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Effects of Hydro-Alcohol Extract of Mistletoe (*Englerina drummomdii Balla ex Polhil & Wiens*) Leaves on Histology of Ovaries and Uterine Tissues of Female Rats

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ABSTRACTS: This paper evaluates the effects of hydro-alcohol extract of *Englerina drummomdii Balla ex Polhil & Wiens* leaves on the histology of ovaries and uterine tissues of female Wistar rats using appropriate standard methods with forty-nine (49) Wistar rats weighing between 160g to 180g were used for the study. Data obtained show that hydro-alcohol extract of *Englerina drummomdii Balla ex Polhil & Wiens* leaves when administered at a dose of 200mg/kg of extract, shows numerous viable follicles. Ovarian tissue shows atretic (AF) and degenerated follicles (DF) when treated with Monosodium glutamate (MSG) alone. And when MSG is co-administered with extract at a dose of 200mg/kg, it causes partial or complete blocks of ovarian tissues. 100mg of extract only caused degeneration of follicles with atretic follicles (AF). When mistletoe extract in low dose (extract 100mg/kg + MSG 800mg/kg) was co-administered with MSG 800mg/kg, it shows recovered secondary follicles (SF), mature ova (MO), germinal and growing follicles follicles, (GF). Mistletoe at low dose, high dose cause degeneration of ovarian and uterine tissues and when extract at 100mg/kg and MSG 800mg/kg is co-administered, it tends to restore the alterations in the histology of both the ovaries and uterine tissues.

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Keywords: Hydro-alcohol, Histology, Ovaries, Uterus, Wistar rats

Reproduction is an important aspect of African culture with the aim to maintain continuity in the family circle (Gbaranor *et al.*, 2021a). Changes in reproductive factors or variables is a common medical concern that affects social life of married people globally (Akinola *et al.*, 2010)). Phytomedicine is a crucial part or aspect of medicine globally that is providing needed alternatives for treating ill-patients. Several plants have been used across the globe by traditionalists for the treatment of various diseases without proper

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documentation especially in Africa (Gbaranor *et al.,* 2021d) and also, herbal medicine is being used across the globe to improve reproductive process (Gbaranor *et al.,* 2021c). Most people in the rural areas and some in the urban areas depends on herbal medicine each time they have medical issues (Gbaranor *et al.,* 2021b). The plant Englerina drummomdii Balle ex Polhill and Wiens is a species of a commonly known plant called mistletoe that belongs to a large family called loranthacae (Gbaranor *et al.,* 2021k). Mistletoe (Englerina drummomdii Balle ex Polhill and Wiens) has a green leaves and fruits and grow on other plants as parasite (Gbaranor *et al.*, 2021f). It is locally called atabe in OgoniLin Niger Delta, Nigeria (Gbaranor *et al*, 2021n). Phytomedicine involves the use of various plant's parts such as leaves, stems, seeds, fruits, barks and roots to treat certain disease at home. Several people have been patronizing herbal medicine to obtain better health care (Gbaranor *et al.,* 2021). The phytochemical analysis of the leaves extract revealed that Englerina drummondii Balle ex Polhill & Wiens contain chemical constituents like flavoids, tannins, saponins, alkaloids, phenols, glycoside, steroids and phytate, polyphenols, steroids and carbohydrates. These bioactive substances could be responsible for the potency and efficacy of the plant (Gbaranor *et al.,* 2021f). Previous study revealed that 60% of the World's populations depend on herbal and traditional medicine, and 85% of the World's developing countries use traditional medicine in caring for diseases (Shuaib, *et al*, 2023). MSG suppresses the female reproductive function in rat possibly by impairing the functions of ovary and uterus (Mondal *et al.,* 2017). MSGs are known to trigger change on reproductive factors like reduced serum levels for trogon, progesterone (El-Beltagy and Elghaweet, 2016). MSGs also triggers changes on reproductive factors by triggering atretic follicles, fragment oocyte, vascular mobbing, and vacuolated stroma in ovary and endometrial glans loss and reduced endometrial thickness (El-Beltagy and Elghaweet, 2016). Hence, the objective of this paper is to evaluate the Effects of hydro-alcohol extract of Mistletoe (Englerina drummomdii Balla ex Polhil & Wiens) leaves on the histology of ovaries and uterine tissues of female Wistar rats.

