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Effects of Aqueous Extract of Turmeric on Estrogen Toxicity on the Histology of the Mammary Gland of Female Wistar Rats

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Abstract:- Estrogen is the most commonly prescribed drug as an oral contraceptives and hormonal replacement therapy in women. The present study was conducted to examine the possible effects of turmeric against Mammary gland toxicity induced by estrogen in female wistar rats. 35 female Wistar rats were assigned into five (7) groups of five (5) rats each after a period of two (2) weeks of acclimatization; the first group which was the control group was the control group, Group 2 received estrogen only, group 3 and group 4 received turmeric and estrogen together, group 5 received turmeric only while group 6 and 7 were pre-treated with turmeric before they were given estrogen orally. At the end of experimental period the animals were weighed and then sacrificed by decapitation under diethyl ether anesthesia. The mammary gland tissues were harvested for histological examination. The histological examination showed that the animals treated with estrogen alone induced ductal epitheliosis while the Concurrent administration of estrogen and turmeric as well as pre-treatment with turmeric retained the normal ductal epithelial status.

Keywords:- *Estrogen, Turmeric, Mammary Gland, Histology.*

I. INTRODUCTION

Oral contraceptive (OC) drugs are tremendously used by women throughout the world to prevent fertilization or to control birth. Estrogens are the most commonly prescribed drugs by far the two major uses are as an oral contraceptive and postmenopausal hormonal replacement therapy (HRT) in women. However, estrogens have been reported to cause several bad 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 and cancers of many organs (Estrogen and cancer website, 2006; Loose & Stancel, 2006).

Over the years, there has being increased scientific research to minimize the health hazards potentiated by some toxin and this was done on phytochemicals being extracted from plant species. The bioactive property of these plants could be attributed to their phyto-constituents such as flavanoids, anthocyanins, vitamins C and E, phenolic compounds, dietary fiber and carotinoids.One of such plant is Tumeric(*Curcuma longa*) whose taxonomic Order is Zingiberales, Family is Zingiberaceae , Genus is Curcuma and Specie is indicum. Turmeric has been subjected to numerous trials and studies and it has been validated and clarified by modern science. 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 conditions 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.

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.

II. MATERIALS AND METHOD

➢ *Materials Used*

- Thirty-five (35) female wistar rats
- Synthetic Estrogen
- Tumeric
- Steel cages
- Troughs
- Weighing balance
- Growers mash feed
- Water
- Syringes
- Cannula
- Laboratory mill
- Laboratory oven
- Measuring cylinder
- Beakers
- Filter paper
- Water bath
- Dissecting set
- Refrigerator

➢ *Procurement, Housing And Care Of Experimental Animals*

Thirty-five (35) adult female wistar rats were obtained from an animal farm in the College of Health Sciences, Nnamdi Azikiwe University, Nnewi, Anambra State, Nigeria and housed in the animal house of the Department of Anatomy, Nnamdi Azikiwe University. 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 .

➢ *Procurement Of Curcuma Longa (Tumeric) And Preparation Of Extract.*

Fresh 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 turmeric extract was prepared by peeling, washing and drying under room temperature. They were 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 becomes concentrated and later preserved in a refrigerator at 4^{o}C prior to use.

➢ *Procurement Of Estrogen*

Estrogens were purchased at Pharmacy in Anambra State Nigeria. They were grounded to fine powder and dissolved in water before administration daily to allow for proper dissolution

➢ *Experimental Design*

The rats were assigned into five (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.
- Group 2 received estrogen 500µg/kg for 6 weeks
- Group 3 received estrogen 500µg/kg 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.
- Group 6 received turmeric 150mg/kg for 2 weeks before receiving estrogens 500µg/kg for 6 weeks.
- Group 7 received turmeric 250mg/kg for 2 weeks before receiving estrogens 500µg/kg for 6 weeks

The administrations were carried out using oral gavage.

➢ *Acute Toxicity Test (LD50)*

This was performed on wistar rats and the Lorke's procedure of LD50 determination was used.

➢ *Phytochemical Analysis Of Turmeric*

Phytochemical screening of tumeric (curcuma longa) was carried out 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.

➢ *Histological Examination*

Twenty four hours after the last administration of extract, the rats were weighed and sacrificed by decapitation under diethyl ether anesthesia, Tissue sections of the Mammary gland were fixed in 10% formaldehyde/PBS for 24 hours at room temperature, cleared in xylene embedded in paraffin wax. Four to five micron (4-5 µm) thick sections were stained with haematoxylin and eosin stain and imaged on a compound light microscope.

