LIVOLIN FORTE® AMELIORATES CADMIUM-INDUCED KIDNEY INJURY IN WISTAR RATS

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LIVOLIN FORTE* UMANJUJE KADMIJUMOM-INDUKOVANO OŠTEĆENJE BUBREGA KOD VISTAR PACOVA

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ABSTRACT

The kidney, which is an integral part of the drug excretion system, was reported as one of the targets of cadmium toxicity. Early events of cadmium toxicity in the cell include a decrease in cell membrane fluidity, breakdown of its integrity, and impairment of its repair mechanisms. Phosphatidylcholine and vitamin E have a marked fluidizing effect on cellular membranes. We hypothesized that Livolin forte® (LIV) could attenuate kidney damage induced by cadmium in rats. Twenty-five adult male Wistar rats were divided into five groups of five rats each: group I (control group) received 0.3 ml/kg/day of propylene glycol for six weeks; group II was given 5 mg/kg/day of cadmium (Cd) i.p for 5 consecutive days; group III rats were treated in a similar way as group II but were allowed a recovery period of 4 weeks; group IV was treated with LIV (5.2 mg/kg/day) for a period of 4 weeks after inducing renal injury with Cd similarly to group II; and group V was allowed a recovery period of 2 weeks after a 4-week LIV treatment (5.2 mg/kg/day) following Cd administration. A significant increase in plasma creatinine, urea, uric acid, and TBARS were observed in groups II and III compared to the control rats. Significant reductions in total protein, glucose, and GSH activity were also recorded. The urine concentrations of creatinine, urea, and uric acid in groups II and III were significantly lower than the control group. This finding was accompanied by a significant decrease in creatinine and urea clearance. Post-treatment with LIV caused significant decreases in plasma creatinine, urea, uric acid, and TBARS. Significant increases in total protein, glucose, and GSH activity of groups IV and V were observed compared to group II. A significant increase in urine concentrations of creatinine, urea, and uric acid and significant decreases in total protein, glucose, and GSH activity were observed in groups IV and V compared to group II. Photomicrographs of the rat kidneys in groups IV and V showed an improvement in the histology of their renal tissue when compared to group II, with features similar to the control rats. Additionally, group III showed an improvement in the histoarchitecture of the kidney compared with group II, although occasional atrophy of some glomeruli and shrinking of renal corpuscles was observed.

SAŽETAK

Bubreg, koji je sastavni deo ekskrecionog sistema lekova, je opisan kao jedan od ciljeva toksičnosti kadmijuma. Rani događaji toksičnosti kadmijuma u ćeliji uključuju smanjenje propustljivosti ćelijske membrane, narušavanje integriteta i oštećenje mehanizama reparacije. Fosfatidilholin i vitamin E imaju izrazit efekat na propustljivost ćelijske membrane. Naša pretpostavka je da Livolin FORTE® (LIV) može ublažiti oštećenje bubrega indukovanu kadmijumom u pacova. Dvadeset pet odraslih muških pacova Vistar soja, su podeljena u pet grupa sa po pet pacova u svakoj: grupa I (kontrolna grupa) primila je 0,3 mL/kg/dan propilen glikola šest nedelja; grupi II je administriran kadmijum (Kd) u dozi od 5 mg/kg/dan i.p. tokom 5 uzastopnih dana; grupa III- pacovi su tretirani na sličan način kao grupa II ali je dozvoljen period oporavka od 4 nedelje; grupa IV je tretirana LIV (5,2 mg / kg / dan) u periodu od 4 nedelje nakon izazivanja renalne povrede sa Kd slično grupi II; i grupa V je dozvoljen period oporavka od 2 nedelje posle 4 nedelje LIV tretmana (5,2 mg/kg/dan) nakon Kd administracije. Značajno povećanje nivoa kreatinina, uree, mokraćne kiseline i TBARS-a u plazmi zabeženo je u grupama II i III odnosu na vrednosti ovih parametara u kontrolnoj grupi. Primećeno je značajno smanjenje ukupnih proteina, glukoze i GSH aktivnosti u grupama IV i V u odnosu na grupu II. U urinu, značajno su povećane vrednosti kreatinina, uree i mokraćne kiseline i značajno smanjenji ukupni proteini, glukoza i aktivnost GSH-a u grupi IV i V u odnosu na grupu II. Fotomikrografije bubrega pacova u grupi IV i V su pokazale poboljšanje histološkog nalaza bubrežnog tkiva u odnosu na II grupu, sa karakteristikama sličnim u kontrolnoj grupi. Pored toga, u grupi III je primećeno poboljšanje u histoarhitekturi bubrega u poređenju sa grupom II, iako je zapažena mestimična atrofija nekih glomerula i skupljanje renalnih korpuskula. Rezultati ove studije ukazuju da LIV administracija ublažuje oštećenje bu-

