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Effect of Hydroalcohol Extract of Englerina Drummondii Balle Ex Polhill & Wiens Leaves and MSG on Oestrous Cycle Of Wistar Rats

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***Abstract*— Herbal medicine is an important part of health care across Africa. Several people have been patronizing herbal medicine to obtain better health care. This study aims to investigate effect of hydroalcohol extract of EnglerinadrummondiiBalle ex Polhill & Wiens (mistletoe) leaves on oestrous cycle onmonosodium glutamate induced alterations in reproductive parameters in female rats.**

Forty-nine female wistar rats weighing between 160g to 180g were used for this study. The animals were divided into 9 groups with five (5) rats per group. Administration of extract lasted for 28 days. Results from our study revealed significance decreased in proestrus, estrus, and diestrus and the number of cycles and significance increased in metestrus.

Statistical analysis was done using SPSS version 24 with ANOVA. P < 0.05 was said to be significant.

***Index Terms*— Effect,Hydroalcohol, Estrous cycle, Reproductive parameters.**

I. INTRODUCTION

Highlight Phytomedicine is an important aspect of medicine that are routinely practiced in Africa (Gbaranor *et al*, 2021).The plant *EnglerinadrummomdiiBalle ex Polhill & Wiens* is commonlyknown plant that belongs to a large family called loranthacae. *EnglerinadrummomdiiBalle ex Polhill & Wiens* has a green leaves and fruits and grow on other plants as parasite.It is commonly called Atabe by the people of Khana in Ogoniland. (Gbaranor *et al*, 2021).

Mistletoe is known to Nigeria, Europe, North Africa, Western and Southern Asia (Jurin*et al.*, 1993). In traditional medicine, the leaves of mistletoe are used to potentiate labour and the extract has been found to exhibit oxytocic activity (Frohne D, Pfander, 1984) on the uterine smooth `muscle (Le O and Zam N, 2008).

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 constituents while some shows similar phytochemical constituents.*EnglerinadrummondiiBalle ex Polhill & Wiens* leaves contained the following phytochemical constituents: tannin, flavonoid, saponin, glycoside, alkaloid, polyphenols, steroids, phytate. and carbohydrates (Gbaranor *et al*, 2021).

Estrous cycle and stages are known by certain morphological alterations that occur in ovaries, vagina and uterus (Goldman *et al*, 2007) and occur during several phases termed pro-estrus, estrus, met-estrus and di-estrus (Hebal and Stromberg, 1986). These stages are mostly identified based on type of cell noticed in vagina smears.

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II. MATERIALS AND METHOD

A. COLLECTION, IDENTIFICATION AND PREPARATION OF PLANT MATERIALS

EnglerinadrummondiiBalle ex Polhill & Wiens (mistletoe) leaves were obtained in the month of August, 2019 from a forest inKhana Local Government Area, Rivers State. The plant was identified and authenticated by Dr.Ekeke Chimezie, department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria.

The *EnglerinadrummondiiBalle ex Polhill & Wiens* leaves were washed and thereafter completely air dried under normal room temperature. The dried leaves were grounded into powder. Three kilograms (3kg) of the grounded powder was placed in jar and 600mils of 70% methanol (hydro methanol) was added. Thereafter, it was properly shaken for three times to ensure the sample absorbed the solvent and it was filtered. The filtrate containing the extract was then put into an evaporating dish and dried in a water bath at a temperature of 45^0c and was constantly observed until it was dried into a paste form.

Base on the study, $LD_{50}$of mistletoe as determined by Mathew *et al*, (2016), is 0.4g/kg (400mg) of body weight was used.

B. EXPERIMENTAL ANIMALS AND MANAGEMENT

Female wistar rats were obtained from the animal house, Faculty of Basic Medical Sciences, University of Port Harcourt. The animals were housed in cages and maintained under natural environmental condition. These animals were fed with normal standard diets that was obtained from Flour Mill Port Harcourt. The animals' weight was between 160g to 180g at the commencement of the study. The animals were weighed before the commencement of the experiment. The research was carried out in accordance with the principles for laboratory animal use and care as found in the European

Community guidelines (European Community Guidelines, 1986).

