ejpmr, 2023, 10(4), 01-10

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EFFECT OF AQUEOUS EXTRACT OF TURMERIC ON ESTROGEN INDUCED HISTOLOGIC TOXICITY OF THE MAMMARY GLAND AND UTERUS OF ADULT FEMALE WISTAR RATS

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Article Received on 23/01/2023 **Article Revised on 12/02/2023** **Article Accepted on 02/03/2023**

ABSTRACT

Estrogen is the most commonly prescribed drug for oral contraceptives and hormonal replacement therapy in women. Over the years, health hazards potentiated by some toxins have been abated using plant species, one of which is Turmeric. This study was conducted to examine the effect of turmeric against mammary gland and uterine toxicity induced by estrogen in adult female wistar rats. 35 female wistar rats weighing between 140g to 155g were assigned into seven groups of five rats each after a period of two weeks of acclimatization; the first group served as the control group, group 2 received estrogen 500ug/kg body weight only for six weeks, group 3 received estrogen 500ug/kg body weight with turmeric 150mg/kg body weight concurrently for six weeks, group 4 received estrogen 500ug/kg body weight and turmeric at 250mg/kg body weight concurrently for six weeks, group 5 received 350mg/kg body weight of turmeric only for six weeks while groups 6 and 7 were pre-treated with 150mg/kg body weight and 250mg/kg of turmeric body weight respectively for two weeks after which they were given 500ug/kg body weight of estrogen for six weeks. All the administrations were given orally once daily. At the end of the experiment, the animals were sacrificed through chloroform inhalation. The mammary gland and uterine tissues were then harvested for histological examination. The histopathological sections of the mammary gland showed ductal epitheliosis and stromal infiltrates of inflammatory cells in the group treated with estrogen alone. But, the pre- and co-administration of turmeric retained the normal ductal epithelial status with the pre-treatment having a better effect. Estrogen also caused a proliferation of the glandular epithelium, increase in the population of stromal cells and thickened endometrial lining of the uterus. The administration of turmeric inhibited these effects in the uterus in a dose dependent manner. As it has been observed in this study, turmeric has the potential to inhibit histologic toxicity induced by estrogen on the mammary gland and the uterus of adult female wistar rats.

KEYWORDS: Estrogen, Turmeric, Mammary gland, Uterus, Histology.

1. INTRODUCTION

Oral contraceptive (OC) drugs are commonly used by women throughout the world to prevent fertilization or to control birth (Madhuri *et al.*, 2007). Estrogens are the most commonly prescribed drugs for postmenopausal hormonal replacement therapy (HRT) in women and oral contraceptives in women (Madhuri *et al.*, 2007). However, estrogens have been reported to produce several side effects such as nausea, vomiting, anorexia, migraine, blurring of vision, mental depression, headache, asthma, endometriosis, fibroids, breast engorgement (fullness and tenderness), increased vaginal secretion (leucorrhoea), edema, cardiovascular and hepatic diseases (Loose & Stancel, 2006). It has been stated that excessive estrogen is trapped in the uterus, ovary or breast due to stagnation in the blood circulation, and overstimulates the cell division leading to cytotoxic effects such as fibroids, cysts or cancers in these organs. After the estrogen hormone binds to its receptors in a cell, it turns on hormone-responsive genes that promote DNA synthesis and cell proliferation (Liehr J.M., 2000). Despite benefits in contraception and hormonal replacement therapy, debates over harmful effects of estrogen have been intensified because of their mammary and uterine impact which is usually considered to be deleterious because of their mitogenic activity (Gerald *et al*., 2015).