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In conclusion, the results of this study indicated that LIV administration ameliorated Cd-induced kidney injury in rats. Thus, LIV represents a prospective therapeutic choice to prevent kidney injury inflicted by Cd exposure.

Keywords: *Cadmium, Livolin forte®, Phosphatidylcholine, Vitamin E, Rat*

brega izazvano kadmijumom (Kd). Dakle, LIV predstavlja potencijalno terapijsko sredstvo u prevenciji oštećenja bubrega uzrokovano izlaganjem kadmijumu.

Klučne reči: *Kadmijum, Livolin Forte®, fosfatidilholine, Vitamin E, pacov*

INTRODUCTION

Heavy metals exposure has become an increasingly recognized source of illness worldwide. Cadmium is a ubiquitous heavy metal in the environment and a known industrial pollutant. Exposure to cadmium through food, water, and occupational sources has been known p to cause a variety of adverse effects. Cadmium is known to cause reproductive disorders, renal and hepatic dysfunction, osteomalacia, neurological impairment, and pancreatic activity changes (1). Additionally, various structures and metabolic processes can be affected, such as nucleic acids, carbohydrates, energy metabolism, protein synthesis, and enzyme systems (2). Inhalation of cadmium causes respiratory stress and injury to the respiratory tract. Emphysema and chronic rhinitis have been linked to high cadmium concentrations in polluted air. Reduction in forced expiratory volume and a high incidence of respiratory distress syndrome were reported among people exposed to cadmium (3). Cadmium was also reported to be injurious to the heart (4). The kidney, which is an integral part of the drug excretion system, was reported as one of the targets of cadmium toxicity (5, 6). Long-term ingestion of cadmium causes kidney damage, the initial signs of which are proteinuria and ß$_2$ microglobulinuria (7).

Early events in cadmium toxicity in the cell include a decrease in cell membrane fluidity, breakdown of its integrity, as well as impairment of its repair mechanisms. All of these changes are associated with a number of disorders, including kidney and neurological diseases, various cancers, and cell death (8, 9). Phosphatidylcholine, particularly phosphatidylcholine rich in polyunsaturated fatty acids, has a marked fluidizing effect on cellular membranes.

Vitamin E, an antioxidant, is presumed to be incorporated into the lipid bilayer of biological membranes to an extent proportional to the amount of polyunsaturated fatty acids or phospholipids in the membrane (10). Because of its hydrophobic nature, vitamin E is readily held within the hydrophilic lipid region of the membrane and lipoprotein where its ability to quench free radicals becomes readily useful (11). As an important antioxidant, Vitamin E may interfere with cadmium toxicity by preventing auto-oxidation of cell membranes.

Livolin Forte® (Mega Lifesciences (Australia) Pty. Ltd.) is a drug that is used in the treatment and management of liver diseases. This agent basically contains phospholipids with vitamins including essential phospholipids-polyunsaturated phosphatidylcholine (300 mg), vitamin B_1 (thiamine mononitrate, 10 mg), vitamin B_2 (riboflavin, 6 mg), vitamin B_6 (pyridoxine HCl, 10 mg), vitamin B_{12} (cyanocobalamin, 10 mcg), nicotinamide (30 mg), and vitamin E acetate (alpha tocopheryl acetate, 10 mg). Livolin forte® (LIV) has been shown to promote a rapid arresting of clinical symptomatology and normalization of biochemical indices in patients with steatohepatitis (12). Additionally, LIV ameliorates the elevation in alanine transaminase in HIV-infected patients when commencing highly active antiretroviral therapy (13). LIV has also been reported to exhibit a significant hepato-protective effect in acute ethanol-induced fatty liver in Wistar rats (14).

