Systems Biology in Reproductive Medicine

ISSN: 1939-6368 (Print) 1939-6376 (Online) Journal homepage: informahealthcare.com/journals/iaan20

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To cite this article: Sarwat Jahan, Natasha Iftikhar, Hizb Ullah, Gul Rukh & Ishtiaq Hussain (2015) Alleviative effect of quercetin on rat testis against arsenic: a histological and biochemical study, Systems Biology in Reproductive Medicine, 61:2, 89-95, DOI: 10.3109/19396368.2014.998350

To link to this article: https://doi.org/10.3109/19396368.2014.998350

Published online: 24 Dec 2014.

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Systems Biology in Reproductive Medicine

http://informahealthcare.com/aan
ISSN: 1939-6368 (print), 1939-6376 (electronic)

Syst Biol Reprod Med, 2015; 61(2): 89–95
© 2015 Informa Healthcare USA, Inc. DOI: 10.3109/19396368.2014.998350

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RESEARCH ARTICLE

Alleviative effect of quercetin on rat testis against arsenic: a histological and biochemical study

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Abstract

The preventive effect of quercetin on arsenic stimulated reproductive ailments in male Sprague Dawely (SD) rats was investigated. Twenty rats were divided into four groups. The first group served as a control and was provided tap water. The second group of rats was treated with sodium arsenite at the dose of 50 ppm in drinking water. The third group served as a positive control and received an oral dose of quercetin (50 mg/kg). In the fourth group, quercetin (50 mg/kg) was co-administered orally with arsenic (50 ppm in drinking water). All the treatments were carried out for 49 days. Arsenic treatment resulted in adverse morphological and histopathological changes in testis of rats including reduced epithelial height and tubular diameter, and increased luminal diameter. In contrast, these adverse effects of arsenic were eliminated by co-administration of quercetin. Additionally arsenic treatment significantly increased testicular thiobarbituric acid reactive substance (TBARS) levels while catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and glutathione reductase (GSR) activities, and plasma and intra-testicular testosterone concentrations, were decreased significantly. Lipid peroxidation (LPO) was significantly suppressed and depleted antioxidant defense mechanism was restored by the quercetin co-treatment. Also quercetin treatment resulted in a marked increase in plasma and testicular testosterone concentrations. On the basis of these findings, it was concluded that quercetin may be used as a potential therapeutic drug against arsenic induced reproductive toxicity.

Abbreviations: SD: Sprague Dawely; TBARS: thiobarbituric acid reactive substance; CAT: catalase; SOD: superoxide dismutase; POD: peroxidise; GSR: glutathione reductase; LPO: lipid peroxidation; ROS: reactive oxygen species; RNS: reactive nitrogen species; PKC: protein kinase C; GSH: glutathione; GPx: glutathione peroxidise

Keywords

Antioxidant enzymes, arsenic, intra-testicular, lipid peroxidation, quercetin

History

Received 18 May 2014
Revised 30 September 2014
Accepted 1 October 2014
Published online 24 December 2014

Introduction

Arsenic is a naturally occurring element that is present in food, soil, and water. In the environment and in the human body, arsenic is found in organic and inorganic compound forms. The major source of arsenic exposure to living organisms is drinking water (0.01–3.7 mg/l). Arsenic exerts its toxic effects by generating reactive oxygen species (ROS) and reactive nitrogen species (RNS) causing oxidative damage to lipids, proteins, and DNA in cells, leading to necrosis [Ahmad et al. 2000; Brochmoller et al. 2000; Garcia-Chavez et al. 2003; Iwama et al. 2001; Nandi et al. 2005].

Besides the toxicity caused by arsenic in other tissues, the effect of sodium arsenite on the male reproductive system has also been documented. Arsenic causes a reduction in testicular weight as well as a dose dependent reduction in accessory sex organ weight [Ahmad et al. 2008]. Exposure to arsenic leads to a reduction in sperm number [Fisch 2008; Jana et al. 2006], sperm viability and motility [Mukherjee and Mukhopadhyay 2009], as well as abnormal sperm morphology [Pant et al. 2004], disruption of DNA replication and repair [Abernathy et al. 1999; Duker et al. 2005; Styblo et al. 2002], and imbalance between antioxidant defense mechanism and free radical production, leading to oxidative stress [Honglian et al. 2004]. Germ cells treated with arsenic degenerate. Arsenic also inhibits androgen production, acting primarily at the level of the pituitary, inhibiting the release of LH and FSH through an estrogenic mode of action [Jana et al. 2006].