➢ *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) will be applied for determining the significance. The acceptable level of significance will be established at $P < 0.05$.

III. RESULTS

➢ *Lethal toxicity test of Estrogen and Aqueous Extract of Turmeric (Curcuma longa).*

The lethal toxicity test of aqueous extract turmeric (Curcuma longa) on wistar rats showed no sign of toxicity at doses as high as 5,000ug/kg while that of estrogen showed high rate of mortality at 1,265ug/kg.

➢ *Phytochemical Analysis of turmeric*

Table 3.1 phytochemical analysis of turmeric

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

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

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Table 3.2 Effect of *Curcuma longa* on body weight on estrogen-induced toxicity

	Initial weight (g)	Final weight (g)	P-value
	MEAN±SEM	MEAN±SEM	
Group A (Positive control)	152.33±9.97	159.48±7.39	0.62 [b]
Group B (500 µg/kg of E2)	146.52±12.91	171.20±4.30	0.10 [b]
Group C (500 µg/kg of E2 + 150mg/kg of *C. longa*)	151.95±5.12	157.70±4.64	0.09 [b]
Group D (500 µg/kg of E2 + 250mg/kg of *C. longa*)	152.70±7.16	159.07±7.50	0.54 [b]
Group E (350 mg/kg of *C. longa*)	149.52±5.62	119.52±5.81	0.07 [b]
Group F (150 mg/kg of *C. longa* + 500 µg/kg of E2)	150.20±3.86	159.45±5.36	0.21 [b]
Group G (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)

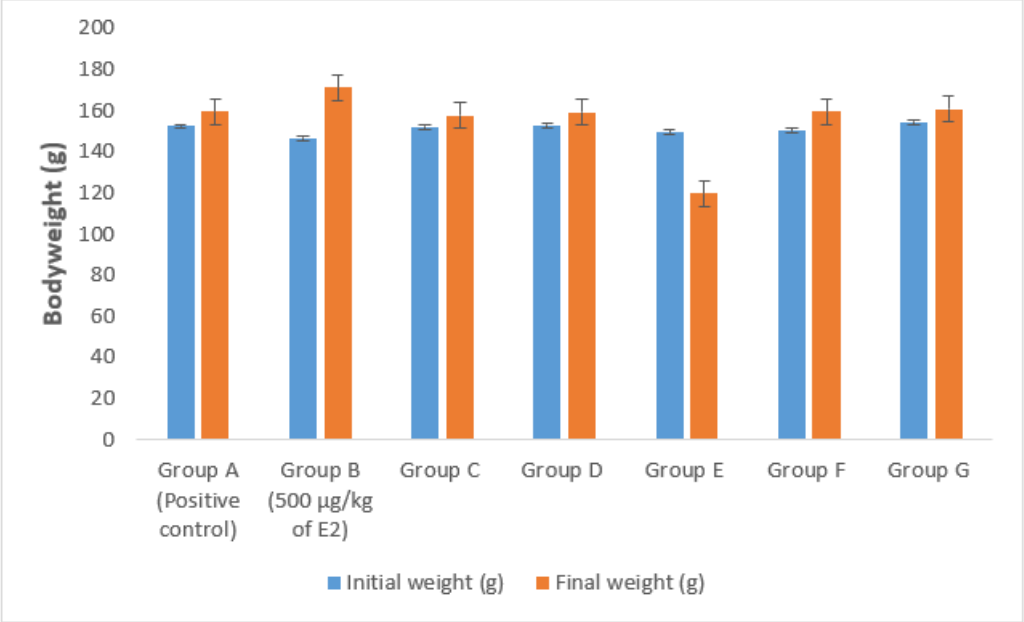
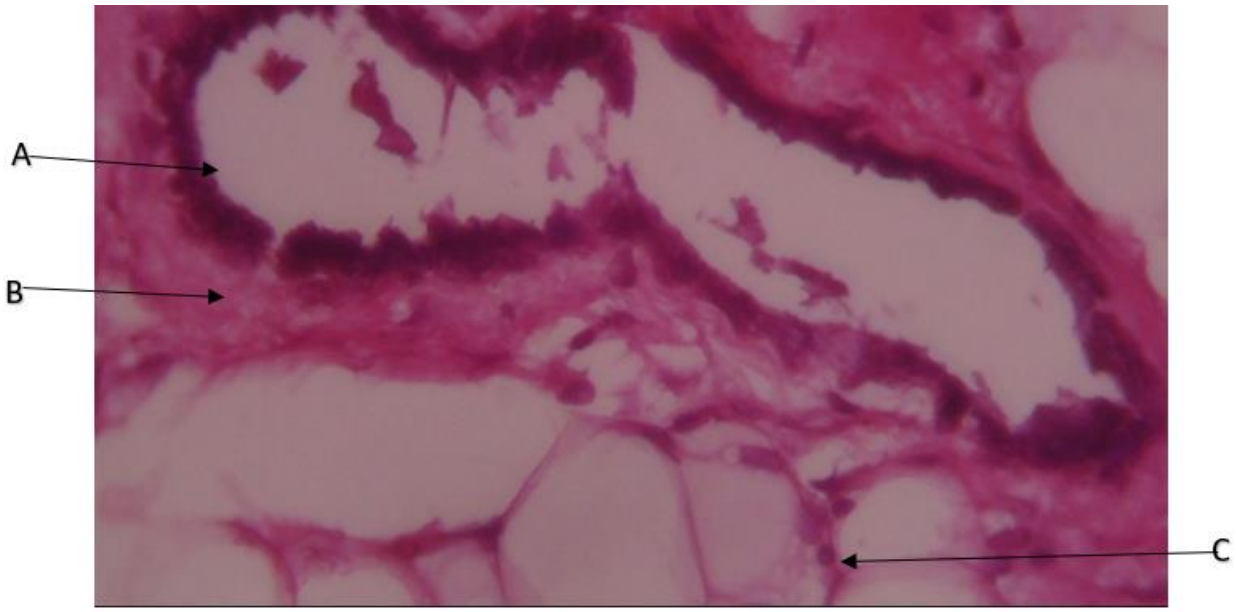