Based on the constituents in LIV, we hypothesized that LIV could attenuate renal damage induced by cadmium in rats. Therefore, this study aimed at investigating the effect of LIV on cadmium-induced kidney injury in Wistar rats.

MATERIALS AND METHODS

Drug and Chemicals

Livolin forte® from Mega Lifesciences (Pakenham, VIC, Australia; batch number 107050), cadmium sulphate from Guangzhou Fischer Chemical Co., Ltd., Guangdong, and propylene glycol (Biovision, Milpitas, CA, USA) were the agents used in this study.

Drug Preparation

Each capsule containing 366 mg of LIV was dissolved in 20 ml of propylene glycol, after which 0.04 ml of the solution (equivalent to 0.78 mg of LIV) was administered orally to a 150 g rat. This dosage is equivalent to 5.2 mg/kg, which is the therapeutic dose of the drug in humans.

Animal Care and Management

Twenty (25) adult male Wistar rats weighing 140 g - 190 g were obtained from the Animal House of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife and allowed to acclimatize in the laboratory for two weeks before the commencement of the study. The

rats were kept under normal environmental conditions with a natural light/dark cycle and were fed a standard rodent pellet diet (Caps Feed PLC, Osogbo, Nigeria) and water *ad libitum*. They were housed individually in separate metabolic cages (Ohaus R Model; Ohaus, Pine Brook, New Jersey, USA) during the experiment to obtain a 24-hr urine sample. The experimental procedures adopted in this study were in strict compliance with Experimental Animal Care and Use of Laboratory Animals in Biomedical Research, College of Health Sciences, Obafemi Awolowo University, Ile-Ife.

Experimental Design

The rats were divided into five (5) groups of 5 rats each as follows: Group I (control group) received 0.3 ml/kg/day propylene glycol orally throughout the course of the study (6 weeks). Group II was administered cadmium (Cd) alone, 5 mg/kg/day (i.p), for 5 consecutive days to induce renal injury. The rats were sacrificed 24 hours after the last day of Cd administration. Group III (recovery group) was treated similarly to Group II, but the rats were allowed a recovery period of 4 weeks (after Cd intoxication) without treatment with LIV. Group IV was treated with LIV (5.2 mg/kg/day) for a period of 4 weeks after inducing renal injury with Cd similarly to Group II. Group V was allowed a recovery period of 2 weeks after a 4-week LIV treatment (5.2 mg/kg/day) following Cd-induced renal injury, after which the rats were sacrificed by cervical dislocation.

Blood samples from all rats were drawn via cardiac puncture, collected into separate EDTA bottles and centrifuged at 4000 rpm for 15 minutes at -4°C. A cold centrifuge (Centurium Scientific, Model 8881) was used in this study. Plasma was obtained and collected into separate plain bottles for the assessment of biochemical parameters. Thereafter, the kidney of each rat was carefully excised and fixed inside 10% formo-saline for histopathological studies.

Measurement of Body Weight

Weekly body weights of the rats were measured with the aid of a digital weighing balance (Hanson, China) to assess weekly weight gain or weight loss.

Measurement of Food Consumption, Water Intake, and Urine Volume

The food consumption, water intake, and urine output of the rat were measured. Water intake and urine volumes were measured with the aid of a measuring cylinder (Volac, Great Britain), while the food consumption was measured with the aid of a digital weighing balance (Hanson, China).

Biochemical Analysis

Levels of creatinine, urea, uric acid, and glucose in the plasma were determined by the use of appropriate biochemical kits purchased from Randox Laboratories (Crumlin, Co. Antrim, UK). The urine concentrations of urea, creatinine, uric acid, protein, and glucose were estimated in the last samples of urine collected from the rats before sacrifice, using the same methods that were used in the analysis of plasma. Creatinine clearance was subsequently calculated using a standard formula.

