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Effect of Quinine on Reproductive Parameters in Male Wistar Rats

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Abstract

Objective: This study was designed to investigate the effect of quinine on reproductive parameters in male Wistar rats.
Methods: Ten male Wistar rats (120 - 140 g) were divided into control (distilled water) and quinine-treated (8.57 mg/kg) groups (5 per group) for hormonal assay, andrological and histopathological studies. The animals were orally treated on daily basis for 50 days. Plasma testosterone level was assayed using Enzyme-linked Immuno-sorbent Assay (ELISA) and semen analysis was done microscopically. Histology of testes was also done. Data were analysed using descriptive statistics and student's t-test at p=0.05. .
Results: Treatment of rats with quinine (8.57 mg/kg) caused no significant ($p>0.05$) change in testosterone level relative to control. Treatment of rats with quinine (8.57 mg/kg) caused no significant ($p>0.05$) reduction in progressive sperm motility, but induced significant ($p<0.05$) reduction sperm count relative to their respective controls.
Conclusion: It can therefore be concluded that quinine probably has a mild deleterious effect on the reproductive function in male rats.

Keywords: Quinine, Rats, Sperm motility, Sperm count, Testosterone.

INTRODUCTION

Quinine is an alkaloid, a naturally occurring chemical compound**.** It is a medication used to treat malaria and babesiosis. This includes the treatment of malaria due to *Plasmodium falciparum* that is resistant to chloroquine when artesunate is not available. [1] Quinine is also the ingredient in tonic water that gives it its bitter taste. [2]

Quinine has been reported to induce decrease in population and diameters of Purkinje cells in rats. [3] Its antiepileptic effect in PTZ model of seizure has been reported. [4] Quinine has been reported to control body weight gain without affecting food intake in male C57BL6 mice. [5] Its additive effect on antidepressant drugs in the forced swimming test in mice has been reported. [6] It has also been reported to induce tinnitus [7] and delay ulcer healing [8] in rats.

However, due to scanty information from literature on the effect of quinine on reproductive parameters in male rats, this study therefore aims at investigating the effect of this antimalaria agent on these aforementioned parameters in male rats.

MATERIALS AND METHODS

Experimental Animals

Adult male rats weighing between 120 – 140 g bred in the Pre-Clinical Animal House of the College of Medicine and Health Sciences, Afe Babalola University were used. They were housed under standard laboratory conditions and had free access to feed and water; they were acclimatized for two weeks to laboratory conditions before the commencement of the experiments. All experiments were carried out in compliance with the recommendations of Afe Babalola University Ethics Committee on guiding principles on care and use of animals.

Drug

Quinine sulphate tablets (Dupen Laboratories, Ltd.) were bought from Danax Pharmacy, Ibadan, Nigeria.

Quinine sulphate (300 mg) was dissolved in 10 mL of distilled water to give a concentration of 30 mg/mL.

The dosage of quinine used in this study was in accordance with that reported by the manufacturer.

Body Weight

Body weight was monitored on weekly basis throughout the duration of the experiment for each rat.

Experimental Design

Ten male rats (120 – 140 g) were randomly divided into two groups, with each consisting of five animals. The two groups were subjected to the following oral treatments once a day for fifty (50) days:

Group I: received 0.5 mL/100 g of distilled water as control group.

Group II: received 8.57 mg/kg of quinine.

Collection of Blood Samples

Twenty four hours (day 51) after the last dosing of the groups, blood samples were collected from all the animals through the medial cantus for the determination of plasma testosterone levels. All the animals were later sacrificed by an overdose of diethyl ether and the testes were removed along with the epididymides for semen analysis.

Hormonal Assay

Plasma samples were assayed for testosterone using the enzyme-linked immunosorbent assay (ELISA) technique using the Fortress kit.

Semen Collection

The testes were removed along with the epididymides. The caudal epididymides were separated from the testes, blotted with filter papers and lacerated to collect the semen.

