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Testicular Toxicity following Subacute Exposure of Arsenic and Mancozeb Alone and in Combination: Ameliorative Efficacy of Quercetin and Catechin

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Abstract

Mancozeb (MZ) is a contact fungicide having low toxicity in non-target species, but its continuous exposure can be harmful. The aim of the present study was to determine the impact of the toxic interaction between MZ and arsenic on the testicular tissue of rats and to compare the amelioration potential of quercetin and catechin against the induced toxicity. Sixty adult rats were randomly allocated into 10 groups with 6 animals in each. A significant ($p<0.05$) decline in TAS, TTH, SOD, CAT, GPx, GR and TTH and a rise ($p<0.05$) in MDA and AOPP-were recorded in testicular tissue of MZ-treated rats in comparison to control. Exposure to different doses of arsenic (10, 50, 100 ppb) also produced a dose-dependent effect on these oxidative biomarkers. Arsenic exposure produces potentiating MZ-induced testicular toxicity in Wistar rats. Testicular damage was further corroborated by extremely severe histopathological changes *viz.,* interstitial as well as sub-capsular congestion, oedema aside from degeneration, necrosis and loss of seminiferous tubules and a drastic deterioration in sperm motility in this group. In contrast, administration of toxicants along with quercetin or catechin markedly attenuated the alterations in oxidative as well as cellular damage biomarkers and testicular histopathological alterations. Our results suggested that simultaneous low dose exposure to arsenic potentiated testicular toxicity induced by MZ. Furthermore, catechin was more potent as compared to quercetin in ameliorating testicular changes induced by concurrent arsenic and MZ exposure.

Keywords: Arsenic, Catechin, Mancozeb, Quercetin, Testicular Damage

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1. Introduction

Fungicides are important tools in the management and control of plant diseases constituting more than 35% of the pesticide trade worldwide. Europe is a prominent market for the sale of fungicides and the latter are applied to protect grains, cereals, fruits and vegetables[1]. Although fungicidal application is a successful plant protection strategy, but their excessive, irrational and indiscriminate use is a potential environmental and human health hazard because of the problems such as residue accumulation, resistance build up and impact on non-target micro-flora associated with their usage[1,2]. Mancozeb (MZ), a commonly used Ethylene-Bis-Dithiocarbamate (EBDC) category fungicide, has been in global commercial use for several decades[3] because of its broad-spectrum fungicidal activity, low toxicity in humans and a relatively short environmental persistence[4]. At present, India is not only a leading producer but also a dominant consumption market accounting for over 28% of international trade. According to national pesticide production data from the Ministry of Chemicals and Fertilizers of India, the country's MZ output in 2018 was 82,000 tons. Mancozeb is a contact fungicide having low acute toxicity[5] despite that, it poses adverse health risks to humans and animals. It has been recommended by researchers that it be considered a formidable developmental and reproductive hazard for humans[6]. MZ inhibits ovarian follicular growth, and compensatory hypertrophy of the contralateral ovary after partial oophorectomy, causes biochemical alterations and alters the oestrous cycle, and fertilizability in rats[7-9]. Likewise in male rats, a reduction in the weight of reproductive organs, testosterone levels, and fertility besides alterations in enzymatic and biochemical constituents of the testis has been recorded[10,11].

Arsenic (As) is the 20th most abundant element in earth's crust, 14th in seawater and 12th in our body[12]. Bangladesh and India (West Bengal) are the worst affected countries where the groundwater arsenic concentrations are above the recommendations set by the WHO[13]. The mechanisms of arsenic toxicity are complex and vary with the chemical form of As. At biochemical level, arsenate substitutes phosphate and result in inhibition of many enzymes and several reactions in the mitochondria, while as arsenite and its methylated form (trivalent organic) react with thiol (-SH) in proteins and inhibit their activity. It also causes inactivation of various cellular enzymes, particularly those involved in cellular energy pathways and DNA replication and repair. Since the trivalent form has higher affinity for thiol groups, it inhibits enzymes like glutathione peroxidase, reductases, thioredoxin and lipoic acids and cysteinyl residues of enzymes[14]. Unbound arsenic also exerts toxicity by producing ROS during their redox cycling and metabolic processes that cause lipid peroxidation by oxidative stress[15]. Its effects may range from acute gastrointestinal toxicity, encephalopathy and peripheral neuropathy to reproductive toxicosis. Besides reduction in testicular weight, oxidative stress mediated peroxidation of lipids and apoptosis of germ cells has also been noted[16-18].

In the face of pervasive environmental pollution, it is a fact that humans are being simultaneously exposed to a variety of chemicals and hence calls for an assessment of possible toxic interactions among them. Prior research clearly indicates that a combination of divergent pollutants casts greater adverse effects on reproductive health in exposed subjects[19-24]. With ever increasing use of MZ globally[3,25] and presence of As[15] in groundwater beyond the permissible point, their combined toxicity in exposed individuals seems a rational presumption. Previous reports suggest that ROS mediated damage is an underlying factor in MZ or As mediated testicular toxicity and infertility[17,26-29]. We designed this study to further elucidate whether toxic interaction between As and MZ potentiates oxidative stress or exacerbates testicular damage inflicted after administration of any one of them in Wistar rats, which may have formidable implications for male fertility in general.

