Testicular Toxicity of Rinbacin in Rats

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Rinbacin is a local Nigerian herbal remedy. The effects of rinbacin on testicular histology were studied in prepubertal rats. Sexually immature male rats, divided into seven per group, were given rinbacin in drinking waters at 0, 26.25 g/l, or 52.50 g/l for 13 weeks, after which the animals were killed and testes excised, weighed, and processed for histologic study. The epididymal sperm number (ESN) was determined. There were no significant effects of either the low or high doses of rinbacin on fluid intake, body weight, testicular weight, and testis-body weight ratio. There was, however, a significant ($p < 0.05$) decrease in the ESN of animals at both doses of rinbacin. Histologic examination of the testes indicated that the high dose of rinbacin induced significant degenerative changes, while the low dose had only a mild effect on testicular histology. Rinbacin decreases the ESN and causes degenerative lesions, especially at the high dose, in prepubertal rats.

Key words rinbacin; testicular toxicity; epididymal sperm number; seminiferous tubule

The testis is the main organ of male reproduction and is suspended in the bilateral compartments of the scrotal sac. Production of spermatozoa from stem cells of the testis is a complex process that requires about 5 weeks in mice and 11 weeks in humans. Chemicals can disturb normal spermatogenesis by direct interaction with target cells within the testis itself or indirectly by interfering with hormonal stimulation or alteration in blood supply.¹⁾

Toxic substances that can damage the cells of the various organs of reproduction and/or impair the hormonal responses affect the quality and/or quantity of sperm cells. The issue of testicular toxicity is of growing concern, and reports from various parts of Europe and the U.S.A. suggest that testicular toxicity may be increasing. Carlsen and coworkers,²⁾ published a meta-analysis of data from the international literature which revealed a significant decrease in sperm concentration and semen volume in otherwise healthy men over the period 1938–1990. Carter *et al.*³⁾ exposed male rats to benomyl, an agricultural antifungal that is metabolized to carben-dazin (a toxic metabolite) and found the pesticide to produce testicular lesions. Many other drugs have been found to produce similar testicular toxicities.

Rinbacin is a registered herbal medicine in Nigeria (registration number 016283). According to one homeopath (personal communication) it consists of 48% roots, 18% seeds, 22% leaves, and 12% flowers. It is claimed to be useful in the management of various disease conditions including bacterial infections, abdominal and menstrual pains, hemorrhoids, diabetes, and miscarriage (unreported data). However, the potential toxicologic actions of this drug have not been reported. Physicians have an increasing interest in understanding the therapeutic and safety aspects of the medicinal herbs that their patients may be using,⁴⁾ and hence there is a need for these studies.

Cases of reproductive failure after prolonged intake of herbal preparations have been anecdotally reported in Nigeria. An increasing number of cases remain undocumented due to poor record keeping in the developing world.⁵⁾ Since rinbacin is widely used in Nigeria because of its wide range of acclaimed pharmacologic properties, it is feared that the

high doses frequently used may be implicated in some undocumented cases of reproductive failure. This study evaluates the testicular actions of rinbacin in sexually immature rats at two doses.

MATERIALS AND METHODS

Extraction Nigerian homeopaths dispense rinbacin in sachets of 5.25 and 10.50 g to be administered in 200 ml of water. Dried samples of rinbacin obtained from a dispensing herbal home in Nnewi, Nigeria, in sachets weighing 5.25 and 10.50 g were extracted in 200 ml of distilled water at a temperature of $22 \pm 3^\circ\text{C}$. The conical flask used for the extraction was covered tightly for 24 h, after which the amber-colored supernatant was filtered off on the second day and refrigerated until use. The extract was administered to rats at doses of 26.25 g/l (low dose) and 52.50 g/l (high dose).

