

Effect of Curcumin on Some Heavy Metals Induced Renal and Testicular Injuries in Male Rats

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Abstract

Back ground Toxic heavy metals in water, air and soil are global problems that are a growing threat to humanity. Heavy metals are widely distributed in the environment and some of them occur in food, water, air and tissues even in the absence of occupational exposure. The antioxidant and protective influences of curcumin on a mixture of some heavy metals (Pb, Hg and Cd) induced renal and testicular injuries in male rats were detected.

Curcumin, a yellow pigment from *Curcuma longa*, is a major component of turmeric and is commonly used as a spice and food-coloring material. It exhibits anti-inflammatory, anti-tumor, and antioxidant properties.

Aim The present study aims to evaluate the antioxidative activities and protective role of curcumin against some heavy metals induced renal and testicular injuries in male rats.

Material and Method

Male Swiss albino rats were orally administrated by curcumin (150 mg/kg B.W.) using suitable stomach tube (eight weeks) before receiving mixture of heavy metals (Pd, Hg and Cd) in drinking water for two weeks. Levels of plasma creatinine, urea, uric acid and serum testosterone concentration were measured, glutathione content and superoxide dismutase activities in kidney and testis tissues were estimated and also histological examinations for kidney and testis tissue were detected.

Results

The results revealed that mixture of heavy metals lead to significant increase in the level of plasma creatinine, urea and uric acid, Meanwhile, significant decrease in serum testosterone concentration. Glutathione content and superoxide dismutase activities in kidney and testis tissues were significantly decrease by using a mixture of heavy metals. But groups administrated with curcumin before administrated with mixture of heavy metals, exerted noticeable amelioration against their damage in most of the biochemical and histological tested parameters.

Keywords: Ionizing radiation, Curcumin, Antioxidant, Liver and kidney tissues.

Introduction

Heavy metallic compounds have emerged as a major class of industrial waste product (1).

Heavy metals are persistent environmental contaminants since they cannot be degraded or destroyed. Heavy metals are chemical elements capable of spreading in the environmental compartments and circulating between them. When passed on to the living organisms cause poisoning or death (2).

According to the toxicity for humans and animals, the metals are divided into groups, heavy metals which have a toxic influence on living organisms included cadmium (Cd), mercury (Hg) and lead (Pb) (3).

Continuous environmental and occupational heavy metals exposure can lead to chronic nephropathy. However, many experimental studies showed that several heavy metals caused renal failure associated with severe histopathological and physiological alterations (4, 5, and 6).

Due to the rapid industrialization and overgrowing urbanization, the toxic effects of

heavy metals on male reproduction system have become a major health concern in the globe (7, and 8). The evidence of the past twenty years have shown a disturbing trend in male reproductive health hazards due to careless use of these chemicals which causes detrimental effects on different organs.

Lead (Pb) is a poisonous metal, which is ubiquitous in both organic (Tetraethyl lead) and inorganic (lead acetate, lead chloride) forms in environment (9) and is emitted from automobile fuels, industrial discharge and paints.

(10) reported that, workers employed in these industries are more exposed to lead than general public. Numerous studies have shown that 15–30% of lead exposure in humans occurs through inhalation and 70–85% with food and drinks from gastrointestinal tract. Lead remains a considerable occupational and public health problem, which is known to cause a number of adverse effects in both men and women.

(11) reported effects in rats include low number of testicular spermatids, low daily sperm production, low epididymal sperm count, abnormal prostatic function and changes in serum level of testosterone. Most of these effects have been reported in workers exposed to lead even at an acceptable level (10 µg/dl) recommended by Occupational Safety and Health Administration (OSHA) in 1993 (12).

Mercury is a naturally occurring metal found in the environment in several forms: elemental organic and inorganic. The elemental form is commonly used in dental fillings and thermometers. The inorganic compounds are used in skin care and medicinal products, while the organic compounds are present in fungicides, paints, and diet (contaminated fish). All forms of mercury are considered poisonous and the chemical and physical forms of mercury determine its absorption, metabolism, distribution, and excretion pathways (13). Chronic exposure either to inorganic or organic mercury can permanently damage the brain, kidneys and developing fetus (14).

