significantly increase in only the group treated with extract 200mg/kg when compareto MSG 800mg/kg only group. However, previous studies revealed the adverse impact on MSG on female reproduction system (Miskowiak*et al.*, 1999, Monaal*et al.*, 2017). There is significant reduction in total level of cholesterol noticed in treated group when compare to control group and MSG only group, suggestthat extract, letrozole and MSG may trigger disorder in lipid production in rats when exposed to high dose for extract and MSG and lower dose for letrozole for long time.

There is increased in SOD in groups treated with extract 400mg/kg and letrozole 0.6mg/kg + MSG. In 200mg/kg + MSG treated group, there is increased in CAT, decreased in MDA, TP and increased in TC.Also, increased SOD may mop up the free radicals to prevent oxidative stress. Increased SOD, CAT and decrease MDA may indicates that the extract is an antioxidant.

Conclusion

Oxidative stress caused by reactive oxygen species (ROS) is noted to cause damage to the body cells when found in excess.The study shows significant decrease in MDA, notable rise in SOD, CAT, TC and decrease in TP. Increased in SOD, CAT and decreased in MDA may indicates that the extract is an antioxidant.

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VI. DECLARATIONS

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