Over the years, there has been increased scientific research to minimize the health hazards potentiated by some toxin and this has been done using phytochemicals extracted from plant species (Shikov *et al.*, 2014). The bioactive property of these plants could be attributed to their phyto-constituents such as flavonoids, anthocyanins, vitamins C and E, phenolic compounds, dietary fiber and carotinoids. One of such plant is Turmeric (*Curcuma longa*). Turmeric has been subjected to numerous trials and studies and it has been validated

and clarified by modern science (Vaughn *et al*., 2016; Gupta et al., 2013; Gul P and Bakht J. 2015). It is commonly used as a spice, but it is also known for its medicinal purposes. It has a long history of use in traditional medicine for the treatment of ailments such as arthritis, heartburn (dyspepsia), joint pain, stomach pain, hemorrhage, diarrhea, intestinal gas, stomach bloating, loss of appetite, jaundice, irritable bowel syndrome (IBS), high cholesterol, a skin condition called lichen planus, skin inflammation from radiation treatment, and fatigue (Wilken *et al.* 2011). Turmeric has also shown to alleviate various forms of male and female reproductive disorders in experimental animals and thus to enhance fertility (Khorsandi *et al*., 2013; Raj A.j. 2018; LI *et al.*, 2011). It was shown to be a potent scavenger of a variety of reactive oxygen species including hydroxyl radicals, nitrogen dioxide radicals, and superoxide radicals (Goel A *et al*., 2008). Based on these evidences, the present study was conducted to examine the effects of turmeric against histologic toxicity of the uterus and mammary gland induced by estrogen in adult female wistar rats.

The uterus or womb is a major female hormone - responsive reproductive sex organ of most mammals including humans. One end, the cervix, opens into the vagina, while the other is connected to one or both fallopian tubes, depending on the species. It is within the uterus that the fetus develops during gestation, usually developing completely in placental mammals such as humans. The mammary gland is a highly evolved and specialized organ present in pairs, one on each side of the anterior chest wall. The organ's primary function is to secrete milk. Though it is present in both sexes, it is well developed in females and rudimentary in males. It is also a vital accessory organ of the female reproductive system.

2. MATERIALS AND METHOD

A. Place of study

This experiment was carried out in the Animal House of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi, Anambra State.

B. Collection and Preparation of turmeric extract

Turmerics were procured from a local market in Onitsha Anambra State, Nigeria and taken to the Botany Department of Nnamdi Azikiwe University Awka, Anambra State Nigeria for identification of its physical characteristics/properties. The turmerics were peeled, washed and dried under room temperature. They were then grinded into fine powder in a blender and soaked in water for 72hours in the ratio of 100 g to 50 ml of water and stirred every 12 hours. Then, the solution was filtered through Whatman No. 1 filter paper. The extract was dried using water bath at a temperature of 40^{o}C till it became concentrated and later preserved in a refrigerator at 4^{o}C prior to use.

C. Phytochemical analysis of turmeric

Phytochemical screening of turmeric (Curcuma longa) was carried out to check for the presence of glycosides, flavonoids, oil, saponins, tannins, carbohydrates and proteins. The phytochemical screening was done using the procedure outlined by Trease and Evans (1996).

D. Collection of estrogen

Estrogens were purchased at Nebechs Pharmaceutical Company in Onitsha, Anambra State, Nigeria. They were ground to fine powder and dissolved in water before administration daily to allow for proper dissolution.

E. Ethical approval

The ethical approval was obtained from Nnamdi Azikiwe University Animal Research Ethics Committee.

F. Experimental animals

Thirty-five (35) adult female wistar rats were obtained from an animal farm at the College of Health Sciences, Nnamdi Azikiwe University, Nnewi, Anambra State, Nigeria and housed in the Animal House of the aforementioned college. They were allowed to acclimatize to laboratory room conditions (12 hour dark/light periods) for two weeks before the onset of the experiment. The rats were fed with rat chow, and water. All the animals received humane care according to criteria outlined in the Guide for the Care and Use of Laboratory Animals, prepared by the National Academy of Science and published by the National Institutes of Health (Garber *et al*., 2011).

G. Acute toxicity test (LD50)

Acute toxicity tests for turmeric and estrogen were performed on wistar rats using the Lorke's procedure of LD50 determination.

H. Experimental design

The rats were assigned into seven (7) groups of five (5) rats each after a period of two (2) weeks of acclimatization.

- Group 1 (Control) received only water and rat chow for 6 weeks.
- Group 2 received estrogen 500µg/kg body weight for 6 weeks.
- Group 3 received estrogen 500µg/kg body weight and turmeric 150mg/kg body weight for 6 weeks.
- Group 4 received 500µg/kg body weight and turmeric 250mg/kg body weight for 6 weeks.
- Group 5 received turmeric 350mg/kg body weight only for 6 weeks.
- Group 6 first received turmeric 150mg/kg body weight for 2 weeks after which estrogen 500µg/kg was administered for the next 6 weeks.
- Group 7 first received turmeric 250mg/kg body weight for 2 weeks after which they received estrogen 500µg/kg body weight for the next 6 weeks.