Nowadays, much emphasis is being laid on using naturally occurring dietary substances for the control and management of toxicities. Phenolic compounds *viz.,* quercetin and catechin which are plant secondary metabolites, are widely distributed in fruits and nuts. Quercetin has potent antioxidant, chelating and free radical scavenging properties which can counter oxidant-induced DNA damage and lipid peroxidation and thus have found application in the treatment of many pathological conditions including cancer, obesity, chronic inflammation, cardiovascular and nervous disorders[30,31]. Similarly, catechin flavonoid rich in flavan-3-ols[32] are an important constituent of tea, apples, red wine and confers numerous health benefits[33]. Both clinical and epidemiological studies have found catechin to be effective as antioxidant, anti-inflammatory, anti-bacterial, anti-viral, immuno-stimulatory, cardio-protective, diuretic, anti-carcinogenic, anti-mutagenic, anti-tumorigenic,

anti-allergic, anti-diabetic and anti-hypertensive agent[34-36]. Versatility of catechin in part due to ortho dihydroxy or ortho trihydroxy moieties in their structure, endows them with profound antioxidant activity as well as their secondary metabolites, semi quinones, are efficient free radical scavengers[37]. Therefore, we also wanted to evaluate and compare the ameliorative efficiency of catechin and quercetin against testicular toxicity induced by combined exposure of MZ and arsenic in Wistar rats.

2. Materials and Methods

2.1 Experimental Design

The animal experiments were conducted on healthy male adult Wistar rats procured from the Indian Institute of Integrative Medicine (CSIR Lab), Jammu. The animals were acclimatized for a week on pelleted feed with access to ad-libitum tap water under standard conditions (25 ± 2 °C temp, 50 ± 15 % relative humidity) and normal photoperiod (12 h light and dark). All the experimental animals were kept under continuous observation for any clinical sign during the entire study period. The use of rat model for the study was approved by ethical committee of the institution (3/IAEC/2021) and experiment was conducted as per standard guidelines. Mancozeb (MZ) used in the study is a commercial formulation as wettable powder (75%) was procured from Indofil Industries Limited, Mumbai, India. All the chemicals used in the study were of analytical grade procured from Sigma Chemical Corp or Hi-Media, Mumbai, etc.

The sub-acute toxicity was induced by using MZ at 1/10th of the median lethal dose (LD_{50} - 800 mg/kg, b. wt) alone and along with different doses of arsenic i.e., 10 ppb, 50 ppb and 100 ppb in drinking water PO (the WHO recommended level of arsenic in drinking water is 10 ppb and in some Asian countries like Bangladesh, the accepted level is 50 ppb). The toxicological study was conducted on sixty adults male Wistar rats, weighing between 180-200 gm. The animals were randomly allocated into 10 groups, with 6 animals in each. Group I served as control, Group II was administered MZ [800 mg/kg, b. wt, oral gavage (PO), for 28 days]. Groups III, IV and V received sodium arsenite (10 ppb, 50 ppb and 100 ppb, respectively) containing drinking water continuously for 28 days. Groups VI, VII and VIII were provided with sodium arsenite at 10 ppb, 50 ppb and 100 ppb respectively, along with MZ (800 mg/kg, b. wt, PO) for 28 days. Groups IX and X were given sodium arsenite at 100 ppb and MZ at 800 mg/kg b. wt, PO along with quercetin (50 mg/kg b. wt, PO) and catechin (50 mg/kg b. wt, PO) respectively for 28 days.

2.2 Collection of Tissue Sample

Animals were sacrificed by cervical dislocation on the 29th day under light diethyl ether anaesthesia. Testes were removed and 1g of tissue was collected in 10 ml ice-cold 0.5M phosphate buffer (pH-7.4) for antioxidant studies. 10% tissue homogenate was prepared for the antioxidant enzyme analysis using a tissue homogenizer (1000 rpm for 5-7 minutes at room temperature). A portion of tissue was also collected in a formal saline solution (10%) for histopathological studies.

2.3 Antioxidant Biomarkers

Different antioxidative biomarkers *viz.* Total Antioxidant Status (TAS) was determined spectrophotometrically by using 2,2 azinobis (3-ethylbenzothiazoline 6-sulphonate) (ABTS) according to the method previously described[38]. Total Thiols (TTH) were determined as per the method described[39] and expressed as millimolar (mM) using reduced glutathione as a standard. The enzymatic parameters *viz.*, Catalase (CAT) and Glutathione Peroxidase (GPx) were determined as per the standard methods[40,41]. The activities of Superoxide Dismutase (SOD) and Glutathione Reductase (GR) were determined as per the method described previously in testicular tissue[42,43]. Similarly, Malondialdehyde (MDA) and Advance Oxidation Protein Product (AOPP) levels in hepatic tissue of different groups were determined using a standard protocol and expressed in nmol of MDA formed/g/h and µM of Chloramine-T, respectively[44,45].