Reduced glutathione (GSH) was determined by the method of Beutler and Kelly (15). Total protein determination was carried out according to the method of Lowry et al. (16) as described by Holme and Peck (17). The level of lipid peroxidation in the renal tissue was determined by estimating the level of thiobarbituric acid reactive substances (TBARS) in the homogenate of the kidneys according to the method of Ohkawa et al. (18).

Histopathological Evaluation

The fixed kidney samples were dehydrated in graded alcohol and embedded in paraffin wax. The samples were then cut into 7-8 µm thick sections and stained with haematoxylin-eosin for photomicroscopic assessment using a Leica DM 750 Camera Microscope at x 400 magnification.

Statistical Analysis

The results obtained were expressed as the mean ± standard error of the mean (S.E.M.) and subjected to one-way analysis of variance (ANOVA). The data were further subjected to a post-hoc test using Student-Newman-Keuls method, and differences with probability values of $p < 0.05$ were considered statistically significant. The statistical analysis was performed with the aid of GraphPad Prism 5.03 (GraphPad Software Inc., CA, USA) and Microsoft Office Excel, 2007 package.

RESULTS

Food Consumption and Body Weight

The food consumption of the control group increased significantly throughout the study period. Similarly, a significant increase in body weight was observed in the control group throughout the course of the study (Figure 1). The food consumption of the experimental rats decreased significantly during the 5 days of treatment with cadmium when compared with their pre-treatment values. This reduction was accompanied by a significant decrease in body weight in groups II and IV. In the remaining weeks of the study, the food consumption of the experimental groups increased significantly compared to the food consumption during the 5-day treatment period but was significantly lower than the pre-treatment values in groups III and IV (Figure 1). The body weight of the experimental groups during the remaining weeks of treatment was not significantly different from their pre-treatment values, except for group IV, which showed a significant decrease in body weight in the 1st, 2nd, and 3rd weeks compared to the pre-treatment value (Figure 2). The body weight of rats in this group was significantly higher during the 4th week than the 1st and 2nd weeks.

Water Intake and Urine Volume

The water intake of the experimental groups dropped significantly during the 5 days of treatment with cadmium

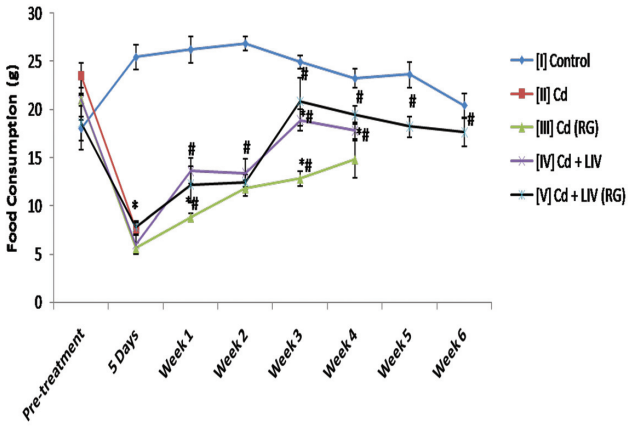

Figure 1: Effect of Livolin forte® on food consumption in cadmium- induced renal injury in rats. Values are given as mean ± SEM (n=5). * = Significantly different from pre-treatment value. # = significantly different from 5 days of treatment with cadmium.

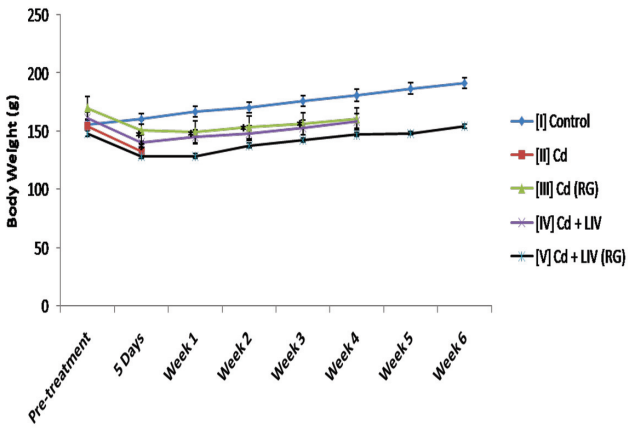

Figure 2. Effect of Livolin forte ® on body weight in cadmium- induced renal injury in rats. Values are given as mean ± SEM (n=5). * = Significantly different from pre-treatment value. δ = Significantly different from the 4th week of treatment.

