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Effect of quercetin and curcumin in rats sub-acutely exposed to cadmium chloride: haemato-biochemical changes, oxidative stress parameters and histopathological changes in intestine, liver and kidney of rats

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ABSTRACT

Quercetin is a flavonoid mostly found in fruits and vegetables. Curcumin is the main natural polyphenol found in the rhizome of *Curcuma longa* and in others *Curcuma* spp.. Individually, quercetin and curcumin had shown to have various pharmacological properties. The increasing level of cadmium in the environment is alarming as cadmium affects the antioxidant defense system with ability to persist in the body for long time. The bioaccumulation of cadmium is well-known, which is dangerous for the health of human and animals after continuous exposure to it. The present experiment was carried out to evaluate the ameliorating effect of quercetin (50 mg/kg daily orally for 28 days) and curcumin (100 mg/kg daily orally for 28 days) alone and in combination of both against cadmium-induced (100 ppm in water for 28 days) alterations in biochemical markers and histological changes in intestine, liver and kidney of rats. Body weight gain in rats of toxicity group during the 4th week of study period was significantly affected by the cadmium. Cadmium exposure significantly increased the levels of AST, ALT, ALP, bilirubin and glucose in serum along with higher level of MDA in intestine, liver and kidney of rats. The administration of quercetin and curcumin in combination as compared to individual treatment along with cadmium exposure had shown significantly lower levels of above parameters. Various histological changes were noticed in intestine, liver and kidney of rats following exposure to cadmium which were improved in rats treated with individual or combined treatment of quercetin and curcumin. Quercetin alone had shown the ameliorating effect against cadmium-induced alteration in kidney of rats. While, combination of quercetin and curcumin has been found to protect the intestine and liver from cadmium-induced damage following sub-acute exposure in rats. However, further study is needed to explore the mechanism of protective effect of the quercetin and curcumin against cadmium-induced changes in intestine, liver and kidney.

Keywords: Quercetin, Curcumin, Cadmium chloride, Oxidative stress, Histopathology, Rats

INTRODUCTION

Cadmium (Cd) is well-recognized environmental pollutant and exposure to it is posing numerous adverse effects in human and animals. Expose to Cd led to cancer and organ system toxicity such as skeletal, gastrointestinal tract, hepatic, urinary, reproductive, cardiovascular, respiratory, and central and peripheral nervous systems. It is also associated with waste water pollution and its discharge into water resulting in adverse effects on living organisms and the environment [1]. Cd is known for long elimination half-life of about 20-30 years risking multi organ toxicity [2].

Reactive oxygen species (ROS) are accountable in Cd-induced harmful health effects as it stimulate the production of ROS due to an inhibitory effect on mitochondrial electron transport chain [3, 4]. Cadmium causes anaemia through destruction of RBCs, [5] reduction absorption of iron from gut and diminished production of erythropoietin (EPO) hormon [6]. Moreover, Cd exposure causes testicular atrophy, renal failure, hepatic damage, hypertension and central nervous system injury [7]. Increased membrane lipid peroxidation in tissues is a key tool of Cd toxicity and damaging the cells antioxidant system and cause injury to cellular components mainly by interaction of metal ions with cell organelles [8].

Oxidative stress mediated induced toxicity by Cd can be reversed using natural or synthetic antioxidants. As herbs provides major source of natural antioxidants in the form of flavonoids and it have greater importance to combating the oxidative stress. Some of naturally extracted molecules like quercetin and curcumin have greater potential to reverse the damage caused by the heavy metals like Cd. Quercetin (QRCT) is a flavonoid compound and is ubiquitously dispersed in fruits and vegetables and act as major part of flavonoids from daily foods. It is widely found in skin of apple, pills of red onion, berries, grains,

tea and red wine. Quercetin is a powerful antioxidant acts by chelation of metal ions, scavenging of oxygen free radicals and guards against lipid peroxidation [9]. Quercetin has many pharmacological properties like antioxidant, neuroprotective, hepatoprotective and protective action on reproductive system [10, 11]. Curcumin (CMN) is a dynamic constituent of *Curcuma longa* (turmeric) derived from rhizomes. Curcumin has wide spectrum of biological activities such as antioxidant, anti-inflammatory, immunomodulatory, antineoplastic and antifungal activity [12, 13, 14]. It is a potent inhibitor of various reactive oxygen species. It exhibits protective effects against oxidative damage as a scavenger for free radicals and prevents lipid peroxidation. Curcumin binds to heavy metals such as Cd and lead and thus has a detoxification effect against heavy metals [14].

Individually quercetin and curcumin has shown potential pharmacological effects against oxidative stress-induced damage to major organs. Quercetin reported to boost the bioavailability of curcumin through enhancing its uptake into human carcinoma cells [15, 16]. Zhang *et al.* (2015) documented that curcumin and quercetin has anti-gastric cancer effect against gastric cancer MGC-803 cells [17]. There may be possibility to enhance the effect by using them in combination. The effects of quercetin and curcumin in combination against Cd induced oxidative damage to intestine, liver and kidney have not been evaluated so far. Thus, present study was planned to evaluate the ameliorating effect of quercetin and curcumin alone and in combination of both against Cd induced alterations in biochemical markers and histopathological changes in intestine, liver and kidney of rats.