2.4 Progressive Sperm Motility

Percent progressive motility of epididymal sperms in control and different treated groups was estimated as per the standard method.

2.5 Histopathological Studies

For recording histopathological alterations in testicular tissues, a small piece of testes from each animal of respective groups was fixed in 10% formalin, embedded in paraffin, sectioned, stained with Haematoxylin and Eosin (H&E) and examined under a light microscope.

2.6 Statistical Analysis

The values are presented in mean ± standard error. The results were analysed by analysis of variance (ANOVA) at a 5% level of significance ($p<0.05$) using the Duncan Multiple Range test (SPSS 21.0).

3. Results

3.1 Effects on Activities of SOD and CAT

Alterations in the mean values of SOD and CAT in control and different treated groups are depicted in Table 1. A significant ($p<0.05$) reduction was observed in SOD and CAT activity in Group II as compared to control. Groups III, IV and V manifested a significant decrease in SOD as well as CAT activity in a dose-dependent manner. Further, in the interaction Groups VI, VII and VIII, a significant curtailment in SOD and CAT levels was seen as compared to III, IV and V respectively. SOD activity in all dual toxicant rats was significantly lower as compared to Group II also indicating significantly enhanced toxicity but CAT levels showed significant depreciation from G II in only G VIII. Group VIII showed a 69.4% and 66.5% fall in SOD and CAT activity respectively as compared to control. Levels of SOD in quercetin as well as catechin treatment groups were significantly higher than that of Group VIII with no significant difference in level of amelioration between the two therapy groups whereas CAT levels were upgraded in a significant manner only by quercetin.

3.2 Activities of GPx, GR and AE

In Group II, a significant ($p<0.05$) reduction was observed in GPx and GR activity as compared to control. Groups III, IV and V represented a decreasing trend in GPx and GR values, but a significant difference was seen in GPx in Groups IV and V whereas GR levels varied significantly only in Group V when compared to control. Combination Groups VI, VII, and VIII witnessed a significant fall in GPx as well as in GR levels in a dose-dependent manner which was not only significantly lower in contrast to their respective individual toxicant treatment group levels but also as compared to Group II concentrations. Group VIII witnessed a 64.3% and 61.3% decline in GPx and GR in activity, respectively, compared to control. While catechin completely repaired changes in GR, quercetin was only partly successful in reinstating deranged GR values. In contrast, quercetin showed somewhat better activity in preventing falls in GPx concentrations even as both the herbal agents were not completely effective in

Table 1. Alterations in Superoxide Dismutase (SOD), Catalase (CAT) and Arylesterase (AE) in testes of rats following repeated administration of Mancozeb (MZ) alone or in-combination with Arsenic (As) and its amelioration with quercetin and catechin

Treatment group	SOD (Unit/ g of tissue)	CAT (μmol H_2O_2 decom./ min / g of tissue)	AE (U/100mg tissue)
Control	433.77^{e}±15.58	1550.75^{e}±15.39	5.55^{e}±0.035
MZ (800mg/kg)	309.73^{d}±12.83	647.39^{b}±37.79	3.85^{c}±0.051
Arsenic (10 ppb)	423.82^{e}±11.64	1519.78^{e}±37.53	5.34^{e}±0.062
Arsenic (50 ppb)	359.39^{d}±20.07	1166.42^{d}±45.36	4.40^{d}±0.165
Arsenic (100 ppb)	295.29^{c}±27.12	1014.55^{c}±24.98	3.91^{c}±0.082
MZ + As (10 ppb)	208.81^{abc}±26.37	626.19^{b}±22.93	3.16^{b}±0.10
MZ + As (50 ppb)	173.81^{ab}±24.33	586.19^{b}±38.10	2.85^{b}±0.058
MZ +As (100 ppb)	132.75^{a}±20.70	520.15^{a}±30.43	2.02^{a}±0.069
MZ+ As (100 ppb) + Quercetin (50 mg/kg)	232.50^{bcd}±15.49	777.24^{b}±33.33	3.54^{c}±0.062
MZ+ As (100 ppb) + Catechin (50 mg/kg)	256.87^{bcd}±9.60	537.69^{a}±11.82	3.65^{c}±0.057

Values are given as mean ± SE of 6 animals

Values having different superscripts (a, b, c, d, e, f) in a column are statistically different from one another at a 5% level of significance

Table 2. Alterations in Total Thiols (TTH), Glutathione Peroxidase (GPx) and Glutathione Reductase (GR) in testes of rats following repeated administration of Mancozeb (MZ) alone or in combination with Arsenic (As) and its amelioration with quercetin and catechin

