

Histological Observations of the Testis of Wistar Rats Following the Oral Administration of Cotecxin (dihydroartemisinin)

¹T. Murdakai, ¹A.A. Buraimoh and ²H.O. Kwanashie

¹Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria

²Department of Pharmacology, Faculty of Pharmaceutical Science,
Ahmadu Bello University, Zaria, Kaduna, Nigeria

Abstract: Cotecxin has been reportedly used in the treatment of malaria with high clinical effect and low toxicity. This study therefore, tried to examine the effects of cotecxin on the histology of the testis of wistar rats. A total of twenty four (24) male wistar rats were the subjects used in this experiment. The wistar rats were divided into three groups with each group containing eight (8) rats. Different concentrations of cotecxin were administered orally to the wistar rats which had an average weight of 150 g. Group I is the control group, Group II received 3.42 mg/kg and Group III were given 17.10 mg/Kg. The duration of administration was seven days. After which four (4) rats from each group were sacrificed on the eighth day. The remaining twelve rats were equally sacrificed on the 15th day and immediately fixed in 10% formalin. The tissues were processed and stained in Haematoxylin and Eosin (H&E). The changes observed on the eighth day in the testis were disarray of the spermatogenic cells and disorientation of the testis. These changes were observed to have been disappearing and normal histological features being restored in those rats sacrificed at the 15th day. It was therefore concluded that cotecxin has negative effect on the histology of the testis during administrations and these effects were reversible some days after stoppage of the administration. This suggests that cotecxin could be safe but its prolong usage may be discouraged.

Key words: Cotecxin, histological observations, oral administration, testis, wistar rats

INTRODUCTION

Almost all healthy male vertebrates have two testes. Plural testes, also called testicle, in animals, the organ that produces sperm, the male reproductive cell, and androgens, the male hormones. In humans, the testes occur as a pair of oval-shaped organs. They are contained within the scrotal sac, which is located directly behind the penis and in front of the anus. Both functions of the testicle are influenced by gonadotropic hormones produced by the anterior pituitary. Luteinizing hormone (LH) results in testosterone release. The presence of both testosterone and Follicle-Stimulating Hormone (FSH) is needed to support spermatogenesis (Michael and Wojciech, 2006; Romer and Parsons, 1977; Skinner *et al.*, 1989). There are two phases in which the testes grow substantially; namely in embryonic and pubertal age. (Scott, 2000) The testes grow in response to the start of spermatogenesis. Size depends on lytic function, sperm production (amount of spermatogenesis present in testis), interstitial fluid, and Sertoli cell fluid production. After puberty, the volume of the testes can be increased by over 500% as compared to the pre-pubertal size. Testicular size as a proportion of body weight varies widely. Testicles

are fully descended before one reaches puberty. (Alan, 2007; Crane and Scott, 2002). Malaria remains the World's most devastating human infection. It is an acute and chronic mosquito borne diseases characterised by fever, chills, anaemia, splenomegaly and damage to other organs such as liver etc. (Aguwa, 1996). Malaria is responsible for an estimated one million deaths annually. (Bouree *et al.*, 1994; Edward, 1998; Herfindal and Gouley, 1996; Rang and Dale, 1994). Although, the mosquito borne infection has been virtually eradicated in the United State, emigration from and travel to endemic regions constitute a continuing health problem (Goodman *et al.*, 1985). Effective and safe drugs, as well as vaccine are still needed to combat this scourge.

Cotecxin contains dihydroartemisinin. This is in category of antimalarials called artemisinins, which are derived from the plant *Artemisia annua*. Artemisinins are very effective in treating falciparum malaria, the species that is most likely to be fatal. Cortecxin also provides rapid and high clinical effects with low toxicity (Wang *et al.*, 2001; White, 1994).

This study was designed in order to examine the possible effects that cotecxin could have on the histology of the testis of wistar rats.

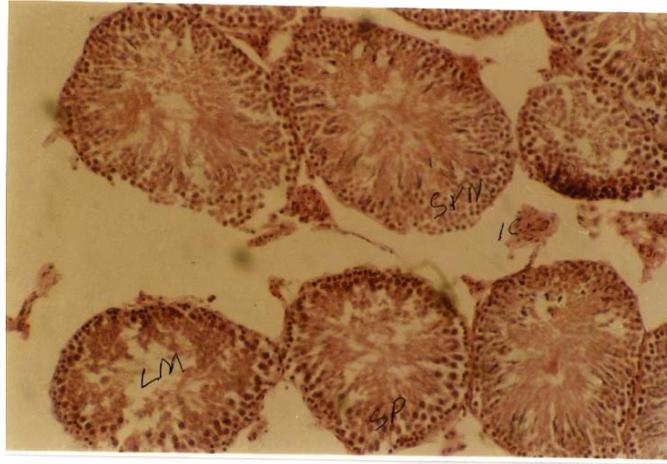


Fig. 1: Section of the testis with normal arrangement of the seminiferous tubules, sperms and Interstitial cells of leydig(IC) H&E Stain Mag. X100 ('Control' group I)