MATERIALS AND METHODS

Experimental animals and design

This study was conducted on 36 SD rats (8-9 weeks of age, 255-270 g weight). *Ad libitum* feed and water were provided throughout the experiment. Rats were well maintained under 23 to 27˚C temperature; 42 to 55% humidity and 12 hours light-dark cycle. The rats were randomly divided to six groups (six rats in each group) into normal control group (C1), toxicity control group (C2), vehicle group (C3), quercetin treatment group (T1), curcumin treatment group (T2) and quercetin and curcumin in combination treatment group (T3). Animals of normal control group given *ad libitum* R.O. drinking water for a period of 28 days. Rats of other groups (C2, C3, T1, T2 and T3) were offered drinking water along with Cd at the level of 100 ppm for 28 days. The vehicle control (C3) group was administered with corn oil and the volume of administration was calculated based on volume of corn oil used per kg of body weight in other groups of treatments (T1, T2 and T3). The stock solution of quercetin was prepared by dissolving 250 mg in 10 mL of corn oil (25 mg/mL) and was given by oral route at the dose rate of 50 mg/kg daily for 28 days to animals of group T1 and T3. Curcumin (500 mg) was dissolved in 10 mL of corn oil (50 mg/mL) and given by oral route at the dose rate of 100 mg/kg daily for 28 days to animals of group T2 and T3. Oral gavage needle with round end was used for administration of quercetin, curcumin and vehicle (corn oil) on daily basis to rats of different groups and live body weight of animals was recorded before application of test substances. The research protocol was agreed by the Institutional Animal Ethics Committee (IAEC), College of Veterinary Science and Animal Husbandry, Junagadh, Gujarat.

Evaluation of haematological and biochemical parameters

Various haematological parameters were analyzed by automated haematology analyzer (Abacus Junior Vet 5, Diatron, Hungary) and biochemical parameters were determined using standard kits on fully automatic biochemistry analyzer (Diatek Health Care Pvt. Ltd, India).

Evaluation of oxidative stress parameters

All oxidative stress parameters like activity of SOD and catalase, and GSH and MDA level in blood and tissues samples of intestine, liver and kidney were evaluated as described previously [18].

Histopathological examination

The formalin fixed tissues of intestine, liver and kidney were embedded in paraffin and processed as per standard procedures. Sectioning of tissue samples was carried out at 5-6 µ thickness with semi-automated rotary microtome (Leica Biosystems, Germany) and were stained with haematoxylin and eosin stain [19].

Statistical analysis

Statistical analyses of all data were carried out using Graphpad Prism 9. Shapiro-Wilk test was used to evaluate the normality of data along with Bartlett's test to confirm the equal variance. Data with normal distribution and equal variance were analyzed by one way analysis of variance (ANOVA) followed by Tukey's HSD test. The data lacking normal distribution or equal variances were analyzed by Kruskal-Wallis test followed by Dunn's test [20].

RESULTS

Symptoms

Noticeable clinical signs of toxicity were not observed in rats of any groups except hair fall upon grooming and diarrhea in Cd treatment and vehicle groups, such effect in T1, T2 and T3 were lesser as compared to toxicity and vehicle groups (C2 and C3). The body weight gain in toxicity group and quercetin treatment group during the whole study period was non-significantly lower as compared to the normal control group. The body weight gain during the whole study period in rats of treatment group T3 (quercetin + curcumin) was at par with normal control group (Figure 1a). During the 4th week, the body weight gain in rats treated with quercetin + curcumin was significantly (p <0.005) higher than that of toxicity group (Figure 1b).

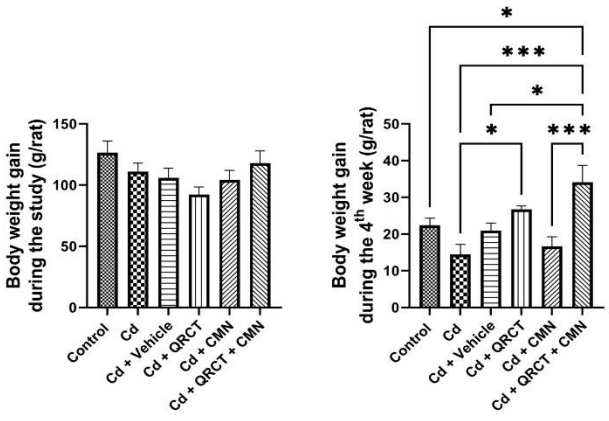


Figure 1: Bogy weight gain (g/rat) during the study period and body weight gain (g/rat) during the 4th week of study period. Data were analyzed by one way ANOVA followed by Tukey's HSD test. * indicates p < .05 and *** indicates p < .005.

Organ body weight ratio

Only kidney body weight ratio was significantly (p <0.05) decreased in toxicity (0.0069 ± 0.0002) and vehicle group (0.0071 ± 0.0002) as compared to that of control group (0.0079 ± 0.0006). Curcumin treatment (T2) partially prevented the change in kidney body weight ratio (0.0074 ± 0.0002).

Haematological and biochemical parameters

Most haematological parameters were not significantly altered upon Cd exposure to rats for 28 days (Table 1) except AST and ALP, which were significantly increased in rats of toxicity group. The quercetin and curcumin alone as well as in combination averted the alterations up to certain extent (Table 2). The levels of AST in all three treatment groups were significantly lower than toxicity group. However, the level of ALP was significantly lower (p <0.05) in rats treated with quercetin + curcumin as compared to that of toxicity group.

