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Histologic sequelae following exposure to turmeric extract on wistar rats ovary and uterus

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Abstract

The regular and single use of turmeric in reference to the reproductive system following several reviews is conflicting, and its effect has not been fully ascertained. The study therefore espoused the histologic sequelae following exposure to turmeric extract on wistar rats ovary and uterus. Ethical clearance was sought from the Research and Ethics Committee in the Faculty of Basic Medical Sciences of the Delta State University prior to the commencement of this research. 24 wistar rats divided into 4 groups of 6 rats each. Group A was the control group while groups B, C and D were the treated groups that received 500, 1000 and 1500mg/kg of turmeric extract respectively. Animals were also sub-sectioned, labeled accordingly into 7th, 14th and 21st days respectively for easy identification and treatment or administration. At the end of each experimental day, animals were weighed and euthanized by cervical dislocation. Ovaries and uterus were dissected and fixed in 10% formal saline solution, following regulated histologic procedures. Prepared Slides were viewed using a digital microscope. The micrographs obtained revealed deleterious effect on the histology and cytology of the ovary and uterus following treatment of turmeric**.** The observed histological distortions to the ovary and uterus have most likely arisen from repeated exposure to tumeric extract; this has proven turmeric to be a harmful substance to the female reproductive system especially women of child bearing age and the regular and continuous consumption of these agents should be regulated.

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Introduction

Turmeric is a commonly used spice, popularly known as *Curcuma* as its active ingredient. It is rhizomatous and herbaceous plant use to attain certain health ameliorating effect in humans (Lestari and Indrayanto, 2014). It possesses antioxidant, anti-inflammatory, antimicrobial, anti-angiogenic and anticancer effects, which ascertain it therapeutic property (Ahmad *et al.,* 2020; Bahrami *et al.,* 2020). It can also boost immunocompetent cells and produce inflammatory cytokines (Moody *et al.,* 2020). On the otherhand its mechanism of action remain unknown in respect to reproduction, but it is a known fact that tumeric is phytoestrogenic in nature (Bachmeiri *et al.,* 2010). It's widely consumed as a traditional medicine despite the lack of awareness of its safety or toxic effects especially to the reproductive system (Jeber and Tawfeek, 2012; Thakur *et al.,* 2009; Ilyas *et al.,* 2019).

Reproduction is a vital biological process for maintenance of species. However, exogenous substances such as herbs can be used to suppress or promote fertility (Harat *et al.,* 2008). A study has proven the beneficial antioxidative effect of curcumin on the testis and ovaries (Jeber and Tawfeek, 2012). In the ovaries, turmeric promotes ovarian viability, folliculogenesis, fecundity and response to hormones. Some studies have also reveal curcumin to mediate cell cycle arrest in many different tumour cells through the regulation of several proteins and cascades (Park *et al.,* 2002; Aggrawal *et al.,* 2006). Jeber and Tawfeek in their study also affirmed to the protective effect of curcumin as against potassium dichromate induced ovary. The beneficial effect of turmeric on dysmenorrhea and endometriosis, as a therapeutic major by way of reducing endometrial cells lining is also documented in the uterus (Utami *et al.,* 2020; Zhang *et al.,* 2013)

Apart from the beneficial effects of curcumin on female reproduction, ominious effects such as decrease of gonadotropin hormones and inhibition of the estrous cycle have been documented (Thakur *et al.,* 2009). Studies have also proven turmeric to be an infertility agent with discoveries showing its relaxant properties that reduces the level of oxytocin thereby reducing the contraction of the uterus which is not healthy for women of childbearing age (Ittipanichong *et al.,* 2003; Manvizhi *et al.,* 2020). Morealso, turmeric has been shown to cause degenerative features of the graafian follicles and germinal epithelium of the ovaries with presence of rudimentary corpus luteam cells (Ray *et al.,* 2011). Considering the aforementioned treatise reviewed, with contracdictory (safety and toxic) effect of the use of turmeric on female reproduction which is not fully studied or ascertained coupled with scanty reports on the anatomic histoarchitecture of uterus and ovary following exposure to the single use of turmeric. The study therefore evaluated the histologic sequelae following exposure to turmeric extract on wistar rats ovary and uterus.