It is not possible to avoid exposure to arsenic because arsenic is present in soil, rocks, and natural water, and animals are exposed to arsenic toxicity through food and water resources. Research is actively pursuing the characterization of natural products which not only protect against toxicity caused by arsenic but also have health benefits. Quercetin, one of the flavonoids, is a potent oxygen free radical scavenger and is

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abundant in fruits and vegetables like apple, onion, mulberry, potatoe, broccoli, tea, peanut, soybean, and red wine [Morales et al. 2006; Wach et al. 2007]. Numerous *in vitro* studies have revealed the diverse biological effects and properties of quercetin. These include inducing apoptosis, antimutagenesis, and modulating cell cycle, while inhibiting protein kinase C (PKC), lipoxygenase, histamine–release, superoxide dismutase (SOD)-like activity, and angiogenesis and angiotensin converting enzyme II [Avila et al. 1994; Formica and Regelson 1995; Murakami et al. 2008; Yang et al. 2006]. Quercetin is perhaps beneficial for human health through the biological effects of its antioxidant activity [Terao and Piskula 1998]. Quercetin has stimulatory effects on sperm quality and reproductive organs in male rats [Taepongsorat et al. 2008]. The protective role of quercetin on reproduction in male animals is still controversial, perhaps because most of the studies have been carried out *in vitro* using cell lines [Tong-liang et al. 2012]. On the basis of available previous literature, it was hypothesized that quercetin may reduce arsenic induced reproductive toxicity within the testis. The main objective of the present study was to investigate the protective effect of quercetin against arsenic induced oxidative stress and histological damage in rat testis.

Results

Histology and morphometric considerations

Histological examination of the control group showed normal spermatogenesis and filled lumen with closely arranged seminiferous tubules. However in the arsenic treated group, the tubular diameter was reduced and interstitial space was wide with loose and dispersed cells arranged in the epithelium (Figure 1). Sloughing in the epithelial layer was also observed. In the arsenic plus quercetin treated group, no sign of arsenic toxicity was observed. The tubular lumen was filled with elongated spermatids and minimal to no sloughing was observed in the epithelium. Quercetin supplement alone showed more positive effects on spermatogenesis. Seminiferous epithelium was thicker, comparable to the control group (Figure 1).

A significant ($p < 0.001$) reduction in the thickness of the tunica albuginea was noted in the arsenic treated group compared to the control group. The thickness of the tunica albuginea was significantly higher ($p < 0.001$) in the arsenic plus quercetin treated animals, compared to the control and arsenic only treated groups. The quercetine only treatment group showed a significant ($p < 0.001$) increase in the thickness of tunica albuginea when compared to the control and arsenic only treated groups (Table 1).

The interstitial space increased ($p < 0.001$) significantly under arsenic toxicity compared to the control group. Quercetin treatment alone and in combination with arsenic showed positive effects. The interstitial space in the quercetin only and arsenic plus quercetin treated animals was significantly reduced ($p < 0.01$, $p < 0.001$, respectively) compared to the arsenic treated animals. The diameter of the seminiferous tubules and epithelial height in the arsenic treated group was

