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Quercetin protects against testicular toxicity induced by chronic administration of therapeutic dose of quinine sulfate in rats

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Abstract

Background: Quinine, a rapidly acting blood schizonticide with a long history of use for the treatment of malaria, is gradually been implicated in reproductive toxicity.
Methods: In this study, testicular and spermatotoxic effects of quinine sulfate (QS) following treatment with an oral dose of 10 mg/kg/day (normal therapeutic dose) for 8 weeks was investigated in male albino rats. Toxicity was evaluated by assessing antioxidant defense capacity and markers of oxidative stress and testicular dysfunction in the testes and epididymal sperm. The possible ameliorative effect of quercetin (QC), when co-administered with QS, was also assessed.
Results: Administration of QS induced oxidative stress in rats. The activities of superoxide dismutase, catalase, and malondialdehyde (a marker of lipid peroxidation) increased ($p<0.05$) both in the testes and epididymal sperm following QS treatment when compared with saline-treated (control) rats. Ascorbic acid levels were significantly reduced, with an insignificant decrease in glutathione and testosterone levels in the QS-treated rats, when compared with control. The spermiogram decreased with increase in total sperm abnormalities in QS-treated rats and was associated with histopathological changes. Our results revealed that QC significantly ameliorated QS-induced testicular toxicity and oxidative stress.
Conclusions: The testicular toxicity of QS is in part due to impairment of testicular antioxidant defense, spermatogenesis and enhancement of lipid peroxidation. Also, the ability of QC to reverse the deleterious effects of QS on the testes and epididymis qualifies it as a potent chemo-protective agent against QS-induced reproductive toxicity.

Keywords: oxidative stress; quercetin; quinine sulfate; testicular toxicity.

Introduction

There is no doubt that chemotherapy has provided immense benefits in the control of microbial and parasitic diseases. The biochemical difference that exists between the infecting micro-organism, and the human host, has made it possible to design drugs which are selectively toxic against the parasites while sparing the host cells. Despite this success, even the most effective antimicrobial or antiparasitic agent may cause unwanted lesions and toxicities. Several studies have revealed that many chemotherapeutic agents produce toxic adverse effects in the male reproductive organs (1–6). Chemotherapy-induced gonadal toxicities are related to the type of drugs used, the total dose and the duration of therapy (1, 7).

Quinine has a long history of use for the treatment of malaria and there are numerous reports on its toxicity in humans (8–13). In a recent study, Osinubi et al. (14) showed that short term administration of quinine sulfate (QS) caused a general destruction coupled with degeneration of cells of the seminiferous epithelium, thus suggesting possible disruption of spermatogenesis.

Oxidative stress has been implicated in the etiology of male infertility (15). Reports have indicated that spermatozoa are particularly susceptible to oxidative stress-induced damage, because their plasma membranes contain large quantities of PUFA (16) and their cytoplasm contains low concentrations of scavenging enzymes (17). Similar studies have also suggested oxidative stress-mediated damage to the sperm plasma membrane, may account for defective sperm function observed in a high proportion of infertility patients (18, 19). Oxidative stress attacks not only the fluidity of the sperm plasma membrane, but also the integrity of DNA in the sperm nucleus (20). Testicular damage following treatment with chemotherapeutic agents to oxidative damage has been reported (1–3, 21, 22). It is therefore reasonable to propose that drug-induced oxidative stress has a substantial role to play in reproductive health. In this

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present study, we investigated the effect chronic administration of a therapeutic dose of QS on antioxidant enzymes and markers of oxidative stress in testis and epididymal sperm. The possible ameliorative effect of quercetin (QC), a known antioxidant, was also determined in this model of oxidative stress.

Materials and methods

Drugs and chemicals

QS was obtained from Generics Limited (Mumbai, India), quercetin (3,3′,4′,5,7-pentahydroxy flavone) dehydrate (QC), thiobarbituric acid (TBA), tricholoroacetic acid (TCA), reduced glutathione (GSH), ascorbic acid (AA), bovine serum albumin (BSA), and Tris base were obtained from Sigma Chemical Co (London, UK). Enzyme immunoassay (EIA) kits for serum testosterone determination were obtained from Immunometrics (London, UK). All other chemicals and reagents were obtained from BDH laboratories Ltd (Poole, UK), Hopkins and Williams (UK).