C. STUDY DESIGN

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

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

Group 3: Administered mistletoe extract only100mg/kg for 28 days

Group 4: Administered mistletoe extract only200mg/kg for 28days

Group 5: Administered mistletoe extract only 400mg/kg for 28 days

Group 6: Administered mistletoe extract 100mg/kg + MSG 800mg/kg for 28 days

Group 7: Administered extract 200mg/kg + MSG 800mg/kg for 28 days

Group 8: Administered extract 400mg/kg + MSG 800mg/kg for 28 days

Group 9: Administered letrozole 0.6mg/kg + MSG 800mg/kg for 28 days

D. DETERMINATION OF ESTROUS CYCLE

The estrous cycle was determined according to methods credited to Goldman *et al.,* (2007). Pro-estrus was identified through smears possessing more of nucleated epithelial cells, Estrus was identified as smears with large amount of cornified epithelial cells,Met-aestrus equal amount of epithelia celland leucocytes whereas di-estrus stage was recognized by smears using presence leucocytes. Vagina smear of female rats were collected within 7.00 to 9.00 hour using micro dropping pipette, normal saline and distilled water. Vagina smear was dropped or placed on microscope slide and examined every day during early hour of the day (7am – 9am). The wet smear was viewed under light microscope.

III. RESULTS

Proestrus stage: The resultsshows that group 1 (normal control or negative control) whencompare to groups treated with MSG 800mg/kg, extract 400mg/kg, 100mg/kg + MSG 800mg/kg and 400mg/kg + MSG 800mg/kg shows notable decreased in pro-estrus stages whereas group 2 (MSG 800mg or positive control) when compare to groups 1(control) and group treated with letrozole 0.6mg/kg + MSG 800mg/kg shows significance increased in the number of proestrus stages (Table1).

Estrusstage: The study revealed that, when the control is compare with group letrozole 0.6mg/kg + MSG 800mg/kg, there is a significance decrease in the number estrus stage. However, when the negative control is compared with the following treated groups,extract 100mg/kg + MSG 800mg/kg, 200mg/kg, letrozole 0.6mg/kg + MSG 800mg/kg, there is a significance decreased in the number estrus stages (Table 1).

Metestrus stage: Again, the results shows that group 1 (control) when compared with groups treated with MSG 800mg/kg, extract 100mg/kg, 200mg/kg, 400mg/kg, 100mg/kg + MSG 800mg/kg, 200mg/kg + MSG 800mg/kg,400mg/kg + MSG 800mg/kg, and letrozole 0.6mg/kg + MSG 800mg/kg, shows significance increased in the number of metestrus stages and MSG 800mg/kg only when compared with control group shows a significance decreased in the number of metestrus stages (Table1).

Diestrus stage: Also, when the control group is compared with the groups treated with MSG 800mg/kg only, 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 significance decreased in the number of diestrus stages while when the group treated with MSG 800mg/kg only is compared with the control, it shows significance increased in the number of diestrus stage (Table 1).

Number of circles, results of 28 days administration of mistletoe extract shows that the control group when compared with the following treated groups MSG 800mg/kg only, extract 100mg/kg, extract 200mg/kg, 400mg/kg, extract 100mg/kg + MSG 800mg/kg, 200mg/kg + MSG 800mg/kg,400mg/kg + MSG 800mg/kg, and letrozole 0.6mg/kg + MSG 800mg/kg, shows a significance decreased in the number of circles and when group 2 (MSG 800mg/kg) is compared with group 1(control), there is a significance increased in the number of circles (Table 1).

IV. DISCUSSION

The study revealed significant decreased inpro-estrus, estrus, and di-estrus stages and significant increase in metestrus stage of estrous cycle.Pro-estrus andestrus stages are regarded as pre-ovulatory phase, while meestrus and diestrus are regarded as post-ovulatory stages ofestrous cycle and decrease in these stages might impact reproductive process in rats. This decrease could arise from interference at level of ovary resulting in decreased oestrogen and progesterone release. When phases of estrous cycle are decreased, it may affect reproductive process of the said animal. However, since ovulation occur during the estrous stage, decrease in this stage due to extract and MSG administration may affect reproductive process.