MATERIALS AND METHOD

Collection, Identification and Preparation of Plant materials Englerina drummondii Balle ex Polhill & Wiens leaves were obtained from a forest in Khana Local Government Area, Rivers State. The plant was introduced to me (researcher) by Prof B.A. Ekeke (Prof of Silviculture and Forestry) of the Forestry Department, Faculty of Agriculture, Rivers State University, Port Harcourt, Nigeria. It was identified and authenticated in the Department of Plant Science and Biotechnology, University of Port Harcourt with the Herbarium Number: UPH/V/1468. Plant extract was carried out according to the method described by Handa *et al*, (2008). LD50 of mistletoe as determined (Matthew, *et al*, (2016) was 0.4g/kg (400mg) of body weight was used.

Ethical committee of University of Port Harcourt approved the research with the Ref number: UPH/CEREMAD/REC/MM71/019

Experimental design: Forty-nine (49) animals were randomly selected and grouped into nine (9) groups with five rats per group. Their initial weight was taken. Group 1 (control), received 5ml/kg of distil water + feed, group 2, received mono sodium glutamate (MSG) 800mg/kg + feed, group 3, received extract 100mg/kg + feed, group 4, received extract 200mg/kg + feed, group 5, received extract 400mg/kg + feed, group 6, received extract 100mg/kg + MSG 800mg/kg + feed, group 7, received extract 200mg/kg + MSG 800mg/kg + feed, group 8, received extract 400mg/kg + MSG 800mg/kg + feed, group 9, received letrozole 0.6mg/kg + MSG 800mg/kg + feed. Administration of extracts was done for 28 days and on 29thday, the animals were sacrifice. Each animal was anaesthetized in desiccator with cotton wool soaked in about 10ml chloroform and the needed organs were harvested.

Samples Collection: Uterus and ovaries were collected and weighed immediately with sensitive weighing balance. After weighing, the ovaries and uterus were fixed or stored in Bruin fluid for Histology examination.

RESULTS AND DISCUSSION

The histology of the normal control revealed normal study showing ovarian cortex with primordial follicles, growing follicles (GF) and mature ovum (MO) and atretic follicles (AF), secondary follicles (SF) at different stages of developments. In fig: 2, the ovarian tissue shows more atretic (AF) and degenerated follicles (DF) when treated with Monosodium glutamate compound. This may be due to monosodium glutamate that affects the microarchitecture of the ovary and this could affect the sex hormones that are produced by follicles. In the group treated with low dose of extract) 100mg of extract only), an ovarian tissue showed degeneration of follicles with atresia (AF) with few viable follicles [mature ovum (MO) is present}. The histology also showed mature ova, growing follicles, corpus albicans and secondary follicles in the group treated with 200mg/kg (medium dose) of extract. In fig. 3, there are few viable follicles (MO) while in fig. 4 there are many numerous viable

follicles. This mean that sex hormones in the group treated with extract 200mg/kg may be improve due numerous mature follicles present. The study revealed an ovarian tissue from extract 400mg treated rats, showing mature ova (MO), growing follicles (GF), secondary follicles (SF) with atretic follicles (AF). Again, ovarian tissues from rats treated with 800mg MSG plus 100 mg extract, showing recovered secondary follicles (SF), mature ova (MO), germinal and growing follicles follicles, (GF).