Semen Analysis

Progressive sperm motility: This was done immediately after the semen collection. Semen was squeezed from the caudal epididymis onto a pre-warmed microscope slide (27^{O}C) and two drops of warm 2.9 % sodium citrate was added, the slide was then covered with a warm cover slip and examined under the microscope using x400

magnification. Ten fields of the microscope were randomly selected and the sperm motility of 10 sperms was assessed on each field. Therefore, the motility of 100 sperms was assessed randomly. Sperms were labelled as motile, sluggish, or immotile. The percentage of motile sperms was defined as the number of motile sperms divided by the total number of counted sperms (i.e. 100). [9]

Sperm viability (Life/dead ratio): This was done by adding two drops of warm Eosin/Nigrosin stain to the semen on a pre-warmed slide, a uniform smear was then made and dried with air; the stained slide was immediately examined under the microscope using x400 magnification. The live sperm cells were unstained while the dead sperm cells absorbed the stain. The stained and unstained sperm were counted and the percentage was calculated. [10]

Sperm morphology: This was done by adding two drops of warm Walls and Ewas stain (Eosin/Nigrosin stain can also be used) to the semen on a pre-warmed slide, a uniform smear was then made and air-dried; the stained slide was immediately examined under the microscope using x400 magnification. [10] Five fields of the microscope were randomly selected and the types and number of abnormal spermatozoa were evaluated from the total number of spermatozoa in the five fields; the number of abnormal spermatozoa was expressed as a percentage of the total number of spermatozoa.

Sperm count: This was done by removing the caudal epididymis from the right testes and blotted with filter paper. The caudal epididymis was immersed in 5 mL formol-saline in a graduated test-tube and the volume of fluid displaced was taken as the volume of the epididymis. The caudal epididymis and the 5 mL formol-salline were then poured into a mortar and homogenized into a suspension from which the sperm count was carried out using the improved Neubauer haemocytometer under the microscope.

Testicular Histology

After removing the testes, they were immediately fixed in Bouin's fluid for 12 hours and the Bouin's fixative was washed from the samples with 70 % alcohol. The tissues were then cut in slabs of about 0.5 cm transversely and were dehydrated by passing through different grades of alcohol: 70 % alcohol for 2 hours, 95 % alcohol for 2 hours, 100 % alcohol for 2 hours, 100 % alcohol for 2 hours and finally 100 % alcohol for 2 hours. The tissues were then cleared to remove the alcohol, the clearing was done for 6 hours using xylene. The tissues were then infilterated in molten Paraffin wax for 2 hours in an oven at 57^{O}C, thereafter the tissues were embedded. Serial sections were cut using rotary microtone at 5 microns (5 μm). The satisfactory ribbons were picked up from a water bath (50^{O} - 55^{O}C) with microscope slides that had been coated on one side with egg albumin as an adhesive and the slides were dried in an oven. Each section was deparaffinized in xylene for 1 minute before immersed in absolute alcohol for 1 minute and later in descending grades of alcohol for about 30 seconds each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 minutes. The slides were rinsed in water, then differentiated in 1 % acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 seconds and rinsed in water for a few seconds, before being immersed in 70 %, 90 % and twice in absolute alcohol for 30 seconds each to dehydrate the preparations. The preparations were cleared of alcohol by dipping them in xylene for 1 minute. Each slide was then cleaned, blotted and mounted with DPX and cover slip, and examined under the microscope. Photomicrographs were taken at x40, x100 and x400 magnifications

Statistical Analysis

The mean and standard error of mean (S.E.M.) were calculated for all values. Comparison between the control and the treated group was done using student's t-test. Differences were considered statistically significant at $p<0.05$.

RESULTS

Effect on Body weight

Treatment of rats for fifty days with quinine (8.57 mg/kg) produced no significant ($p>0.05$) changes in body weight throughout the duration of treatment (Figure 1).

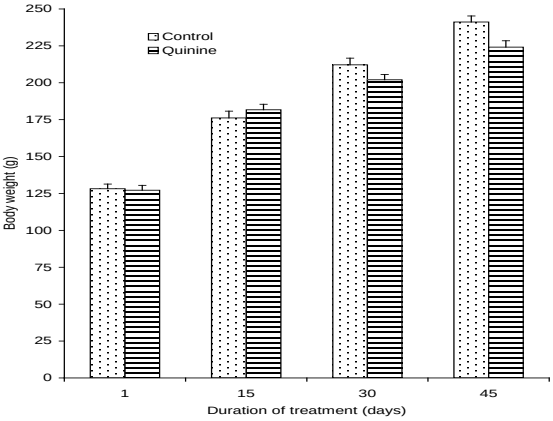

Figure 1: Body weight changes in control rats and rats treated with quinine for 50 days (n=5, *$p<0.05$)

Effect on Plasma Testosterone Level

reatment of rats for fifty days with quinine (8.57 mg/kg) produced no significant ($p>0.05$) change in testosterone level relative to control (Figure 2).