Animals and treatment

Twenty-four male adult albino rats of the Wistar strain, weighing between 220 g and 240 g were obtained from the primate colony of the Department of Biochemistry, University of Ibadan. Rats were fed on commercial pelleted diet (Bendel Feeds Ltd, Ewu/Benin, Nigeria) and drinking water *ad libitum*, maintained under standard laboratory conditions and subjected to natural photoperiod of 12 h light/12 h dark cycle.

Rats were randomly assigned into three groups of eight rats/group. Rats in group 1 served as controls and received normal saline (0.7 mL/kg). Rats in group 2 were treated with QS (10 mg/kg body weight/day) for 8 weeks. QC (10 mg/kg body weight) was co-administered with QS (10 mg/kg body weight/day) for 8 weeks to rats in group 3. All treatments were given via the oral route.

Sample collection

Rats in all groups were fasted overnight, weighed and sacrificed 24 h after the last treatment by cervical dislocation. Testes, epididymis, seminal vesicles and ventral prostate were removed, cleared of adhering tissues, washed in 0.9% NaCl, blotted on filter paper and weighed. Both testes of each animal were used for subcellular fractionation, biochemical and histological studies.

Estimation of epididymal sperm count and motility

Epididymal sperm was obtained by mincing the epididymis in normal saline and filtering through a nylon mesh. The sperm concentration was determined using the improved Neuber hemocytometer after dilution in 0.9% NaCl (ratio 1:200). For the sperm motility assay, a small drop of semen was placed on a slide and the diluents (normal saline and buffered 2.9% sodium citrate solution) were added dropwise until the desired dilution was obtained. A cover slide was put on the smear of the sodium citrate diluted semen and was used for the estimation of spermatozoa motility. Observation was done at ×100 magnification within 2–4 min of their isolation from the epididymis and data were expressed as percentages.

Determination of daily sperm production and testicular sperm number

Daily sperm production (DSP) was determined using seven frozen left testes from control and treated rats according to Joyce et al. (23). Briefly, after the testes were removed and weighed, they were homogenized for 3 min in 25 mL of physiological saline containing 0.05% (v/v) Triton X-100. Sample aliquots (5.5 µL) were then placed on the hemocytometer and counted twice at ×100 magnification under the microscope, to determine the average number of spermatids/sample. These values were used to obtain the total number of spermatids/testis and this number was then divided by the testes weight to give spermatids/g of testes. Developing spermatids spend 4.61 days in rats. Thus, the values for the number of spermatids/testis were divided by 4.61 to obtain daily sperm production.

Sperm morphology and percentage viability assay

After sacrifice, the rat testicles were removed immediately and placed in well insulated ice box maintained at 0–4°C. The epididymis were trimmed off the body of the testes and the semen samples were collected from the caudal epididymis through an incision with a scalpel blade into the lumen and using two to four drops of 2.9% buffered fluid. Aliquots of sperm suspension were stained using Wells and Awa stains for morphological examination and 1% eosin B and 5% nigrosine in 3% sodium citrate dehydrate solution for live-dead ratio.

Biochemical assays

The testes and caudal epididymis were homogenized in 1.15% KCl (1:10 w/v) using a Potter-Elvehjem homogenizer. The resulting homogenate was first centrifuged at 2500 *g* for 10 min to remove nuclear fractions and later at 10,000 *g* for another 10 min, to obtain the mitochondrial fraction (MF) and post-mitochondrial supernatant (PMS) for biochemical estimations. These procedures were carried out at 4°C. The level of superoxide dismutase (SOD) activity was determined, based on the ability of this enzyme to inhibit the spontaneous oxidation of adrenaline to adrenochrome, as described by Misra and Fridovich (24). Catalase (CAT) activity was assessed according to the method of Clairborne (25). The assay was based on the ability of CAT to induce the disappearance of H_2O_2, which was followed spectrophotometrically. The level of reduced glutathione (GSH) was estimated according to the method described by Jollow et al. (26) and lipid peroxidation determined as malondialdehyde (MDA) using the procedure of Vashney and Kale (27). Ascorbic acid (AA) concentration was estimated according to the method of Jakota and Dani (28) and protein determination was by Lowry et al. (29).