FIG 1 effect of *Curcuma longa* on body weight on estrogen-induced toxicity



MICROGRAPH 1 (CONTROL). Composed of: A, duct, B, Fibrocollagenous Stroma and C, Fat tissue (H&E x 400).

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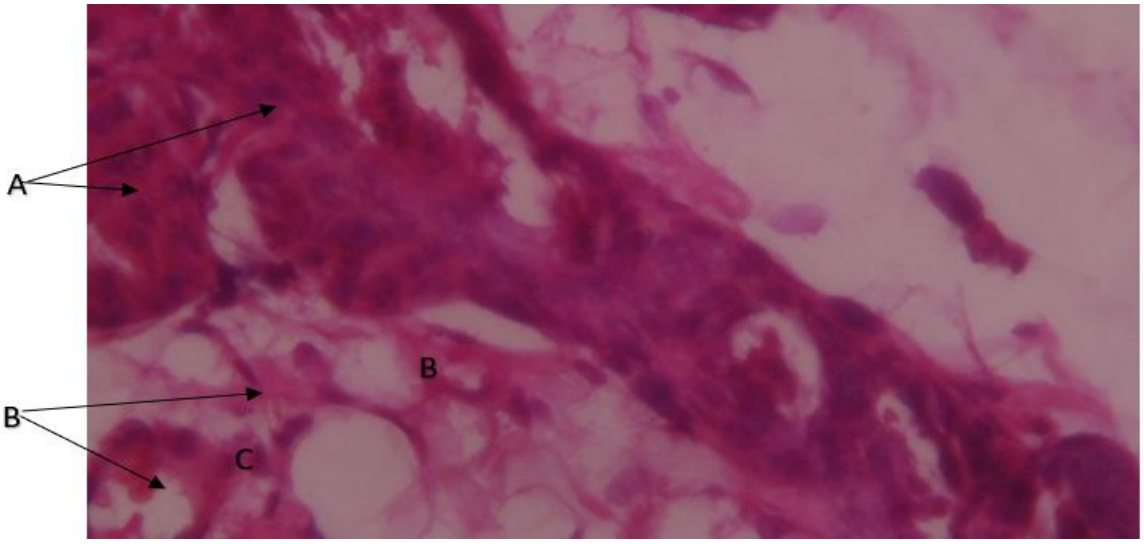
