



Journal of
**Pharmacology and
Toxicology**

ISSN 1816-496X



Academic
Journals Inc.

www.academicjournals.com

Histological Evaluation of the Rats Testis Following Administration of a Herbal Tea Mixture

M.B. Maina, S.H. Garba and T.W. Jacks
Department of Human Anatomy, College of Medical Sciences,
University of Maiduguri, Maiduguri, Nigeria

Abstract: This research was carried out as a preliminary study to determine the histological effect of a herbal tea mixture on the rat testis. A total of 25 adult male albino rats of the Wister strain were used, they were randomly divided into five groups of five rats each. Group I served as control, while rats in groups II-IV were administered 2, 4 and 8 g kg⁻¹ body weight of the herbal tea, respectively for 28 days. Rats in group V were administered 8 g kg⁻¹ of the herbal tea for 28 days and allowed to stay for 14 days post treatment to observe for reversibility, persistence or delayed occurrence of toxic effects. At the end of the experimental periods, the animals were sacrificed and the weights of the testes recorded, fixed and processed for routine histological technique. Administration of the herbal tea to rats showed a significant increase in body weights, but testicular weights were unaffected. Histological examination of the rat's testis revealed interstitial edema and congestion of blood vessels in the testes of the treated rats. Withdrawal of the herbal tea for 14 days showed a slight degree of recovery in the rats. These findings suggests that the histological organization of the testis can significantly be altered with continuous and increase use of the herbal tea mixture. Further studies to determine the effect of the tea on the morphometry, biometry and hormonal profile of the rat's testes following long term exposure will be useful.

Key words: Rats, testis, tea, interstitial edema, congestion

INTRODUCTION

The consumption of herbal tea has become a global tradition because of its numerous documented and undocumented health benefits. Primitively, tea was processed from the leaves of *Camellia sinensis* by the Chinese but today, it is consumed and processed from the mixture of different medicinal herbs widely around the world.

Most of the popular anti-lipidemic herbal tea used today are processed from the mixture of different herbs including; *Gynostemma pentaphyllum*, *Radix polygoni multiflori*, *Semen cassiae*, Green tea and *Folium nelumbinus*. These herbs have been shown from various studies to have both medicinal and toxic effect. *Radix polygoni multiflori* is one of the most important and widely used Chinese tonic herbs, it is a tonic for the vital organs and blood and it is used in the treatment of malaria, in clearing toxins from the blood and in relieving constipation. Research has also shown that *R. polygonum multiflori* significantly increase superoxide dismutase (SOD) activity and also inhibits b-monoamine oxides (b-MAO). *Cassiae seimen* helps to remove heat from the liver, improve vision, moisturizes the intestine and eases the bowels. Green tea which contains polyphenols helps in easing the effects of alcohol, act as a stimulant, cures blotchiness, quenches thirst, eliminates indigestion, cures beriberi disease, prevents fatigue and improves urinary and brain functions. *Folium nelumbinus* is believed to relieve summer heat, invigorate splenic functions and arrest bleeding by reducing heat in blood.

Corresponding Author: Sani Hyedima Garba, Department of Human Anatomy, College of Medical Sciences, University of Maiduguri, Nigeria Tel: 234-08054807002/234-080-235-489-52

Toxicity studies carried out on the effect of medicinal plants on the testis have demonstrated various degree of toxicity; chronic exposure to *Mancozeb* has demonstrated gonadal toxicity to male rats marked by biochemical and pathological changes in the gonads (Kackar *et al.*, 1997). Dietary (0.5%) level exposure to methylxanthines caffeine, theobromine or theophylline in 4-6 weeks old male rats have shown to cause severe testicular atrophy with oligospermatogenesis. Despite the various toxic effects of herbs on testicular activity other herbs have been reported to be beneficial e.g., the protective effects of *Hibiscus sabdariffa* (Rossele) and *Zingiber officinale* (ginger) against Cisplatin-induced reproductive toxicity in rats has also been reported by increasing the activities of testicular antioxidant enzymes and restoration of sperm motility (Amr and Hamza, 2006).

Because the consumption of herbal tea is increasing arising from the promises of great medicinal miracles made by sale representatives of the manufacturers of these products in Nigeria. This study was design to investigate the effect of the herbal tea mixture on the histology of the rat's testis.