The administrations were carried out using oral gavage, once daily.

I. Animal Sacrifice and Sample Collection

Twenty four hours after the last administration of the extract, the rats were weighed and sacrificed by chloroform inhalation. Tissue sections of the Mammary gland and uterus were fixed in 10% formalsaline for histological examination.

J. Histological examination

Portions of the mammary gland and Uterus were fixed in 10% formalsaline for 24 hours at room temperature, and then prepared into 4-5 µm thick paraffin-embedded sections. These were stained with hematoxylin-eosin and imaged on a compound light microscope.

K. Statistical analysis

Statistical Package for the Social Sciences (SPSS; Version 20) was used for data analysis, and the results expressed as mean ± SEM. One way analysis of variance (ANOVA) was applied for determining the significance. The acceptable level of significance was established at $P < 0.05$.

3. RESULTS

A. Lethal Toxicity Test of Estrogen and Aqueous Extract of Turmeric (Curcuma longa).

The lethal toxicity test of aqueous extract of turmeric (Curcuma longa) on wistar rats showed no sign of toxicity at 5,000ug/kg body weight while that of estrogen showed signs of toxicity at 1,265ug/kg.

Table 1: Phytochemical analysis of turmeric.

Constituent	Indication
Alkaloids	+
Carbohydrates	-
Reducing Sugar	-
Flavonoids	++
Glycosides	+
Saponins	+
Taninns	+
Proteins	-
Oils	-
Terpenoids	++

Key: ++ = present; + = present (in trace amount); − = absent

Table 2: Mean Body Weight (Initial and Final) in Gram.

	Initial weight (g)	Final weight (g)	P-value
	MEAN±SEM	MEAN±SEM	
Group 1 (Positive control)	152.33±9.97	159.48±7.39	0.62 [b]
Group 2 (500 µg/kg of E2)	146.52±12.91	171.20±4.30	0.10 [b]
Group 3 (500 µg/kg of E2 + 150mg/kg of *C. longa*)	151.95±5.12	157.70±4.64	0.09 [b]
Group 4 (500 µg/kg of E2 + 250mg/kg of *C. longa*)	152.70±7.16	159.07±7.50	0.54 [b]
Group 5 (350 mg/kg of *C. longa*)	149.52±5.62	119.52±5.81	0.07 [b]
Group 6 (150 mg/kg of *C. longa* + 500 µg/kg of E2)	150.20±3.86	159.45±5.36	0.21 [b]
Group 7 (250 mg/kg of *C. longa* + 500 µg/kg of E2)	154.37±6.32	160.70±6.86	0.54 [b]

Data was analyzed using T-test, and values considered significant at $p<0.05$. SEM: Standard error of mean, a (significant), b (not significant), E2 (Estrogen).

There was no significant difference in the body weights in all the groups ($p>0.05$) when the initial body weights were compared to the final body weights.

B. Histopathological findings of the mammary gland

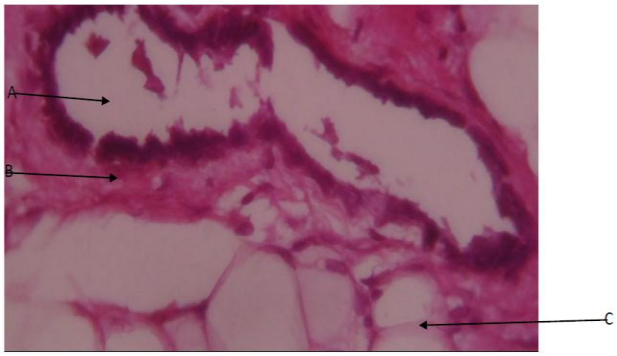

Plate 1: Rat mammary gland. Control. Composed of: A, duct, B, fibrocollagenous stroma and C, fat tissue (H&E x 400).