Materials and methods

Ethical consideration

Ethical clearance was sought from the Research and Ethics Committee in the Faculty of Basic Medical Sciences of the Delta State University prior to the commencement of this research.

Study Design

This study adopted an experimental design model.

Sample Size and Grouping

The 24 wistar rats were divided into 4 groups of 6 rats each. Group A was the control group while groups B, C and D were the treated groups that received 500, 1000 and 1500mg/kg of turmeric extract respectively. Animals were also sub-sectioned, labeled accordingly into 7^{th}, 14^{th} and 21^{st} days respectively for easy identification and treatment or administration.

Animal Model

Twenty four (24) adult female Wistar rats, with a body weight ranging between 180g - 200g were used as experimental model. Aimals were obtained from the animal holding facility of College of Health Science in Delta State University of Abraka. Delta State, Nigeria. They were acclimated for 5 days and were fed twice daily with unrestricted access to food and water under standard conditions (12 hours of light and dark cycle and temperature of about 28 - 31°C).

The experiment was conducted for a period of 3 weeks (21 days) (Ahama and Odokuma, 2022).

Sample size

A total number of 24 female adult Wistar rats were used for this study.

Group A: Six (6) Wistar rats received food and distilled water only (Control)
Group B: Six (6) Wistar rats received 500mg/kg of turmeric extract, food and water only
Group C: Six (6) Wistar rats received 1000mg/kg of turmeric extract, food and water only
Group D: Six (6) Wistar rats received 1500mg/kg of turmeric extract, food and water only.

Plant Extract (Tumeric) and its Preparation

Turmeric (Curcuma longa) rhizome was used for the study. Obtained from a local market in Delta State, Nigeria and chopped into small bits, sundried and crushed to fine powder in a blender. Fifty grams (50g) of the powdered form was cold macerated with 0.5litre of 80% methanol in water for 72 hours at an ambient temperature (26 - 28°C). The resultant mixture was filtered using Whatman filter paper (No. 1) and the filtrate concentrated to dryness in vacuo at 40°C using water bath to give 9g (18% weight) of a semi-solid extract. The semi-solid mixture extract was stored in a refrigerator at 4°C until use (Thakur *et al.,* 2009; Ibraheem *et al.,* 2018).

Determination of Administered concentration of the Drug

The doses of turmeric used for this study were 500mg/kg, 1000mg/kg and 1500mg/kg respectively; this was selected based on the knowledge that the LD_{50} of turmeric has been evaluated to be 5000mg/kg body weight in Adult Wistar rats (Mohammed *et al.,* 2016)

Animal Euthanasia

At the end of each experimental day which was stipulated for 7, 14 and 21 days. The animals were weighed and euthanized by cervical dislocation. Ovaries and uterus were dissected out, placed in tissue cassette and fixed in 10% formal saline solution.

Histological procedures

Fixed tissue cassette are processed manually under standard histological procedures which entails several stages, from fixation using 10% formal saline, tissue processing comprises four sections: dehydration which involve the use of series changes of the tissue in an increasing concentration of 90% to absolute alcohol, clearing which entails series changes of the tissue in xylene, infiltration involving series changes of the tissue in paraffin wax and embedding emcompassing preparation of tissue block, using a mold. Sectioning is then carried out using a microtome at a thickness of 5 to 7um, sectioned ribbon are then exposed to haematoxylin and eosin staining pattern and then mounted using Dibutylphthalate Polystyrene Xylene. Photomicrography is then conducted (Ahama and Odokuma, 2022)

Photomicrography

Prepared Slides were viewed and tissue images were captured using digital microscope "CARL ZEISS (Primo Star)" of about 8.3 mega pixel camera, connected to laptop. The micrographs obtained were used to evaluate the histology and cytology of the ovary.

Result

Ovarian Histology

Sections of the ovary show ovarian tissue with tunica albuginea beneath a germinal epithelium lining the cortex with several corpora luteal, also seen are smaller primordial, primary and secondary and graffian follicle in the cortical region. The corpora luteal are composed of luteinized granulosa cell, which possess abundant deeply eosinophilic cytoplasm and round to oval vesicular nuclei located centrally. These cells are separated by a thin fibro-vascular stroma. Blood vessels are also present in the medullary region of the ovary. Result obtained at day 7 depicts features of normal ovarian tissue in all groups with varying doses. At day 14, the sections show a normal histoarchitecture of the ovary except for group C and D with doses 1000 and 1500mg/kg respectively which depict features of marked ovarian fibrosis, atrophy and follicular arrest. Similar report was also visible at day 21.