Serum testosterone determination

Serum testosterone was measured by EIA, following manufacturer's instructions provided in the assay kits (Immunometics, London, UK).

Histopathology

Rat testes were fixed in Bouin's fluid, dehydrated in graded alcohol and embedded in paraffin. Fine sections were obtained, mounted on glass slides and counter-stained with hematoxylin & eosin (H & E) for light microscopic analyses.

Statistical analysis

All data were expressed as mean±standard deviation (SD). Differences between the groups were determined by one-way analysis of variance (ANOVA) and post hoc testing was performed for intergroup comparisons using Student's t-test. Values were regarded as significantly different at $p<0.05$.

Results

Antioxidant status in testes and epididymal sperm of quinine -treated rats

Table 1 shows the antioxidant status in testes and epididymal sperm of quinine-treated rats. The activity of SOD significantly ($p<0.05$) decreased in the mitochondrial fraction of testes of rats treated with QS, while the decreases observed in the PMS of the testes, as well as in the epididymal sperm, were not significant when compared with the control. The activity of CAT, on the other hand, significantly decreased both in the mitochondrial fraction and post-mitochondrial supernatant of the testes and also in the epididymal sperm in the QS treated rats. The QS-induced decrease in CAT activity was, however, significantly ameliorated by QC when co-administered both in the testes and epididymis. The preservation of SOD activity by QC, however, was only significant in the mitochondrial fraction of the testes of the QS-treated rats. Also, QS decreased GSH levels both in the testes and epididymis, but these values were not significantly different from control. Similarly, QC significantly prevented the QS-induced decrease in AA concentration in the mitochondrial fraction of the testes and epididymal sperm, but prevented GSH concentration non-significantly in these tissues. Administration of QS to rats significantly increased MDA levels, both in the testes, and the epididymal sperm, when compared with controls. Co-administration with QC significantly lowered QS-induced increase in MDA level.

Table 1 Effect of quercetin on testicular and sperm antioxidant systems in quinine-treated rats.

	Control	QS, 10 mg/kg	QS+QC, 10 mg/kg
SOD			
Testes (MF)	2.29±0.01	1.20±0.43[a]	2.20±0.51[b]
(PMS)	2.36±0.76	1.97±0.82	2.29±0.71
Epididymal sperm	2.29±0.42	1.72±0.62	1.96±0.71
CAT			
Testes (MF)	1.15±0.01	0.97±0.02[a]	1.11±0.3[b]
(PMS)	1.35±0.01	1.02±0.05[a]	1.35±0.03[b]
Epididymal sperm	1.43±0.02	1.21±0.01[a]	1.29±0.01[b]
GSH			
Testes (MF)	1.03±0.04	0.81±0.52	1.46±0.82
(PMS)	1.26±0.61	0.98±0.40	1.09±0.81
Epididymal sperm	0.97±0.03	0.71±0.62	0.96±0.77
Ascorbic acid			
Testes (MF)	0.21±0.02	0.17±0.03[a]	0.21±0.02[b]
(PMS)	0.71±0.02	0.43±0.01[a]	0.57±0.02
Epididymal sperm	0.22±0.02	0.15±0.03[a]	0.37±0.10[b]
MDA			
Testes[c] (MF)	28.5±13.4	64.5±16.3[a]	35.8±6.4[b]
(PMS)	13.3±2.5	58.8±13.3[a]	20.4±6.4[b]
Epididymal sperm[d]	23.8±4.7	50.7±8.2[a]	30.8±7.5[b]

The data are expressed as mean±SD for eight rats. [a]Values differ significantly from control ($p<0.05$). [b]Values differ significantly from QS alone ($p<0.05$). [c] and [d], MDA expressed as µmol/mg tissue and µmol/mg protein, respectively. MF, mitochondrial fraction; PMS, post mitochondrial supernatant. SOD activity (Units/mg protein), CAT activity (µmol H_2O_2 consumed/min/mg protein), GSH level (µmol/g tissue), ascorbic acid level (mg/g tissue).