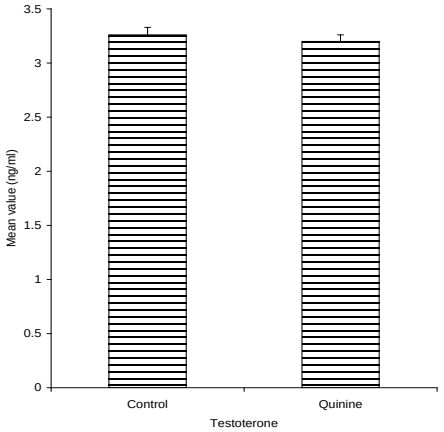

Figure 2: Effect of treatment of rats for 50 days with quinine on plasma testosterone level (n=5, *$p<0.5$)

Effect on Sperm Characteristics

The effect of 50 days treatment of rats with quinine (8.57 mg/kg) on sperm characteristics are shown in Figures 3 and 4.

Treatment of rats with quinine (8.57 mg/kg) for 50 days produced no significant ($p>0.05$) changes in progressive sperm motility, percentage of viable sperms, percentage of abnormal sperms, but induced significant ($p<0.05$) reduction in sperm count relative to their respective controls.

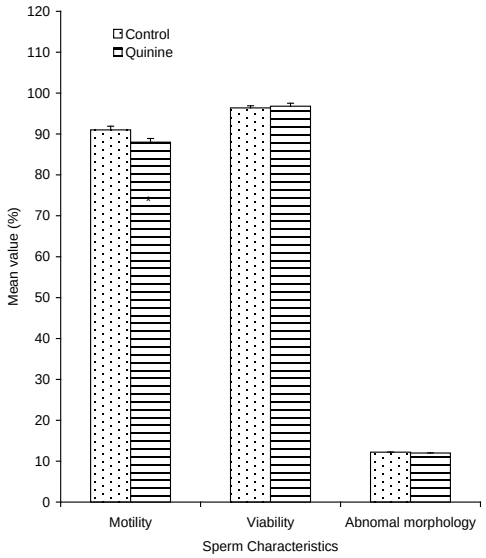

Figure 3: Spermogram showing the effect of 50 days treatment of rats with quinine on sperm characteristics (n=5, *p <0.05)

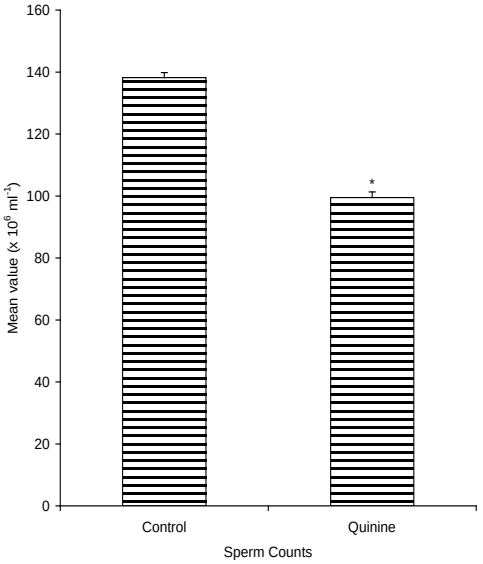

Figure 4: Spermogram showing the effect of 50 days treatment of rats with quinine on sperm count (n=5, *p<0.05)

Histopathological Effect

Figures 5 and 6 respectively show the transverse sections through the testes of control rat and rat treated with quinine (8.57 mg/kg) for fifty days.

Treatment of rats with quinine (8.57 mg/kg) caused no visible lesion in the testes of rats, which is similar to what was observed in the control rats.

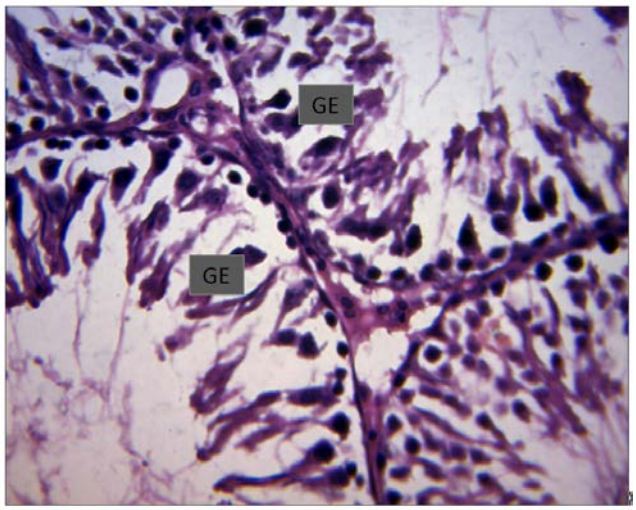

Figure 5: Effect of 50 days treatment of rat with distilled water (control) on rat's testis (×400). Photomicrograph showing normal viable germinal epithelium (GE) with no visible lesion seen.