Inorganic mercury is widely used in certain types of batteries (usually mercuric oxide) and continues to be an essential component of fluorescent light bulbs and thermometers (15). The effects of mercury poisoning are determined by the amount and rate of absorption as well as the physical and chemical properties of the mercury compound. However, each form of mercury exhibits a different pattern of toxicity, and variation exists between species. The kidney, liver, gastrointestinal system, and central nervous system are the main target sites of mercury toxicity (15).

(16) reported that, the primary target organ for inorganic salts of mercury is the kidney hence it is known as nephrotoxic agent. Because of the high bonding affinity between mercury and sulfur, mercury binds to metallothioneins and small molecular weight thiols such as cysteine (17) and glutathione (18).

Mercury is known to increase the intracellular levels of reactive oxygen species such as superoxide (19) and hydrogen peroxides (20), which induce oxidative stress, resulting in tissue damaging effects (21). Alteration in renal glutathione (GSH) contents is an important feature in the expression of mercury nephrotoxicity (22).

Cadmium (Cd) is one of the most important environmental and occupational metallic toxicants and is widely dispersed in the environment. High level exposure to this toxic heavy metal is usually

the result of environmental contamination from human activities. Exposure to Cd can cause both acute and chronic tissue injury and can damage various organs, including liver and kidney in human beings and experimental animals (23). The generation of reactive oxygen species (ROS) followed by development of oxidative stress in the target organs is one of several mechanisms through which cadmium exerts its toxicity (24).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are oxygen and nitrogen containing molecules constitute the main category of free radicals. At high concentrations, free radicals can be harmful to living organisms. Some ROS damage biomolecules indirectly. For example, H₂O₂ and O₂·- initiate DNA and lipids damage by interaction with transition metal ion, in particular iron and copper, in the metal-catalysed Haber-Weiss reaction, producing OH. It is the most electrophilic and reactive of the ROS (25). OH can be produced by ultraviolet, ionizing radiations and some heavy metals. This radical is considered the most frequently damaging species. It has been estimated that the OH is responsible for 60–70% of the tissue damage caused by heavy metals (2). Moreover, .OH has great ability to react with almost any molecule in the vicinity of where it is generated (26). Chemical nature and reactivity of free radicals in biological systems has been recently reviewed (27). Once formed ROS and RNS can produce a chain reaction. The transfer of the free radical to a biological molecule can be sufficiently damaging to cause bond breakage or inactivation of key functions. The organic ROO· can transfer the radical from molecule to molecule causing damage at each encounter. Thus, a cumulative effect can occur, greater than a single ionization or broken bond.

Human and all of the aerobic organisms have a very efficient defense network of antioxidants against oxidative stress. An antioxidant can be defined as a molecule or an element that, when present at low concentrations compared to those of the oxidizable substrate, significantly combat, delays and inhibit oxidation of that substrate, thus, prevent free radicals from damaging healthy cells (28 and 29). Under normal condition, cells have well coordinated and efficient endogenous antioxidant defense systems, which protect against the injurious effects of oxidants.

From the viewpoint of mechanistic functions, antioxidant defense mechanisms can be classified into the following five lines of defenses: preventing antioxidants, scavenging antioxidants,

repair and *de novo* antioxidants, adaptive antioxidants, and finally cellular signaling messenger (29 and 30). The first line of defense is the preventing antioxidants which act by suppressing the formation of ROS and RNS by reducing H₂O₂ and lipid hydroperoxides that are generated during lipid peroxidation, to water and lipid hydroxides, respectively, or sequestering pro-oxidant metal ions such as iron and copper by some binding of proteins (e.g., transferrin, metallothionein). The second line of defense can be described as the scavenging antioxidants which exist to intercept, or scavenge free radicals and remove active species rapidly before attacking biologically essential molecules. The third line of defenses is various enzymes which function by repairing damages, clearing the wastes, and reconstituting the lost function. The adaptation mechanism is considered the fourth line of defense, in which appropriate antioxidants are released at the right time and transported to the right site in right concentration. Some antioxidants constitute the fifth line of defense by functioning as a cellular signaling messenger to control the level of antioxidant compounds and enzymes (31 and 32).

Mammals are endowed with antioxidant defense system that includes nonenzymatic antioxidants enzymatic activities. However, these systems are not always fully operative. Therefore, diet derived antioxidants become particularly important in diminishing cumulative oxidant damage and a number of dietary antioxidants have been reported to decrease free radiation attack on biochemical (33).