MATERIALS AND METHODS

Materials

Haematoxylin, Eosin, potassium alum, sodium iodate, glycerol, ethanol, xylene, Canada balsam, egg albumin, acetic acid, formaldehyde, hot air oven, microtome, hot plate, L-mould and plate, Vanox-T Olympus microscope, sample bottles, dissecting sets, wooden chucks, glass slides, cover slips, beakers, water bath, refrigerator, oven, dissecting board, paraffin wax, sodium bicarbonate and hydrochloric acid.

Test Article

The herbal tea was obtained from retail outlets located within Maiduguri metropolis, Maiduguri, Borno state. The herbal tea purchased contained ≥ 8.5 mg of gypnoside as its main ingredients with each bag weighing 1.5 g.

Preparation of Herbal Tea

A total of 2 g of the herbal tea was accurately weighed and emptied into a container containing 2 mL of distilled water. The mixture was allowed to soak for 15 min before it was administered to the rats.

Animal and Husbandry

This study was carried out in the Departments of Human Anatomy and Human Physiology, University of Maiduguri, Nigeria, between January and October, 2007. A total of 25 normal male Wister albino rats weighing between 120 and 200 g and 3-4 months of age were used for this study. They were purchased from the animal house unit of the Department of Pharmacology and Pharmaceutical Sciences, University of Jos, Plateau State, Nigeria. Following an acclimatization period of 2 weeks, the rats were individually identified by colour tattoo and weighed. The rats were kept in plastic cages at room temperature with 12 h light/dark cycle. They had access to standard laboratory diet (Sanders Nigeria Limited, Kaduna) and drinking water *ad libitum*.

Experimental Design

This study was conducted according to the US Environmental protection Agency Health effects test guidelines for repeated dose 28-day oral toxicity study in rodents. The herbal tea mixture was orally administered daily. The 25 rats were weighed and randomly divided into 5 groups of 5 rats per

dosage group (I-V). Group I served as control and were administered distilled water, groups II, III and IV were administered 2, 4 and 8 g kg⁻¹ of the herbal tea, respectively for 28 days while group V served as the recovery group and were administered the highest dose (8 g kg⁻¹) of the herbal tea for 28 days and allowed 14 days post treatment to observe for reversibility, persistence or delayed occurrence of toxic effects. At the end of the experimental period, body weights of all rats were taken and recorded. The rats were then sacrificed and the testis obtained was cleaned of any adherent tissue, the wet weight taken and preserved in Bouin's fluid for routine histological processing and subsequent histopathological examination.

Histological Analysis

The testes obtained were carefully dissected out, weighed, fixed in Bouin's fluid and embedded in Paraffin. The testicular tissues were then sectioned at 5 µm and stained with Haematoxylin and Eosin and mounted in Canada balsam. Light microscopic examination of the sections was then carried out.

Statistical Analysis

Numerical data obtained from the study were expressed as the Mean±SD. Differences among means of control and treated groups were determined using statistical package (GraphPad Instat). A probability level of less than 5% (p<0.05) was considered significant.

RESULTS

Effect of Herbal Tea on Body and Testicular Weights

Statistical analysis of body weights recorded in this showed an increase in the body weights of all the rats administered with the herbal tea but significant increases (p<0.05) were only observed in the body weight of rats administered with 2 and 4 g kg⁻¹ of the herbal tea. The testicular weights of all the rats in all the groups showed no significant difference between the rats in the control group and the treated ones (Table 1).

Histological Findings

Light microscopic study of the sections obtained from the rats in the control group showed normal histological arrangements of blood vessels, Leydig cells and a seminiferous tubule with normal lumen (Fig. 1). Administration of 2, 4 and 8 g kg⁻¹ of the herbal tea mixture for 28 days to the rats showed testicular sections having mild/severe interstitial edema, congestion of blood vessels within the testis, seminiferous tubules that contained distorted germ cells and some aggregations of spermatogenic cells within the lumen (Fig. 2-4) with the severity increasing with the doses administered. The testes

Table 1: Effect of the administration of herbal tea mixture on the rat's testicular and body weights

Groups	Doses of extract (g kg ⁻¹)	Weight of left testis (mg)	Weight of right testis (mg)	Initial body weight (g)	Final body weight (g)	Body weight difference (g)	Weight change (%)
I	0	1.22±0.01	1.22±0.02	135.7±00.91	184.2±03.25*	48.5	26.33
II	2	1.25±0.08	1.26±0.08	169.8±18.03	231.5±10.61*	61.7	26.65
III	4	1.23±0.08	1.23±0.18	175.7±02.12	225.5±12.02*	49.8	22.08
IV	8	1.35±0.08	1.25±0.09	147.5±13.51	218.9±29.91	71.4	32.61
V	8 + 14 days post recovery	1.31±0.12	1.32±0.12	138.8±24.40	201.9±31.61	63.1	31.25