MATERIALS AND METHODS

This experiment was carried out in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Experimental animals: A total of twenty four (24) adult male wistar rats were obtained from the Department of Pharmacology, Faculty of Pharmaceutical Sciences and the experiment was conducted in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria. The animals were fed with standard diet and water and were allowed to adapt to the laboratory environment in the Department of Human Anatomy for two weeks in order to acclimatize. The duration of the experiment was fifteen (15) days; but drug (cotexcin) administration lasted for seven (7) days.

Experimental design: The wistar rats were divided into three (3) groups with each group containing eight (8) rats. Different concentrations of cotexcin were administered orally to wistar rats with an average weight of 150 g. Group I was the control, which received distilled water, Group II received 3.42 mg/kg and Group III were given 17.10 mg/Kg of Cotexcin. The duration of administration was seven days. After which four (4) rats from each group were sacrificed on the eighth day and the testes fixed immediately in 10% formalin. The remaining twelve rats were equally sacrificed on the 15th day and immediately fixed in 10% formalin. The route administration was orally.

Tissue processing and staining: The wistar rats were sacrificed by anesthetizing them in a suffocating chamber using chloro form, they were then dissected and the testes

were removed, and immediately fixed in 10% formalin. The testes were then transferred into an automatic tissue processor where they went through a process of dehydration in an ascending grades of alcohol (ethanol) 70, 80, 95% and absolute alcohol for 2 changes each. The tissues were then cleared in Xylene and embedded in paraffin wax. Serial sections of 5 micron thick were obtained using a rotary microtome. The tissue sections were deparaffinised, hydrated and stained using the routine (H&E) haematoxylin and eosin staining method (Gurr, 1962). The stained sections were examined under the light microscope. The Micrographs of the slides were taken in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria.

RESULTS AND DISCUSSION

The testes are oval organs about the size of large olives that lie in the scrotum, secured at either end by a structure called the spermatic cord. The testicles (also called testes or gonads) are the male sex glands. They are located behind the penis in a pouch of skin called the scrotum. The testicles produce sperm and testosterone. The testicles are located outside the body because sperm develop best at a temperature several degrees cooler than normal internal body temperature. Most men have two testes. The testes are responsible for making testosterone, the primary male sex hormone, and for generating sperm. Within the testes are coiled masses of tubes called seminiferous tubules. These tubules are responsible for producing the sperm cells through a process called spermatogenesis. (Elaine, 2004; Scott, 2000).

Cortexcin is an artemisinin derivative, which is a potent inhibitor of early ring stage parasites and has shown to be well tolerated, as well as, much more

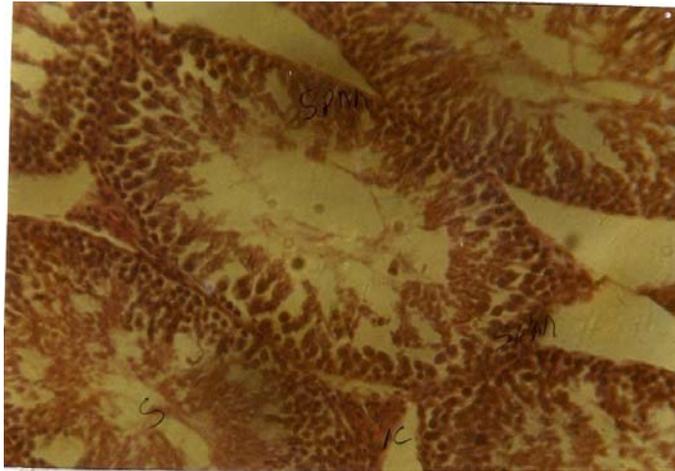


Fig. 2: (SPM): Section of the testis sacrificed at 8th day of administration with slight disorientation of the Spermatonic cells; (SM): reduction in the number of spermatozoain the central lumen of seminiferous tubule. H&E X 100 (group II)