Curcumin, a yellow pigment from *Curcuma longa*, is a major component of turmeric and is commonly used as a spice and food-coloring material. It exhibits anti-inflammatory (34), antitumor, and antioxidant properties (35). Curcumin is a low molecular-weight polyphenol, first chemically characterized in 1910, with the molecular formula of C₂₁H₂₀O₆. It is generally regarded as the most active constituent of and comprises 2–8% of most turmeric preparations. It has long been used as the yellow spice in Indian food and as a naturally occurring medicine for the treatment of inflammatory diseases (36).

Curcumin has antioxidant, radioprotective, antibacterial, antifungal, antiviral, antiinflammatory, antiproliferative, proapoptotic and antiatherosclerotic effects, exerting medicinal benefits for arthritis, allergy, asthma, inflammatory bowel disease, nephrotoxicity, psoriasis, diabetes, Alzheimer's disease, multiple sclerosis, cancer,

neurodegenerative and cardiovascular disease(37 and 38).

It is known that curcumin prevents the formation of ROS and scavenges free radicals and it protects cells from peroxidative stress. Curcumin not only exhibits antioxidative and free radical scavenging properties, but also enhances the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px) (39).

The present study is designed to investigate the possibility that the administration of curcumin would have a beneficial effect on these heavy metals-induced renal and testicular injuries.

Material and Methods

Forty eight male albino rats weighing (125 ± 10 g) were obtained from the laboratory Animal Colony, Ministry of Health and Population, Helwan, Cairo, Egypt.

Metals and Drugs:

lead (Pb), Mercury (Hg) and cadmium (Cd) were purchased from El Gomhoria Company for Drugs and Chemical Industries, meanwhile, Curcumin was kindly provided from Government Pharmaceutical Organization. Cairo, Egypt.

Methods:

Preparation of the basal diet:

Basal diet was prepared to meet the rats nutrient requirements according to methods of (40). It constituted of 10% protein, 3404 K. Calorie Energy, 5 % fiber, 7.5 fat, 0.5% vitamins and minerals mixture, 0.3 % salt (Sodium Chloride).

Experimental Design:

The present experiment was carried out on forty eight male albino rats (125 ± 10 g), that were housed in plastic cages at a room temperature maintained at 25 C°. All rats were kept under normal healthy conditions and allowed to water and basal diet freely for one week before starting the experiment for acclimatization and then the rats were divided into main four groups as follows:

Group - I: Rats served as control and received vehicle, corn oil only (5 ml/kg body weight), without any heavy metals.

Group - II: Rats received curcumin dissolved in corn oil at a dose of 150 mg/5 ml/kg body weight for two months by gastric gavages, five times weekly.

Group - III: Rats received a mixture of heavy metals (Pb, Hg and Cd) for two weeks dissolved in their drinking water as follows: 40 ppm Pb, 20 ppm Hg, 40 ppm Cd.

Group - IV: Rats received curcumin using stomach tube (dose of 150 mg/5 ml/kg body weight for two months, five times weekly) and

stopped before received to the same drinking solution given to the third group.

After two weeks, blood samples were taken from orbital venous plexus under total anesthesia with diethyl ether.

These blood samples were collected and used for determination of creatinine, urea, uric acid and testosterone levels using suitable kits reagents (41, 42, and 43, respectively).

Kidney and testis homogenates were obtained using a tissue homogenizer. The homogenates (1:10 w/v) were prepared using a 100 mM KCl buffer (7:00 pH) containing EDTA 0.3 mM. All homogenates were centrifuged at 1500 rpm for 30 min at 4°C and the supernatants were used for the biochemical assays.

Determination of superoxide dimutase (SOD) activity tissue samples were homogenized in the ratio of 1/10 (w/v) in phosphate buffer (PH 7.4) and centrifuged at 5000 g for 30 min. The supernatant was carefully separated, the 3/5 (v/v) chloroform and ethanol were added. This mixture was centrifuged at 5000Xg for 2 h. The supernatant was used for the determination of SOD. This assay involves Xanthine oxidase used as superoxide generator. The protein concentration of the same supernatant was measured by the method of (44) and the results were expressed as unit per mg protein that inhibit the rate nitroblue tetrazolium (NBT) reduction by 50%.

Determination of reduced glutathione (GSH) content of tissue samples were determined by the method of (45). Tissue samples were homogenized in the metaphosphoric acid solution and colored by DTNB. The results were expressed as micromoles per mg protein.