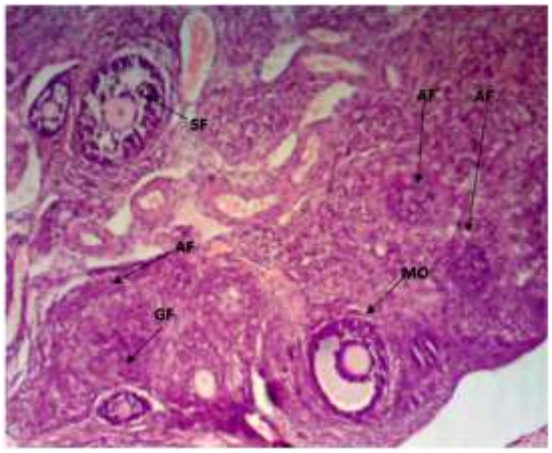

Fig: 1: Group 1 photomicrograph section of normal control tissue Showing ovarian cortex with primordial follicles, growing follicles (GF) and mature ovum (MO) and atretic follicles (AF), secondary follicles (SF) at different stages of developments.

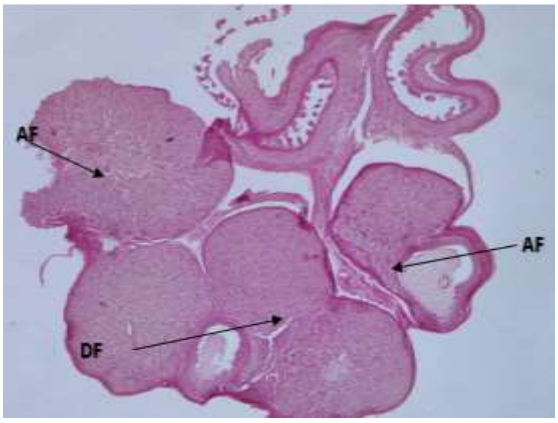

Fig: 2: Group 2. Photomicrograph section of an ovarian tissue from rats treated with Monosodium glutamate compound showing atretic (AF) and degenerated follicles (DF).

In fig. 5, there are atretic follicles while in fig. 6 the secondary follicles are recovery. This recovery of secondary follicles could be that the extract cushions the effects of MSG on the ovary and this may improve fertility. The histology of the group treated with Monosodium glutamate 800mg and 200mg extract of an ovarian tissue from rats showing atretic (AF) and degenerated follicles (DF). This atretic and degenerative follicles found in this group may be due to increase in the dose of extract from 100mg/kg to 200mg/kg.

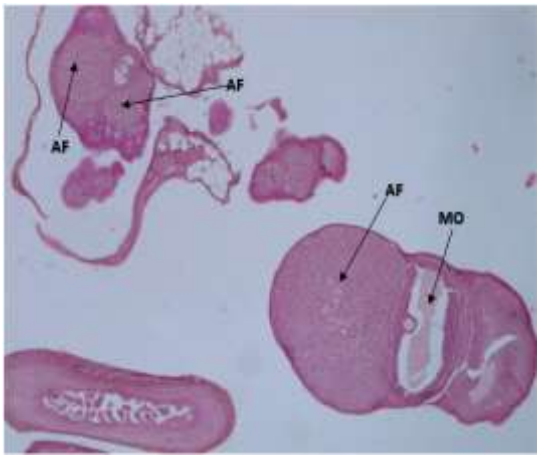

Fig. 3: Group 3 Photomicrograph section of an ovarian tissue from Wister rats treated with 100mg of extract only. Section showed degeneration of follicles with atresia (AF). Few viable follicles [mature ovum (MO)] are present.