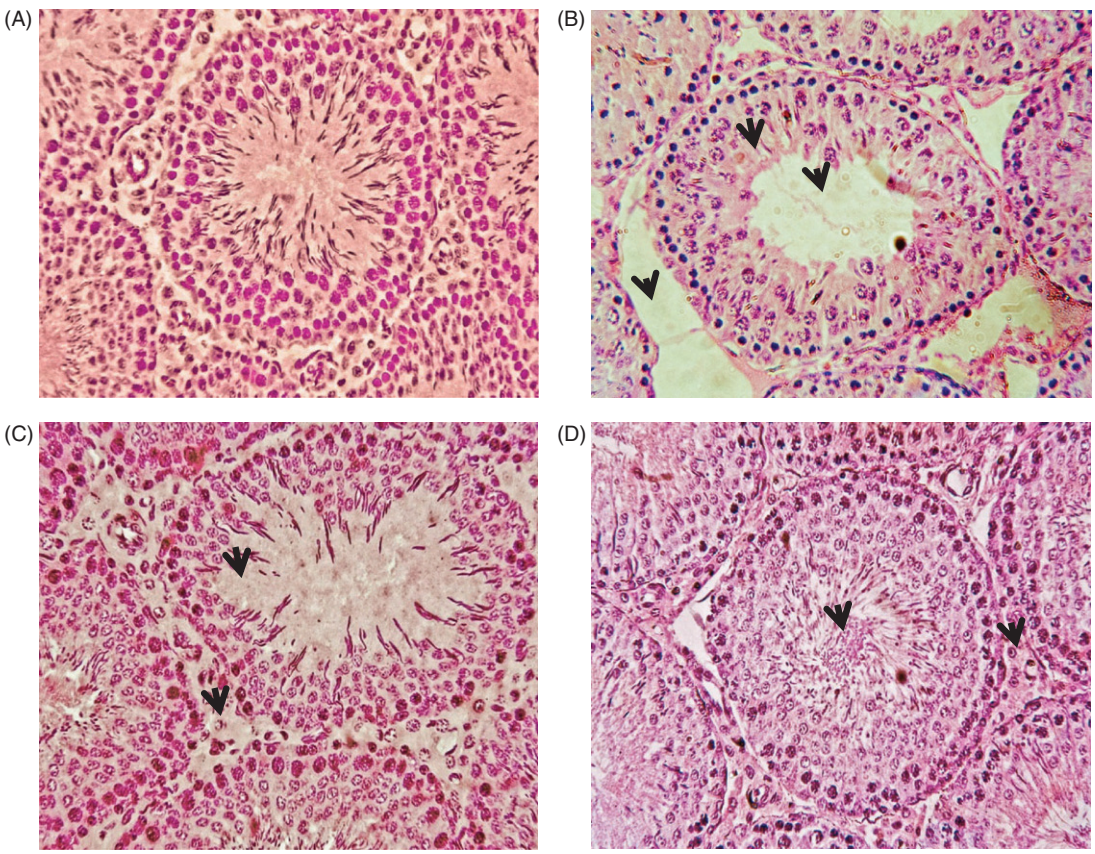

Figure 1. Photomicrograph of seminiferous tubules. (A) control, (B) arsenic treated showing tubules with empty lumen, sloughing of the epithelial layer (arrow) with scattered sertoli cells and degenerated interstitial space (arrow), (C) arsenic + quercetin showing seminiferous tubule with elongating spermatids with a little degeneration in interstitial space (arrow) and less sloughing in epithelial layer of cells (arrow), and (D) quercetin showing filled lumen and compact seminiferous tubules with less interstitial space. Magnification ×40.

also significantly reduced compared to the control group. Co-treatment with quercetin caused no significant reduction in the tubular diameter and epithelial height (Table 1). The tubular lumen was significantly higher ($p<0.001$) in the arsenic treated group as compared to the control and arsenic plus quercetin treated groups (Table 1).

Arsenic treatment yielded a significant reduction ($p<0.001$) in the number of spermatogonia, primary spermatocytes, secondary spermatocytes, and spermatids as well as leydig cells compared to the control group. The number of different cells in the arsenic plus quercetin treated group were similar to the control group. Cell count was also significantly high ($p<0.001$) in the quercetin only treated group as compared to the arsenic treated group (Table 2).