Treatment Group	TTH (μM)	GPx (Unit/ g of tissue)	GR (nmol of NADPH/min)
Control	$3.33^{c}\pm0.046$	$160.79^{e}\pm3.61$	$33.72^{e}\pm0.55$
MZ (800mg/kg)	$1.84^{ab}\pm0.209$	$126.87^{d}\pm5.94$	$26.76^{cd}\pm1.20$
Arsenic (10 ppb)	$3.22^{c}\pm0.218$	$153.93^{e}\pm6.72$	$32.66^{de}\pm0.93$
Arsenic (50 ppb)	$2.65^{b}\pm0.280$	$135.97^{d}\pm3.26$	$29.07^{de}\pm1.43$
Arsenic (100 ppb)	$2.46^{b}\pm0.292$	$86.82^{bc}\pm5.52$	$21.31^{bc}\pm1.54$
MZ + As (10 ppb)	$2.01^{ab}\pm0.296$	$82.53^{bc}\pm8.81$	$20.79^{b}\pm0.38$
MZ + As (50 ppb)	$1.78^{ab}\pm0.244$	$66.16^{ab}\pm5.89$	$19.13^{b}\pm1.80$
MZ +As (100 ppb)	$1.53^{a}\pm0.243$	$57.37^{a}\pm6.53$	$13.05^{a}\pm0.82$
MZ+ As (100 ppb) + Quercetin (50 mg/kg)	$1.96^{ab}\pm0.332$	$125.63^{d}\pm8.08$	$21.34^{bc}\pm2.04$
MZ+ As (100 ppb) + Catechin (50mg/kg)	$2.01^{ab}\pm0.295$	$95.79^{c}\pm5.73$	$28.61^{de}\pm1.06$

Values are given as mean ± SE of 6 animals
Values having different superscripts (a, b, c, d, e, f) in a column are statistically different from one another at a 5% level of significance

counteracting the dual toxicant challenge when compared with levels in control animals. Similarly, the activity of AE witnessed a significant ($p<0.05$) decline in Group II and a dose-dependent decline in Groups III, IV and V which was significant only in Groups IV and V as compared to control. Interaction between toxicants in Groups VI, VII and VIII caused a significant fall in AE activity as compared to any single treatment with toxicants (Groups II, III, IV and V). Although activities of catechin and quercetin were at par in significantly upgrading AE levels after dual toxicant exposure, a complete restoration was not seen in comparison to control values (Table 2).

Table 3. Alterations in Total Antioxidant Status (TAS), Malondialdehyde (MDA) and Advanced Oxidation Protein Product (AOPP) in testes of rats following repeated administration of Mancozeb (MZ) alone or in combination with Arsenic (As) and its amelioration with quercetin and catechin

Treatment Group	TAS (mM)	MDA (nmole of MDA formed/gm/hr)	AOPP (μM of Chloramine-T)
Control	$21.61^{f}\pm0.160$	$26.04^{a}\pm1.28$	$1.23^{a}\pm0.066$
MZ (800mg/kg)	$18.09^{de}\pm0.280$	$39.11^{bc}\pm2.99$	$1.34^{b}\pm0.077$
Arsenic (10 ppb)	$19.72^{e}\pm0.112$	$27.42^{ab}\pm1.52$	$1.25^{a}\pm0.020$
Arsenic (50 ppb)	$17.93^{de}\pm0.511$	$38.64^{ab}\pm0.72$	$1.48^{c}\pm0.037$
Arsenic (100ppb)	$16.91^{d}\pm0.097$	$48.74^{cd}\pm2.81$	$1.52^{c}\pm0.066$
MZ + As (10ppb)	$14.00^{c}\pm0.588$	$40.57^{c}\pm3.74$	$1.55^{bc}\pm0.027$
MZ + As (50ppb)	$13.76^{bc}\pm0.307$	$49.75^{cd}\pm4.01$	$1.60^{d}\pm0.017$
MZ +As (100 ppb)	$11.66^{a}\pm0.512$	$61.90^{e}\pm4.29$	$1.74^{d}\pm0.130$
MZ+ As (100ppb) + Quercetin (50 mg/kg)	$12.09^{ab}\pm0.508$	$67.33^{e}\pm1.63$	$1.42^{bc}\pm0.042$
MZ+ As (100ppb) + Catechin (50mg/kg)	$14.70^{c}\pm0.535$	$70.08^{e}\pm2.71$	$1.30^{ab}\pm0.032$

Values are given as mean ± SE of 6 animals
Values having different superscripts (a, b, c, d, e, f) in a column are statistically different from one another at a 5% level of significance

3.3 Effect on Levels of TAS and TTH

Mean values of TAS and TTH in testicular tissue of control and different treatment groups are presented in Table 2 and 3. A significant ($p<0.05$) decline in TAS and TTH activity was noticed in Group II in comparison to control. Groups III, IV and V showed a fall in TAS and TTH levels in a dose-dependent manner as compared to control. The Groups VI, VII, VIII treated with the combinations of toxicants registered a decrease in TAS and TTH levels as compared to Groups III, IV and V respectively, but combination groups had significant decrease in only TAS levels but not TTH levels when compared to the respective levels in Group II. Group VIII showed a 45.9% and 54.1% fall in TAS and TTH activity respectively as compared to control. Quercetin was not significant in alleviating depression in TAS or TTH levels however catechin significantly improved TAS but not TTH.