Phytochemical Analysis Tests for the chemical constituents alkaloids, flavonoids, essential oils, saponin, sugar, protein, and lipid were carried out using the methods of Trease and Evans,⁶⁾ and Odebiyi and Sofowara.⁷⁾

Animals Twenty-one male albino rats obtained from the Toxicology Unit of the Department of Pharmacology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, were used for this study. The animals ranged in age from 3 to 5 weeks, and weighed between 36 and 42 g. The rats were housed under standard housing conditions at a temperature of $22 \pm 3^\circ\text{C}$ and a 12-h light/12-h dark cycle. The animals were housed singly, and provided with water and feed (rat pellets, Pfizer Pharmaceuticals PLC, Ikeja, Nigeria) *ad libitum*.

Determination of LD₅₀ The acute toxicity study was carried out using the method of Litchfield and Wilcoxon.⁸⁾ Seven groups of 10 rats each were administered 100, 200, 400, 800, 1600, 3200, and 6400 mg/kg of the extract intraperitoneally, and the number of deaths per group observed for 24 h. The LD₅₀ was calculated from the graph of percentage mortality (converted to probit) against the log dose of the extract. The antilog of the corresponding log dose of probit 5 was chosen as the LD₅₀. The Department of Pharmacology

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Table 1. Mean Daily Fluid Intake, Body Weight, Testis Weight, and Testis-Body Weight (TBW) Ratio in Drug-Exposed Rats

Treatment	Fluid intake (ml/rat/d)	Initial body weight (g)	Final body weight (g)	Testis weight (g)	TBW ratio (g/kg)
Control	31.47 $\pm$ 3.57	40.16 $\pm$ 1.10	180.44 $\pm$ 6.92	1.93 $\pm$ 0.09	10.78 $\pm$ 0.64
Low dose	26.69 $\pm$ 2.99	36.51 $\pm$ 3.51	179.23 $\pm$ 3.93	1.93 $\pm$ 0.16	10.80 $\pm$ 0.90
High dose	25.16 $\pm$ 2.74	41.89 $\pm$ 1.81	179.26 $\pm$ 8.11	2.11 $\pm$ 0.10	11.94 $\pm$ 0.81

Values are expressed as mean $\pm$ S.E.M. for groups of 7 rats.

Ethical Committee approved the study.

Experiment Three weight-matched experimental groups of seven animals each were used for the study. The first group received 26.25 g/l (low dose) and the second group 52.50 g/l of the rinbacin extract (high dose) in drinking water, while the third group was given distilled water only (control), for a period of 13 weeks. The volume of fluid ingested by the animals was measured daily, and the body weights were measured weekly.

At the end of the experimental period, the animals were weighed, killed under chloroform anesthesia, and the testes weighed. The epididymal sperm number (ESN) was counted in each of the animals after quadruplicate aliquots were obtained from each sample. Sperm was removed from the epididymis by a modification of the method described by Blazak *et al.*⁹⁾ The epididymis was minced and placed into a blender containing sodium bicarbonate-formalin diluting fluid,¹⁰⁾ and homogenized for 5 min. The sperm was counted using a hemocytometer.

Histology The testes were harvested, fixed in Bouin's fluid for at least 48 h, processed by the paraffin wax impregnation method with an automatic tissue processor, and sections (5 μ m thick) were cut using a rotary microtome. The sections were stained with hematoxylin and eosin (H & E), and examined by light microscopy. Photomicrographs of sections were taken.

Statistical Analysis Fluid intake, animals body weights, testicular weights, testis-body weight ratios, and ESN were analyzed using one-way analysis of variance (ANOVA), while differences between treatment groups were tested using the Scheffe multiple comparison method,¹¹⁾ with 0.05 representing the level of significance.

RESULTS

The drug extract gave positive reactions to alkaloids, flavonoids, and essential oils, in a ratio of 3:2:1. The intraperitoneal LD₅₀ of the extract was determined to be 3.18 g/kg.

There were no significant differences in body weights at the end of the experimental period among the three rat groups (Table 1). All the animals showed a progressive increase in body weight over the course of the experiment. The volume of fluid ingested during the experimental period was similar among the three groups (Table 1). Testis wet weight and relative weight were statistically equal in the control and rinbacin groups (Table 1). The mean ESN showed a dose-dependent decrease with rinbacin administration (Fig. 1).