Biochemical analysis

The concentration of catalase (CAT) was unremarkable when the arsenic only treated group was compared to the control group. However, a significant increase ($p<0.001$) in CAT activity was observed in the quercetin only treated animals as compared to the control animals. In the arsenic plus quercetin treated group there was a significant increase ($p<0.01$) in CAT as compared to the arsenic treated group. SOD and peroxidise (POD) concentrations were significantly reduced in the arsenic treated group compared to the control group ($p<0.001$, $p<0.01$, respectively). A significant increase in SOD ($p<0.001$) and POD ($p<0.01$, $p<0.001$) was noted in the quercetin and arsenic plus quercetin treated groups as compared to arsenic only treated group (Table 3). Similarly, a significant reduction ($p<0.05$) in glutathione reductase (GSR) content was found in the arsenic treated group compared to the control group. Quercetin treatment alone or in combination with arsenic, caused a significant increase ($p<0.001$, $p<0.05$) in GSR content compared to the arsenic alone treated group. The level of thiobarbituric acid reactive substance (TBARS) was significantly higher ($p<0.01$) in the arsenic only treated group compared to the control group. However in the quercetin only and arsenic plus quercetin treated groups the levels of TBARS were significantly reduced ($p<0.001$) compared to the arsenic treated group and the control group ($p<0.05$) (Table 4).

Mean testicular and plasma testosterone concentrations in the control, arsenic, quercetin, and arsenic plus quercetin treated groups are shown in Table 5. The mean plasma and intra-testicular testosterone concentrations in the arsenic treated group were significantly reduced ($p<0.001$, $p<0.01$) when compared to the control group. Quercetin by itself or in combination with arsenic resulted in a significant increase in the mean plasma testosterone ($p<0.05$, $p<0.001$) and intra-testicular testosterone concentrations ($p<0.001$) compared to arsenic only treated group. The concentration of plasma testosterone in the arsenic plus quercetin treated group was comparable to that of the control group (Table 5).

Discussion

The present study was designed to investigate the protective effects of quercetin against the toxic effects induced by sodium arsenite in testes of adult male Sprague Dawley (SD) rats. Histopathological results revealed that arsenic treatment caused marked damage and morphological changes in the seminiferous tubules. The group of rats treated with arsenic showed a significant reduction in seminiferous tubule diameter, seminiferous tubule epithelial height, tunica albuginea height, and increased tubular lumen [Manna et al. 2008]. Sodium arsenite causes toxicity through a process known as arsenolysis by binding to the sulfhydryl and carbonyl groups of proteins and replacing phosphate moieties [ASTDR 1990; Hall 2002]. This leads to the impairment of spermatogenesis, steroidogenesis, and a reduction in the number of testicular cells. These detrimental effects were ameliorated by the

Table 1. Mean ± SEM interstitial space, tunica albuginea height, seminiferous tubule diameter, seminiferous tubule epithelial height, and tubular lumen (μm) of testis in control and treated groups after 49 days of treatment.

Groups ($n=5$)	Interstitial space (μm)	Tunica albuginea height (μm)	Seminiferous tubule diameter (μm)	Seminiferous tubule epithelial height (μm)	Tubular lumen (μm)
Control	9.06 ± 0.24	26.48 ± 0.73	177.22 ± 4.64	76.05 ± 0.98	12.85 ± 0.96
Arsenic	$11.27 \pm 0.25^{***}$	$14.90 \pm 0.27^{***}$	$164.05 \pm 1.89^{*}$	$24.96 \pm 0.23^{***}$	$65.03 \pm 0.94^{***}$
Quercetin	$10.08 \pm 0.26^{*++}$	$28.55 \pm 1.00^{+++}$	$179.31 \pm 3.50^{++}$	$78.05 \pm 0.53^{+++}$	$14.05 \pm 0.47^{+++}$
Arsenic + Quercetin	$9.83 \pm 0.21^{+++}$	$20.26 \pm 0.82^{***+++}$	$176.02 \pm 0.97^{+}$	$51.29 \pm 0.81^{***+++}$	$21.04 \pm 0.59^{***+++}$

$n=5$/group; Values are expressed as mean ± SEM; *, *** indicate significance from the control group at $p<0.05$ and $p<0.001$ probability level, respectively; +, ++, +++ indicate significance from the arsenic group at $p<0.05$, $p<0.01$, and $p<0.001$ probability level, respectively.

Table 2. Mean ± SEM number of different cell types in each seminiferous tubule per field of control and treated rats after 49 days of treatment.