Table 1: Haematological parameters of rats under different treatments

Parameters	Treatment groups					
	Control	**Cd**	**Cd + Vehicle**	**Cd + QRCT**	**Cd + CMN**	**Cd + QRCT + CMN**
HB (g/dL)	15.47 ± 0.20^{a}	14.57 ± 0.71^{a}	14.63 ± 0.30^{a}	15.17 ± 0.22^{a}	14.77 ± 0.15^{a}	14.88 ± 0.22^{a}
PCV (%)	44.05 ± 0.95^{a}	41.59 ± 1.57^{a}	41.44 ± 1.03^{a}	44.22 ± 0.78^{a}	43.21 ± 1.01^{a}	44.76 ± 0.78^{a}
TEC (10^{6}/µl)	8.87 ± 0.25^{ab}	8.52 ± 0.49^{a}	8.55 ± 0.34^{a}	9.40 ± 0.21^{ab}	8.92 ± 0.17^{ab}	9.59 ± 0.24^{b}
TLC (10^{3}/cmm)	10.36 ± 0.78^{a}	9.74 ± 1.24^{a}	9.62 ± 0.91^{a}	9.53 ± 0.38^{a}	9.69 ± 0.24^{a}	8.72 ± 0.46^{a}
MCV (fl)	49.67 ± 0.67^{b}	47.50 ± 0.96^{ab}	47.17 ± 0.91^{a}	47.00 ± 0.63^{a}	48.00 ± 0.68^{ab}	46.83 ± 0.70^{a}
MCHC (%)	36.02 ± 0.87^{b}	36.05 ± 0.57^{b}	35.57 ± 0.60^{b}	34.35 ± 0.63^{ab}	34.57 ± 0.49^{ab}	33.30 ± 0.41^{a}
MCH (pg)	17.48 ± 0.45^{c}	17.10 ± 0.28^{bc}	16.78 ± 0.38^{bc}	16.18 ± 0.36^{ab}	16.47 ± 0.30^{abc}	15.58 ± 0.38^{a}
Lymphocyte (%)	83.67 ± 2.38^{ab}	80.87 ± 2.21^{a}	81.12 ± 1.31^{a}	87.03 ± 1.14^{b}	85.12 ± 1.66^{ab}	85.95 ± 1.69^{ab}
Monocytes (%)	2.52 ± 0.61^{a}	3.47 ± 0.88^{a}	3.32 ± 0.14^{a}	2.05 ± 0.59 ^{a}	1.77 ± 0.49^{a}	2.15 ± 0.41^{a}
Neutrophils (%)	13.80 ± 1.88^{a}	15.67 ± 1.58^{a}	14.52 ± 1.97^{a}	10.87 ± 1.40^{a}	13.10 ± 1.80^{a}	11.87 ± 1.49^{a}

ANOVA followed by Tukey's HSD test. Values with different superscript in rows differ significantly (P<0.05).

Table 2: Biochemical parameters of rats under different treatments

Parameters	Treatment groups					
	Control	**Cd**	**Cd + Vehicle**	**Cd + QRCT**	**Cd + CMN**	**Cd + QRCT + CMN**
ALT (IU/L)	51.85 ± 2.95^{a}	67.48 ± 3.29^{ab}	62.61 ± 1.81^{ab}	64.99 ± 5.21^{b}	62.16 ± 6.13^{ab}	72.02 ± 3.91^{b}
AST (IU/L)	79.84 ± 5.99^{a}	119.35 ± 16.43^{b}	94.78 ± 5.31^{ab}	83.61 ± 5.96^{a}	83.35 ± 5.66^{a}	82.36 ± 0.95^{a}
ALP (IU/L)	169.40 ± 13.78^{a}	215.75 ± 13.34^{b}	185.73 ±14.34^{ab}	190.63 ± 11.58^{ab}	175.75 ±11.63^{ab}	160.17 ± 9.59^{a}
BUN (mg/dL)	20.83 ± 0.91^{ab}	23.15 ± 0.99^{b}	19.92 ± 0.73^{a}	21.14 ± 0.53^{ab}	21.97 ± 0.47^{ab}	20.85 ± 0.86^{ab}
Creatinine (mg/dL)	0.34 ± 0.04^{a}	0.40 ± 0.09^{a}	0.35 ± 0.05^{a}	0.36 ± 0.08^{a}	0.28 ± 0.02^{a}	0.25 ± 0.05^{a}
Total protein (g/dL)	5.03 ± 0.21^{ab}	4.61 ± 0.17^{a}	4.77 ± 0.11^{ab}	4.75 ± 0.15^{ab}	5.14 ± 0.13^{b}	5.00 ± 0.07^{ab}
Total Bilirubin (mg/dL)	0.21 ± 0.01^{a}	0.35 ± 0.02^{d}	0.32 ± 0.02^{cd}	0.26 ± 0.02^{ab}	0.29 ± 0.01^{bc}	0.25 ± 0.01^{ab}
Blood glucose level (mg/dL)	116.50 ± 5.3^{a}	142.50 ± 8.1^{bc}	134.50 ± 7.6^{abc}	123.17 ± 2.8^{ab}	145.00 ± 5.3^{c}	134.17 ± 8.1^{abc}

ANOVA followed by Tukey's HSD test. Values with different superscript in rows differ significantly (P<0.05).

Oxidative stress parameters

Various oxidative stress parameters determined from blood, intestine, liver and kidney are depicted in figure 2, 3, 4, 5, respectively.

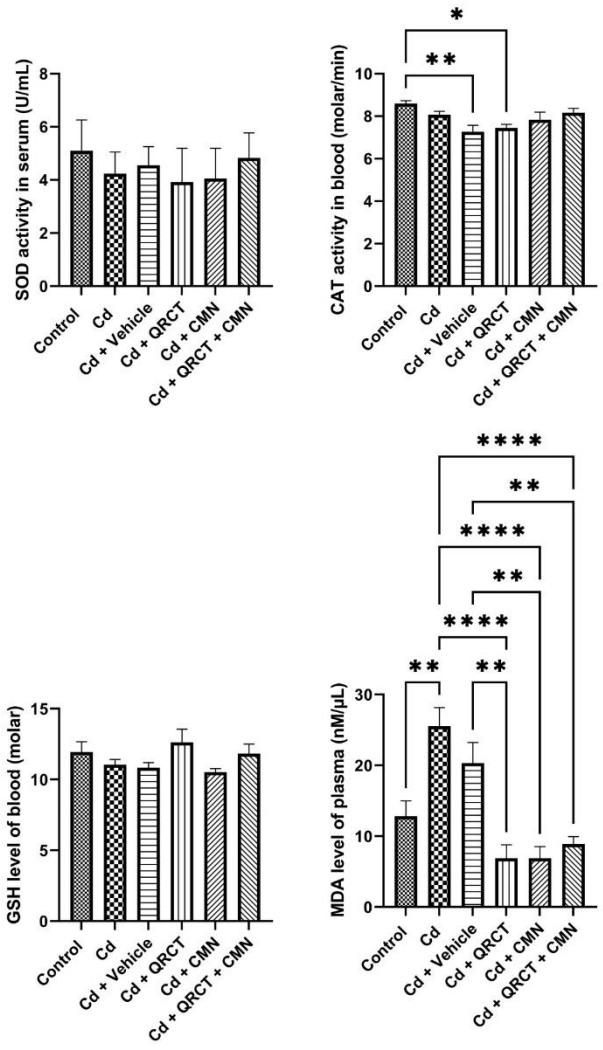
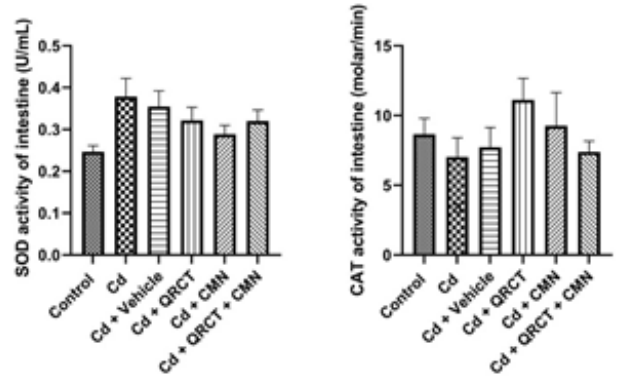