Histological assays:

Also, kidneys and testis were quickly removed, immersed in 10% formalin, dehydrated and embedded in paraffin, sectioned at 4 μ m, stained with hematoxylin and eosin (H&E) and evaluated by light microscopy.

Images representative of typical histological profile in control and all treated groups were captured with the aid of Motic imaging software.

Statistical analysis

Data are expressed as Mean \pm SE. Data were assessed by paired t-test (46, and 47).

Results:

Data presented in Table 1 represent the levels of plasma creatinine, urea and uric acid concentrations of the different experimental groups. There was significant alteration in renal function in rats exposed to heavy metals (group 3) in comparison to control as indicated by significant

increases of creatinine (+151.4%), urea (+82.4%) and uric acid (+64.4%) concentrations, meanwhile, significant decrease in serum testosterone concentration (-50.0%).

Insignificant changes in the levels of plasma creatinine, urea, uric acid and testosterone concentrations were observed in rats treated with heavy metals plus curcumin.

In group 3 (Treated group with heavy metals) (table 2), the levels of GSH in kidney (-27.8%) and testis (-24.0%) were significantly decreased. In addition, significant decreases in the level of SOD in kidney (-40.0%) and testis (-27.0%) were noted.

Insignificant changes in the levels of GSH and SOD of both kidney and testis were observed in rats treated with heavy metals plus curcumin (group 4).

Histopathological examination of the kidney and testis specimens showed severe alterations in rats exposed to heavy metals (group 3).

Renal tubular dilatations with congestion of blood vessels with hemorrhage and degeneration of renal corpuscles were noted (Fig. 1B–F) compared to the normal structure of control group (Fig. 1A). Also, most of the seminiferous tubules of testes in this group showed complete absence of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa and loss of spermatogenesis process (Fig. 2B–D) in comparison with normal structure of seminiferous tubules in control mice (Fig. 2A).

Administration of curcumin protected the kidney and testis of rats exposed to heavy metals as evidenced by appearance of normal structures of kidney, specially renal corpuscles, (Fig. 1G) and seminiferous tubule of testis (Fig. 2E). Additionally, the histopathological evaluation of curcumin treated rats (group 4) showed normal renal corpuscle (Fig. 1H) and seminiferous tubule (Fig. 2F) structures.

Discussion:

It is well known that heavy metals are widely distributed in environment and some of them can cause physiological, biochemical and histological disorders. Humans are exposed to these metals from numerous sources, including contaminated air, water, soil and food. Therefore, the evaluation of toxic potentials of metals is important for the risk assessment of human beings ordinarily exposed to these substances.

Different scientific studies indicated that the degree of toxic manifestation of different metals depends on dose, duration, route of administration and other physiological factors, especially nutrition

(48). The present work demonstrates that rat chronically intoxicated with a mixture of some heavy metals display a pronounced impairment in kidney function which is confirmed by the enhancement of plasma creatinine, urea, uric acid and decrease of testosterone levels and histopathological alterations.

Several studies demonstrated a significant enhancement of blood creatinine, urea, uric acid and decrease testosterone concentrations, renal and testicular histological alterations in experimental animals intoxicated with Pb, Hg, Cd.(49, 50, and 51).

The present study indicated that the administration of heavy metals to rats produced testicular damage, which led to spermatogenic arrest. Similar observations were noted in experimental animals exposed to heavy metals (7, 8, and 4). The potential toxicity of heavy metals caused alteration in sperm morphology, count, motility as well as biochemical disruptions of enzymes and hormones (48).

(51) demonstrated that Pb at both low and high doses induced lipid peroxidation in liver, and heart lipid peroxidation was observed in rats treated with a high dose of Pb.

Toxicity is manifest in male reproductive system by deposition of Pb in testes, epididymis, vas deferens, seminal vesicle and seminal ejaculate. Pb has an adverse effect on sperm count and retarded the activity of alive sperm. Moreover, motility as well as prolonged latency of sperm melting both in exposed person and experimental animals were observed after Pb exposure (53).

Hg is a spermatogenic, steroidal- and fetotoxic agent. Hg exhibited structural alteration of testicular tissue along with biochemical change. The control testis of albino rat showed sharp localization of ACPase, ATPase and ALKPase in PTM, spermatogenic cell and Leydig cell membrane (54). When Hg and its compound, methyl mercury chloride, affected these membrane-bound hydrolytic enzymes in rats it resulted in sharp decrease of these enzymes, co-related with progressive degeneration of peritubular membrane. Hg also caused the structural and functional disintegration of these enzymes due to its high affinity towards the enzyme's (SH) group (49).

