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Research Article

THE PROTECTIVE EFFECTS OF *TURMERIC* ON TESTICULAR TISSUES, AFTER TREATMENT WITH *METRONIDAZOLE* IN ADULT MALE WISTAR RATS

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ABSTRACT
Metronidazole has been shown to exert some negative effects on sperm analysis and testis structure in some previous studies while turmeric has been shown to have no side effects despite being a therapeutic agent with multiple beneficial functions such as its anti-oxidant effects, potent cancer chemopreventive actions, hepatoprotective effects, and antimicrobial effect amongst a host of many others. This study evaluated the protective effects of turmeric on testicular tissues after treatment with metronidazole in varying doses in adult male wistar rats. Twenty adult male wistar rats with weight range of between 165-180g were assigned into four groups A, B, C and D of 5 animals each. Group D served as the control group and were orally administered water and feed only, the experimental groups A, B and C were orally administered 200mg of metronidazole, 400mg of metronidazole and 400mg of metronidazole as well as 400mg of turmeric respectively for duration of twenty-eight days. Twenty four hours after the last administration, the animals were anaesthetized under chloroform inhalation and dissected. The testis were harvested, weighed and underwent tissue processing. The histological results revealed that the therapeutic dose of 200mg of metronidazole caused mild sloughing off of testicular tissues, following administration for 28 days, while the high dose of 400mg for same duration showed severe sloughing off of testicular tissues but the 400mg turmeric exhibited protective effect on the testicular tissues following 400mg metronidazole administration. This study therefore suggests that effects of metronidazole on the testicular tissues could also contribute to male infertility, and therefore that turmeric, which has anti-sloughing off property, be included in the diet of individuals on metronidazole administration, at least for as long as the medication lasts.

Keywords: *Metronidazole, Testis, Turmeric, Protective, Wistar Rats*

INTRODUCTION
Metronidazole, a 5-nitroimidazole drug widely used in veterinary and human medicine for the treatment of trichomoniasis, giardiasis, amebiasis and anaerobic bacterial infections (Frey and Löscher, 1996), as developed in 1960 (Ligha and Paul 2011).
Metronidazole is 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole B.P. It appears as a white to brownish cream crystalline substance with melting point 159-162^{0}C. Solubility in water at 20^{0}C is 1g/100mL; in ethyl alcohol, 0.5g/100mL; in chloroform, 0.4g/100mL; slightly soluble in ether and soluble in dilute acids. When reconstituted as Metronidazole IV for infusion, it has a pH of between 4.8 and 5.2. Each mL contains metronidazole B.P. 5mg, anhydrous citric acid B.P 0.4mg, sodium phosphate B.P 1.5mg and sodium chloride B.P 7.4mg as well as mL contains 0.135mmol of sodium. Its mode of action has not been fully elucidated.
Metronidazole should be stored in a cool (15^{0}c – 25^{0}c) dry place, protected from light as well as out of reach of children. Heat and moisture may cause drug to be less active. Side effects of metronidazole are nausea, diarrhoea and metallic taste in mouth. Intravenous administration is commonly associated with thrombophlebitis. Infrequent adverse effect include; hypersensitivity reaction (rash, itch, flushing, fever);

headache, dizziness, vomiting, glossitis, stomatitis, dark urine and paraesthesia. High dose or long term systematic treatment with metronidazole is associated with the development of leukopodia, neutropathy and/or CNS toxicity (Rossi *et al.,* 2006).

Metronidazole has been shown to have negative effects on sperm analysis and testis structure. About 165mg/kg/day and 500mg/kg/day of metronidazole was administered for 30days orally, and it was concluded that metronidazole can reduce germinal epithelium volume and the number of spermatocytes and spermatids (Ali *et al*., 2010).

Studies were carried on the effect of metronidazole toxicity on reproduction, spermatogenesis, plasma gonadotrophin and testosterone in rats, and it was reported that the action of metronidazole on spermatogenesis and sexual hormones in rats was suppressive and had hazardous effects on the germ and Leydig cells after penetration into the blood-testis barrier of the rats (Sohrabi *et al*., 2007).