3.4 Effect on Levels of MDA and AOPP

The mean values of MDA and AOPP increased significantly in Group II as compared to the control group. In Groups III, IV and V, the MDA and AOPP activity increased in a dose-dependent manner when compared to control. The toxicant interaction Groups VI, VII and VIII showed a significant increment in MDA as well in AOPP values compared to the respective single toxicant Groups III, IV and V levels. Group VIII represented a 137.7% and 41.5% rise in MDA and AOPP levels respectively as compared to control. Catechin and quercetin failed to amend MDA levels however; significant betterment was seen in AOPP levels in amelioration groups. Moreover, quercetin was partially restored whereas catechin completely corrected deterioration in AOPP concentrations as compared to the control concentrations (Table 3).

3.5 Epididymal Progressive Sperm Motility

The mean per cent motility of sperms from epididymis in control and different groups is studied immediately after collections of epididymis under a light microscope. There was a progressive decline in sperm motility in all single toxicant treatment groups as compared to the control and it ranged between 80-90 %. After combined toxicant administration a further decline was seen, and motility varied between 67.5% in Group VI to 56.66% in Group VIII in a dose-dependent fashion. After antioxidant supplementation, there was marked improvement in sperm motility which became 78 and 79.9 % in Groups IX and X respectively.

3.6 Histopathological Alterations in Testes

Testes of control rats were lined by a thick capsule layer and contained healthy seminiferous tubules separated by thin fibrous connective tissue (Figure 1(a)). Interstitium also had Leydig cells which had round nuclei and moderate amounts of cytoplasm. Seminiferous tubules were lined by intact basement membranes and housed normal populations of cells in various stages of spermatogenesis. Round spermatogonia and primary spermatocytes were recognized. Spermatids and mature spermatozoa were also present towards the lumen of the seminiferous tubules. Testis of Group II revealed moderately severe pathological changes which included congestion, interstitial oedema, and degeneration of seminiferous tubules along with a reduction in their number (Figure 1(b)). The number of sperm cells was significantly reduced due to degeneration and necrosis of seminiferous tubules. Group III presented a somewhat similar picture to that seen in the control group and apart from only mild interstitial congestion, nothing abnormal could be detected (Figure 1(c)). Group IV showed congestion and oedema, particularly in areas adjoining the capsule (Figure 1(d)). Degeneration and necrosis in seminiferous tubules were also noted. Similarly, Group V revealed sub-capsular oedema, testicular haemorrhage, and degeneration in seminiferous tubules (Figure 1(e)). Lesions in Group VI consisted of interstitial congestion along with degeneration, necrosis and loss of seminiferous tubules (Figure 1(f)).

Subcapsular oedema besides congestion, severe degeneration and necrosis of seminiferous tubules was seen in Group VII rats (Figure 2(a)), whereas coagulation necrosis was seen to affect a significant number of seminiferous tubules in almost all rats of Group VIII (Figure 2(b)). Also, necrotic fibrillar debris replaced stages of developing spermatozoa inside several seminiferous tubules while many tubules appeared atrophied, hyalinised, and devoid of any viable sperms in most animals in G VIII (Figure 2(b)). In addition, calcification was seen in necrotic tubules in some rats