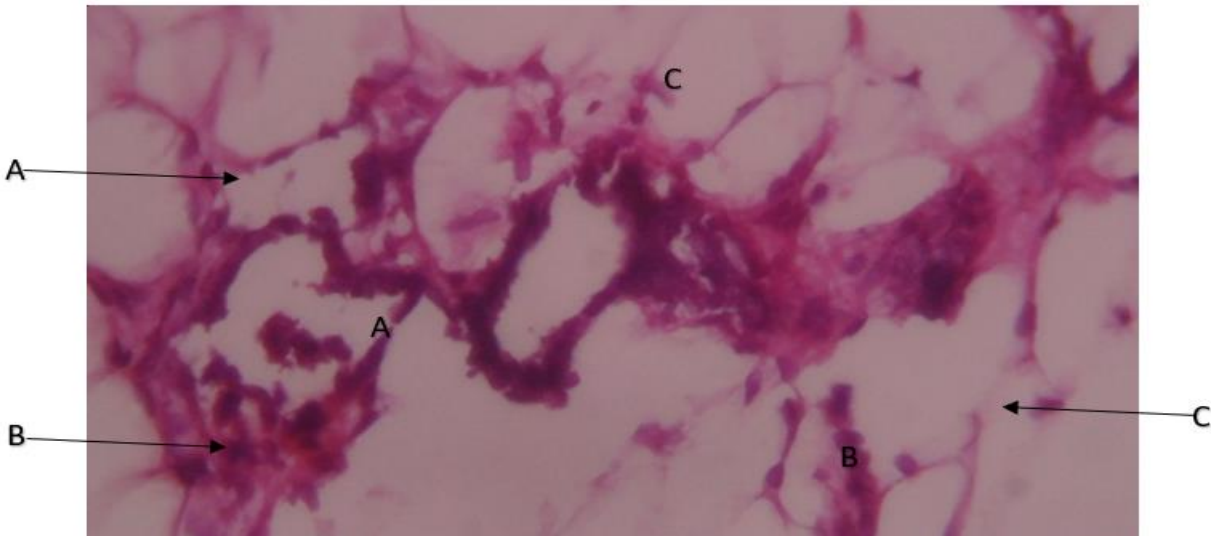
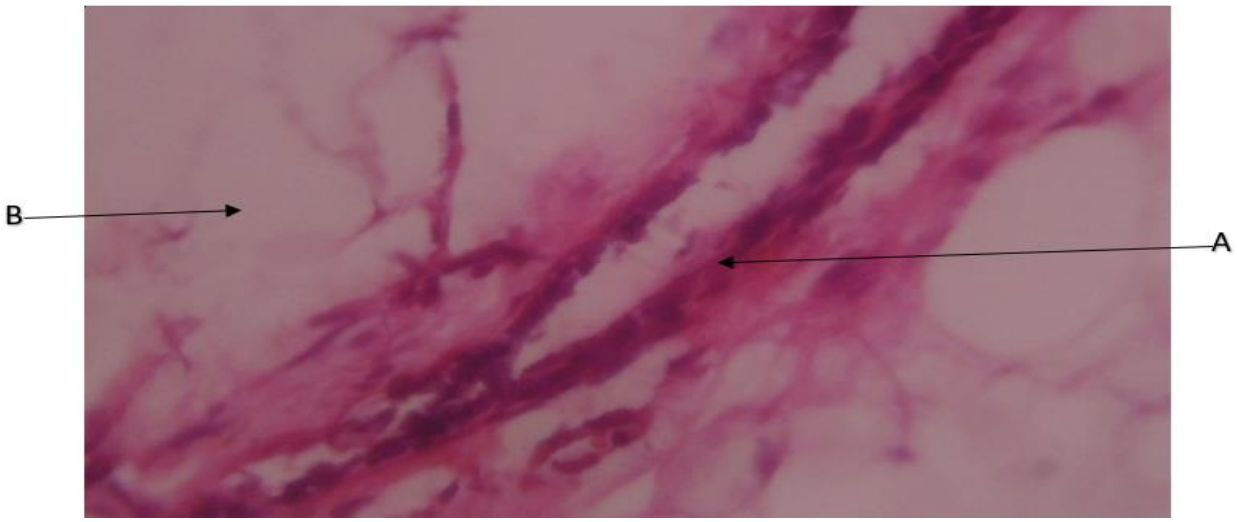


MICROGRAPH 2 (TREATED WITH ESTROGEN ONLY 500Uug/kg ONLY) COMPOSED OF: A, SECRETORY DUCTAL EPITHELIOSIS, B VASCULARIZED FAT TISSUE AND C, STROMAL LYMPHOTIC INFILTRATES (H&E x 400).