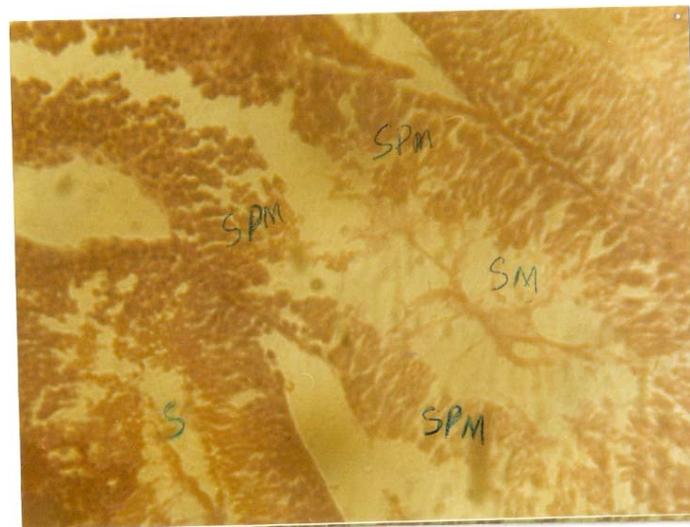


Fig. 3: (SP): Section of the testis sacrificed at the 8th day after drug administration, with disorientation of the spermatogenic cells; (SM): reduction in the number of central spermatozoa and enlargement of the interstitial cells of leydig H&E Mag. X100(group III)

effective than other anti- malaria drugs. It has been reportedly used in the treatment of malaria with high clinical effect and low toxicity (Wang *et al.*, 2001; White, 1994).

The results for wistar rats in group I, where only distilled water was administered showed normal features of the histology of the testes (Fig. 1) while the wistar rats in group II, that received 3.42 mg/kg and sacrificed at the 8th day of administration, showed slight disorientation of the Spermatonic cells (SPM) and reduction in the number of spermatozoa(SM) in the central lumen of seminiferous tubule (Fig. 2). In group III ,where the wistar rats received a dose of 17.1 mg/kg, sacrificed at the 8th day

after drug administration, there were disorientation of the Spermatogenic cells (SP) ; reduction in the number of central spermatozoa (SM) and enlargement of the interstitial cells of leydig (Fig. 3). The groups II and III which received 3.42 and 17.1 mg/kg, respectively showed some degree of degeneration (damage) in the histology of the testes (Fig. 2 and 3) when compared with group I (Control group) that showed normal architecture of the histology of the testes without any degeneration of the sperm cells (Fig. 1).

The degenerated changes observed in treated groups (groups I and II) were seen to have disappeared and normal histological features being restored in those rats

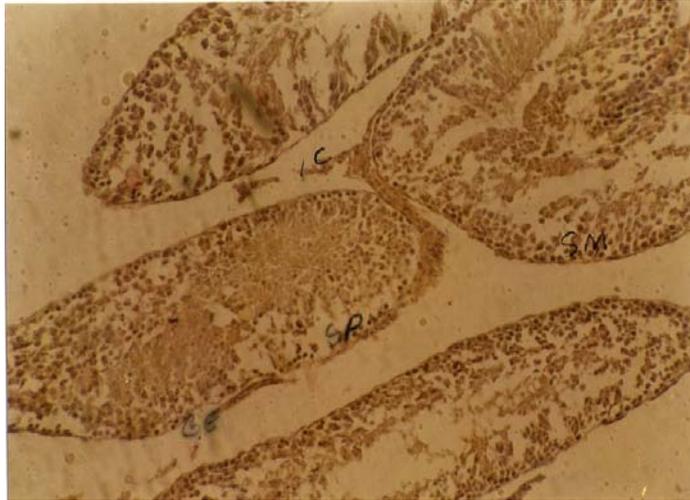


Fig. 4: (SP): Section of the testis sacrificed at the 15th day with disorganization of spermatogenic cells; (GE): degeneration of the germinal epithelium; (SM): slight vacuolation appearing between the epithelium and the spermatogenic cells and few matured spermatozoa H&E Mag. X100 (group II)