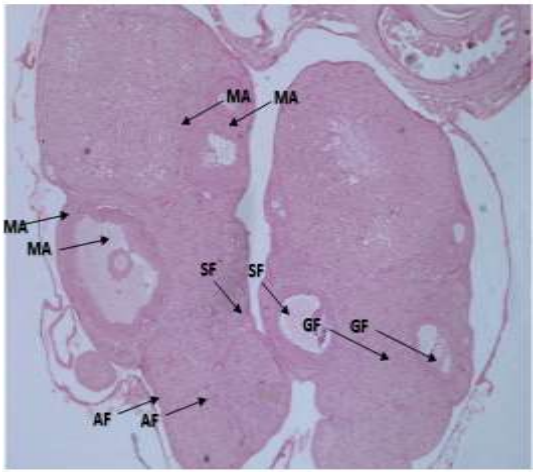

Fig. 4: Group 4 Photomicrograph section of an ovarian tissue from wistar rats treated with 200mg of extract showing mature ova, growing follicles, corpus albicans and secondary follicles.

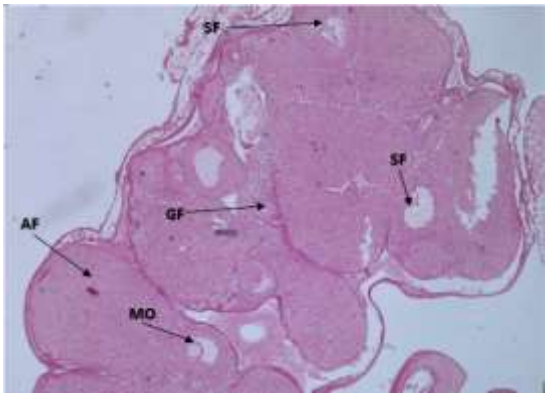

Fig.5 Group 5 Photomicrograph section of an ovarian tissue from extract 400mg treated rats, showing mature ova (MO), growing follicles (GF), secondary follicles (SF) with atretic follicles (AF).

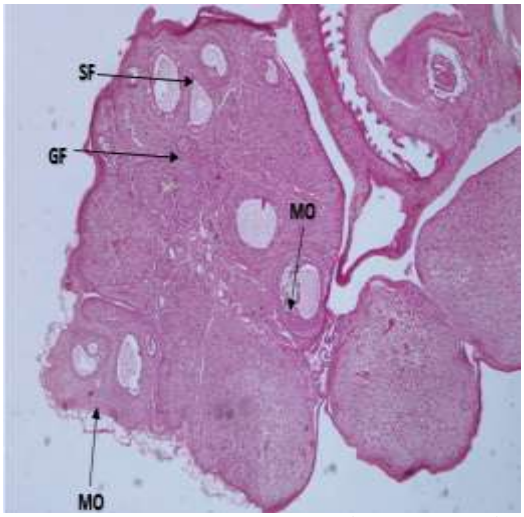

Fig.6 Group 6 Photomicrograph section of ovarian tissues from rats treated with 800mg MSG plus 100 mg extract, showing recovered secondary follicles (SF), mature ova (MO), germinal and growing follicles follicles, (GF).

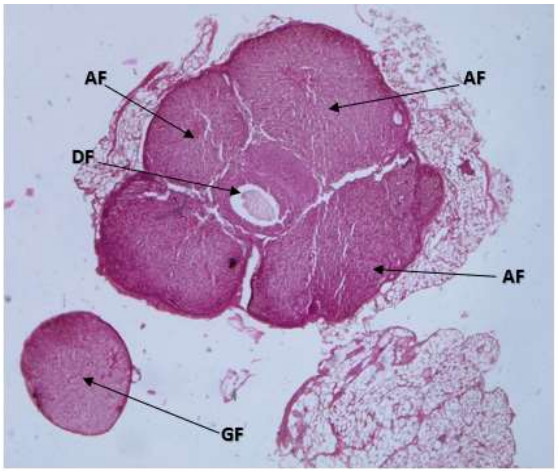

Fig.7 Group 7 Photomicrograph section of an ovarian tissue from rats treated with Monosodium glutamate compound 800mg and 200mg extract showing atretic (AF) and degenerated follicles (DF).