Groups ($n=5$)	Spermatogonia	Primary Spermatocytes	Secondary Spermatocytes	Spermatids	Leydig cells/cm^2
Control	44.73 ± 1.30	39.20 ± 1.61	30.66 ± 1.86	49.13 ± 3.42	4.21 ± 0.35
Arsenic	$30.00 \pm 0.72^{***}$	$22.33 \pm 0.82^{***}$	$19.00 \pm 0.77^{***}$	$26.55 \pm 1.23^{***}$	3.39 ± 0.24
Quercetin	$46.00 \pm 1.43^{+++}$	$40.00 \pm 1.22^{+++}$	$31.66 \pm 1.65^{+++}$	$50.76 \pm 2.17^{+++}$	$5.21 \pm 0.25^{+++}$
Arsenic + Quercetin	$40.60 \pm 1.43^{+++}$	$34.00 \pm 1.57^{+++}$	$25.36 \pm 1.20^{+}$	$42.76 \pm 2.63^{+++}$	3.86 ± 0.22

$n=5$/group; values are expressed as mean ± SEM; *** indicate significance from the control group at $p<0.001$ probability level; +, +++ indicate significance from the arsenic group at $p<0.05$ and $p<0.001$ probability level, respectively.

Table 3. Specific activities of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in the testis of control and treated groups after 49 days of treatment.

	Activity of		
Groups ($n = 5$)	CAT (U/mg protein)	POD (nanomole)	SOD (U/mg protein)
Control	7.57 ± 0.54	1.93 ± 0.54	4.25 ± 0.28
Arsenic	4.28 ± 0.34	0.05 ± 0.02**	2.04 ± 0.48***
Quercetin	22.38 ± 2.24***+++	$1.82 \pm 0.44^{++}$	$5.34 \pm 0.75^{+++}$
Arsenic + Quercetin	$11.98 \pm 0.80^{++}$	$1.31 \pm 0.37^{+++}$	$4.31 \pm 0.71^{+++}$

$n = 5$/group; Values are expressed as mean ± SEM; **, *** indicate significance from the control group at $p < 0.01$ and $p < 0.001$ probability level, respectively; ++, +++ indicate significance from the arsenic group at $p < 0.01$ and $p < 0.001$ probability level, respectively.

Table 4. Thiobarbituric acid reactive substance (TBARS) and glutathione reductase (GSR) activity in testis of control and treated groups after 49 days of treatment.

Groups ($n = 5$)	TBARS (nM TBARS/ min/mg tissue)	GSR (nM NADPH oxidized/ min/mg tissue)
Control	16.45 ± 1.15	2.03 ± 0.32
Arsenic	23.36 ± 1.45**	0.83 ± 0.05*
Quercetin	$14.87 \pm 0.12^{+++}$	3.31 ± 0.28**$^{+++}$
Arsenic + Quercetin	12.06 ± 0.64*$^{+++}$	$1.57 \pm 0.17^{+}$

$n = 5$/group; Values are expressed as mean ± SEM; *, ** indicate significance from the control group at $p < 0.05$ and $p < 0.01$ probability level, respectively; +, +++ indicate significance from the arsenic group at $p < 0.05$ and $p < 0.001$ probability level, respectively.

Table 5. Mean ± SEM testicular (ng/g tissue) and plasma (ng/ml) testosterone concentration in control and treated groups after 49 days of treatment.

Groups ($n = 5$)	Testicular T levels (ng/g tissue)	plasma T levels (ng/ml)
Control	96.79 ± 17.32	2.65 ± 0.51
Arsenic	47.11 ± 7.88**	1.02 ± 0.23***
Quercetin	$112.78 \pm 22.48^{+++}$	$2.17 \pm 0.22^{+}$
Arsenic + Quercetin	$101.29 \pm 23.70^{+++}$	3.58 ± 0.57*$^{+++}$

$n = 5$/group; values are expressed as mean ± SEM; *, **, *** indicate significance from the control group at $p < 0.05$, $p < 0.01$, and $p < 0.001$ probability level, respectively; +, ++, +++ indicate significance from the arsenic group at $p < 0.05$, $p < 0.01$, and $p < 0.001$ probability level, respectively.

treatment of quercetin. These results corroborate the previous findings of Izawa et al. [2008] and Taepongsorat et al. [2008]. Izawa et al. [2008] reported that testicular damage caused by DEPs in mice can be reduced by quercetin. Taepongsorat et al. [2008] reported that quercetin prevented this retention and increased the number of spermatozoa along with the tubular area of seminiferous tubules. It was assumed that these effects of quercetin depend upon dose and time duration of quercetin treatment.