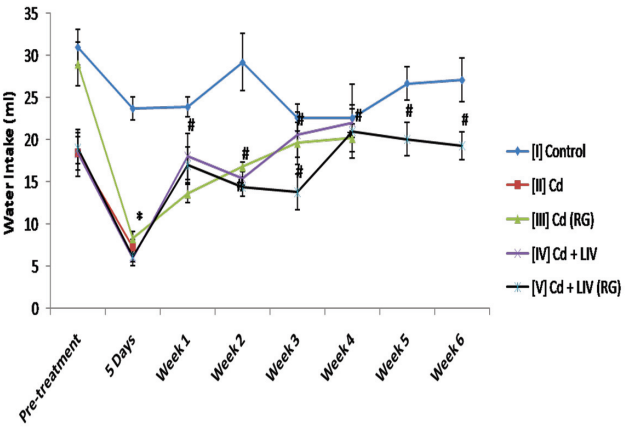

Figure 3. Effect of Livolin forte® on water intake in cadmium- induced renal injury in rats. Values are given as mean ± SEM (n=5). * = Significantly different from pre-treatment value. # = Significantly different from 5 days of treatment with cadmium

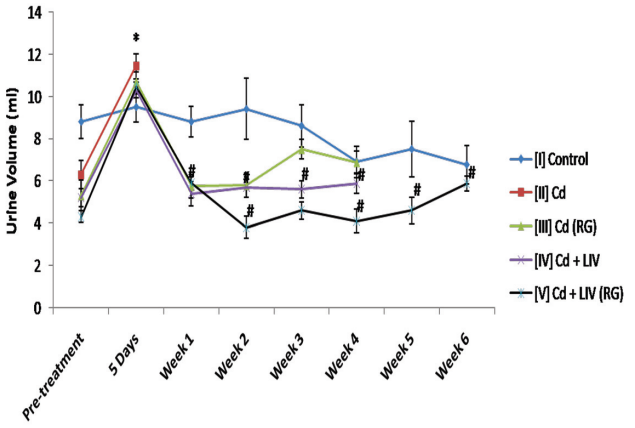

Figure 4. Effect of Livolin forte® on urine volume in cadmium- induced renal injury in rats. Values are given as mean ± SEM (n=5). * = Significantly different from pre-treatment value. # = Significantly different from 5 days of treatment with cadmium.

compared to their pre-treatment values (Figure 3). This reduction was accompanied by an increase in urine volume, which was significantly higher than the pre-treatment values (Figure 4). During the remaining weeks, a significant increase in water intake was observed in the experimental groups compared to the water intake during the 5-day treatment period with cadmium (Figure 3). However, the water intake in group III decreased significantly compared to the pre-treatment value.

Plasma Creatinine, Urine Creatinine, and Creatinine Clearance

A significant reduction in urine creatinine was observed in groups II and III compared to the control rats (Table 2). This finding was accompanied by an increase in the plasma concentration of creatinine (Table 1). The creatinine clearance in these groups dropped significantly compared to the control group and groups IV and V (Figure 5). However, the creatinine clearance and plasma and urine concentrations in groups IV and V were not significantly different from the control rats. Groups III, IV, and V showed a significant increase in the urine creatinine concentration compared to group II (Table 2). The plasma concentration of creatinine decreased significantly in these groups compared to group II. A significant increase in creatinine clearance was observed in these groups compared to group II (Figure 5).