Turmeric, a perennial herb is a plant that is characterized by its tall, reed-like stems and underground rhizome systems. The rhizome or root, whose chief constituent flavour are the *turmer one* and *curcumin*, commonly known curry powder are used as spice or food additive when cooking foods like meat and fish sauces, pepper soups and vegetable dishes.

The most important feature of turmeric is that it has no side effects despite being a therapeutic agent with multiple beneficial functions. It can also act as a scavenger of free radicals (Salama and El-Bahr, 2007; Khanna, 1999).

Turmeric is considered to be an effective antioxidant against oxidative tissue damage. It can significantly inhibit the generation of reactive oxygen species, both in vitro and in vivo (Joe and Lokesh, 1994).

In addition, turmeric is also considered to be a potent cancer chemopreventive agent (Duvoix *et al*., 2005; Agarwal and Prabakaran, 2005).

Pharmacological actions of turmeric includes: hepatoprotective effects, anti inflamatory activity, anticarcinogenic effect, anti-oxidant effect, gastrointestinal effect, antimicrobial effect and cardiovascular effect.

Turmeric serves a number of medicinal benefits when taken in a dose less than 500 mg. For best results and to minimize side effects, healthcare providers suggest avoiding a dose over twice or thrice the recommended dose (i.e. 1500 mg). Turmeric roots can be taken in a dose of up to 3g. Turmeric has no interactions with drugs because of the possibility of additive anti-platelet activity, and so caution should be taken with respect to concurrent use of *curcumin* with anticoagulants as well as with medications and dietary supplements known to have anti-platelet activity. The American Herbal products Association classify turmeric as a menstrual stimulant and some sources recommend avoiding *curcumin* in pregnancy. Its use is not recommended during breastfeeding, as effects on breastfeeding infants are unknown. Turmeric should be avoided in patients with bleeding disorders and bile duct obstruction and should be used only under the supervision of a physician in patients with gallstones (Jiang, 2007).

The testis of an adult male wistar rat descends at 4-6 weeks of age while it weighs about 1.8g (Suckow *et al*., 2005).

The study therefore is to investigate the protective effects of turmeric as a natural antioxidant on the testicular tissues, after an initial treatment with metronidazole in adult male wistar rats.

MATERIALS AND METHODS

Breeding of Animals and Duration of Experiment

Twenty (20) adult male wistar rats were used in this study which were obtained from a local farm at Nsukka, Enugu State, Nigeria and kept in the animal house of the Department of Anatomy, Faculty of Basic Medical Science, College of Health Sciences, Nnamdi Azikiwe University, Anambra State, Nigeria. The animals were acclimatized for a period of two (2) weeks, after which the substances for test were administered for a period of twenty-eight (28) days while the entire study lasted for six (6) weeks.

Materials for Study

The materials used for this experiment includes the following:

Twenty (20) adult male wistar rats, Metronidazole, Turmeric powder, Growers mash, Standard cages which were four (4) in number, Electronic weighing balance with an accuracy range of 100g, Syringes and canula for administering the extracts, Compound light microscope, Slides, 10% formalin and Distilled water.

Drug Preparation

Metronidazole; produced by M&B Pharmaceuticals, with an expiration date of 2018, were purchased from God'swill Pharmacy at Nnewi, Anambra State, Nigeria in the month of June, 2014. It was grounded to fine powder and dissolved in a known quantity of water 10 minutes before administration daily to allow proper dissolution.

Turmeric powder; which was produced and packaged in India by TRS Asia's Finest Foods with expiration date of 2016 was purchased from the main market at Nnewi town. Before administration, the turmeric powder was weighed and prepared into solution form.

Experimental Protocols

Twenty (20) apparently healthy adult male wistar rats were assigned to four (4) different cages in a group of (5) animals each for acclimatization, for a period of two (2) weeks, prior to the commencement of the experiment. Group D served as the control group and received distilled water and feed only while Groups A, B and C served as the test groups and received 200mg/kg body weight/day of metronidazole, 400mg/kg body weight/day of metronidazole and 400mg/kg body weight/day of metronidazole as well as 400mg/kg body weight/day of turmeric respectively for twenty eight days. Twenty-four (24) hours after the last administration, the animals were inactivated by chloroform vapour inhalation and dissected. The testes were then harvested and trimmed to a size of 3mm 3mm and then fixed in 10% formalin for 1 hour, for preservation of normal physiological condition for histological processing.