Also, monosodium glutamate (MSG) significantly decreases the phases of estrous cycle and the numbers of cycle. However, this substance (MSG) that caused decreased in these parameters may interfere with or have toxic effect on reproductive processes.

The study revealed that the extract significantlydecreased thenumber of cyclesin all treated groups when compare to control group. Again, only control group that is notably increased when compared to the monosodium glutamate (MSG)only treated group. These decreased in number of cycles could be due to hormonal imbalance.The female estrous cycle of wistarrats takes about 4 to 5 days to complete a cycle.

When extract alone, at a dose of 400mg/kg was given, it significantly decreased the proestrus phase of the cycle and when co-administered with monosodium glutamate 800mg/kg and extract at dose of 100mg/kg and 400mg/kg, it also

decreases the proestrus phase. This could suggest that extract at high dose may be the cause of the decrease in proestrus stage of the estrous cycle.

Table 1: Effect of hydroalcohol extract of Englerina *drummondii Balle ex Polhill & Wiens* on oestrous cycle on MSG in induced alterations in reproductive parametersin rats

Group	Proestrus (days) Mean ± SEM	Estrus (days) Mean ± SEM	Metestrus (days) Mean ± SEM	Diestrus (days) Mean ± SEM	No of cycles (in 24 days) Mean ± SEM
Normal control	5.00 ± 0.32	8.40 ± 0.25	8.20 ± 0.20	6.20 ± 0.58	5.00 ± 0.32
MSG 800MG/KG	[a]2.60 ± 0.98	10.40 ± 1.08	[a]13.00 ± 1.64	[a]1.60 ± 0.81	[a]3.00 ± 0.32
Extract 100mg/kg	3.20 ± 1.24	8.60 ± 0.75	[a]14.20 ± 1.24	[a]2.20 ± 0.66	[a]3.40 ± 0.25
Extract 200mg/kg	3.40 ± 0.25	7.00 ± 1.05	[a]15.80 ± 0.75	[a]1.40 ± 0.51	[a]3.20 ± 0.20
Extract 400mg/kg	[a]2.60 ± 0.40	7.40 ± 1.81	[a]14.80 ± 0.97	[a]3.00 ± 0.84	[a]3.40 ± 0.25
MSG + Extract 100mg/kg	[a]2.67 ± 0.62	[b]7.00 ± 1.27	[a]15.17 ± 1.45	[a]1.00 ± 0.68	[a]2.5000 ± 0.34
MSG + Extract 200mg/kg	3.33 ± 0.61	[b]7.00 ± 1.09	[a]15.33± 1.15	[a]2.33 ± 0.84	[a]2.33 ± 0.33
MSG + Extract 400mg/kg	[a]2.50 ± 0.62	8.83 ± 1.64	[a]13.33 ± 1.28	[a]0.83 ± 0.40	[a]2.83 ± 0.31
MSG + Letrozole 0.6mg/kg	[b]4.83 ± 0.40	[ab]5.00 ± 0.52	[a]16.17 ± 0.70	[a]2.00 ± 0.63	[a]2.50 ± 1.62

a = $p < 0.05$ when compared with normal control
b = $p < 0.05$ when compared with the MSG (800mg) only treated grou

V. CONCLUSIONS

Reproduction is an important process among all groups across the world and several medicinal plants has been employed to secure the process. *EnglerinadrummondiiBalle ex Polhill & Wiens*when administered decreases the proestrus, estrus, diestrus stages of the estrous cycle and increases the metestrus stage of the estrous cycle of the wistar rats.

The extract alone and when co-administered with monosodium glutamate also decreases the number of cycles of the wistar rats. This decrease in the stages and number of cycles of the estrous cycle of wistar rats may affect reproductive process.

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