*Significant result with p≤0.05

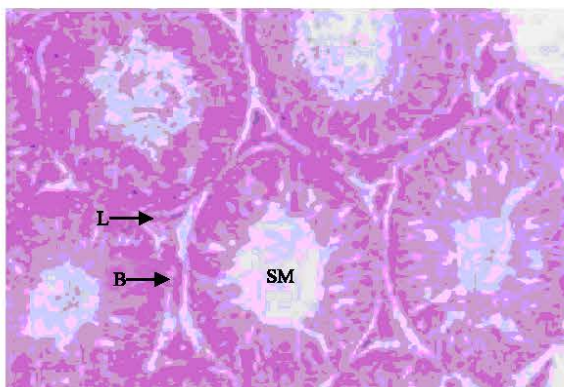


Fig. 1: Section of normal testis showing Blood vessels (B), Leydig cells (L) and seminiferous tubule (SM). H and E stain (Mag x 100)

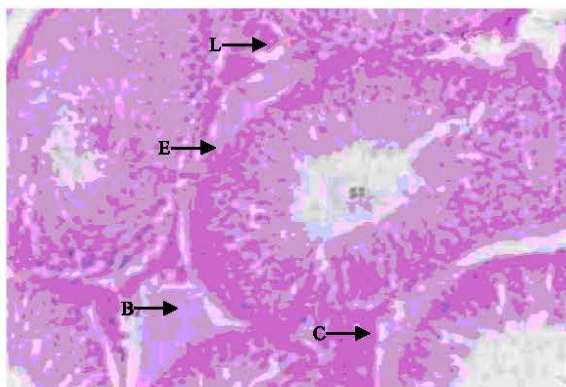


Fig. 2: Testis of a rat administered with 2 g kg⁻¹ of the herbal tea showing. Leydig cells (L), congestion of blood vessels (C), Edema (E) and seminiferous tubule (ST). H and E stain (Mag x 200)

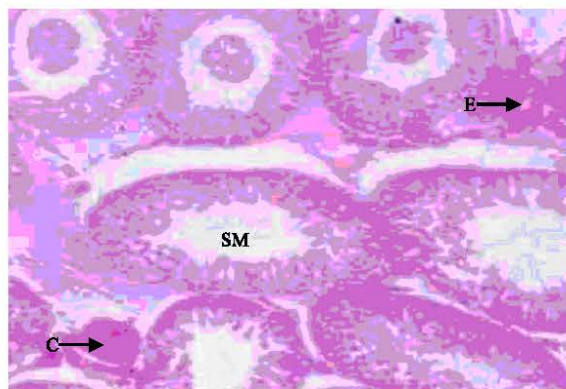


Fig. 3: Testis of a rat administered with 4 g kg⁻¹ Leydig cells (L), congestion of blood vessels (c), Edema (E) and seminiferous tubule (SM). H and E stain (Mag x 100)

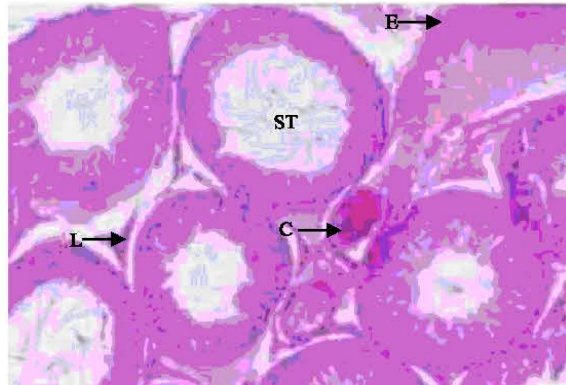


Fig. 4: Testis of a rat induced with 8 g kg^{-1} . Showing congestion of blood vessels (C), Edema (E) and seminiferous tubule (SM). H and E stain (Mag x 100)