Testicular histopathological evaluation using light and electron microscopy showed that Cd produced an extensive germ cells apoptosis in Sprague–Dawley rats (55).

(56) showed that the administration of Cd caused marked morphological changes in the form

of swelling, congestion, hemorrhage and necrosis in testes of Sprague–Dawley rats. High dose of Cd exposure caused rapid testicular edema, hemorrhage and necrosis. Cd exerted deleterious effect on the vascular structure of testis that may be the result of varying degrees of Cd induced ischemia. Degeneration of testicular tissue after different doses of Cd exposure caused rupture of blood vessels (57). Also, histologically testes showed dose dependent seminiferous epithelial necrosis, degeneration and loss of spermatozoa in albino rats exposed to Cd (58).

The decrease in serum testosterone levels observed in heavy metals treated rats may reflect direct effects of the metal at the testis as this metal accumulates in this tissue. Similar alteration in testosterone concentrations have been reported (59), the study indicating a disruption of the regulatory mechanism of the hypothalamic-pituitary axis. In addition, administration of curcumin might have relieved the reduction of testosterone and/or may have triggered their synthesis that in turn attenuated the oxidative damage caused by cadmium or its metabolites. The final step in the biosynthesis of testosterone is the reduction of androstendione by the enzyme, 17-ketosteroid reductase (60). The significant decrease of this enzyme in heavy metals intoxicated rats interprets the decreased level of testosterone in such group. However, administration of curcumin increased the level of this enzyme and subsequently increased testosterone level in curcumin treated group, this is according to the fact that 17-ketosteroid reductase enzyme is protein in nature, that damaged like other proteins by heavy metals and treatment with curcumin attenuates the damage effect on the tissue protein content and hence on the enzyme.

In rats treated with heavy metals there were significant decreases in the levels of GSH and SOD in kidney and testis tissues.

Glutathione, a tripeptide present in the majority of cells, is responsible for hydrophilic xenobiotics conjugation. GSH serves many vital physiological functions including protection of cells from reactive oxygen species (ROS), detoxification of exogenous compounds, and amino acid transport (61, and 62). Sulphydryl group of glutathione is essential for its antioxidant activity against some forms of ROS in cells (63).

Much of the pathology is associated with the decrease in intracellular GSH concentration (64). Therefore, GSH concentration is important for survival of the cells. The most important protective mechanism for free radical scavenging and

inhibition of electrophilic xenobiotics attack on cellular macromolecules involves tripeptide glutathione (63). Due to nucleophilic thiol group, it can detoxify substances in one of three ways: (1) conjugation catalyzed by glutathione-S-transferases (GST), (2) chemical reaction with a reactive metabolite to form a conjugate and (3) donation of proton or hydrogen atom to reactive metabolites or free radicals. Regarding the role of glutathione in the protection against oxidative stress and detoxification of xenobiotics, its availability in the reduced form (GSH) may be a key factor in maintenance of health. It has been established in several different animal models, as well as in humans, that a decrease in GSH concentration may be associated with aging and pathogenesis of many diseases (65, and 66).

Superoxide dismutases (SODs) belong to a family of antioxidant enzymes that catalyze the dismutation of superoxide to yield hydrogen peroxide and oxygen (67). SOD is essentially a protective enzyme which scavenges the superoxide ions produced as cellular by-products during oxidative stress (68). Its decreased activity can lead to adverse effects because superoxide anions are extremely toxic and may accumulate in the cells. Many studies indicate that heavy metals act as catalysts in the oxidative reactions of biological macromolecules therefore the toxicities with these metals might be due to oxidative tissue damage (69, and 70).

Enhanced generation of ROS can overwhelm cells' intrinsic antioxidant defenses, and result in a condition known as "oxidative stress". Cells under oxidative stress display various dysfunctions due to lesions caused by ROS to lipids, proteins and DNA. Consequently, it is suggested that metal-induced oxidative stress in cells can be partially responsible for the toxic effects of heavy metals (71).

Antioxidants may play an important role in abating some health hazards of heavy metals in connection with an interaction of physiological free radicals (health effects). So, multiple mechanisms may be responsible for ROS production in toxic metal exposure. Among them, alterations in thiol status, increased lipid peroxidation, production of ROS, and damage to cell's antioxidant defense systems are well known for all redox-active and inactive elements (72).