Tissue Processing

For easy sections study under the compound light microscope, the tissues' fixation was done in 10% formalin, the tissues were then dehydrated in ascending grades of alcohol of 50%, 70%, 90% and 100% for one to two (1-2) hours each but twice in 100% alcohol to ensure that dehydration was fully achieved. The tissues were then cleared in xylene for a period of one to two (1-2) hours to remove the alcohol from the tissue and then impregnated with two changes of molten paraffin wax at a temperature of 56^0C before embedding to solid form for sectioning. Sections of a thickness guage of about 5 microns was done with the aid of a rotatory microtome and then these slices of sections were picked up with a pair of forceps and then placed on slides made sticky by starch. The tissue sections were deparaffinised and hydrated in a water bath bath of about 50-55^0C, the tissues were stained using the routine haematoxylin and eosin methods. The stained slides were then wiped clean of dirt with 70% alcohol, with two (2) drops of mountant added before covering with a cover slip for examination under a compound light microscope.

RESULTS AND DISCUSSION

Results

Table 1: Comparative summary of rat and organ weights, semen morphology and count of all the groups respectively

Parameters	Group A n =4	Group B n =4	Group C n=4	Group D n =4	F-value n =4	P-value n =4
Testis WT	5.61±0.37	5.59±0.40	5.52±0.29	5.27±0.03	0.828	0.503
Total sperm count	38.30±7.08	41.83±16.45	39.87±16.45	23.43±7.73	1.284	0.344
% Normal morphology	57.33±7.51	72.33±2.52	62.67±2.52	68.50±2.89	17.937	0.001*

Initial rat WT	185.00±12.91	185.00±12.91	182.50±9.57	165.00±10.0	2.841	0.083
Final rat WT	215.00±12.91	215.00±12.91	210.00±8.17	195.00±10.00	2.867	0.081

Physical Observations

During the period of administration of the solutions, the adult rats in groups A, B and C appeared less active than those in the group D.

Body Weight Changes

An increase in body weight was also noticed in the rats of groups A, B and C than those of group D and below is a table as well as a bar chart showing this finding.

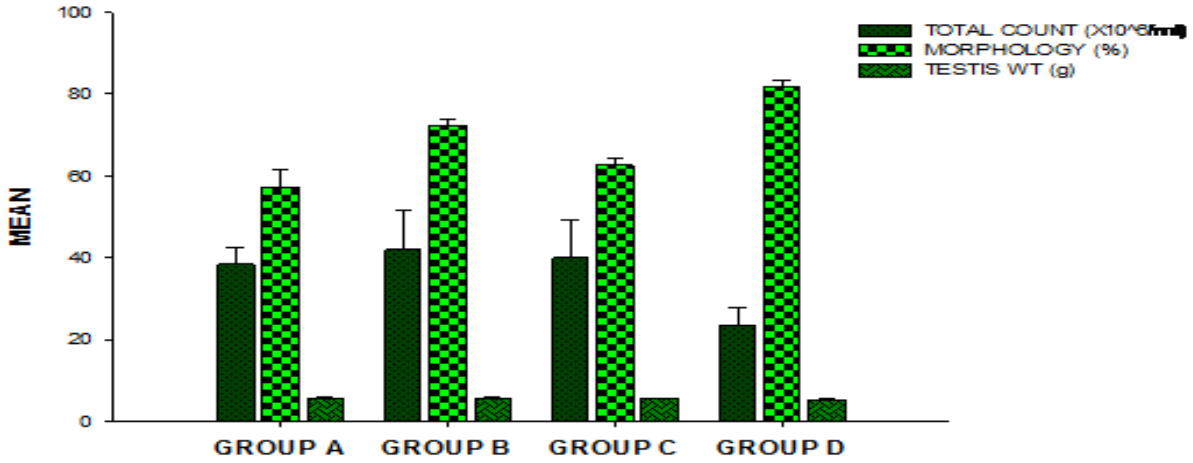

Figure 2: Bar chart showing the testis weight, sperm count and morphology of the different groups