Effect of quercetin on relative organ weights, spermiogram and testosterone level in quinine-treated rats

The relative weights of the testes and accessory sex organs, namely, the epididymis, seminal vesicles and prostate gland, are presented in Tables 2 and 3. There was no significant change between the relative weights of the testes, epididymis and prostate gland in the control and the QS-treated groups and between the QS-treated group and the QS plus QC-treated groups. QS, however, produced a significant ($p<0.05$) increase in the relative weight of the seminal vesicle when compared with the control. There were significant reductions in sperm count, sperm motility and live-dead count in the QS-treated rats, when compared with control. These values were significantly higher in the groups that received QS plus QC, when compared with the group that received QS only. Administration of QS to rats produced a slight decrease in plasma testosterone level, but significantly reduced the DSP and testicular sperm number (TSN) when compared with controls. QC significantly prevented QS-induced decreases in DSP and TSN, but not plasma testosterone levels in the rats. No significant tail abnormalities of sperm were recorded in the groups. QS-treated rats exhibited significantly higher bent tail abnormalities when compared with the control group. QC also ameliorated these QS-induced sperm abnormalities.

Table 2 Effect of quercetin on relative organ weights, spermiogram and testosterone level in quinine-treated rats.

	Control	QS, 10 mg/kg	QS+QC, 10 mg/kg
Testes, g/100 g	0.98±0.32	0.90±0.22	0.96±0.27
Epididymis, g/100 g	0.12±0.01	0.11±0.01	0.10±0.01
Seminal vesicle, g/100 g	0.31±0.07	0.44±0.02[a]	0.36±0.11
Prostate gland, g/100 g	0.17±0.06	0.25±0.07	0.20±0.03
Sperm count, %	85.6±4.61	55.2±7.21[a]	73.0±9.12[b]
Sperm motility, %	84.0±5.52	58.0±8.41[a]	76.0±6.71
Live-dead ratio, %	92.0±2.71	75.8±9.13[a]	90.6±6.82[b]
Abnormality, %	17.25±1.12	28.13±1.32[a]	22.85±0.83
TSN	50.35±2.61	29.12±3.21[a]	45.86±3.62[b]
DSP	8.92±1.62	4.62±1.51[a]	6.84±2.11[b]
Testosterone	5.21±0.87	4.98±0.76	5.10±1.01

The data are expressed as mean±SD for eight rats. [a]Values differ significantly from control ($p<0.05$). [b]Values differ significantly from QS alone ($p<0.05$). QS, quinine sulfate; QC, quercetin. TSN (testicular sperm number) as 10^6/g testis, DSP (daily sperm production) as 10^7/g testis and testosterone level is expressed as mol/L.

Table 3 Effect of quercetin on morphological characteristics of sperm cells in quinine-treated rats.

	Bent tail	Curved mid-piece	Bent mid-piece	Headless tail	Tailless head
Control	4.3±0.0	4.3±1.3	3.0±1.4	3.0±1.4	4.0±0.8
QS, 10 mg/kg	7.3±1.7[b]	7.0±0.8	5.5±1.9	4.0±1.2	4.8±1.5
QS+QC, 10 mg/kg	5.8±1.5	5.0±1.0	4.2±0.5	4.6±1.1	3.8±0.5

The data are expressed as mean±SD for eight rats. [a]Values differ significantly from control ($p<0.05$). [b]Values differ significantly from QS alone ($p<0.05$). QS, quinine sulfate; QC, quercetin.

Effect of treatments on testis histology

Representative photomicrographs of testes from quinine sulfate and quinine sulfate plus QC-treated animals are shown in Figure 1. The testes of control rats showed normal seminiferous tubules, with an adequate number of sperm cells. Histopathological changes observed in the testis of QS-treated rats included disruption of testicular basement membrane of seminiferous tubules and loss of spermatozoa. Sections of QS plus QC-treated testes revealed seminiferous tubules, with well organized structure and which were functionally active with many spermatozoa.