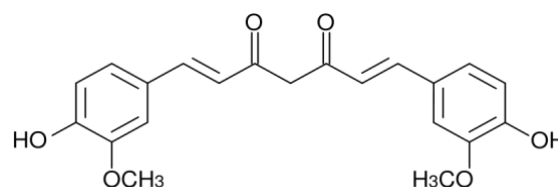


Figure 1. Curcumin.

Curcumin is known to protect biomembranes against peroxidative damage. Peroxidation of lipids is known to be a free-radical-mediated chain reaction, leading to the damage of the cell membranes and the inhibition of peroxidation by curcumin is mainly attributed to the scavenging of the reactive free radicals involved in the peroxidation. Most of the antioxidants have either a phenolic functional group or a diketone group. Curcumin is a unique antioxidant, which contains a variety of functional groups, including the β -diketo group, carbon-carbon double bonds, and phenyl rings containing varying amounts of hydroxyl and methoxy substituents (Figure1) (73).

The central argument is whether the phenolic or the central methylenic hydrogen in the heptadienone moiety is responsible for its antioxidant activity. (74) concluded that curcumin is a super H-atom donor by donating the H-atom from the central methylenic group rather than from the phenolic group in acidic and neutral aqueous and acetonitrile solutions. On the other hand, (75) and (76) proposed that curcumin is a classical phenolic chain-breaking antioxidant, donating H-atoms from the phenolic group. (77) have also claimed that the phenolic group is essential for the free-radical-scavenging activity and that the presence of the methoxy group increased the activity.

In conclusion, the present results showed that, treating heavy metals-intoxicated rats with curcumin significantly protected the kidney and testis structures and functions as compared to the controls. These observations were confirmed by insignificant alterations in the levels of plasma creatinine, urea, uric acid, testosterone concentrations and GSH and SOD of both kidney and testis. This study therefore suggests that curcumin may be useful preventive agents against the effect of the studied heavy metals at least partly due to their antioxidant properties.

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Table 1: Levels of plasma creatinine, urea, uric acid and testosterone concentrations (mean± SD) in controls, curcumin, heavy metals and heavy metals plus curcumin treated rats.

Groups	Plasma creatinine (mg/dL)	Plasma urea (mg/dL)	Plasma uric acid (mg/dL)	Serum testosterone (ng/ml)
Control G.	0.37±0.03 (100%)	13.95±1.38 (100%)	1.88±0.13 (100%)	1.4±0.1 (100%)
Curcumin G.	0.36±0.025 (-2.7%)	15.0±1.4 (+7.5%)	1.7±0.11 (-9.5%)	* 1.8±0.16 (+28.5%)
Heavy metals G.	*** 0.93±0.05 (+151.4%)	*** 25.45±3.09 (+ 82.4%)	*** 3.09±0.31 (+ 64.4%)	*** 0.7±0.05 (-50.0%)
Curcumin + heavy metals G.	0.44±0.032 (+18.9%)	14.88±2.46 (6.7%)	2.2±0.23 (+17.0%)	1.1±0.08 (21.5%)

Each value represents the mean of 12 rats ± SE.

Significant different from the corresponding control at $P<0.01$ *, $P<0.01$ ** and $P<0.01$ ***.

Table 2: Levels of kidney GSH, kidney SOD, testis GSH and testis SOD (mean± SD) in controls, curcumin, heavy metals and heavy metals plus curcumin treated rats.

Groups	Kidney GSH (Imol/gtissue)	Kidney SOD (U/mg tissue)	Testis GSH (Imol/g tissue)	Testis SOD (U/mg tissue)
Control G.	3.42±0.30 (100%)	4.58±0.38 (100%)	9.73±0.94 (100%)	142.33±11.33 (100%)
Curcumin G.	3.5±0.27 (+2.3%)	5.0±0.4 (+9.0%)	10.0±0.87 (+2.7%)	150.0±13.4 (+5.4%)
Heavy metals G.	*** 2.47±0.48 (- 27.8%)	*** 2.75±0.66 (- 40.0%)	*** 7.40±1.01 (- 24.0%)	*** 103.97±5.79 (- 27.0%)
Curcumin + heavy metals G.	3.20±0.42 (- 4.4%)	3.97±0.51 (- 13.3%)	8.28±0.861 (-14.9%)	126.17±10.44 (-11.4%)

Legends as in table (1).