Figure 2: Oxidative stress parameters in blood of rats under different treatments. Data of SOD were analyzed by Kruskal-Wallis test followed by Dunn's test. Other data were analyzed by one way ANOVA followed by Tukey's HSD test. * indicates $p < .05$, ** indicates $p < .01$ and **** indicates $p < .001$.



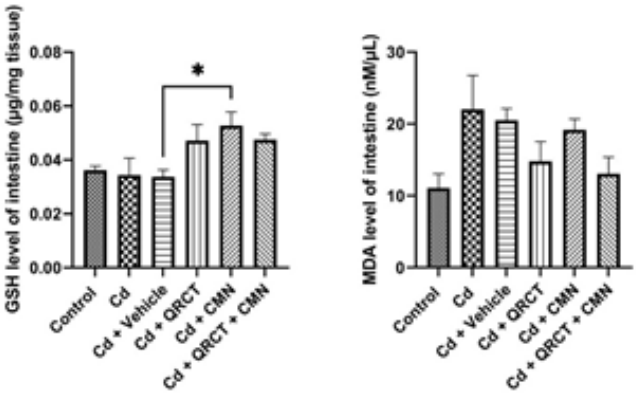


Figure 3: Oxidative stress parameters of intestine tissue of rats under different treatments. Data of GSH and MDA were analyzed by Kruskal-Wallis test followed by Dunn's test. Other data were analyzed by one way ANOVA followed by Tukey's HSD test. * indicates $p < .05$.

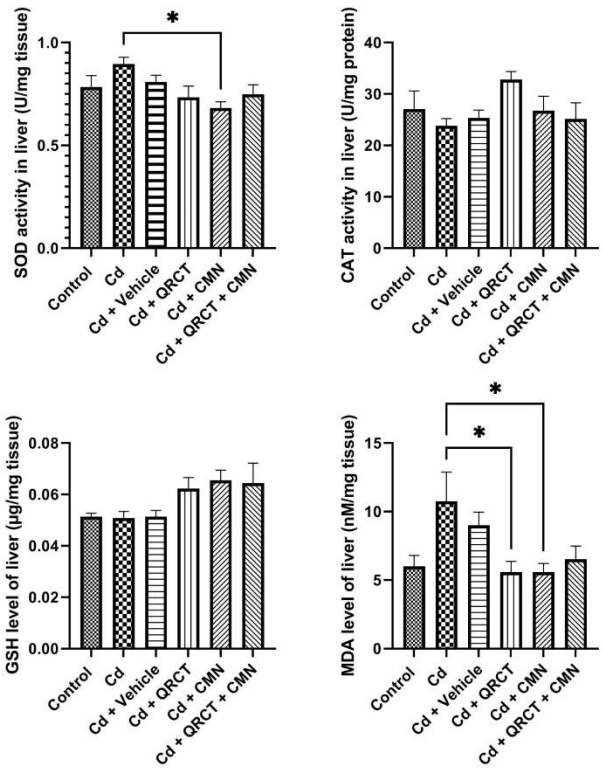


Figure 4: Oxidative stress parameters of liver tissue of rats under different treatments. Data of GSH were analyzed by Kruskal-Wallis test followed by Dunn's test. Other data were analyzed by one way ANOVA followed by Tukey's HSD test. * indicates $p < .05$.

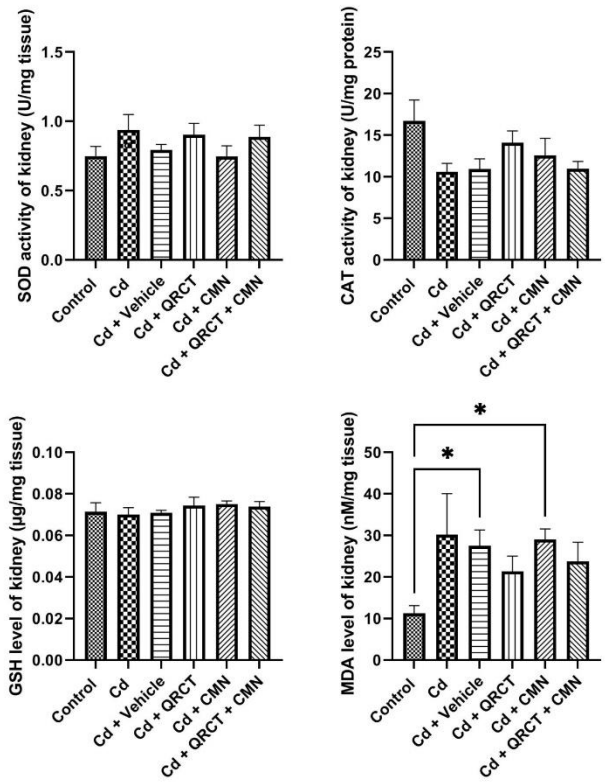
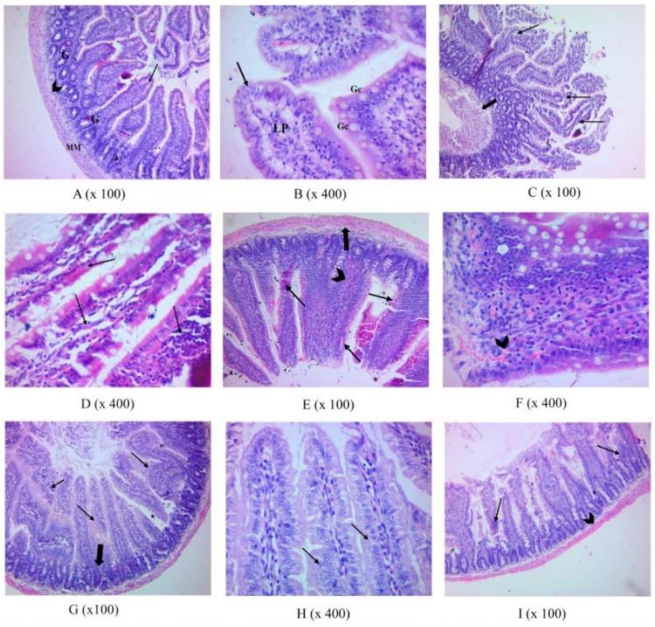