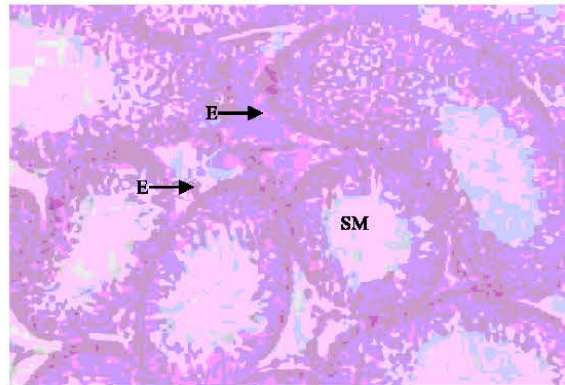


Fig. 5: Testis of a rat administered with 8 g kg^{-1} . Leydig cells (L), Edema (E) and seminiferous tubule (SM). H and E stain (Mag x 100)

of the rats administered 8 g kg^{-1} and allowed to stay for 14 days post treatment showed slight degree of recovery by the mild interstitial edema and distorted germ cells observed when compared with the rats administered 8 g kg^{-1} (Fig. 5).

DISCUSSION

The present study was undertaken to assess the possible effects of the administration of an herbal tea mixture containing *Gynostemma pentaphyllum*, *Radix polygona multiflora*, *Semen cassiae*, Green tea and *Folium nelumbinus* for 28 days, on the rat testes. Administration of the herbal tea mixture at a lower dose of 2 g kg^{-1} did not alter the body weight while higher doses of 4 and 8 g kg^{-1} caused a significant increase in the body weights of the rats. The lack of effect on body weight at 2 g kg^{-1} agrees with a similar study of Kao *et al.* (2000a), where, (-) Epigallocatechin-3-gallate a major component of green tea was proved to be the main chemical involved in the reduction of food intake leading to a reduction in body weight. The increase in the body weights observed in rats treated with 4 and 8 g kg^{-1} of the herbal tea mixture is in disagreement with results obtained from other studies

indicating one of the component tea (green tea) for the maintenance of appropriate body weight (Kao *et al.*, 2000b). Testicular weight or size generally establishes the normalcy of testes, enabling experimentally induced changes in testicular size to be assessed (França and Russell, 1998). The results show that the herbal tea did not alter weights of the testes showing the normalcy of the testis but histological examination proved testicular toxicity. The testis is an extremely heterogeneous organ, containing numerous compartments and cell types and mainly consists of the seminiferous tubules in which spermatogenesis takes place and the space between the tubules, the interstitium, which has, among others, an endocrine function (Kim and Yang, 1999).

Normal testis weight varies only modestly within a given test species (Schwetz *et al.*, 1980; Blazak *et al.*, 1985). This relatively low inter animal variability suggests that absolute testis weight should be a precise indicator of gonadal injury. However, damage to the testes may be detected as a weight change only at doses higher than those required to produce significant effects in other measures of gonadal status (Berndtson, 1977; Foote *et al.*, 1986; Ku *et al.*, 1993). This contradiction may arise from several factors, including a delay before cell deaths are reflected in a weight decrease (due to preceding edema and inflammation, cellular infiltration) or Leydig cell hyperplasia. Blockage of the efferent ducts by cells sloughed from the germinal epithelium or the efferent ducts themselves can lead to an increase in testis weight due to fluid accumulation (Hess *et al.*, 1991; Nakai *et al.*, 1993), an effect that could offset the effect of depletion of the germinal epithelium on testis weight. Thus, while testis weight measurements may not reflect certain adverse testicular effects and do not indicate the nature of an effect, a significant increase or decrease is indicative of an adverse effect.

Though testicular weights were not affected, histological examination showed testicular sections having mild/ severe interstitial edema, congestion of blood vessels within the testis, seminiferous tubules that contained distorted germ cells and some aggregations of spermatogenic cells within the lumen. Various studies have also reported the occurrence of interstitial testicular edema when various doses of the components constituting the herbal tea mixture were administered and most of the researchers linked the toxicity to factors such as testosterone (Maddocks and Sharpe, 1989), Human chorionic gonadotrophin (hCG) or endogenous LH (Bergh *et al.*, 1990), direct effect of GnRH agonists, vascular abnormality (Bergh *et al.*, 1988), inflammation, salt retention and lymphatic obstruction (Robbins *et al.*, 2004). Though this research did not look into such factors it may be imperative to mention that the factors mentioned above should not be ruled out as the causative factors. The vascular congestion observed in this study suggests that the herbal tea administered to the rats probably causes impaired venous out flow in the rat's testis and thus, resulted to congestion and interstitial edema. The effects observed could be said to be dose dependent, as the degree of the effects was seen to be progressive with an increase in the dosage administered. The effects observed could be said to be reversible since the rats in the recovery group sacrificed 14 days after receiving the herbal tea mixture showed very mild sign of recovery with mild interstitial edema as the predominant histological effect.