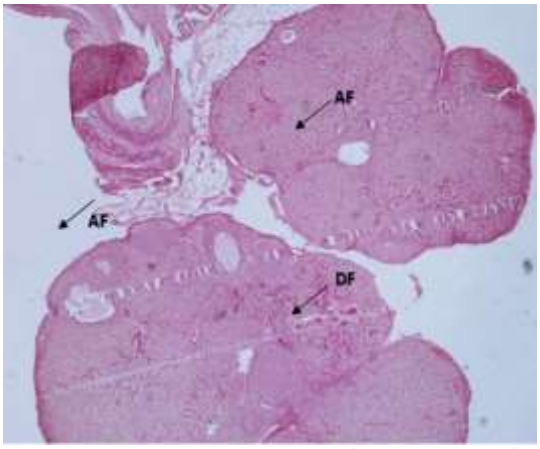

Fig.8 Group 8 Photomicrograph section of an ovarian tissue from rats treated with Monosodium glutamate compound (800mg) and 400mg extract showing atretic (AF) and degenerated follicles (DF).

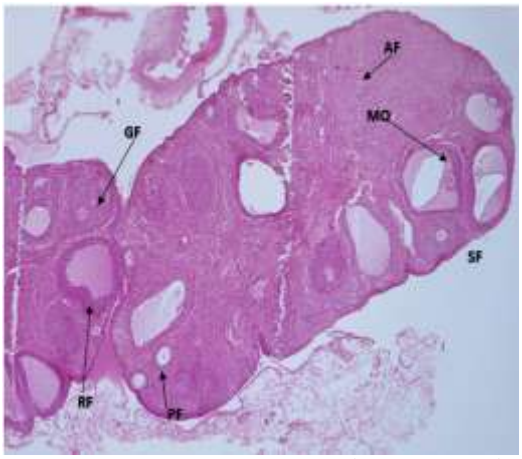

Fig. 9 Group 9 Photomicrograph section of ovarian tissues from rats treated with Letrozole plus 800mg monosodium glutamate, showing recovered secondary follicles (SF), mature ova (MO), germinal and growing follicles follicles, (GF).

In fig. 8 the level of degenerated follicles is high and more obvious when compared with fig. 7. In fig. 9 there are recovered secondary follicles (SF), with mature ova (MO), when treated with Letrozole plus 800mg monosodium glutamate. This indicate that letrozole could improve fertility.

In the uterus, the histology shows healthy endometrium in progestational phase with coiled gland (CG), lined with pseudostratified columnar cells of the endometrium and columnar calls of the uterine glands, and enlarged gland (EG) of the uterus in the control group. In fig. 11, the uterine endometrium showed glandular tissue fibrosis (TF) and glandular epithelial tissues lesion, (ETL). This fibrosis may be due to the substance MSG. In fig. 12, the uterine tissue showed coiled epithelial (CE) and enlarged glandular (EGE) epithelial tissues at progestational phase when treated with 100mg extract. In both figs. 13 and 14, showed tissues damage (TD) with glandular epithelial tissues lesion (GTL) and cellular distortions (CD) when 200mg/kg and 400mg/kg extract respectively. This damage could be due to dose dependent. Again, in fig. 15, when MSG + 100mg extract was administered, the uterine tissues showed restoration of coiled (CG) and enlarged glandular epithelial tissues (EG) with no cellular damage and in figs. 16 and 17, the endometrial tissues showed cystic glandular epithelium (CGE) with tissues degeneration (TD) and cellular distortions (CD) in both groups when treated with 200mg extract + MSG and 400mg/kg extract + MSG 800mg/kg, In addition, the group treated with 400mg/kg extract + MSG has cystic tissue fibrosis (CTF) and degenerations, (TD). In fig.18, the uterine tissue showed glandular epithelial tissues with coiled (CG) and enlarged glands, (EG), when treated with lectrozole + MSG