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



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

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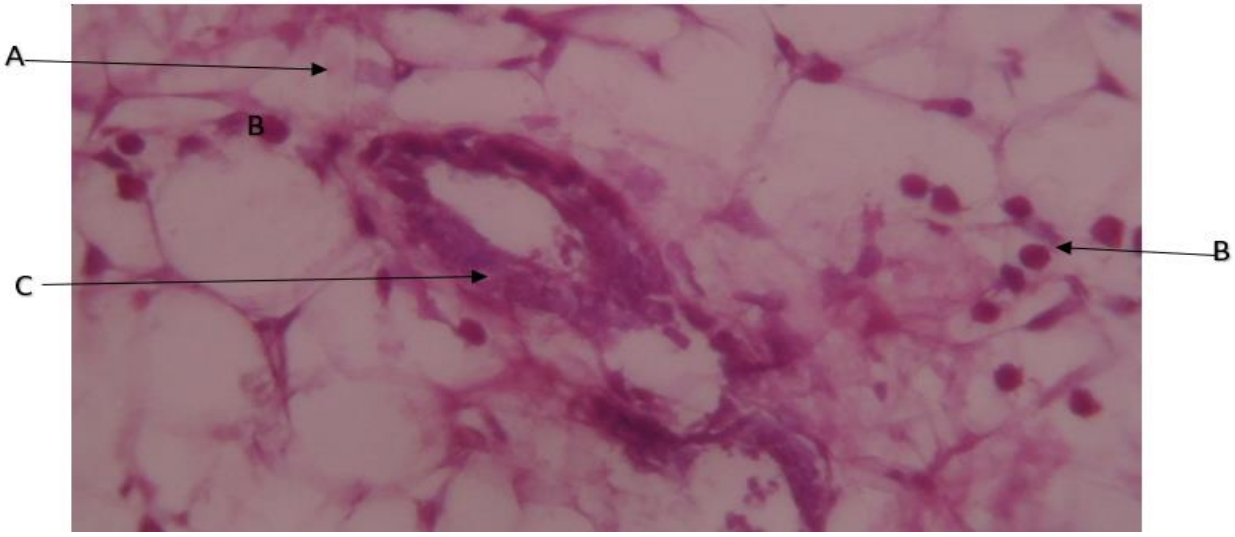
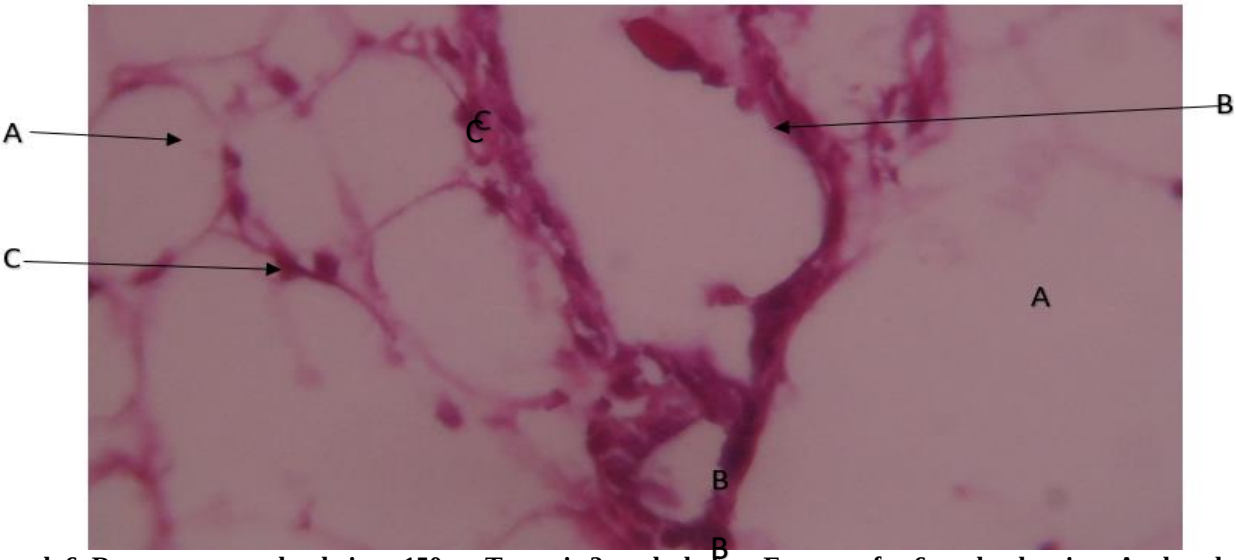
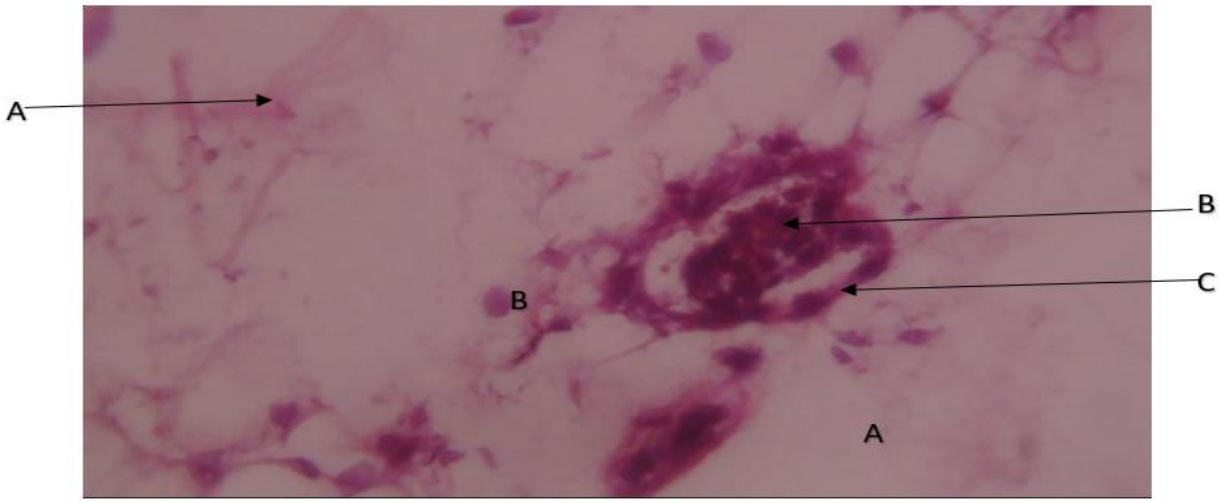