Figure 5: Oxidative stress parameters of kidney tissue of rats under different treatments. Data of CAT and MDA were analyzed by Kruskal-Wallis test followed by Dunn's test. Other data were analyzed by one way ANOVA followed by Tukey's HSD test. * indicates $p < .05$.

Histopathological examination

The microscopic lesions observed in intestine, liver and kidney of rats of different treatment groups are as shown in figure 6, 7 and 8, respectively.



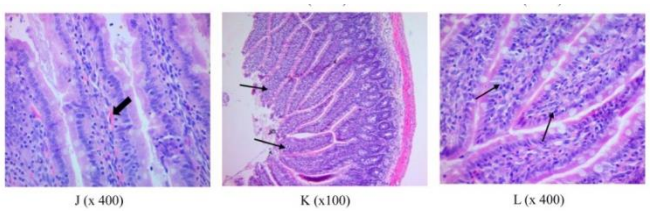


Figure 6: Microscopic view of intestine of rats of different groups (H & E, x 100, x 400). (A, B) Intestine of C1 group - normal structure of intestinal villi (thin arrow) with intact lamina propria (LP), goblet cells (Gc), glands (G), muscularis mucosae (MM) and submucosa (arrow head); (C, D) Intestine of group C2 - degenerative changes in intestinal villi (thin arrow) (epithelial layer, lamina propria), glands, submucosa and muscularis mucosae (thick arrow); (E, F) Intestine of group C3 - degenerative changes in intestinal villi (thin arrow) (epithelial layer), glands, intact submucosa and muscularis mucosae (thick arrow), mild haemorrhagic lesion in lamina propria (arrow head); (G, H) Intestine of group T1 - mild degeneration of epithelial layer of intestinal villi (thin arrow) and glands (thick arrow), intact submucosa and muscularis mucosae as compared to toxicity group (C2); (I, J) Intestine of group T2 - mild degenerated epithelial layer of intestinal villi (thin arrow) and mild haemorrhagic lesion in lamina propria (thick arrow) as compared to toxicity group (arrow head) (C2); (K, L) Intestine of group T3 - almost normal structure of intestinal villi (thin arrow) and glands, intact submucosa and muscularis mucosae as compared to toxicity group (C2).

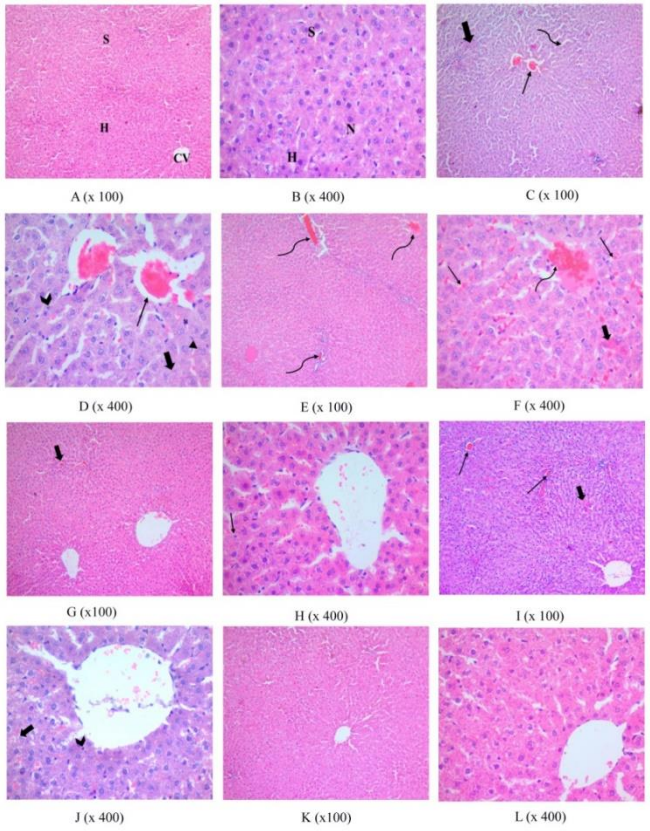


Figure 7: Microscopic view of liver of rats of different groups (H & E, x 100, x 400). (A, B) Liver of group C1 - normal histological architecture of liver with hepatocytes (H), nuclei (N), central vein (CV), sinusoids (S); (C, D) Liver of group C2 - degeneration of hepatocytes (thick arrow), central vein congestion (thin arrow), increase in sinusoidal space, pyknotic nuclei (arrow head) and fragmentation of nuclei (triangle) as compared to control group (C1); (E, F) Liver of group C3 - degeneration of hepatocytes (thick arrow), increase in sinusoidal space, hemorrhages (thin arrow), central vein congestion

(curved arrow); (G, H) Liver of group T1 - normal arrangement of hepatocytes with mild venous congestion (thick arrow), and pyknotic nuclei (thin arrow) as compared to toxicity group (C2); (I, J) Liver of group T2 - mild degeneration of hepatocytes (thick arrow) as compared to toxicity group (C2), venous congestion (thin arrow), pyknotic nuclei (arrow head); (K, L) Liver of group T3 - almost normal structure.