Results of the present study revealed a significant decrease in the activity of POD, SOD, and GSR in rats treated with sodium arsenite compared to their control. It was found that arsenic exposure caused an increase in lipid peroxidation. The activity of POD, SOD, and GSR was reduced, while the increase in LPO in rats treated with arsenic was in agreement with a previous study in which it was reported that arsenic reduces antioxidant enzymes [Flora 1999]. Reduced levels of POD and SOD might lead to the increase in the generation of ROS and reactive nitrogen species (RNS), which cause oxidative damage by increasing lipid peroxidation in testes leading to a disruption in spermatogenesis and steroidogensis. Conversely, reduced GSR levels may indicate that glutathione (GSH) is not regenerated in a sufficient amount that is required for the removal of H_2O_2. This accumulation of H_2O_2 results in oxidative stress and damage to the tissue. As presented above, CAT activity was reduced in the arsenic treated group as compared to the control group but this reduction was not significantly different as compared to the control group. This is not in accord with previous literature. However, CAT levels were significantly higher in the arsenic plus quercetin treated group as compared to the arsenic alone treated group. This suggests a protective role of quercetin against the arsenic induced reduction in CAT within the testis. Additionally, it was found that the concentration of intratesticular testosterone was reduced in rats treated with sodium arsenite in accord with previous literature [Jana et al. 2006]. This decrease in the concentration of intratesticular testosterone in rats treated with arsenic may be due to the decrease in the androgenic enzyme activity as previously reported [Jana et al. 2006]. The concentration of intratesticular testosterone in quercetin plus arsenic treated rats was comparatively high. These findings show that quercetin may have a role in inducing androgenic enzyme activity which needs to be determined.

Sodium arsenite is a chief contributory factor damaging the male reproductive organs, leading to reduced spermatogenesis, testicular testosterone production. This is mediated by disturbing the testicular antioxidant defense system [Sarkar et al. 2003] by increasing ROS that leads to impaired steriodogenesis. Reduced androgen levels due to depleted testis antioxidant enzymes results in reduced spermatogenesis. In our experiment, reduction in the tissue antioxidant enzymes as well as testosterone concentration in the arsenic treated animals was observed. It was speculated that depleted antioxidant enzyme levels may have reduced the testicular testosterone concentration by some unknown mechanism that needs to be determined.

First lines of defense are provided by antioxidant enzymes (SOD, CAT, POD, and GSR) which prevent biological molecules (lipids, proteins, DNA) from damage and decrease lipid peroxidation by inhibiting ROS formation. Hydrogen

peroxide (H_2O_2), nitric oxide (NO), superoxide anion (O_2^-), and hydroxyl radical (OH) are central reactive oxygen and nitrogen species that are involved in tumorigenesis and mutagenesis [Imlay 1988; Wiseman 1996]. SOD counteracts the toxic effects of the superoxide anion. Levels of antioxidant enzymes are important because dismutation of the superoxide anion to form H_2O_2 is catalyzed by SOD while H_2O_2 is converted to water molecules by CAT and glutathione peroxidase (GPx) while reduced GSH is used as an electron donor in such reactions [Josephy et al. 1997; Usoh et al. 2005]. Similarly, the level of GSH is retained by the thiol containing non-protein compound GSR. GSR regenerates GSH (reduced form) from GSSG (oxidized form) for the constant activity of GPx [Williams and Ford 2004]. NADPH oxidases are specialized enzymes that can generate superoxide anion which can be eliminated by CAT, POD, and SOD, decreasing LPO to protect spermatozoa from oxidative stress [Aitken and Brindle 1995].