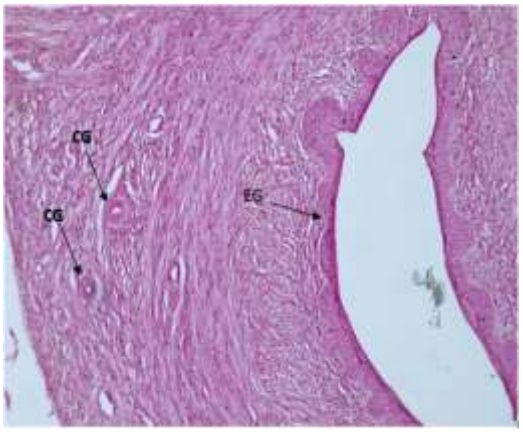

Fig.10 Group 1 Photomicrograph of a section of uterus from showing endometrium in progestational phase with coiled gland (CG) and enlarged gland (EG).

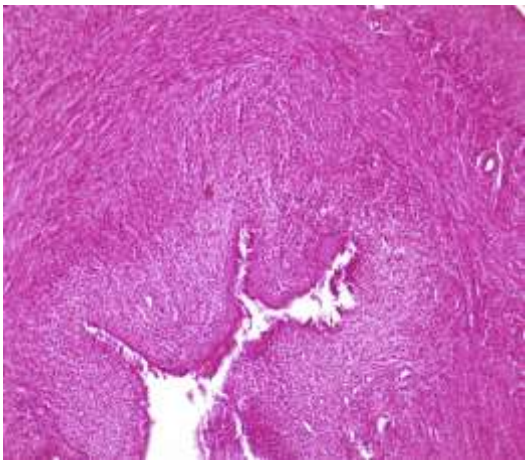

Fig.11 Group 2 Monosodium glutamate (800mg kg bw) treated rats. Photomicrograph section of the uterine endometrium showed glandular tissue fibrosis (TF) and glandular epithelial tissues lesion, (ETL).

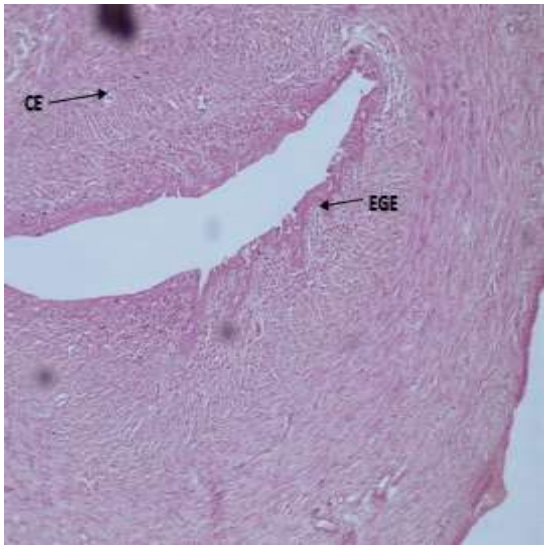

Fig.12 GRP 3 100mg extract treated rats. Photomicrograph section showed coiled (CE) and enlarged glandular (EGE) epithelial tissues at progestational phase**.**

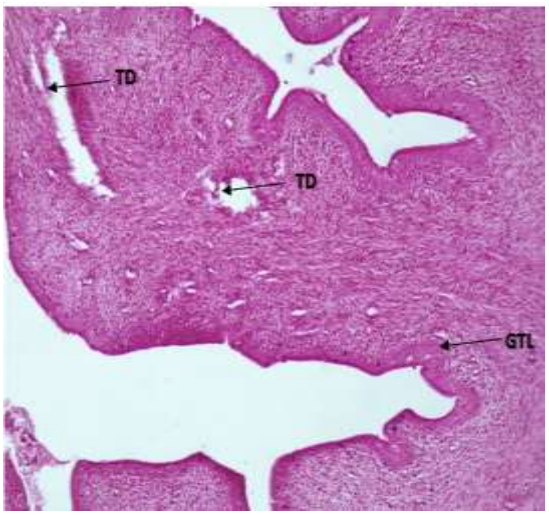

Fig. 13. Group 4: 200mg extract alone (group 4) treated rats. Photomicrograph section showed tissues damage (TD) with glandular epithelial tissues lesion (GTL).