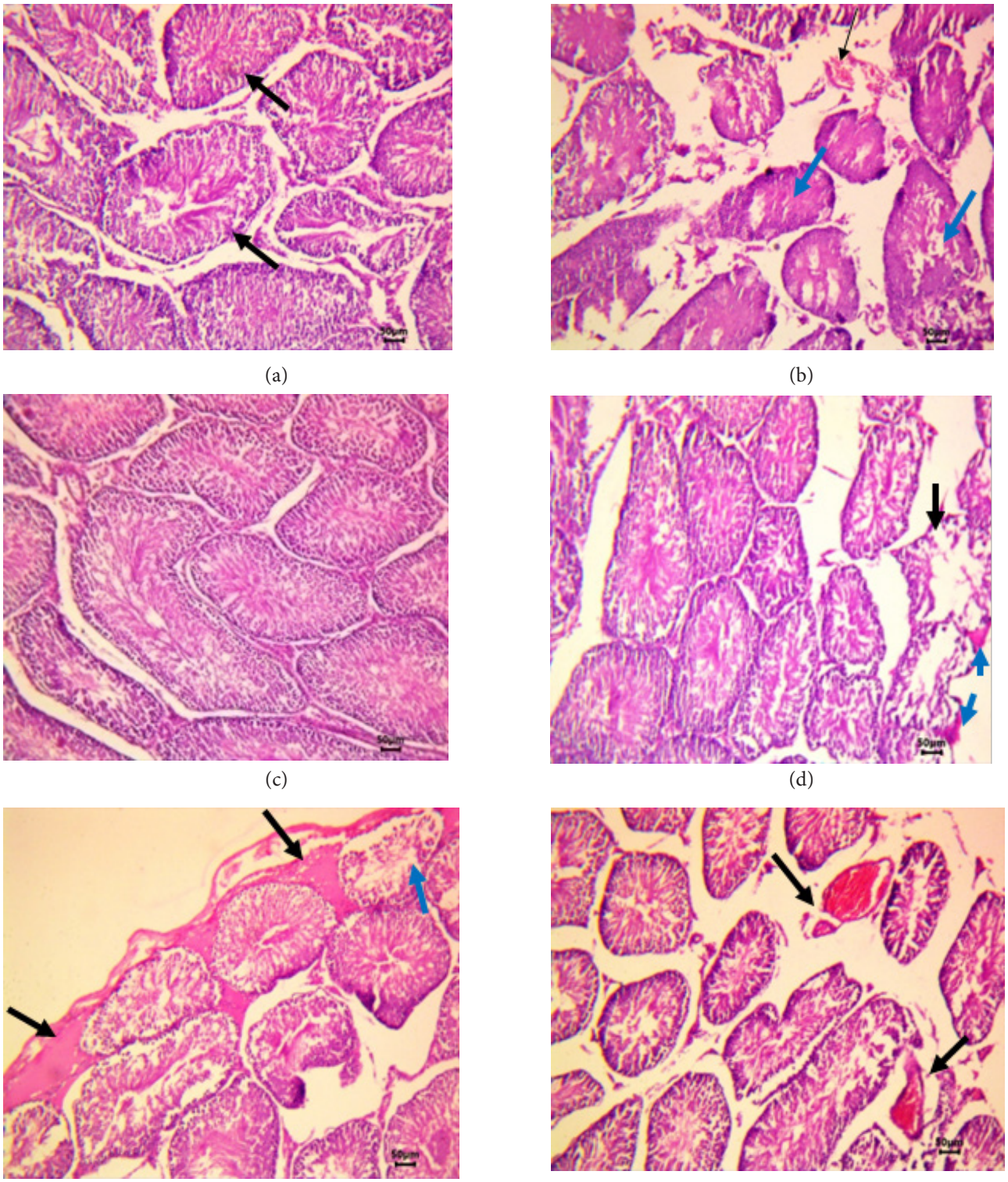


Figure 1. **(a)** Seminiferous tubules (black arrows) in testes of control rats showing normal stages of spermatogenesis and scant interstitium. **(b)** Rat testes in Group II with haemorrhage (thin arrow) reduced number of seminiferous tubules containing degenerating or necrotic contents (blue arrows) and increased interstitial space. **(c)** Normal seminiferous tubules in testes of Group III rats. **(d)** Mild subcapsular oedema (blue arrows) and degenerative changes (black arrow) in adjoining seminiferous tubules in Group IV rats. **(e)** Accumulation of oedema beneath lining capsule (black arrow) along with degeneration in seminiferous tubules (blue arrow) in Group V rats. **(f)** Testes from Group VI rats showing interstitial congestion (arrows).