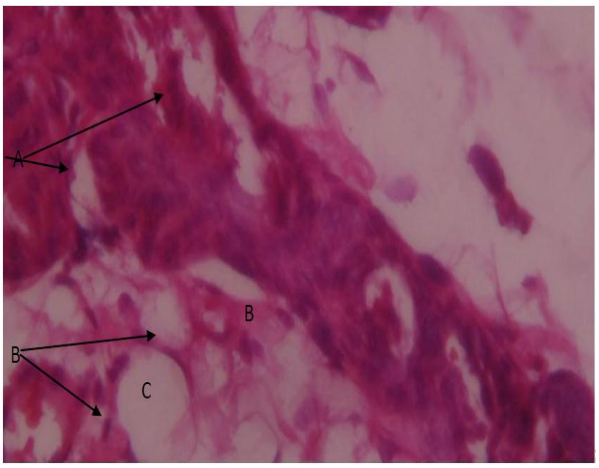

Plate 2: Rat mammary gland given 500ug/kg of Estrogen only showing: A, secretory ductal epitheliosis, B vascularized fat tissue and C, stromal infiltrates of inflammatory cells. (H&E x 400).

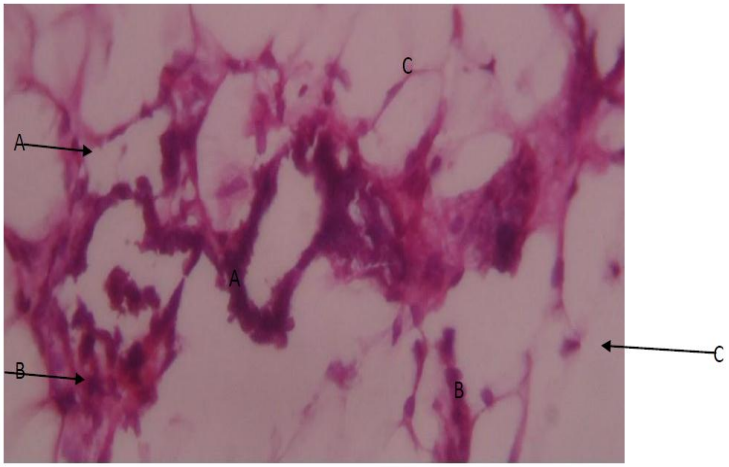

Plate 3: Rat mammary gland given 500ug/kg of Estrogen + Turmeric 150mg only Showing: A, ductal epithelium B, stromal lymphocytic infiltrates and C, abundant fat tissue (H&E x 400).

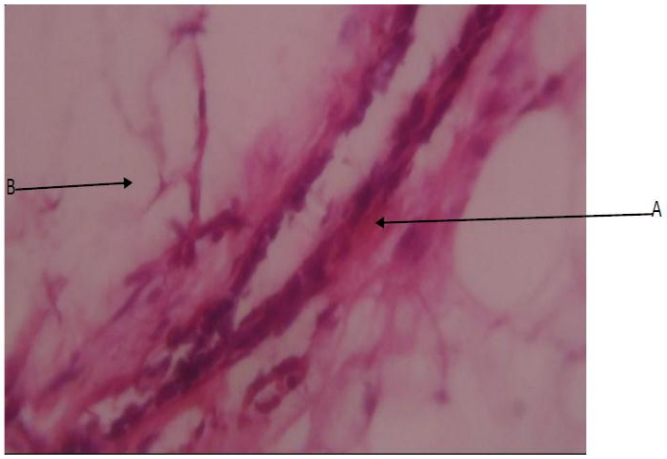

Plate 4: Rat mammary gland given 500ug/kg of Estrogen + Turmeric 250mg only showing: A, ductal epithelium and B, abundant fat tissue (H&E x 400).