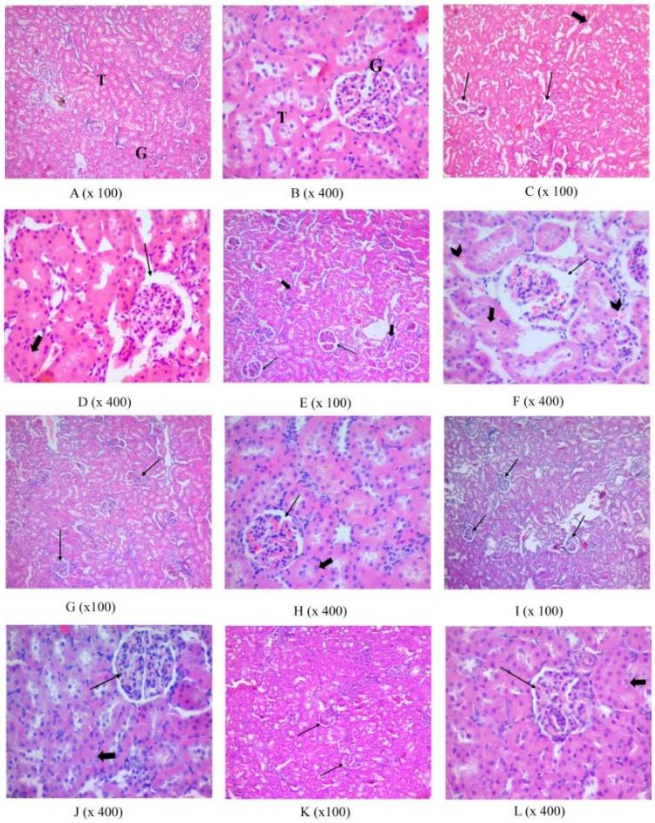


Figure 8: Microscopic view of kidney of rats of different groups (H & E, x 100, x 400). (A, B) Kidney of group C1 - normal architecture of glomeruli (G) and proximal and distal convoluted renal tubules (T); (C, D) kidney of group C2 - shrunken glomeruli with increase space in Bowmen's capsule (thin arrow) and degeneration in renal tubules (thick arrow); (E, F); Kidney of group C3 - shrunken glomeruli (thin arrow) with increase space in Bowmen's capsule and degeneration in renal tubules (thick arrow) with hemorrhage (arrow head) (G, H) Kidney of group T1 - almost normal glomeruli (thin arrow), mild cloudy swelling of renal tubules (thick arrow) as compared to toxicity group (C2); (I, J) Kidney of group T2 - almost normal glomeruli (thin arrow) along with mild cloudy swelling of renal tubules (thick arrow) as compared to toxicity group (C2); (K, L) Kidney of group T3 - almost normal glomeruli (thin arrow) but with mild cloudy swelling of renal tubules (thick arrow) as compared to toxicity group (C2).

DISCUSSION

Heavy metals are inorganic elements and generation of oxidative stress plays a major role behind heavy metal toxicity. Cadmium (Cd) is ubiquitous in nature and known as one of the most toxic metals, in relation to both environmental contamination and toxicity to animals and human. Low dose of Cd in the present study did not affect the feed consumption. However, higher dose of Cd (500 ppm, P.O., 12 weeks) has been reported to cause severe stomach irritation and vomiting with massive fluid imbalance and wide spread gastrointestinal and organ damage [21]. EI-demerdash *et al.* (2009) reported that Cd exposure increases the risk of diabetes mellitus, which enlightens the weight loss in rats [22]. Low heavy metals cause dysfunction of glucocorticoids as it plays a major role in glucose control as well as carbohydrate, lipid and protein metabolism [23]. Lower liver body weight ratio was reported upon Cd exposure at high level in rats[24]. However, in the present study, 100 ppm Cd could not alter the weight of liver which might be due to variation in dose and exposure duration. In the present study, kidney body weight ratio in Toxicity group was significantly reduced which is supported by findings of Ojo *et al*. (2014) who also observed significant reduction in weight of kidney in Cd-exposed rats[25]. Similar to our findings related to decreased kidney weight in rats exposed to Cd, Brzoska *et al.* (2003) reported reduced kidney weight in rats exposed to Cd at 5 mg/kg, P.O. with symptoms of structural, but not functional, damage to the glomeruli[26]. The reduced kidney weight and kidney body weight ratio in Cd-exposed rats in the present study was also reflected in histopathological finding viz. shrunken glomeruli with increase space in bowmen's capsule and degeneration in renal tubules.

Cadmium is highly toxic and binds quickly to extracellular and intracellular protein and disrupts membrane and cell function. In the present study, Cd exposure did not affect the haematological parameters which were similar to the previous reports [27, 28]. In contrast to the findings in the present study, previous studies reported anemia due to loss of membrane functions through oxidative damage [20, 30, 31, 32]. The anaemia was mainly caused due to damage to the kidney and insufficient production of erythropoietin (EPO) and also inflammatory condition and loosening tight junction of duodenum might be responsible for reduced absorption of iron. The anemia might be developed due to the accumulation of non-essential toxic metal in haematopoitic organs of the body like liver, kidney and spleen [33].