Plasma Urea, Urine Urea, and Urea Clearance

The plasma urea concentration in groups II and III increased significantly compared to the control rats (Table 1). A significant decrease in the concentration of urea in the urine of groups II and III was observed compared to the control group (Table 2). A decrease in urea clearance in these groups was observed compared to the control group and groups IV and V (Figure 6). In contrast, urea clearance in groups IV and V was not significantly different from the

Table 1. Effect of Livolin forte® on plasma biochemical indices in cadmium-induced renal injury in rats

	I (Control)	**II** (Cd only)	**III**(Cd (RG))	**IV** (Cd + LIV)	**V** (Cd + LIV (RG))
Creatinine (mg/dl)	0.88 ± 0.02	1.90 ± 0.07*	1.16 ± 0.06*β	0.85 ± 0.06β§	0.85 ± 0.05β§
Urea (g/l)	42.25 ± 0.97	136.6 ± 10.16*	76.06 ± 4.95*β	43.69 ± 2.81β§	41.35 ± 2.69β§
Uric acid(mg/dl)	1.61 ± 0.38	5.51 ± 0.45*	3.04 ± 0.32*β	2.07 ± 0.34β	1.65 ± 0.21β§
Total protein (g/l)	48.14 ± 1.36	35.04 ± 1.86*	41.91 ± 1.85*β	46.29 ± 1.14β	44.18 ± 1.45β
Glucose (mg/dl)	5.31 ± 0.86	15.98 ± 0.58*	8.65 ± 0.56*β	5.84 ± 0.69β§	5.18 ± 0.91β§

Values are given as mean ± SEM (n=5). * = Significantly different from control. β = significantly different from Cd. § = significantly different from Cd (RG) ($p<0.05$). LIV, Livolin forte; Cd, cadmium; RG, recovery group.

Table 2. Effect of Livolin forte® on urine biochemical indices in cadmium-induced renal injury in rats

	I (Control)	**II**(Cd only)	**III**(Cd (RG))	**IV**(Cd + LIV)	**V**(Cd + LIV (RG))
Creatinine (mg/dl)	48.10 ± 1.11	14.63 ± 1.51*	30.94 ± 1.24*β	45.56 ± 2.40β§	47.22 ± 1.48β§
Urea (g/l)	203.7 ± 19.17	63.54 ± 4.46*	127.7 ± 6.79*β	173.3 ± 4.73β§	182.4 ± 15.08β§
Uric acid (mg/dl)	24.61 ± 3.68	5.86 ± 0.59*	19.66 ± 1.24β	22.15 ± 0.41β	26.51 ± 2.26β
Total protein (g/l)	26.07 ± 2.52	41.23 ± 0.70*	28.34 ± 0.83*β	24.83 ± 2.48β	26.18 ± 0.75β
Glucose (mg/ml)	1.10 ± 0.20	1.99 ± 0.04*	1.35 ± 0.16*β	1.08 ± 0.31β	1.15 ± 0.1β§

Values are given as mean ± SEM (n=5). * = Significantly different from control. β = significantly different from Cd. § = significantly different from Cd (RG) ($p<0.05$). LIV, Livolin forte; Cd, cadmium; RG, recovery group.

control group. The plasma and urine concentration of urea in groups IV and V was not significantly different from the control rats. The concentration of urea in the urine of groups III, IV, and V was significantly elevated compared to group II (Table 2). The plasma concentration of urea decreased significantly in these groups compared to group II. The urea clearance of these groups increased significantly compared to group II (Figure 6).

Uric Acid in Plasma and Urine

The concentration of uric acid in the urine of group II was significantly lower than the control rats (Table 2). This finding was accompanied by a significant increase in the plasma concentration of uric acid (Table 1). The urine concentration of uric acid in groups III, IV, and V was significantly higher than in group II but not significantly different from the control group. The plasma concentration of uric acid in groups IV and V decreased significantly compared to group II but was not significantly different from the control group (Table 1). The plasma concentration of uric acid in group III was significantly higher than the control rats but was significantly lower than group II.

Plasma and Urine Total Protein and Glucose

There was a significant decrease in the plasma concentration of total protein in groups II and III compared with