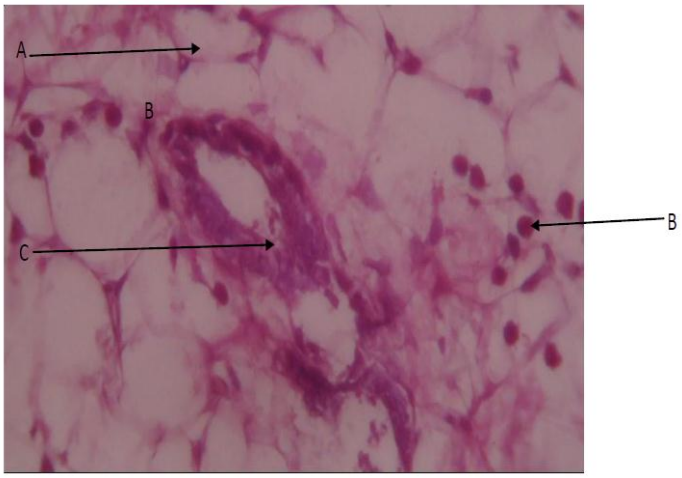

Plate 5: Rat mammary gland given Turmeric 350mg only Showing: A, abundant fat tissue, B, infiltrates of lymphocytes and C, normal ducts (H&E x 400).

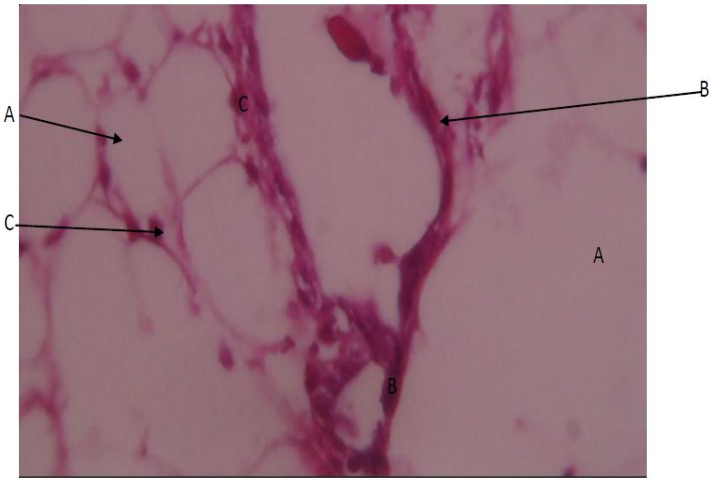

Plate 6: Rat mammary gland given 150mg Turmeric 2 weeks before500ug/kg of Estrogen for 6 weeks showing: A, abundant fat clusters, B, normal ducts and C, stromal lymphocytic infiltrates (H&E x 400).

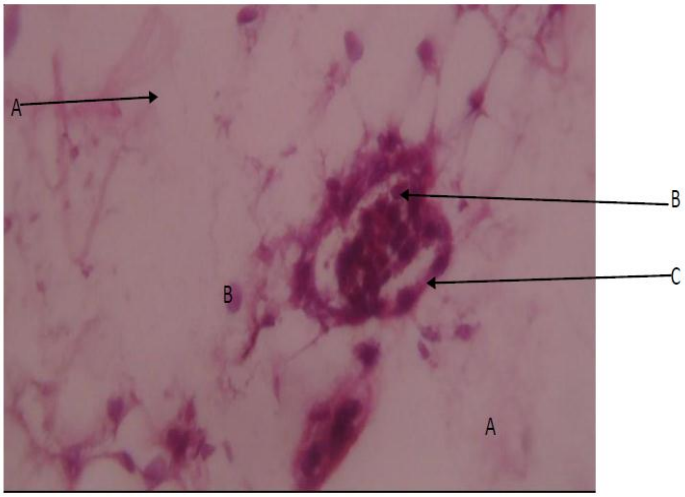

Plate 7: Rat mammary gland given 250mg Turmeric 2 weeks before 500ug/kg of Estrogen for 6 weeks showing: A, abundant fat clusters, B, stromal lymphocytic infiltrates and C, normal ducts (H&E x 400).