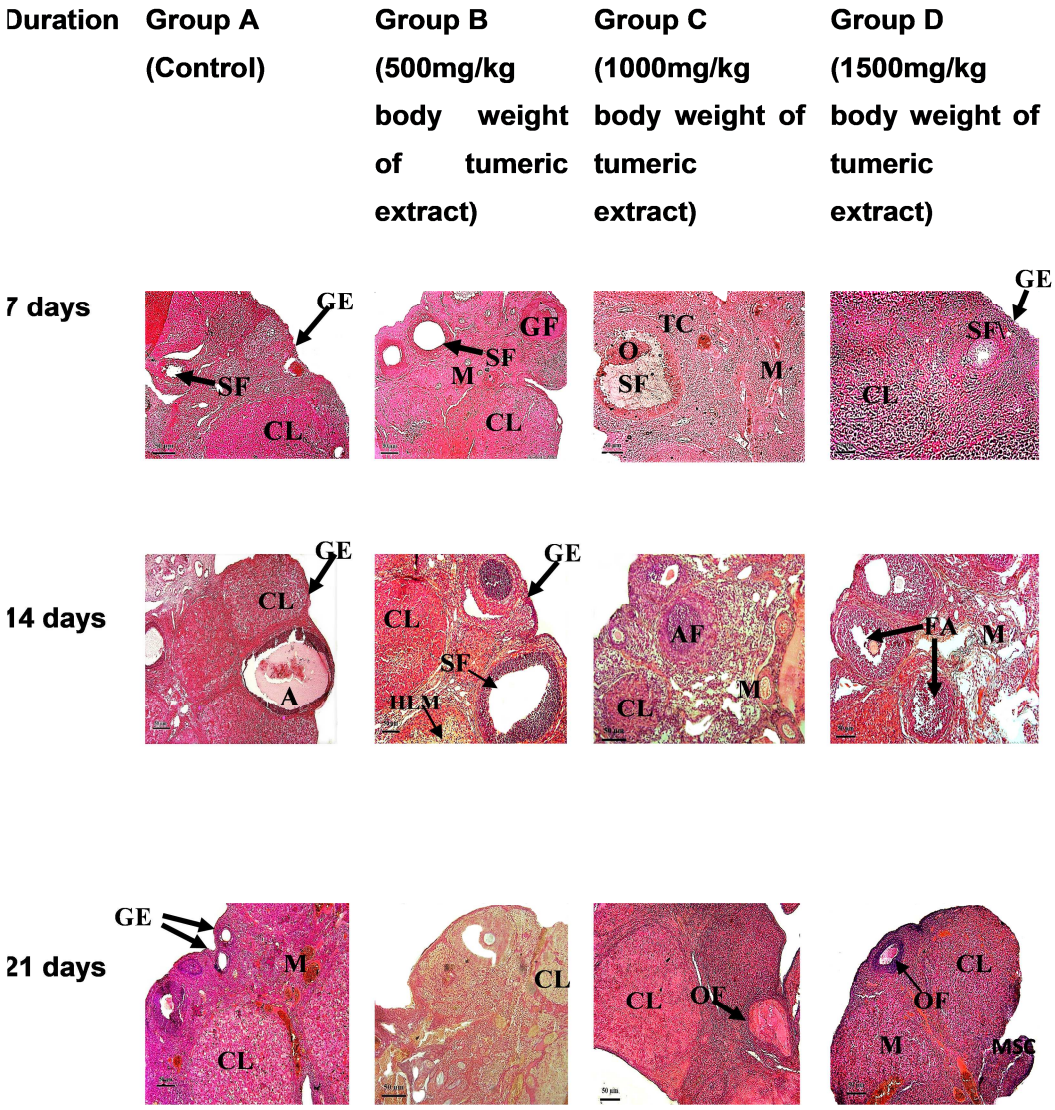

Fig. 1. Histological effects on the ovary of experimental animals exposed to different graded doses of tumeric ectract for 7, 14 and 21 days. H & E (x100).