Histological Findings

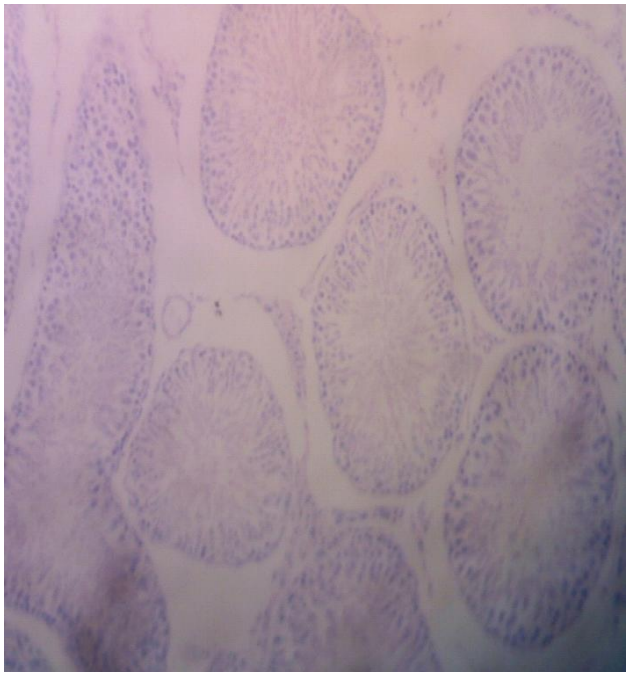

Figure 3:Micrograph 1 (Group A) H&E section of testis of the experimental Group A administered with 200mg of metronidazole; showing mild sloughing off of the germ cells with magnification ×100

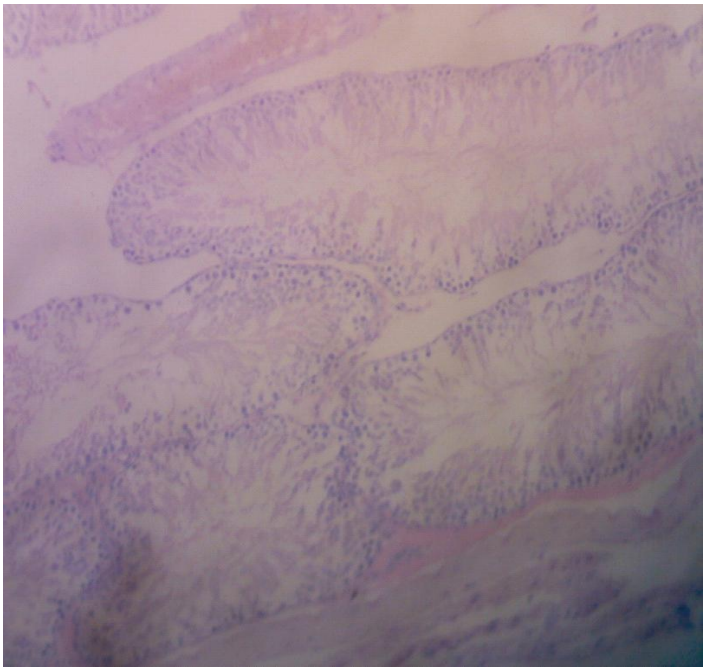

Figure 4: Micrograph 2 (Group B) H&E section of testis of the experimental Group B administered 400mg of metronidazole; showing severe sloughing off of germ cells, slight vacuolization between the epithelial and spermatogenic cells with magnification ×100