C. Histopathological findings of the uterus

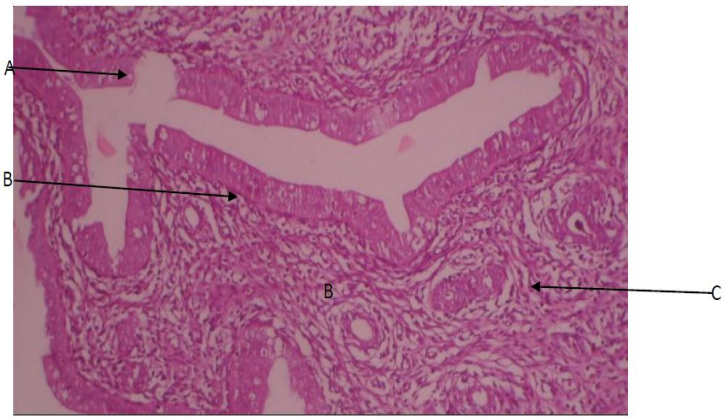

Plate 8: Rat uterus. Control. Composed of: A, endometrial lining, B, stroma and C, glands (H&E x 100).

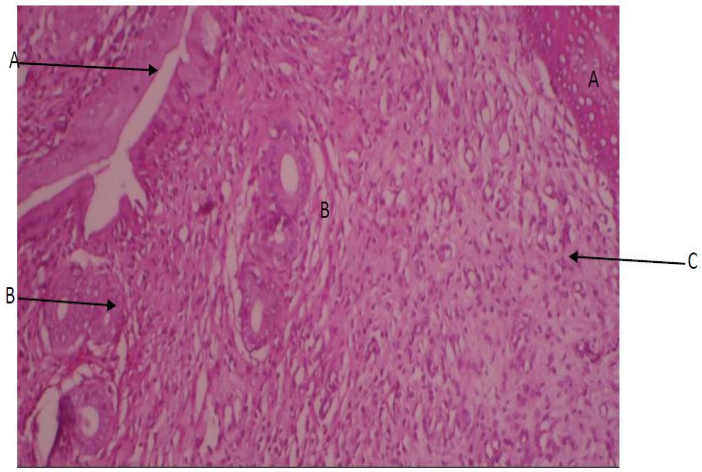

Plate 9: Rat uterus given 500ug/kg of estrogen only showing A: Thick endometrial lining and B, glandular proliferation and C, oedematous stroma and increased cell population (H&E x 100).

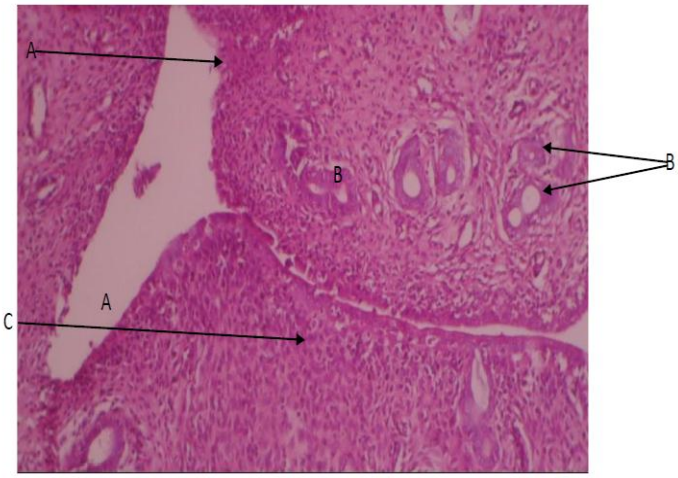

Plate 10: Rat uterus given 500ug/kg of Estrogen + 150mg Tumeric showing: A, thinned endometrial lining, B, normal stroma and C, thickened glandular epithelium (H&E x 100).

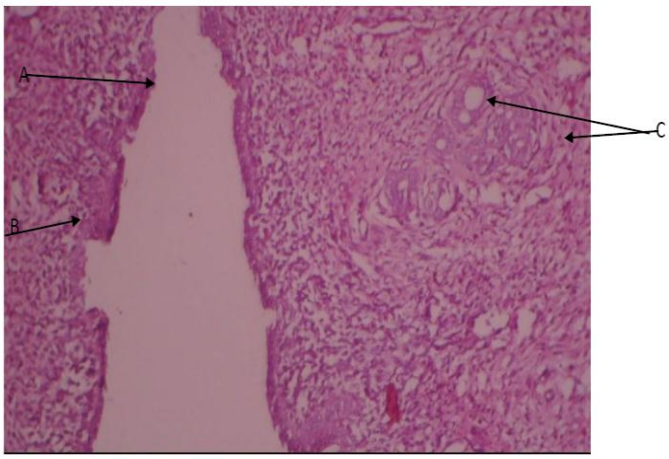

Plate 11: Rat uterus given 500ug/kg of Estrogen + 250mg Turmeric showing: A, normal endometrial lining, B, normal stroma and C, thickened glandular epithelium (H&E x 100).