Figure 2 shows photomicrographs of testes from the different experimental groups. The control group demonstrated normal testicular histology with a high population of interstitial cells (Fig. 2A). Testes from the low-dose rinbacin group

Fig. 1. Mean ESN of Drug-Exposed Rats

Values are expressed as mean $\pm$ S.E.M. for groups of 7 rats. * Significantly decreased compared to control ($p < 0.05$). ** Significantly decreased compared to both control and low-dose group ($p < 0.05$).

Fig. 2. Photomicrographs of Rat Testis Stained by H&E

(A) Control with normal testicular structure, (B) low-dose group rat testis showing mild edema and hyperemia, (C) high-dose group rat testis demonstrating severe edema, necrosis, and spermatogenic arrest. Magnification $\times 200$.

showed a slight change from the normal histologic features (Fig. 2B), with mild edema and hyperemia, and an appreciable population of interstitial cells. There was no tubular damage, disintegration of spermatocytes, necrosis, or spermatogenic arrest; spermatogenesis was evident in most tubules (Fig. 2B). Testicular sections from the high-dose rinbacin group showed severe edema and hyperemia (Fig. 2C). Necrosis and spermatogenic arrest were evident in some seminiferous tubules. The interstitial cell population was markedly reduced, and there were indications of reduced spermatogenesis in some of the tubules (Fig. 2C). Other organs were grossly normal at necropsy.

DISCUSSION

Rinbacin in drinking water did not produce significant effects on fluid ingestion, body weight, or testicular weight. In spite of the absence of effect of rinbacin on testicular weight, both the low- and high-dose levels of the drug significantly reduced mean ESN. The human male has a relatively low sperm count; the number of sperm per ejaculate is typically only between 2- and 4-fold higher than that at which fertility is significantly impaired. In contrast, the number of sperm in a rat or rabbit ejaculate is many times (up to 1000-fold) that which will produce maximum fertility. The epididymal sperm count can be reduced by as much as 90% in the rat without significantly affecting fertility. Thus a reduction in sperm concentration that did not alter rat fertility might have an important effect on human fertility.

Drugs that affect the testicular functions affect the quality and quantity of spermatozoa. Certain characteristics make the testis a target organ of toxicity for drugs, chemicals, and metals: 1) its rapidly growing and dividing tissues allow drugs (for example anticancer drugs) to induce specific testicular damage; 2) its limited supply of blood means that some metals can cause ischemic damage by reducing blood flow; and 3) specific and specialized biochemical pathways, like the utilization of lactate as a source of energy, may constitute an avenue for chemical toxicity or interference in testicular functions.

Medicinal herbs contain a variety of chemicals that are toxic when consumed at high doses, although little is known about their toxicity. Even in the presence of balanced endocrine secretions to maintain the size and integrity of the reproductive tissues, many parts of the male reproductive system are very sensitive to and adversely affected by medicinal plants.¹²⁾ Chronic administration of rinbacin at the dose of 52.50 g/l in rats resulted in pathological changes in the testicular structure, which included necrosis, edema, and hyper-

emia with a concomitant reduction in the interstitial cell population. These changes were associated with spermatogenic arrest in some of the tubules.

Lim and Miller¹³⁾ found that carbendazin, a metabolite of benomyl, produced different testicular pathologic changes in prepubertal (young) and pubertal (mature) rats. Intraperitoneal carbendazin caused sloughing of seminiferous tubular epithelium in adult rats, with little or no damage in young rats. The current study evaluated the testicular toxicity of rinbacin in prepubertal rats. Further studies using adult rats are necessary to ascertain whether the testicular toxicity of rinbacin is age dependent.

From these findings, rinbacin induces testicular damage at high doses, which causes a reduction in ESN in rats. There is ample possibility that one or more of the chemical constituents cause the testicular toxicity. It would be interesting to isolate this culprit moiety, and characterize it with a view to elucidating the mechanism of testicular toxicity. A reduction in sperm count is known to affect fertility more in human than in other animals. On the other hand, some agents associated with widespread testicular damage in rats have been known to be nontoxic in human models due to rapid biotransformation. An epidemiological study on men taking rinbacin is necessary to clarify the effects of this preparation on human testis.

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