In the present study, AST and ALP in the serum of Cd-exposed rats were significantly increased suggesting Cd-related injury to the liver or other organs. The results were promising with the sightings of Haouem *et al*. (2013) who reported that Cd at 150 mg/L, P.O. for 4 weeks in rats resulted alterations in of AST and ALP [24]. Toppo *et al*. (2015) reported that Cd exposure at 200 ppm/kg, P.O. for 28 days in rats resulted elevated level of AST and ALP [34]. The increase in AST in the study may be explained by the leakage enzymes from liver cytosol to blood circulation due to hepatocellular injury, which caused increased cell membrane permeability. The increase in alkaline phosphatase activities associated with hepatic toxicity [35]. The non-significant reduction in increased level of ALT and significant reduction in levels of AST and ALP in rats treated with quercetin and curcumin alone as well as in combination of both were observed in the present study. Quercetin (50 mg/kg, 28 days) has been reported to have benefit over Cd-induced (5 mg/kg P.O., 28 days) oxidative injury in rat hepatic tissue as observed by Prabu *et al*. (2011) [36]. Curcumin (100 and 200 mg/kg) has been reported to lower the serum ALT activity to 52-53% ($P < 0.05$) and AST to about 62% ($P < 0.05$) in CCl_4-induced (0.2 mL/kg i.p.) liver damage in rats. Similar to our findings, curcumin and quercetin in combination have shown ameliorating effect against nicotine-induced alteration in ALT, AST and ALP [37].

The low level of Cd exposure in the present study might not able to affect the BUN and creatinine levels due to less kidney damage as compared to kidney damage reported following Cd exposure at higher dose. However, Lee *et al.* (2014) evaluated the Cd-induced nephrotoxicity and found that Cd treatment at 25 mg/kg, P.O. for 6 weeks significantly increased the BUN level [38].

Bilirubin is released into circulation by breakdown of haemoglobin. It is transported from the spleen to the liver and excreted into bile.

Causes of hyper bilirubinemia include increased haemolysis, genetic errors, jaundice, ineffective erythropoiesis, and xenobiotics induced damage. In the present study, increased bilirubin level in Toxicity group might be due to hepatic dysfunction or injury due to oxidative stress [39, 40]. Alhazza (2008) also reported that Cd exposure (2.5 mg/kg, S.C. 4 times a week, 8 weeks) caused significant increase in total bilirubin after 6 and 8 weeks of exposure to rats [41].Quercetin, curcumin and in combination of both decreased the bilirubin level indicating hepatoprotective effect. Quercetin has been reported to have ameliorating effect against the Cd (5 mg/kg, p.o., 28 days) induced increased serum bilirubin in rats [36].

Cadmium-induced hyperglycemia in the present study was similar to findings by Thalib *et al*. (2017) who reported that Cd exposure (3 mg/L in drinking water, 4 weeks) in mice significantly increased the blood glucose level which might be due to triggering of damage to pancreatic tissue resulting in a decrease in insulin production and the subsequent decrease in insulin causes the disruption in membrane permeability for glucose, so that entry of glucose into cell will be prevented [42]. Shanbaky *et al*. (1978) explained that Cd was responsible for increase in release of catecholamines and plays a major role in carbohydrate metabolism [43]. Epinephrine induces hepatic glycogenolysis, and prevents insulin release [44]. Nilsson *et al*. (1986) demonstrated that Cd accumulation in pancreatic tissue promoted β-cell dysfunction and affected insulin release [45]. However, group treated with quercetin and group treated with combination of quercetin and curcumin showed reduced blood glucose level which indicates antidiabetic effect of quercetin.

In the present study, increased blood MDA level was suggesting of increased lipid peroxidation by Cd exposure. At the same time, SOD, catalase and GSH were slightly decreased upon Cd exposure, suggesting Cd caused partial reduction of antioxidant enzyme activity and increased the risk for oxidative stress. Cadmium causes damage to the erythrocytes as it binds to the erythrocytic membrane and plasma albumin and erythrocytes are more prone to oxidative damage due to the high oxygen tension, presence of poly-unsaturated fatty acids and iron, as a strong catalyst for free radical reactions [46]. Sarkar *et al*. (1997) documented Cd-induced (2.18 mM $CdCl_2$/kg i.p., for 3 days) elevation of lipid peroxidation in rats [47]. Messaoudi *et al*. (2010) reported significant decrease in activities of catalase (CAT), glutathione peroxidise (GSH-Px) and the total glutathione (GSH) contents in erythrocytes and increased superoxide dismutase (SOD) activity upon Cd treatment at 200 ppm level, P.O. for 5 weeks in rats [48]. Ogunrinola *et al*. (2016) reported that exposure to Cd (100, 200 and 300 ppm, P.O., for 6 weeks) in rats resulted in significant ($p<0.05$) decrease in SOD activity in plasma and erythrocytes in a dose-dependent manner [49].

Cadmium enters body mainly through food and drinking water, therefore, the intestinal tract is at high risk of Cd intoxification [50]. Xenobiotics like Cd known to cause oxidative stress and can cause necrosis or apoptosis of the enterocytes, inflammatory response and disrupt the tight junctions in the intestines leading to the disruption of intestinal barrier and the amplification of Cd absorption [51, 52]. In the present study, Cd resulted in increased MDA level in intestine due to lipid peroxidation and activity of SOD enzyme was increased which may be a compensatory action for increased production of free radicles upon continuous exposure directly to Cd. Non-significant decrease in activity of catalase and GSH level were observed upon Cd exposure in intestine tissue. This increases the susceptibility of intestine tissue to the oxidative damage as there was more production of H_2O_2 by increased SOD activity. However, SOD activity was increased in the present study which indicates body's reaction for combating the increased ROS production. In the present study, quercetin and curcumin alone as well as in combination produced the protective effect on oxidative damage by partially reducing the increased activity of SOD, lipid peroxidation and improved the catalase activity and GSH level in intestine. Quercetin (50 mg/kg, P.O., 15 days) has been reported to have protective effect against the radiation-induced (24 hour of irradiation) enteritis and colitis in rats through reduction in lipid peroxidation and increased the serum total antioxidant status (TAS) level. Quercetin showed protective effect on ileum and colon tissues in rats by decreasing oxidative stress and inflammatory response [53]. Moine *et al*. (2018) reported that quercetin restored the GSH levels in the intestinal tissue against the GSH depleting drugs [54]. Menozzi *et al*. (2009) reported dose-independent protecting effect of oral curcumin (50, 100, and 300 mg/kg) against indomethacin (20 mg/kg) induced enteritis in the rat [55].