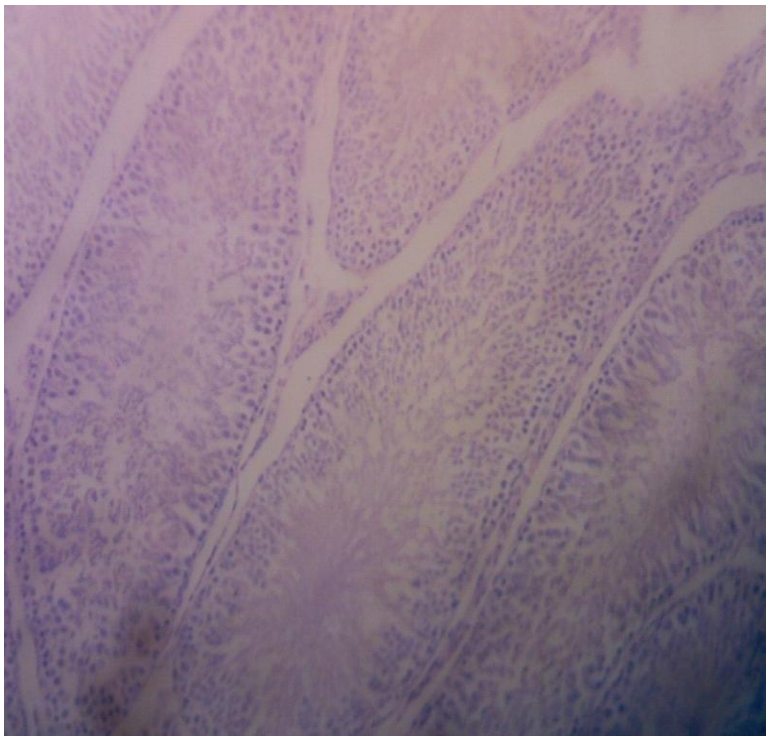

Figure 5: Micrograph 3 (Group C)H&E section of testis of the experimental Group C administered 400mg of metronidazole & turmeric; showing mild sloughing off of germ cells, slight disorientation of spermatogenic cell with magnification ×100

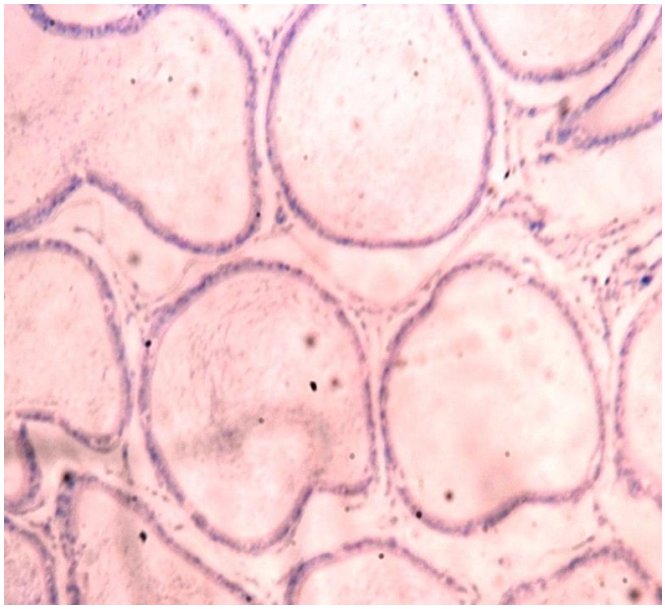

Figure 6: Micrograph 4 (Group D) H&E section of Testis of the control group D administered with only feed and water; showing seminiferous tubules with germ cells at different stages of maturation with magnification ×100 and ×400

Discussion

The present study reports the protective effects of turmeric on the testis of adult male wistar rats, treated with 200mg and 400mg therapeutic doses of metronidazole. The observation of the initial and final body weights of the rats, showed no significant increase or decrease in their weights. The testis weights also showed an insignificant decrease in weight in the groups administered metronidazole alone, this does not correspond to the findings of (El-Nahas and El-Ashmawy, 2004; Samah, 2012), whose works showed that metronidazole administration decreased significantly the weights of the testis; this may owe to the dose difference and duration of metronidazole administration in the research, which lasted for 28 days as against higher doses and duration used by previous researchers. Histological findings revealed that the therapeutic dose of 200mg/kg body weight of metronidazole caused mild deletion or sloughing off of testicular tissues, following administration for 28 days, while the high dose of 400mg for same duration showed severe sloughing off of testicular tissues as shown in the photomicrographs. However, turmeric exhibited protective effect on the testicular tissues of rats following metronidazole administration for 28 days. This could be due to the anti-oxidative, anti-inflammatory and anti carcinogenic property of its active component *curcumin*.

Conclusion

This study has therefore shown that the effect of metronidazole on the testicular tissues could also contribute to the increased rate of male infertility in our world today, and so it is therefore suggested that turmeric, which has anti-sloughing off property, be included in the diet of individuals on metronidazole administration, at least for as long as the medication lasts, it is also recommended that it forms a part of our daily diet because of its anti-oxidative property.

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