Micrograph 5.Rat mammary gland given Tumeric 350mg only showing: A, abundant fat tissue, B, infiltrates of lymphocytes and C, normal ducts (H&E x 400)



Micrograph 6. Rat mammary gland given 150mg Tumeric 2 weeks before Estrogen for 6 weeks showing: A, abundant fat clusters, B, normal ducts and C, stromal lymphocytic infiltrates (H&E x 400).



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

IV. DISCUSSION

The result in (Table 3.1) 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 Tannins have been reported to prevent the development of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them. 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 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. Flavonoid and saponin have also been shown to have antioxidant activities which could have helped in reducing the oxidative stress effect as a result of methotrexate toxicity. 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 exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties. Presence of these phytochemicals in this plant confirms the pharmacological usefulness of turmeric.

From the result in table 3.2 , there was a non-significant increase in the body weight of groups A,B,C,D,F AND G while group E which was given 350mg/kg of turmeric alone had a non-significant decrease in their body weight when their initial body weight was compared to their final body weght.

The mammary gland of the group one which served as the control composed of ducts lined by low columnar epithelial cells supported by fibrocollagenous connective tissue stroma and adipocytes (fat cells). Treatment of rats with estrogen 500ug/kg alone induced ductal epitheliosis (proliferation) and stromal infiltrates of inflammatory cells. Administration of turmeric 350mg/kg alone induced stromal mobilization of lymphocytes (cells of the immune system). Concurrent administration of estrogen and tumeric as well as pre-treatment with tumeric two weeks before estrogen in graded concentration retained the normal ductal epithelial status in a dose-dependent manner, with the pre-treatment having the better effect.

V. CONCLUSION

In conclusion, the treatment of Estrogen-induced toxicity with aqueous extract of turmeric had a beneficial effect on reducing the proliferation of the cells of the mammary glands by inducing stromal mobilization of lymphocytes (cells of the immune system). This study thereby encourages the inclusion of turmeric in our diets.

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