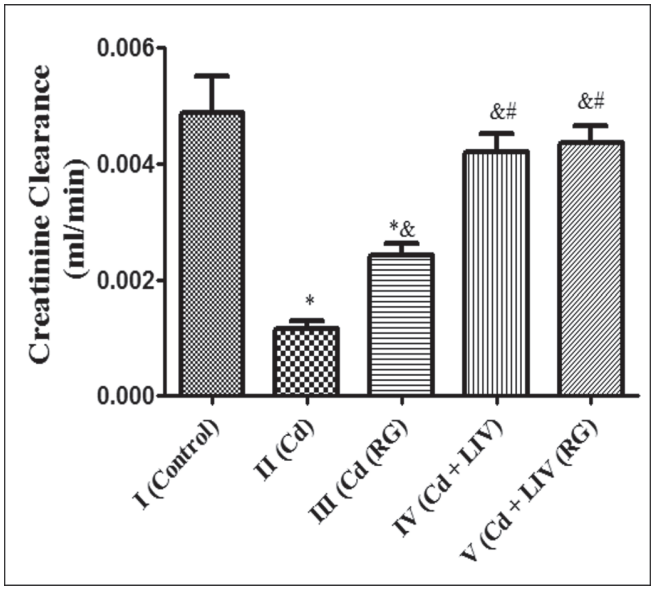

Figure 5. Effect of Livolin forte® on creatinine clearance in cadmium-induced renal injury in rat. Values are given as mean ± SEM (n=5). * = Significantly different from control. & = significantly different from Cd. # = significantly different from Cd (RG) ($p<0.05$)

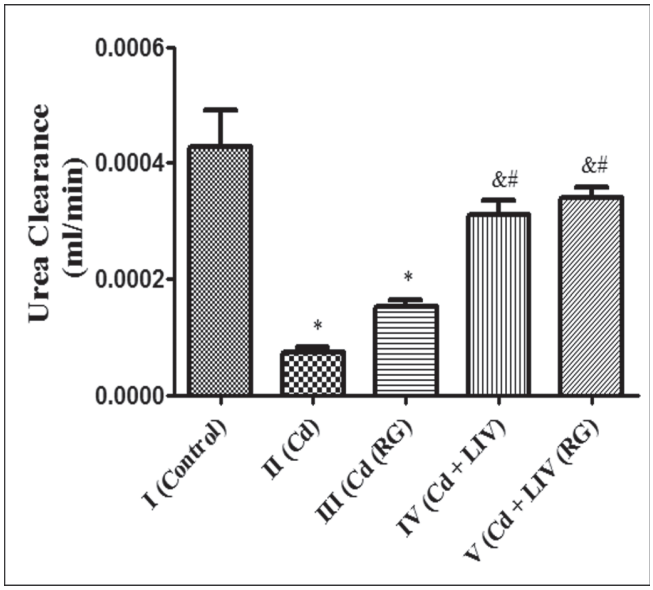

Figure 6. Effect of Livolin forte® on urea clearance in cadmium-induced renal injury in rats. Values are given as mean ± SEM (n=5). * = Significantly different from control. & = significantly different from Cd. # = significantly different from Cd (RG) ($p<0.05$)

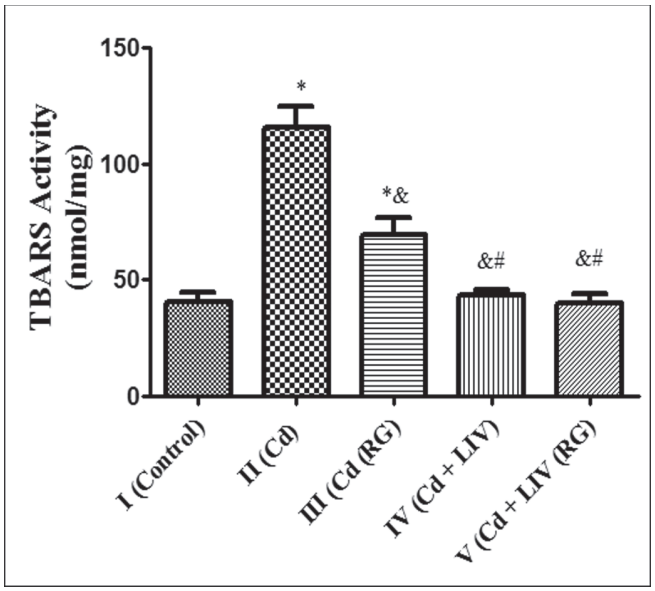

Figure 7. Effect of Livolin forte® on malondialdehyde level in cadmium-induced renal injury in rats. Values are given as mean ± SEM (n=5). * = Significantly different from control. & = significantly different from Cd. # = significantly different from Cd (RG) ($p<0.05$)