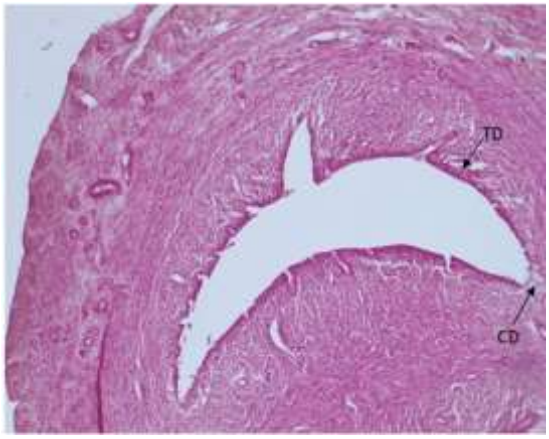

Fig 14. Group 5. 400mg extract alone treated rats. Photomicrograph section showed glandular epithelial tissues damage (TD) and cellular distortions (CD).

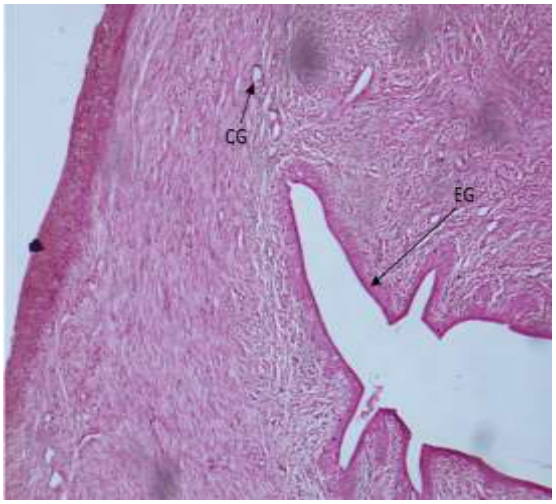

Fig 15. Group 6 MSG + 100mg extract. Photomicrograph section of uterine tissues showing restoration of coiled (CG) and enlarged glandular epithelial tissues (EG) with no cellular damage.

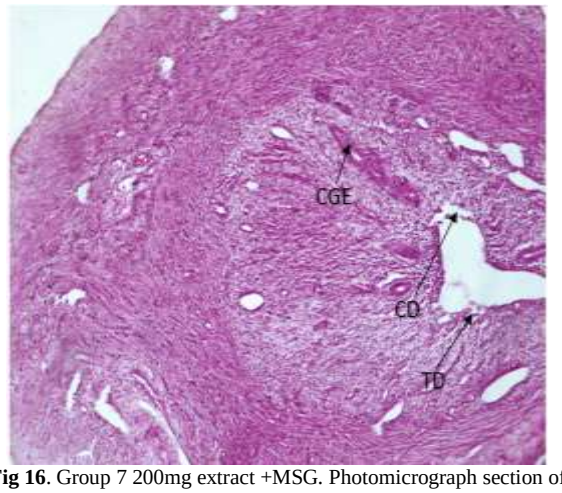

Fig 16. Group 7 200mg extract +MSG. Photomicrograph section of endometrial tissues showed cystic glandular epithelium (CGE) with tissues degeneration (TD) and cellular distortions (CD).