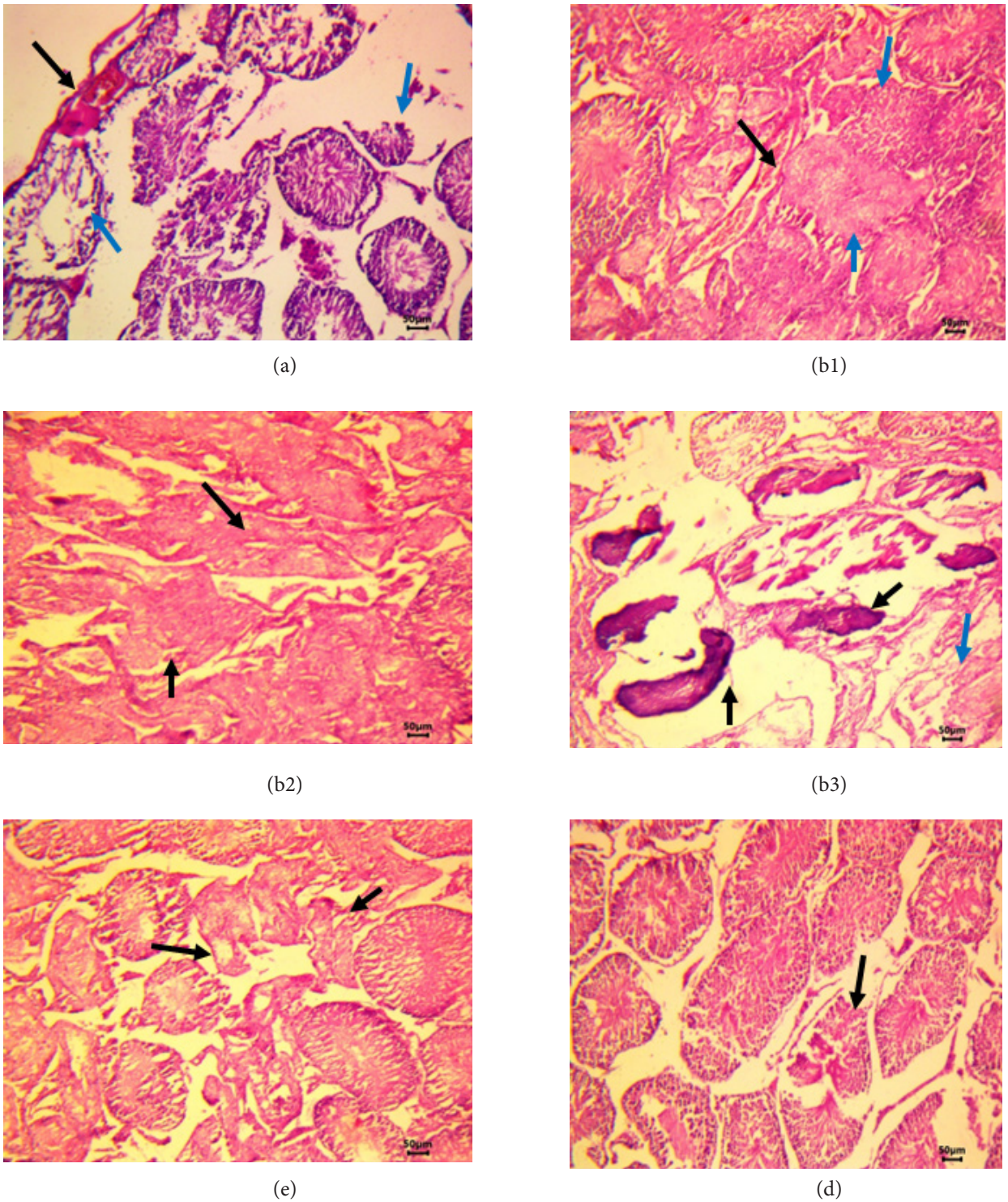


Figure 2. **(a)** Subcapsular oedema (black arrow) along with degeneration and necrosis of seminiferous tubules (blue arrows) in testes of Group VII rats. **(b1)** Showing congestion (black arrow) along with degeneration and necrosis of contents of seminiferous tubules (blue arrows) in Group VIII. **(b2)** Show severe necrosis and hyalinisation of seminiferous tubules (arrows) in Group VIII rats. **(b3)** Indicated destruction of seminiferous tubules in Group VIII and replacement with eosinophilic fibrillar mass (blue arrow) and calcification (black arrows). **(c)** Degeneration and necrosis of seminiferous tubules (black arrows) in Group IX rats. **(d)** Group X rats showing mild degeneration (arrow) of seminiferous tubules.

(Figure 2(b)). The degree of pathological changes was partially ameliorated in Group IX when compared to that seen in Group VIII. Group IX however still showed necrosis of many seminiferous tubules (Figure 2(c)). On the contrary, appreciably fewer severe changes mainly comprising of mild degeneration in seminiferous tubules were found in the testicles of Group X rats (Figure 2(d)).

4. Discussion

Exponential increments in the production, use and disposition of chemicals have profoundly impacted the environment and continue to subject human health to newer unforeseen imperils[11]. Agricultural practices often overlook the scientific literature which clearly implicates it for causing subtle health effects including neurotoxicity,[46] carcinogenicity[3], genotoxicity[47] and reproductive toxicity in human and animals[27,28]. Their exposure has been clearly linked with decreased sperm count, sperm head aberrations and sterility[48]. Similarly, exposure to arsenic has been reported that arsenic exposure damages testicular tissue[49,50]. Also, numerous investigations have documented the involvement of ROS and inactivation of the sulfhydryl group in As or MZ-induced testicular toxicity[17,18,27]. However, little scientific information exists on the hazards of simultaneous MZ and As exposure on testicular tissue and its management utilizing natural antioxidants. So, the present work investigated the amelioration of cumulative oxidative damage by concurrent As and MZ exposure using quercetin and catechin.

Oxidative stress, the imbalance between the generation of free radicals and antioxidant capacity is an important underlying mechanism of damage caused to the male reproductive system especially the testes in cases of xenobiotics toxicities and other diseases and is a potential cause of male infertility. The high susceptibility of testicular tissue to oxidative insult stems from intense mitotic activity requiring high mitochondrial oxygen consumption and very high proportions of polyunsaturated fatty acids in mammalian sperms. In the present study, treatment with either MZ or As decreased the activity of antioxidant enzymes viz, SOD, CAT, GPx, GR and AE apart from causing a decline in TTH as well as TAS and increased peroxidation of lipids and proteins. SOD, CAT and GPx are antioxidant enzymes which form the first line of defence against ROS. Therefore, curtailment in their activity limits the scavenging of free radicals by the primary antioxidant system. Our results are consistent with those of Girish *et al.*, who revealed that MZ significantly decreased GSH, SOD and CAT and increased LPO in testes and epididymis of Wistar rats[27,28,51,52]. Evidence of As overdose suppressing antioxidant defence system has come from a host of previous studies[17,18,53].