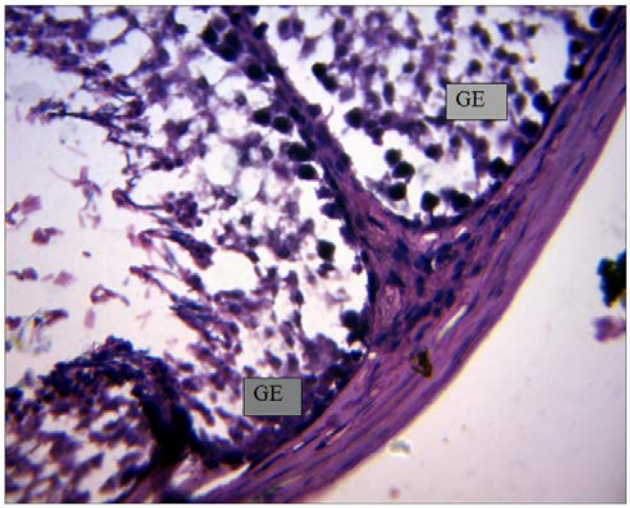

Figure 6: Effect of 50 days treatment of rat with quinine (8.57 mg/kg) on rat's testis (x400). Photomicrograph showing normal viable germinal epithelium (GE) with no visible lesion seen.

DISCUSSION

This study has shown that the treatment of rats with quinine caused no significant changes in body weight of rats. This suggests that quinine has no effect on the catabolism of lipids in the adipose tissue, resulting in insignificant changes in body weight. Contrary results were reported by [11] in *Persea americana* leaf extracts treated rats.

The drug caused no significant change in testosterone level. This probably indicates that quinine has no effect on the hypothalamus-pituitary-gonadal axis, since it has been reported that hypothalamus-pituitary-gonadal axis increases Leydig cell numbers and stimulates their

testosterone production through up-regulating LH. [12] Contrary report was given by [13] in rats treated with *Fumaria parviflora* leaf extract.

Quinine caused no significant change in sperm motility. This suggest that the drug has no effect on fertilizing capacity, since it has been reported that sperm motility is of importance with regard to sperm fertilizing capacity. [14] Contrary report was given by [15] in rats treated with *Pueraria tuberosa* root extract.

There was no significant change in sperm viability after treatment of rats with quinine, which probably indicates that the drug has no significant effect on fertility capacity, since it has been reported that generally, fertility capacity is positively correlated to percentage liveability of the sperm cells. [16] Contrary result was reported by [17] in isolated tetracyclic steroid treated rats.

There was no significant change in the percentage of morphologically abnormal sperm cells after treatment of rats with quinine, which probably indicates that the drug has no significant effect on fertility, since it has been reported that sperm morphology is an essential parameter that reflects the degree of normality and maturity of the sperm population in the ejaculate and correlates with fertility. [18] Defects of the head and mid-piece have been classified as primary defects of spermatogenesis [19], and arise during testicular degeneration. [20] Primary defects of spermatogenesis are more likely to be associated with decreased fertility. [19] Contrary result was reported by [21] in flavonoid Cuscutae treated rats.

Sperm count, motile sperm count and normal sperm morphology have been reported as indices of male fertility. [22] The decrease in sperm count induced by quinine in the treated rats could be an indication that this drug has the potential to alter the processes of spermatogenesis by causing damage to important testicular cells such as Sertoli cells. [23] Similar report was given by [24] in *Camellia sinensis* extract treated rats.

Photomicrographs revealed that rats treated with quinine presented with normal germinal epithelium with no visible lesion. This suggests that quinine has no toxic effect on the exocrine function of the testes at histological level. Similar result was reported by [25] in rats treated with Cadmium chloride.

CONCLUSION

In conclusion, this study has shown that quinine has a mild spermatotoxic or antispermatogenic effect in male rats, considering these findings in animal model, it is recommended that moderation should be exercised in the consumption of this drug by those taking it for therapeutic purpose.

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