Keys CL: Corpus luteum, M: medulla, PF: Primary follicle, SF: secondary follicle, O: Oocyte, OF: ovarian fibrosis, AT: atrophied follicle, FA: arrested follicle, HLM: hemosiderin laden macrophages, A: antrum, TC: theca cells, GE: germinal epithelium

Grouping of Rats

Administration days	Group A (Control)	Group B	Group C	Group D
7th day	2 rats	2 rats	2 rats	2 rats
14th day	2 rats	2 rats	2 rats	2 rats
21st day	2 rats	2 rats	2 rats	2 rats

Uterine Histology

Section of the uterus shows its three layers: perimetrium, myometrium and endometrium. The endometrium is lined with simple columnar epithelium containing numerous tubular glands that are sparsely distributed. Also seen are connective tissues. The myometrium is composed of smooth muscle layers. Seen also is the perimetrium which is composed of thin connective tissue layer. Result obtained at day 7 depicts features of normal ovarian tissue in all groups with varying doses. At day 14, the sections show a normal histoarchitecture of the ovary except for group D with a dose of 1500mg/kg respectively which depicts features of endometrial atrophy. Similar report was also visible at day 21.

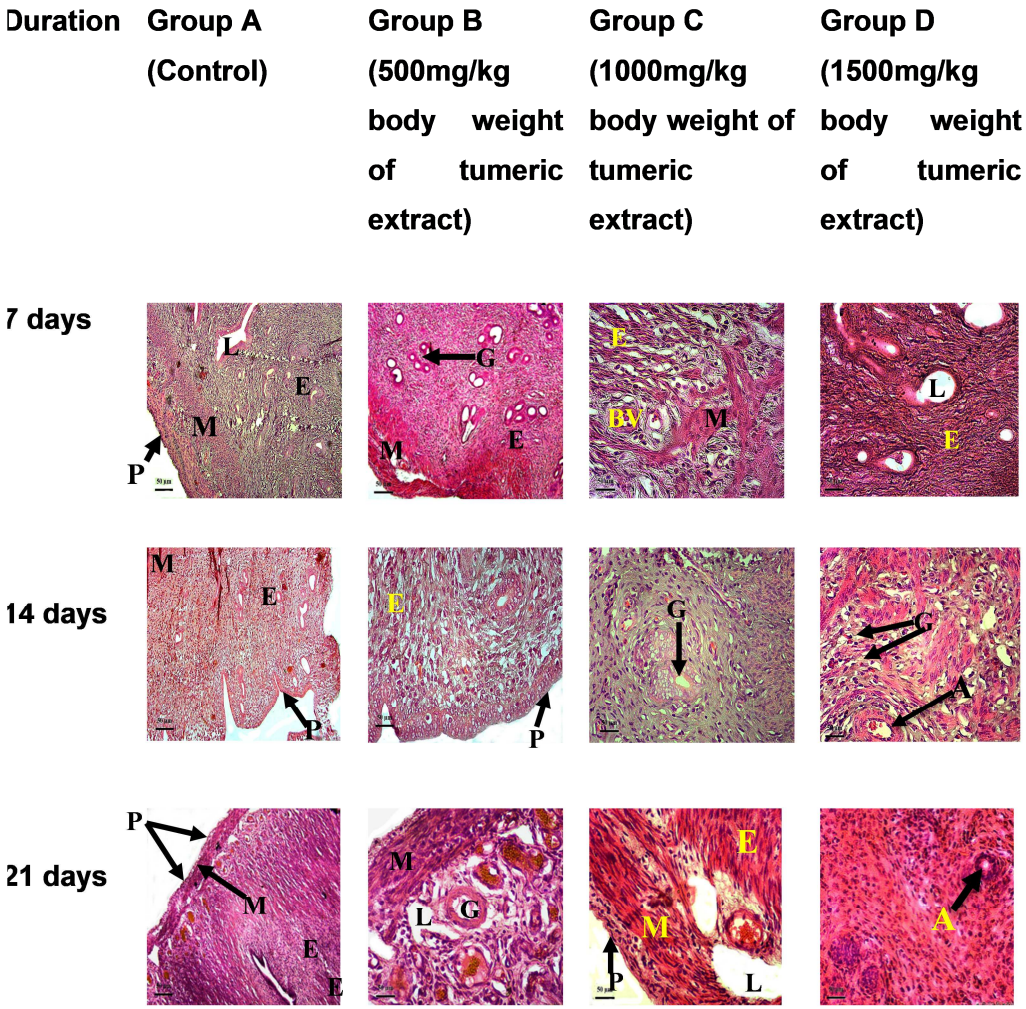

Fig. 2. Histological effects on the uterus of experimental animals exposed to different graded doses of tumeric extracts for 7, 14 and 21 days. H & E (x100)

Keys: E- endometrium, P- perimetrium, M- myometrium, G- glands (Endometrial), L- uterine lumen, BV- blood vessels, A- atrophy (glands)

Discussion

The study on the beneficial property of tumeric are enormous, but none has detailed the anatomic histoarchitectural effect on the ovary and uterus, considering the regular and single use of turmeric, as food additive over a long term and also the uncertainty of its safe or toxic nature is been questioned (Marchiani *et al.,* 2014). Therefore the index study evaluated the effect of acute and sub-acute effect of turmeric extract on the ovarian and uterine histology (Rauf *et al.,* 2018). In the in vivo study, acute exposure of turmeric displayed unremarkable (normal) changes in the ovarian and uterine section of the index study. This may be attributed to the short term exposure of the administered dose of turmeric extract and also might been owe to the fact that tumeric must have undergo first pass effect (Wang, 2017). The outcome obtained at this day can also be traceable to reduction of drug potency by intestinal enzymes in the gut wall (Yan *et al.,* 2010).

The ovarian and uterine response of turmeric (Curcumin) at day 14 revealed a remarkable feature at higher doses of administration of turmeric extract. This agrees with a study conducted by Thakur *et al.*, were it was observed that tumeric possess anti-infertility property, showing a significant increase in the estrogen level and induce inhibitory effect on the FSH and LH, resulting to ovulatory dysfunction, although anatomic feature was not studied (Thakur *et al.,* 2009).