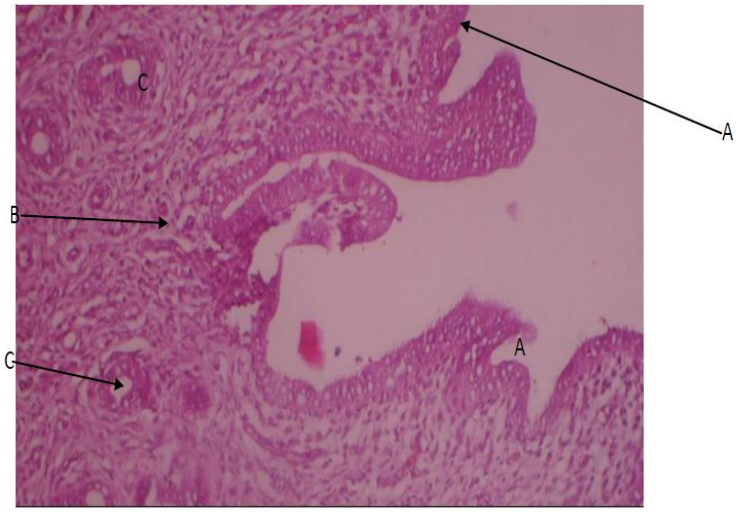

Plate 12: Rat uterus given 350mg Tumeric only showing: A, Normal Endometrial lining, B, normal Stroma and C, normal glands (H&E x 100).

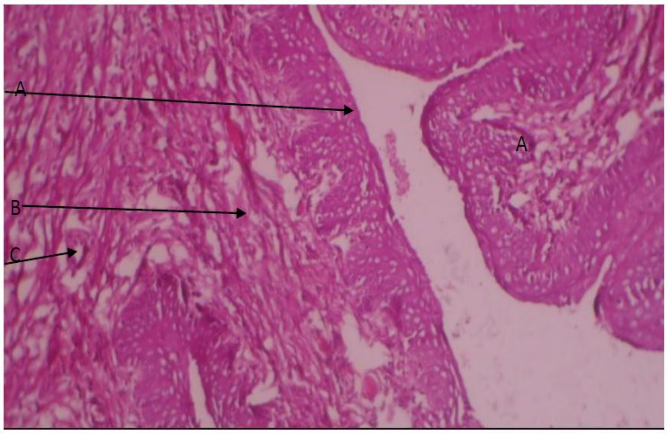

Plate 13: Rat uterus given 150mg Turmeric for 2 weeks before 500ug/kg of Estrogen for 6 weeks showing: A, thickened lining, B, normal stroma and C, glands (H&E x 100).

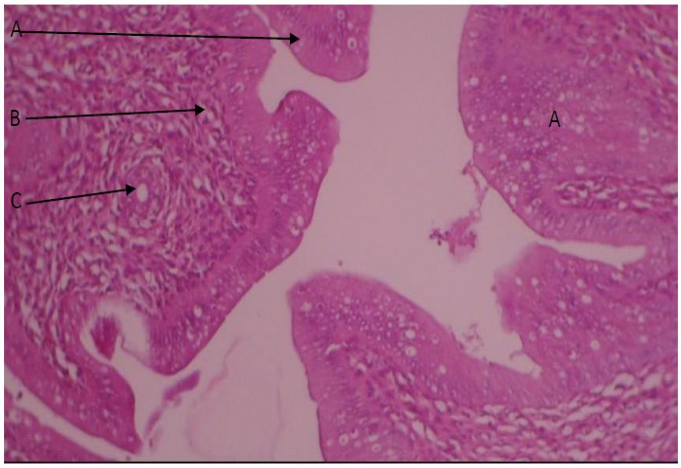

Plate 14: Rat uterus given 250mg Turmeric for 2 weeks before500ug/kg of Estrogen for 6 weeks showing A, normal endometrial lining, B, normal stroma and C, normal endometrial gland. (H&E x 100).