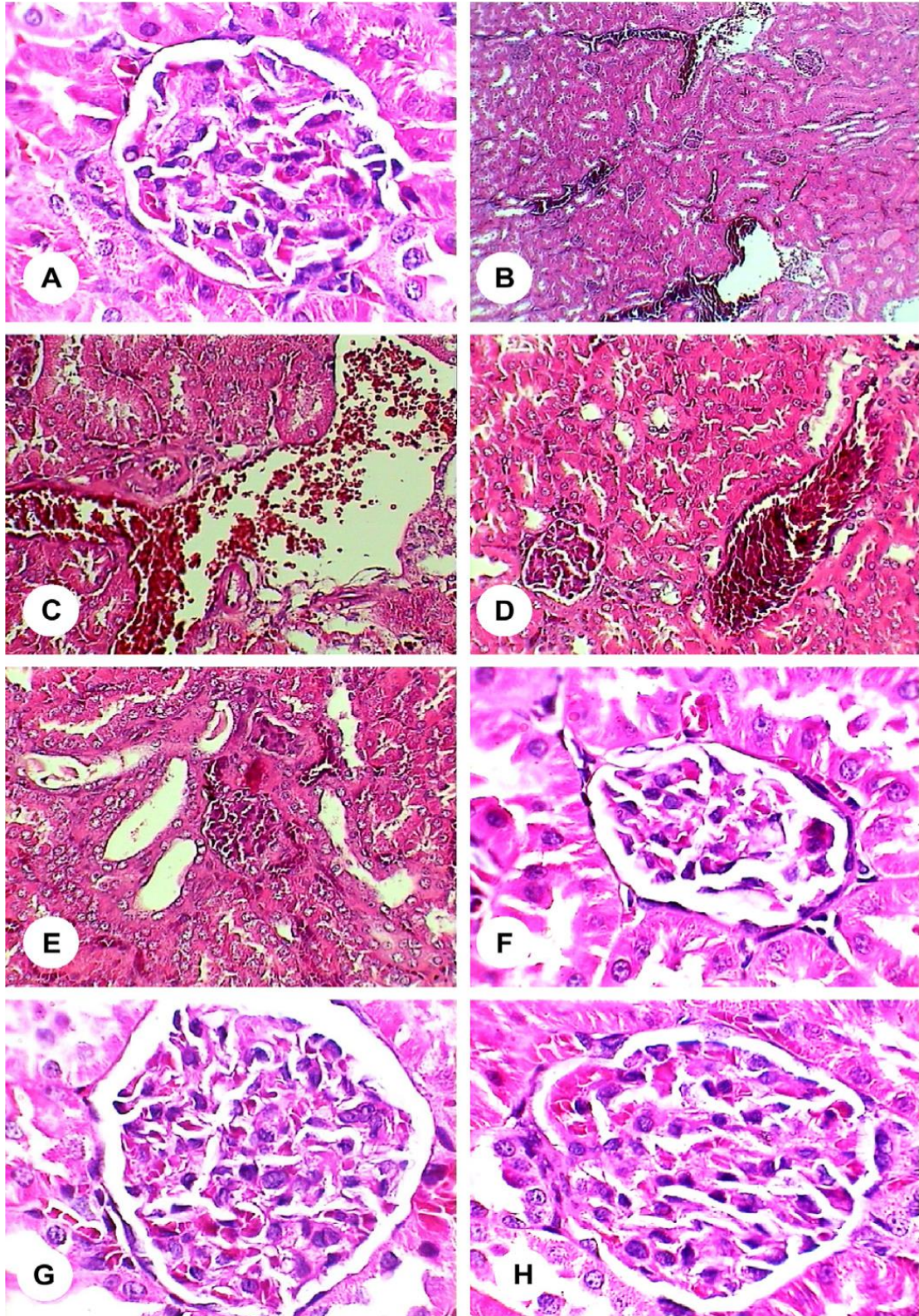


Figure 1 (A–H) Histological changes of kidney in each group. (A) Normal structure of renal corpuscle in control rats (1000 \times). (B) Renal cortex and medulla structure of heavy metals treated rats (100 \times). (C–E) Renal cortex structures of heavy metals treated rats (400 \times). (F) Renal corpuscle structure of heavy metals treated rats (1000 \times). (G) Renal corpuscle structure of heavy metals plus curcumin treated rats (1000 \times). (H) Renal corpuscle structure of curcumin treated rats (1000 \times).