Liver after Cd exposure in the present study showed significant increase in MDA level suggesting of increased level of lipid peroxidation due to damage to the hepatocytes. Simultaneously, liver showed non-significant increase in SOD activity and decrease in catalase activity upon Cd exposure, but on GSH level in liver was not affected upon Cd exposure. In our study, after continuous exposure to low level of Cd, SOD and CAT activity were unable to protect the hepatocytes as there might be reduction in elimination of H_2O_2. The findings of Dzobo and Naik (2013) support our results as Cd treatment in rats (1.67 mg/kg/day, i.p., for 15 days) increased the SOD activity and decreased CAT activity [56]. Enhanced lipid peroxidation gives rise to higher MDA levels in liver and indicate failure of antioxidant defense system to stop formation of excessive free radicals. Cadmium-induced increase in lipid peroxidation might lead to increase in activity of SOD, as SOD switches to remove excess ROS. The reduced catalase activity in the present study, might be a result of metal deficiency, as exposure to Cd. Cadmium (50 mg/L in drinking water for 12 weeks) decreases the levels of iron (Fe) in liver of rats as reported by Jurczuk *et al*. (2004) and as Fe is a major constituent of the active site of catalase, a decrease in Fe might result in a decrease in catalase activity [57]. Cadmium-induced reduction in hepatic catalase activities reflect diminished capacity to remove H_2O_2 in response to Cd in the mitochondria and microsomes.

In the present study, quercetin treatment was able to lower the MDA level and SOD activity in liver, simultaneously increased catalase and GSH activity clearly indicating the protective effect on hepatic tissue. Curcumin treatment significantly decreased the SOD activity, significantly increased the GSH level and partially improved the catalase activity, thus providing protective action on oxidative damage caused by the Cd exposure. Quercetin and curcumin in combination also significantly reduced the lipid peroxidation and protected the liver tissue. Quercetin efficiently quenches free radicals, inhibits lipid peroxidation and protects the hepatic tissue from the Cd-induced oxidative damage [58]. Quercetin also enhances the GSH dependent protection and prevents the depletion of thiols during oxidative stress [59]. Curcumin act as bifunctional antioxidant and directly react with ROS and also to induce an upregulation of several cytoprotective and antioxidant proteins [60]. Curcumin helps in reduction of oxidative stress by scavenging ROS, preventing the denaturation of antioxidant enzymes and reducing the oxidative stress marker. The reduced levels of lipid hydroperoxides and elevated levels of vitamin C and E levels upon quercetin treatment in Cd-exposed rats were also reported previously [61]. The treatment with curcumin has been documented to

ameliorate the Cd-induced decline in SOD, GSH and marked increase in MDA level in liver tissue of rats [62].

The nephrotoxic property of Cd might be facilitated by the release of Cd-metallothionein (MT) complex from damaged hepatocytes and following filtration in glomerulus into the urinary space, it is endocytosed by the proximal tubular cells and undergoes degradation by the lysosomes, resulting in the release of Cd [63]. In kidney, proximal tubules act as primary target for Cd toxicity and which result in renal impairment [64]. It inactivates the enzyme by direct binding of active sites containing SH groups, [65] or dislodgment of metal cofactors from active sites [66]. Depletion in SOD, CAT, GPx and GST activities in kidney of Cd-treated rats might be related to diminished synthesis of enzymes or inactivation of enzyme protein. In present study, kidney showed significant increase in MDA level indicating increased lipid peroxidation and non-significant increase in SOD activity, significant decrease in catalase activity upon Cd exposure. However, on GSH level Cd exposure had no significant effect. Our results were in agreement with the findings of Messaoudi *et al*. (2009) related to the increased MDA level, SOD activity and decreased catalase activity and GSH level in Cd exposed in rats (200 ppm, P.O., 35 days) [67]. Cadmium toxicity pathway may involve increased production of nitric oxide (NO) [68, 69]. Excess NO reacts with superoxide anion and produce peroxynitrite radicals which were responsible for nitration of cellular macromolecules and depletion of intracellular GSH [70, 71]. In the present study, quercetin and curcumin alone as well as in combination non-significantly reduced the SOD activity and non-significantly increased the catalase activity and also stimulated the GSH level along with reduced lipid peroxidation indicating protection against Cd-induced oxidative stress. Quercetin reported to have powerful antioxidant, cytoprotective effects and prevents the endothelial apoptosis caused by oxidants [72]. It prevents iron-catalyzed Fenton reaction by chelating transition metal ions like iron [73]. Quercetin (50mg/kg) treatment attenuated Cd-induced (5 mg/kg 4 weeks) oxidative stress in kidney of rats through reduction in lipid peroxidation and restoration of non-enzymatic and enzymatic antioxidants like SOD, CAT, GPx, GST, GR and G_6PD [74]. Tarasub *et al*. (2011) documented that curcumin (250 mg/kg, P.O., 5 days) had protective action against Cd (Cd acetate 200 mg/kg, P.O., 5 days) induced nephrotoxicity in rats [75]. In the present study, quercetin and curcumin alone as well as in combination non-significantly reduced the SOD activity and non-significantly increased the catalase activity and also stimulated the GSH level along with reduced lipid peroxidation indicating protection against Cd-induced oxidative stress.

CONCLUSIONS

Sub-acute exposure to 100 ppm level of cadmium had altered the biochemical parameters along with lipid peroxidation in intestine, liver and kidney of rats. The cadmium also produced marked histological changes in the organs. Quercetin alone could be able to protect the kidney up to certain extent from toxic effect of cadmium. However, quercetin and curcumin in combination had shown moderate protective effect against cadmium-induced changes in the intestine and liver.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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