Quercetin, a dietary flavonoid, extracted from edible plants, has anti-oxidant capacity, including anti-inflammatory responses, anti-allergic, antiulcer, antimicrobial activities, and hepatoprotective and neuroprotective effects [Hollman and Katan 1999; Pu et al. 2007; Ross and Kasum 2002]. Quercetin, an extensively distributed bioflavonoid, has protective and beneficial effects on human health. However, the effects of quercetin on reproduction and fertility in males remain contentious because most studies have been carried out *in vitro* using cell lines which revealed that quercetin incubation with human semen caused a permanent and dose-dependent decrease in sperm viability and motility [Khanduja et al. 2001]. The increase in the tubular epithelial height and sperm number may be due to the proliferative capacity of quercetin as reported previously [Van Der Woude et al. 2005]. It has been reported that quercetin induces cell proliferation acting through the estrogen receptor (ER), however the exact mechanism needs to be determined [Van Der Woude et al. 2005].

Quercetin co-administrated with arsenic resulted in a significant increase in CAT, POD, SOD, and GSR activities while a decrease in TBARS in testicular tissue of male rats at the dose of 50 mg/kg. These findings are in accord with previous literature reporting that quercetin increases antioxidant enzyme levels (GPx, SOD, CAT, GSH) and reduces lipid peroxidation [Ciftci et al. 2012]. Conversely, many findings have confirmed that quercetin improves enzymatic and non-enzymatic levels, preventing oxidative stress and damage caused by toxic metals and compounds like arsenic, cadmium, and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In the present study, quercetin significantly reduces oxidative stress in the testis and epididymis of male rats. Arsenic intoxication results in the increase production of ROS, which results in the reduced level of GSR as observed in the arsenic alone treated group. However, elevated levels of GSR in the arsenic and quercetin treated group compared to arsenic alone treated group showed enhanced regeneration of GSH from GSSG which is required for the continued activity of GPx. These results confirmed previous findings in which supplementation with 25, 50, and 75 mg/kg quercetin was used against ethanolic intoxication. This resulted in increased GSR activity by increasing the GSH/GSSG ratio [Molina et al. 2003]. Quercetin exerts its anti-oxidant effects by various mechanisms like scavenging free radicals, chelating metal ions like iron, and reducing LPO levels by blocking xanthine oxidases sustaining redox balance [Cheng and Breen 2000; Da-Silva et al. 1998].

Quercetin co-administered with sodium arsenite, yielded a significant increase in intratesticular testosterone. Our findings corroborate previous literature in which quercetin supplemented with TCDD resulted in significantly elevated serum testosterone levels [Ciftci et al. 2012]. These results suggested that quercetin elevates testosterone not only in serum, but also in testicular tissue. In conclusion, the present study suggests that sodium arsenite is responsible for reproductive organ intoxication and quercetin supplementation with arsenic serves to attenuate adverse effects of arsenic by inhibiting ROS and RNS generation.

Materials and Methods

Adult male SD rats (70 and 90 days old and 210 ± 10 g body weight) were obtained from the Animal Facility of Animal Sciences Department, Quaid-I-Azam University, Islamabad. Animals were kept in stainless steel cages in groups (five rats/cage), at a temperature of 20–25°C. All the rats were kept under 12/12 h dark/light cycle and fed with standard laboratory food, and tap water was available *ad libitum*. Animal handling, treatments, and succeeding scarifice was approved by the Department of Animal Sciences, Quaid-I-Azam University.

The animals were divided into four groups: first group served as a control and was provided tap water. The second group was treated with sodium arsenite at the dose of 50 ppm in drinking water. The third group served as positive control and received oral doses of quercetin (50 mg/kg). In the fourth group, quercetin (Catalog No Q4951, Sigma Aldrich, Germany) was co-administered orally at the dose of 50 mg/kg with arsenic (50 ppm in drinking water). Selection of the dose of quercetin (50 mg/kg body weight) was in accord with the previous study of Milton et al. [2013] in which quercetin at the same dose was effective against cadmium induced oxidative stress and toxicity in the cardiac tissue of rats [Prabu et al. 2013]. All the treatments were carried out for 49 days. The animals were sacrificed by decapitation at the 50th day; trunk blood was collected for plasma isolation in heparinized syringes. Blood was centrifuged at 3000 rpm for 10 min; plasma was separated and stored at −20 °C until analyzed. Testicular tissues were dissected out. Left testicular tissue was fixed in sera for histological processing, while the right testis was stored at −80 °C for estimation of antioxidant enzymes.