4. DISCUSSION

The acute toxicity test was carried out using lorke's method and the result the result obtained for that of aqueous extract of turmeric agrees with the finding of Yaundani (2017) who reported the LD50 of turmeric to be more than 5000mg/kg body weight while that of estrogen showed signs of toxicity at 1,265ug/kg which is similar to the work of Govind (2008) that reported the same signs of toxicity at 1200ug/kg body weight.

The phytochemical analysis of turmeric shows the presence of glycosides, saponins, tannins, terpenoids, alkaloids and flavonoids. The presence of this phytochemicals confirmed the medicinal properties of the turmeric plant. Tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues (Aboagye *et al.*, 2019). Tannins have been reported to prevent the development of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them (Bilić *et al*., 2016). The presence of tannins in turmeric supports the traditional medicinal use of this plant in the treatment of different ailments. Another secondary metabolite compound observed in turmeric was alkaloid. One of the most important properties of alkaloids is their toxicity against cells of foreign organisms. Their activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Czapski *et al.*, 2014). Saponin was also found to be present in turmeric extracts and has supported the usefulness of this plant in managing inflammation. Flavonoids, another constituent of turmeric extracts, have exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek *et al*., 2002). Presence of these phytochemicals in this plant confirms the pharmacological usefulness of turmeric. These findings here corresponds with the works of Pawar (2015) and Sawant (2013).

The histological sections of the mammary gland of the control group showed ducts lined by low columnar epithelial cells supported by fibrocollagenous connective tissue, stroma and adipocytes (fat cells). Ductal epitheliosis (proliferation) and stromal infiltrates of inflammatory cells were observed in group 2 animals, administered with estrogen alone. This is similar to the results from several studies that have linked the use of exogenous estrogen to the risk of initiating mammary gland toxicity (Stahlberg *et al*., 2004; Lai *et al*., 2011; Turkoz *et al*., 2013) Administration of turmeric alone induced stromal mobilization of lymphocytes (cells of the immune system). Concurrent administration of estrogen and turmeric as well as pre-treatment with turmeric two weeks before estrogen in graded concentrations retained the normal ductal epithelial status in a dose-dependent manner, with the pre-treatment having the better effect. This correlates with the work of Hiroshi *et al*., (2016) who reported that turmeric reduced tumorigenesis in mammary glands of irradiated rats through the inhibition of the proliferation of preneoplastic cells or tumor origin cells.

Going further, the histological section of the uterus of the control rats showed that it is composed of normal endometrial mucosal lining surrounding the uterine cavity, and deep to the mucus membrane is the endometrial stroma composed of fibrous and connective tissue cells. Embedded in the stroma are the endometrial glands. Thickened endometrial lining, proliferation of the glandular epithelium, and increase in the population of stromal cells as well as stromal oedema are all indicative of estrogen-induced toxicity observed in the group treated with estrogen only. These effects are similar with the reports of many authors (Liehr J.M., 2000; Pandey *et al*., 2006; Cain J.L., 2001), who elucidated that estrogen induced uterine toxicity. Estrogen - induced toxicity has also been observed in the liver and ovaries of rats after administration of estrogen at different doses (500 and 750 µg/kg, orally) for 8 and 12 weeks (Madhuri *et al*., 2007). Concurrent administration of estrogen and turmeric in graded concentrations caused a reduction in

the mucosal lining thickness and stromal oedema in a dose-dependent manner. Pre-treatment of rats with turmeric two weeks before estrogen induced thickening of the mucosal lining (crowding) and maintenance of the normal status of the glandular epithelium, culminating in the maintenance of the normal uterine size. This effect was in a dose – dependent manner. Our findings here are similar to the work of Yilmaz *et al* (2018) who reported that turmeric has a protective effect against cyclophosphamide-induced cytotoxic effects of the uterus, which he stated could be attributed to the anti-inflammatory and anti-oxidants properties of turmeric.

Our findings from this study showed that turmeric has both protective and preventive effects against estrogen-induced histologic toxicity of the mammary gland and uterus.

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