The decline in the levels of TTH may be due to the disruption of the protein thiol groups by both MZ[48] and arsenic[14]. A continuing decline in sperm motility was seen in a dose-dependent manner in As treated groups which was in step with the declining antioxidant status in these rats which is in sync with recent findings[54]. In a similar fashion, MZ also caused a reduction in motile sperm. MZ caused a decrease in sperm motility and count with an increase in lipid peroxidation and reduced GSH content as per other reports[55]. Pathological changes in individual toxicant groups included congestion, subcapsular oedema and seminiferous tubular degeneration and reduction in their concentration which were proportional to the oxidative stress generated. Results of many other workers also support these findings[17,27,51,56]. In a study by Kackar *et al.*, it was depicted that chronic oral administration of MZ to male rats caused noticeable increase in testes weight and decrease in epididymis weight along with lesions like degeneration in seminiferous tubules as well as epididymal tubules and a loss of sperms.[57] Concurrent exposure to pesticides or metals has produced more abundant toxic effects as compared to any single entity in many prior studies[19,21-24].

Co-administration of MZ and As further suppressed the antioxidant defence response which was depicted as a greater reduction in SOD, CAT, GPx, GR, AE and higher elevation in MDA and AOPP levels than that of the individual toxicant groups. The marked increase in lipid peroxidation in the combined toxicant groups in our work probably resulted from greater inactivation of antioxidant defence enzymes, which can be the causative factor for damage to cellular membrane and cytoplasmic membrane structures. The enhanced oxidative stress caused by As and MZ together in testicular tissue was further confirmed by severe histopathological changes like congestion, haemorrhages, sub-capsular oedema, reduced number of seminiferous tubules and degeneration, necrosis, increased interstitial hyalinization and calcification which worsened with increasing dose of As in simultaneous exposure groups. Furthermore,

a greater reduction in sperm motility was seen in dual toxicant groups in a dose-dependent manner. The more pronounced morphological alterations in the combination groups are consistent with other investigations which used dual or multiple toxicants[19,21-24].

Since oxidative stress and DNA damage are considered as major factors leading to male infertility, it is rational to search for dietary ingredients possessing natural oxidative stress inhibiting potential for development of antioxidant therapy for infertility[29]. The present study evaluated the antioxidant prowess of quercetin *vis a vis* that of catechin in counteracting the combined toxicity after exposure to MZ and As. Quercetin is a well-known antioxidant with protection potential for testes against As poisoning[16] whereas it modulated inflammation and oxidative stress mediated renal damage in rats treated with diclofenac[58]. A study by Arslan *et al*.,[59] supported its protective role in alleviating lead-induced oxidative stress-mediated pathological changes in Japanese quails. Equally, cadmium-induced renal damage in rats was prevented by catechin supplementation[32].

Administration of quercetin significantly refined SOD, CAT, GPx, AE and AOPP levels whereas catechin improved SOD, GPx, GR, AE, TAS and AOPP contents in the combined exposure group and the latter displayed better ameliorative response against the oxidative injury after combined toxicity. Even though both herbal agents improved sperm motility, histopathological observations reinforced catechin's superior protecting properties for testicular tissue over that of quercetin in shielding testes from oxidative damage as significantly milder testicular changes, mainly degeneration, were manifested in catechin treated group against severe degeneration and necrosis of testicular parenchyma seen in quercetin administered animals. A report by Akinmoladun *et al*.,[60] however found that quercetin fared better than catechin in protecting against rotenone-induced testicular and splenic toxicity even though both caused remarkable improvement in testicular weight, splenic weight, and depleted oxidative enzymes in rats. But Kayode *et al*., found supplementation with catechin very effective in attenuating inflammation and oxidant damage in liver, kidney and testes after combined toxic effects of As and deltamethrin.[20]

The study by Girish *et al*., illustrated protective effect of forskolin against MZ-induced reproductive toxicity in adult male Wistar rats with improvement in GSH, SOD, CAT levels and increased LPO activity in testes and epididymis after forskolin supplementation.[27] Similarly, a report by Sharma *et al*., suggested coenzyme Q_{10}, and vitamin E guarded against As generated sub-acute testicular toxicity and DNA damage in Swiss Albino mice since GSH, TTH, SOD and total protein levels were improved by coenzyme Q_{10} and vitamin E in arsenic treated group.[53] Results from many other studies also serve as proponents for use of antioxidants therapy against reproductive toxicity and male infertility[29,52,56].

5. Conclusion

In nutshell, the present investigation studied the impact of combined toxic effects of MZ and As on testicular tissue and compared antioxidant ability of quercetin and catechin for the amelioration of induced toxic damage in a rat model. Co-exposure to toxicants substantially increased ROS generation which subsequently incurred greater testicular damage thus highlighting that the persistence of As and MZ in the environment could pose an ecological risk to male fertility in local inmates of that area. Meanwhile, quercetin and catechin significantly mitigated the combined harmful effects of MZ and As on the testes by modifying oxidative stress and suppressing histological changes. Although both quercetin and catechin proved to be useful in alleviating testicular toxicity, catechin was more effective in reducing the load of oxidative damage which was also validated by histopathological assessment which showed a better reduction in the severity of testicular lesions.

6. References

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