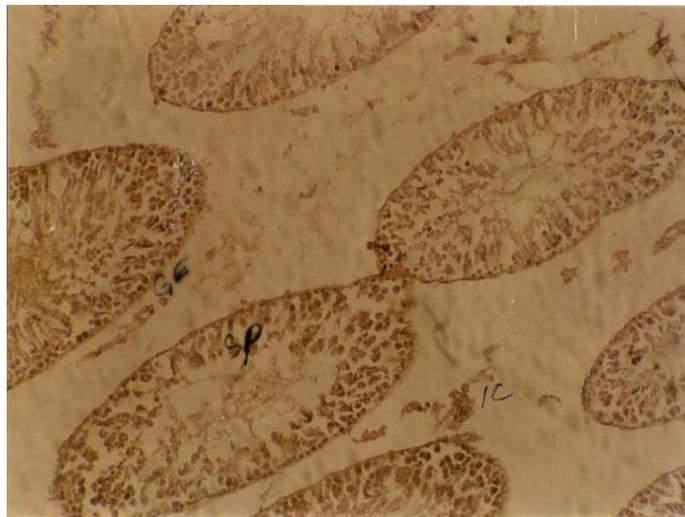


Fig. 5: (SM): Section of the testis, sacrificed at 15th day with absence of matured spermatozoa; (IC): the central lumen of seminiferous tubules are occupied by immature cells; (GE): regeneration of the germinal epithelium; the bridging of gap between the epithelium and the spermatogenic cells H&E X100 (group III)

sacrificed at the 15th day (that is those wistar rats that were allowed to stay for seven more days after termination of administration of drugs; (Fig. 4 and 5). Based on our observations, we therefore conclude that although cotexcin administration could have negative effects on histology of the testes as shown in (Fig. 2 and 3); these negative effects were reversible (regenerated) one week after stoppage of administration (Fig. 4 and 5). This suggests that cotexcin could be safe, as indicated in its low toxicity, but It's prolong usage may be discouraged.

ACKNOWLEDGMENT

The authors wish to acknowledge the Authority of Ahmadu Bello University, Zaria, for given a conducive environment for this study.

REFERENCES

Aguwa, C.N., 1996. Therapeutic Basis of Clinical Pharmacology in the Tropics. 2nd Edn., Optimal Publishers Enugu, Nigeria, pp: 190-201.

- Alan, W., 2007. The Human Body as an Evolutionary Patchwork. Princeton.edu, Retrieved from: <http://richarddawkins.net/article,865>.
- Bouree, P., P.H. Taugourdeau and V. Ng-Anh, 1994. Malaria, Smithkline Beecham Publications, Brentford, Middle Sex, TW89BD, Great-Britain, pp: 1-40.
- Crane, J. and R. Scott, 2002. Eubalaena Glacialis. Animal Diversity Web. Retrieved from: <http://animaldiversity.ummz.umich.edu/site/accounts/information/Eubalaena-glacialis.html>.
- Edward, C.R.W., 1998. Davidson's Principal and Practice of Medicine, 17th Edn., Churchill Livingstone, pp: 151-155.
- Elaine, N.M., 2004. Human Anatomy and Physiology. 6th Edn., Retrieved from: <http://www.ehow.com/facts-5521847-function-anatomy-human-testes.html#ixzz1VsszSqbq>.
- Goodman, A.G., T.W. Rall, A.S. Nies and P. Taylor, 1985. Drugs used in the Chemotherapy Malaria. The Pharmacological Basis of Therapeutics. 7th Edn., Macmillan, pp: 978-995.
- Gurr, E., 1962. Staining Animal Tissue, Practical and Theoretical. 1st Edn., pp: 99, Leonard Hill Books Limited, London.
- Herfindal, E.T. and D.R. Gouley, 1996. Textbook of Therapeutics. Drugs and Disease Management. 3rd Edn., Williams and Wilkins company Baltimore, Maryland. U.S.A., 1244: 1005-1018.
- Michael, H.R. and P. Wojciech, 2006. Histology Text and Atlas. 5th Edn., Lippincott Williams & Wilkins.
- Rang, H.P. and M.M. Dale, 1994. Pharmacology. 4th Edn., ELBS Churchill Livingstone, pp: 116-120, 850-861.
- Romer, A.S. and T.S. Parsons, 1977. The Vertebrate Body. Philadelphia, Holt-Saunders International, PA, pp: 385-386.
- Scott, F.G., 2000. Developmental Biology Online Textbook, 6th Edn., Sinauer Associates, Inc. of Sunderland (MA).
- Skinner, M., R. McLachlan and W. Bremner, 1989. Stimulation of sertoli cell inhibin secretion by the testicular paracrine factor PModS. Mol. Cell. Endocrinol., 66(2): 239-249. Doi: 10.1016/0303-7207(89)90036-1.
- Wang, W., W. Yang and S.T. Micha, 2001. Efficiency of dihydroartemisinin melfloquine on acute uncomplicated falciform malaria. Chinese Med. J., 114(6): 612-613.
- White, N.J., 1994. Artemisinin. Trans. Roy. Soc. Trop. Med. Hyg., pp: 88.