Discussion

QS has been implicated in reproductive toxicity (14, 30). Although, the mechanism remains unclear, it could be proposed, however, that one of the possible ways by which QS elicits a wide array of side effects, may be due to its ability to generate free radicals during metabolism by the liver and other tissues of the body. This is based on the fact that several studies have shown that exposure to a variety of pro-oxidants in vivo, has the propensity to induce significant oxidative damage in testicular tissues (31, 32), and various experimental models have described the implications of oxidative stress on testicular physiology and function (33–35). The present study, therefore, attempts to give an insight into the biochemical implications of dose and duration dependent administration of QS, vis-à-vis, the extent of oxidative damage in the testes and epididymal sperm in rats.

The results from this investigation agree with recent studies implicating quinine in reproductive toxicity and further suggest the involvement of oxidative stress in the testicular dysfunction induced by this drug. This toxicity or adverse effect was observed at normal therapeutic doses in rats treated for 8 weeks. We observed a decrease in the antioxidant defense system in the testicular tissues of rats treated with QS in this study. The enzymatic and non-enzymatic antioxidants are the natural defense against free radical mediated tissue damage in several organs including testes (36). Oxidative damage occurs when the production of reactive oxygen species (ROS) overwhelms the antioxidant defense mechanisms (37). ROS are key agents in the cytotoxic effects in spermatozoa; in addition to their direct effect on cellular constituents, they cause oxidative stress by decreasing the enzymatic defenses of the testes (38). In this present study, the activities of SOD and CAT were significantly decreased in the testes and epididymal sperm following QS administration in the rats. SOD, which spontaneously dismutates superoxide radical to H_2O_2 and molecular oxygen, is considered the first line of defense against the deleterious effect of oxyradicals in cells. The decrease in the activity of CAT may reflect the inability of the testes and epididymal sperm to eliminate H_2O_2 produced by the activation of QS and its metabolites. This may also be

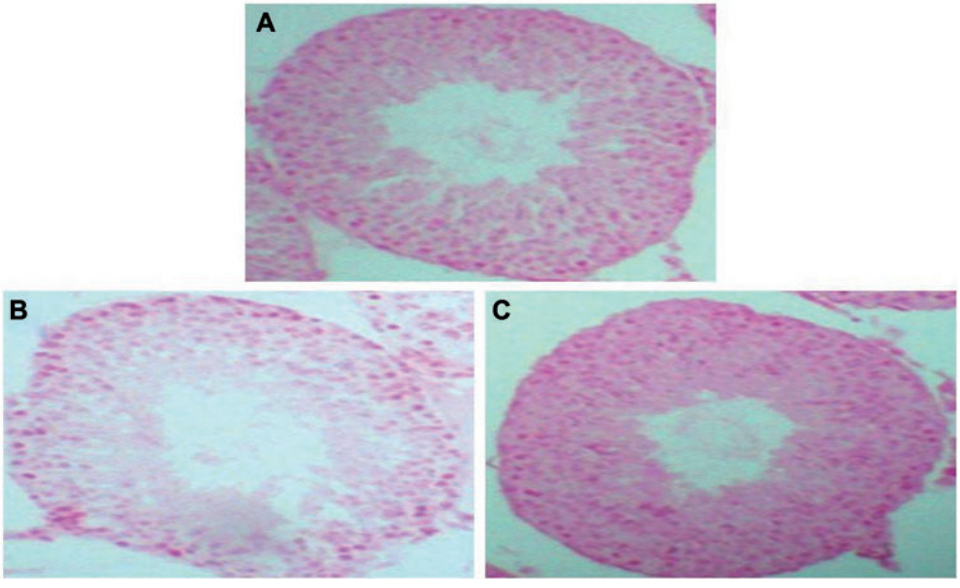

Figure 1 Photomicrographs of section (×250) of rat testes from: (A) Saline-treated (control) group with normal architecture and sperm cells; (B) quinine sulfate (10 mg/kg/day) treated rats for 8 weeks showing marked degeneration of seminiferous tubules; (C) quinine sulfate plus quercetin (10 mg/kg/day) treated group for 8 weeks showing seminiferous tubules with well organized structure and moderate number of spermatozoa.