In conclusion, the findings revealed that the histological organization of the testis can significantly be altered with continuous and increase use of the herbal tea. But further studies to determine the effect of the tea on the morphometry, biometry and hormonal profile following long term exposure will be useful.

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of Ibrahim Wiam and Justus Jibrin of the Departments of Veterinary Anatomy and Human Pharmacology, University of Maiduguri, Nigeria.

REFERENCES

- Amr, A. and A.A. Hamza, 2006. Effects of roselle and ginger on cisplatin-induced reproductive toxicity in rats. *Asian J. Androl.*, 8: 607-612.
- Bergh, A., J.E. Damber and A. Widmark, 1988. Hormonal Control of Testicular Blood Flow, Microcirculation and Vascular Permeability. In: *The Molecular and Cellular Endocrinology of the Testes*, Serono Symposia, Cooke B.A. and R.M. Sharpe (Eds.). Raven Press, New York, pp: 123-134.
- Bergh, A., J.E. Damber and A. Widmark, 1990. A physiological increase in LH may influence vascular permeability in the rat testis. *J. Reprod. Fertil.*, 89: 23-31.
- Berndtson, W.E., 1977. Methods for quantifying mammalian spermatogenesis: A review. *J. Anim. Sci.*, 44: 818-833.
- Blazak, W.F., T.L. Ernst and B.E. Stewart, 1985. Potential indicators of reproductive toxicity, testicular sperm production and epididymal sperm number, transit time and motility in Fischer 344 rats. *Fundam. Applied Toxicol.*, 5: 1097-1103.
- Foote, R.H., E.C. Schermerhorn and M.E. Simkin, 1986. Measurement of semen quality, fertility and reproductive hormones to assess dibromochloropropane (DBCP) effects in live rabbits. *Fundam. Applied Toxicol.*, 6: 628-637.
- França, L.R. and L.D. Russell, 1998. The Testes of Domestic Animals. In: *Male Reproduction: A Multidisciplinary Overview*. Regadera, J. and Martinez-Garcia (Eds.). Churchill Livingstone, Madrid, pp: 197-219.
- Hess, R.A., B.J. Moore, J. Forrer, R.E. Linder and A.A. Abuel-Atta, 1991. The fungicide benomyl (methyl 1- (butylcarbamoyl)-2-benzimidazolecarbamate) causes testicular dysfunction by inducing the sloughing of germ cells and occlusion of efferent ductules. *Fundam. Applied Toxicol.*, 17: 733-745.
- Kackar, R., K. Srivastava Mithilesh and B. Raizada Rajenda, 1997. Indication of gonadal toxicity to male rats after chronic exposure to mancozeb. *Ind. Health*, 35: 104-111.
- Kao, Y.H., R.A. Hiipakka and S. Liao, 2000a. Modulation of endocrinesystems and food intake by green tea epigallocatechin gallate. *Endocrinol.*, 141: 980-987.
- Kao, Y.H., R.A. Hiipakka and S. Liao, 2000b. Modulation of obesity by a green tea catechin. *Am. J. Clin. Nutr.*, 72: 1232-1234.
- Kim, I. and H. Yang, 1999. Morphometric study of the testicular interstitium of rats during postnatal development. *The Korean J. Anat.*, 32: 849-858.
- Ku, W.W., R.E. Chapin, R.N. Wine and B.C. Gladen, 1993. Testicular toxicity of Boric Acid (BA): Relationship of dose to lesion development and recovery in the F344 rat. *Reprod. Toxicol.*, 7: 305-319.
- Maddocks, S. and R.M. Sharpe, 1989. Interstitial fluid volume in the rat testis androgen dependent regulation by the seminiferous tubules? *J. Endocrinol.*, 120: 215-222.
- Nakai, M., B.J. Moore and R.A. Hess, 1993. Epithelial reorganization and irregular growth following carbendazim-induced injury of the efferent ductules of the rat testis. *Anat. Rec.*, 235: 51-60.
- Robbins, L., Stanley and S. Cotran Ramzi, 2004. *Pathologic Basis of Disease: Hemodynamic disorders, Thromboembolic Disease and Shock*. 7th Edn., Saunders, Philadelphia, Pennsylvania, pp: 120-124.
- Schwetz, B.A., K.S. Rao and C.N. Park, 1980. Insensitivity of tests for reproductive problems. *J. Environ. Pathol. Toxicol.*, 3: 81-98.