The homogenization of testicular tissue was carried out in a homogenizer in sodium phosphate buffer. The homogenate was centrifuged at 12,000 rpm for 15 min at 4 °C. Supernatant was separated and used for the estimation of antioxidant enzymes and estimation of intratesticular testosterone concentration.

Histology

Testis and epididymis were fixed in sera and were embedded in paraffin. Five μm thick sections were cut out of paraffin block by using Richert microtome (820 H, USA). Sections

were then affixed to pre-cleaned albumenized glass slides and stretched at 60 °C on a Fisher slide warmer. Slides were transferred to a paraffin oven for the next 12 h for the complete deparafinization. Slides were stained with hematoxylin and eosin and were examined under a Nikon optishot research microscope (Japan) equipped with an automatic micro photographic system. Every 25th section was studied and different parameters of 25 seminiferous tubules in each section were measured.

Biochemical analysis

Testicular tissue (180 mg) was homogenized in 10 volumes of 100 mM KH2PO4 buffer containing 1 mM EDTA, pH 7.4 and centrifuged at 12,000 × g for 30 min at 4 °C. The supernatant was collected and used for the following assays. The assay was repeated two times and mean values were reported.

Catalase activity was determined by the method of Chance and Maehly [1955]. Changes in absorbance of the reaction mixture at 240 nm were determined after one min. One unit of CAT activity was defined as an absorbance change of 0.01 as units/min.

Superoxide dismutase activity was estimated by the method developed by Kakkar et al. [1984]. The amount of chromogen formed was measured by recording the color intensity at 560 nm. Results are expressed in units/mg protein.

Peroxidase activity was determined by the method developed by Chance and Maehly [1955]. Changes in absorbance of the reaction solution at 470 nm were determined. One unit of POD activity was defined as an absorbance change of 0.01 as units/min. The enzyme activity of GSR was quantitated at 25 °C by measuring the disappearance of NADPH at 340 nm and was calculated as nM NADPH oxidized/min/mg protein using molar extinction coefficient of 6.22 × 103/M cm using the method of Carlberg and Mannervik [1975].

The amount of TBARS as an index of lipid peroxidation was assessed by measuring optical density of the supernatant at 535 nm using spectrophotometer against a reagent blank using the method of Wright et al. [1981] as modified by Iqbal et al. [1996]. The results were expressed as nM TBARS/min/mg tissue at 37 °C using a molar extinction coefficient of 1.56×10^5/M cm.

Protein estimation

The total protein content of homogenate and supernatant of testicular tissue was determined by total protein kit (AMP Diagnostics, Austria) using bovine serum albumin as a standard.

Hormone analysis

Plasma and testicular testosterone concentrations in testicular homogenates were determined by using Enzyme Linked Immuno Sorbant Assay (ELISA) kits (ELISA kit, Amegnix, Inc, Burlingame, CA, USA). All were quantified in a single assay. The testosterone concentration of the specimen and controls run concurrently with the standards can be calculated from the standard curve. Intra-assay percent coefficient of variation (% CV) was 8.74.

Statistical analysis

All the data were shown as Mean ± SEM. One way analysis of variance (ANOVA) followed by Tukey's test was used for comparison of different groups using Graph pad prism 5 software. Level of significance was set at $p < 0.05$.

Acknowledgement

This piece of work was funded by the Department of Animal Sciences, Quaid-i-Azam University Islamabad, Pakistan.

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

Author contributions

Led the design, conceived the study, performed the experimental work, and analyzed the results: SJ; Conceived an initial part of the study, performed the experiment, histology, and helped in compiling the results: NI, GR; Performed antioxidant enzymes estimation: HU; Provided quercetin and helped in writing the results: IH; Wrote the paper with input from all other authors: HU, NI.

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