due to enzyme inactivation caused by excess ROS production in the mitochondria and microsomes of the testes and spermatozoa (39). CAT protects SOD against inactivation by H_2O_2 (40, 41). The reduction in SOD activity, therefore, in the QS treated rats, may be as a result of the accumulation of H_2O_2. SOD level in spermatozoa positively correlates with sperm motility (42). The decreased activities of these antioxidant enzymes and the resultant accumulation of ROS and increased oxidative stress in the testes and epididymal sperm cells, account for the increase in lipid peroxidation and reduced sperm motility observed in this study. The increased MDA formation induced by QS was also associated with GSH depletion in the testicular tissues of the rats, although this was not statistically significant. GSH has been shown in rat sperm mitochondria to play a significant role in the peroxyl scavenging mechanism and in maintaining sperm motility (43); reductions in its level may have contributed to the reduced sperm motility and morphology in this study as also reported by Lenzi et al. (44).

The present study further reveals a significant reduction in the levels of AA in the testes and epididymal sperm following QS treatment for 8 weeks (10 mg/kg/day). The implication of this is a decrease in the ability of AA to effectively scavenge relevant ROS and RNS, as well as recycle α-tocopheryl radical back to α-tocopherol (45, 46). The ability of AA to also regenerate other smaller molecular antioxidants, such as GSH, water and β-carotene, from their respective radical species (47) is suppressed by QS. These may also account for the increased lipid peroxidation, decreased SOD and CAT activities and sperm motility recorded in this study.

Furthermore, QS-induced testicular toxicity was associated with mild decreases in serum testosterone levels. The values in the QS-treated group, though lower, were not significantly different from the control, and this is similar to that observed with cisplatin (4). Even though total testosterone levels appear not to be significantly affected, testosterone production may be reduced in rats with seminiferous tubular damage (8, 48). In this study, the administration of QS produced discernable histopathological alterations in the testes, with significant loss in spermatids and spermatozoa. The alteration in antioxidant status and subsequent accumulation of ROS, may lead to the destruction of seminiferous epithelium and the loss of germinal elements, resulting in a decrease in the DSP and evident by the significant decrease in caudal sperm count and TSN observed in the QS-treated rats in this study. The total number of sertoli cells accounts for most of the variability (<85%) in DSP in adult rats (49). Sperm motility was also significantly decreased, owing to increased peroxidative damage as mentioned earlier. The marked increase in the frequency of abnormal sperms among the QS-treated rats, also indicates a significant effect on sperm development. The decrease in the overall motility and progressive motility in the caudal epididymis was accompanied by decreases in some motion parameters. The sperm tail encompasses the structural components directly involved in sperm motility. In this study, head abnormalities (vacuolation), mid-piece and tail abnormalities and end-piece details (extension) were observed in the QS-treated groups. All these may also account for the decrease in live-dead counts. These histopathological alterations did not significantly affect the relative weights of the testes and other accessory organs, although they appear reduced.

The present study also investigated the protective effect of QC, a known antioxidant/flavonoid derived from certain plants foods (50–52) against the observed QS testicular toxicity. Co-administration of QC alongside QS treatment decreased lipid peroxidation products and improved sperm motility and morphology. QC treatment also exerted a significant sparring effect on the activities of SOD and CAT and prevented GSH and AA depletions. Thus, the decreased antioxidant defense system, observed in the QS treated rats, may be primarily due to free radical overproduction leading to oxidative stress.

In conclusion, we have explored the ability of duration-dependent treatment with QS to induce reproductive toxicity and subsequently ascertained the protective role of QC when co-administered. Since QS is still a widely utilized drug in humans for the treatment of malaria, we suggest that the prescription of antioxidants be considered alongside, since QS may deplete antioxidant stores. This would largely ameliorate QS-induced testicular damage and other reported toxicities associated with its use.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
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