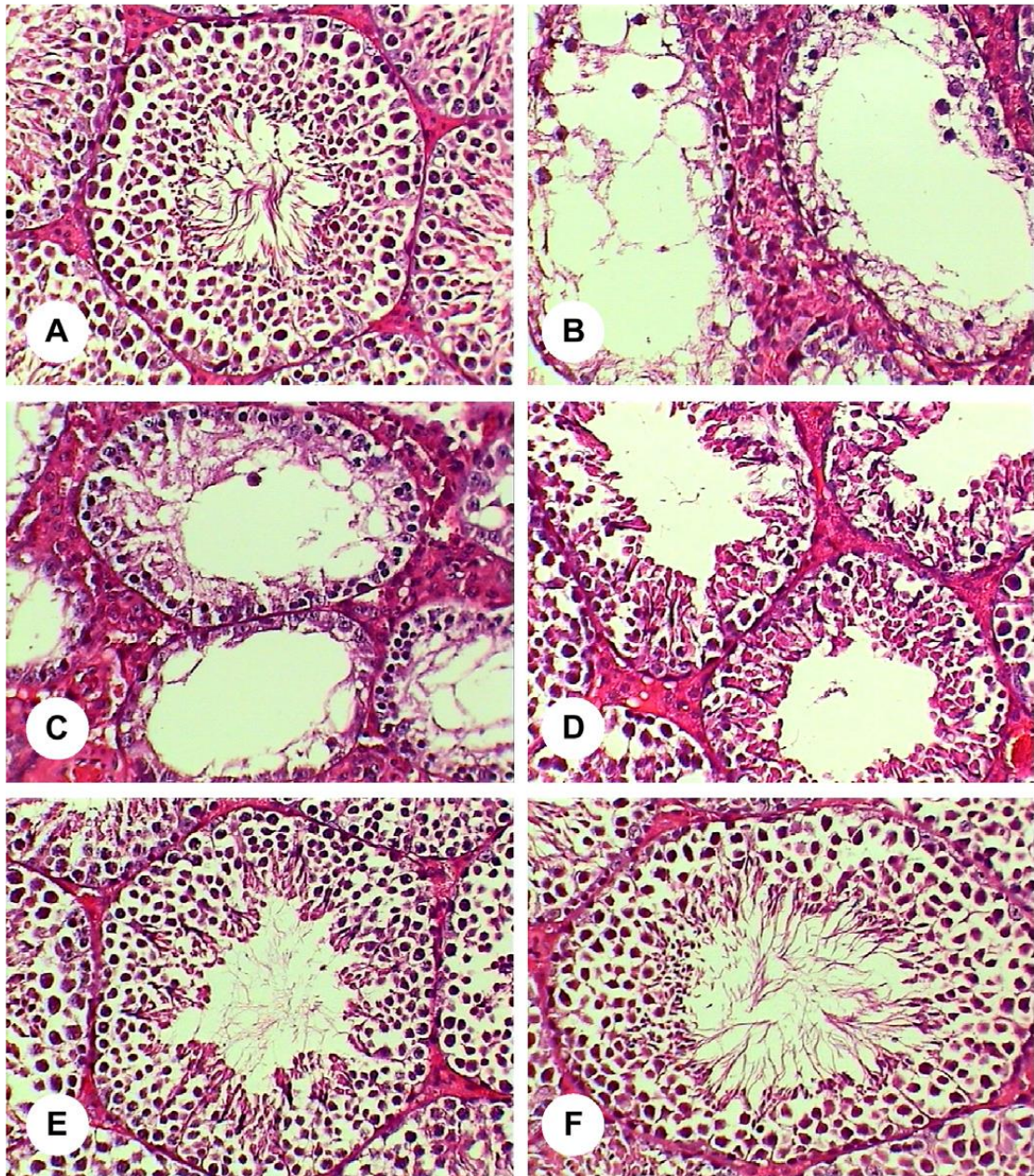


Figure 2 (A–F) Histological changes of testis in each group. (A) Normal structure of seminiferous tubule in control rats (400 \times). (B–D) Seminiferous tubules structure of heavy metals treated rats (400 \times). (E) Seminiferous tubule structure of heavy metals plus curcumin treated rats (400 \times). (F) Seminiferous tubule structure of curcumin treated rats (400 \times).