This can also be attributed to the mode of action of tumeric which is known for its phytoestrogenic property to act similarly as endogenous estrogen on the reproductive system. Also it isa fact that the pratical importance of phytoestrogens lies with their ability to alter the biological response to endogenous estrogen (Bachmeiri *et al.,* 2010). Outcome obtained is also similar to a study conducted by Itthipanichpong *et al.*, where it was observed that curcuminoids cause a relaxant effect on the smooth muscle of the uterus via receptor dependent and independent mecahnisms [14]. This is supported by a study where the relaxant effect of cucurmin was assessed on rat tracheal smooth muscles (Emani, 2017).

Further exposure of turmeric to the ovary and the uterus at day 21, depicts significant histoarchitectural changes following administration of turmeric extract. This was in accordance with the study conducted Naz, where contraceptive property of tumeric was ascertained. Although the study didn't entailed anatomic architecture of the ovary but show inhibitory and contraceptive effect of tumeric on the ovary (Naz, 2011). This is however contrary to a study conducted by Reddy's study where turmeric was observed to have ameliorating effect as against polycystic ovarian syndrome induced by letrozole [26]. Similar upshot was also seen Yan's study where curcumin exert a protective effect against premature ovarian failure in mice (Yan, 2018). However upshot obtained from the index study on the uterus is contrary to a study conducted by Eze Where the effect of turmeric is was also assessed in the uterus and kidney and it was observed that curcumin was able to protetct the uterine and nephritic cells of female rats induced with leiomyoma (Eze, 2021).

Conclusion

The observed histological distortions to the ovary and uterus have most likely arisen from repeated exposure to tumeric extract; this has proven turmeric to be a harmful substance to the female reproductive system especially women of child bearing age and the regular and continuous consumption of this agent should be regulated.

Recommendation

Finding from the study will help to educate women of reproductive age on the accurate, safe and effective use of turmeric. Also the result obtained will enlighten the public on its use, which should start from food and drug agencies. This will aid to reduce the misconception or misinformation on the use of turmeric, to minimal level.

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