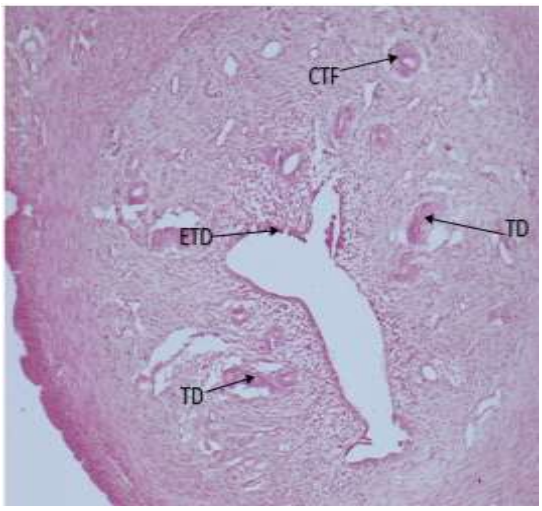

Fig 17. Group 8 MSG +200 mg extract treated rats. Photomicrograph section of uterine endometrial tissues showed glandular epithelial tissues distortion (ETD), cystic tissue fibrosis (CTF) and degenerations, (TD).

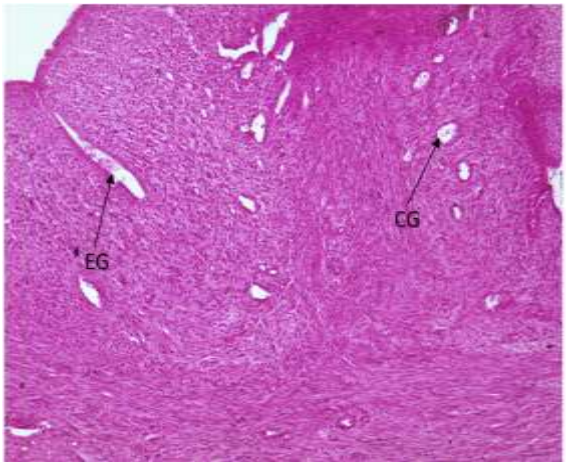

Fig 18. GRP 9 lectrozole +MSG treated rats. Photomicrograph section showed glandular epithelial tissues with coiled (CG) and enlarged glands, (EG).

In the uterus, the histology shows healthy endometrium in progestational phase with coiled gland (CG), lined with pseudostratified columnar cells of the endometrium and columnar calls of the uterine glands, and enlarged gland (EG) of the uterus in the control group. In the group treated with Monosodium glutamate (800mg kg bw) treated rats. Photomicrograph section of the uterine endometrium showed glandular tissue fibrosis (TF) and glandular epithelial tissues lesion, (ETL). This fibrosis may be due to the substance MSG. The animals treated with 100mg extract the histology showed coiled epithelial (CE) and enlarged glandular (EGE) epithelial tissues at progestational phase. When 200mg/kg extract and 400mg/kg extract was administered to the rats, the histology showed tissues damage (TD) with glandular epithelial tissues lesion (GTL) and cellular distortions (CD). This damage could be due to dose dependent. Again, when MSG + 100mg extract was administered, the photomicrograph section of uterine tissues showed restoration of coiled (CG) and enlarged glandular epithelial tissues (EG) with no cellular damage. However, rats treated with 200mg extract + MSG and 400mg/kg extract + MSG 800mg/kg, the photomicrograph section of endometrial tissues showed cystic glandular epithelium (CGE) with tissues degeneration (TD) and cellular distortions (CD) in both groups. In addition, the group treated with 400mg/kg extract + MSG has cystic tissue fibrosis (CTF) and degenerations, (TD). The group treated with lectrozole + MSG**,** the photomicrograph section showed glandular epithelial tissues with coiled (CG) and enlarged glands, (EG).

Conclusion: Mistletoe at low dose, high dose cause degeneration of ovarian and uterine tissues and when extract at 100mg/kg and MSG 800mg/kg is co-administered, it tends to restore the alterations in the histology of both the ovaries and uterine tissues.

Declaration of Conflict of Interest: The authors declare no conflict of interest

Data Availability Statement: Data are available upon request from the first author or corresponding author.

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