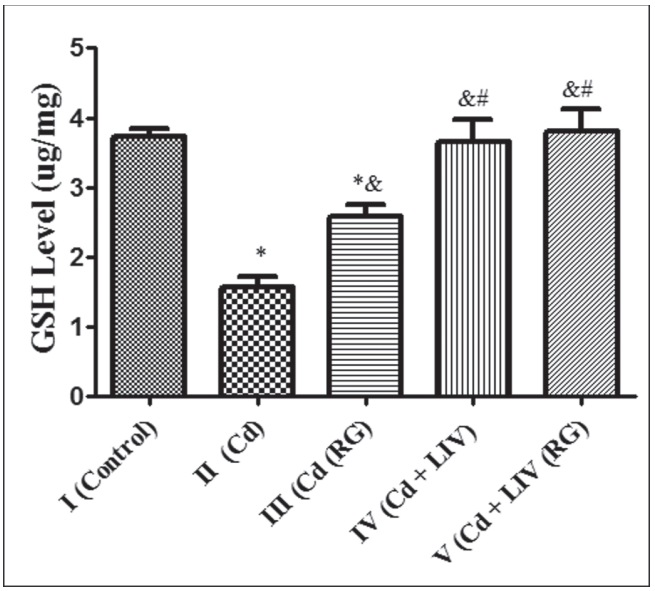

Figure 8. Effect of Livolin forte® on reduced glutathione level in cadmium –induced renal injury in rats. Values are given as mean ± SEM (n=5). * = Significantly different from control. & = significantly different from Cd. # = significantly different from Cd (RG) ($p<0.05$).

the control rats (Table 1). The urine concentration of total protein in group II was significantly higher than the control rats and the other experimental groups (Table 2). The plasma total protein concentration in groups III, IV, and V was significantly higher than group II.

The plasma glucose concentration in groups II and III was significantly elevated compared to the control rats (Table 1). Groups III, IV, and V showed a significant decrease in plasma concentration of glucose compared to group II. The plasma glucose concentration in groups IV and V was not significantly different from the control group. The urine glucose concentration in group II was significantly higher than the control rats and the other experimental groups (Table 2). Groups IV and V showed a significant decrease in urine glucose concentration compared to group III.

Reduced Glutathione and Thiobarbituric Acid Reactive Substances

A significant reduction in the reduced glutathione (GSH) level was observed in group II compared to the control rats and the other experimental groups (Figure 8). Additionally, the GSH level in group III dropped significantly compared to the control rats and groups IV and V. The thiobarbituric acid reactive substances (TBARS) level in group II increased significantly compared to the control rats (Figure 7).

Photomicrographs of the Kidneys

Photomicrographs of the kidneys from group II animals showed distorted renal corpuscles with atrophic glomeruli, severe cloudy swelling of the proximal convoluted tubules with renal tubular destruction, and severe loss of cellular constituents of the tubules compared to the control group, which showed intact renal corpuscles and normal-appearing glomeruli, tubules, and intact Bowman's spaces (Figure 9). Group III showed an improvement in the histoarchitecture of the kidneys compared to group II, although occasional atrophy of some glomeruli and shrinking of renal corpuscles was observed. Group IV exhibited histoarchitectural improvement of the renal cortices compared to Groups II and III. No evidence of atrophic or shrunken glomeruli was observed. The Bowman's spaces were intact with undamaged epithelial linings of the Bowman's capsules. The kidneys in Group V had features that were similar to the control rats (Figure 9).

DISCUSSION

Reduced growth rate is one of the symptoms indicative of toxicity in animals who were administered cadmium (19, 20). In this study, the food consumption of the experimental rats decreased significantly during the 5 days of treatment with cadmium compared to their pre-treatment values. This observed change is consistent with previously published reports (21, 22).

One of the hormones that plays a role in the regulation of appetite is ghrelin. Ghrelin is produced primarily by the stomach and proximal small intestine. In addition to the intestine, ghrelin has been identified in other peripheral tissues, such as the pancreas, ovaries, and adrenal cortex.

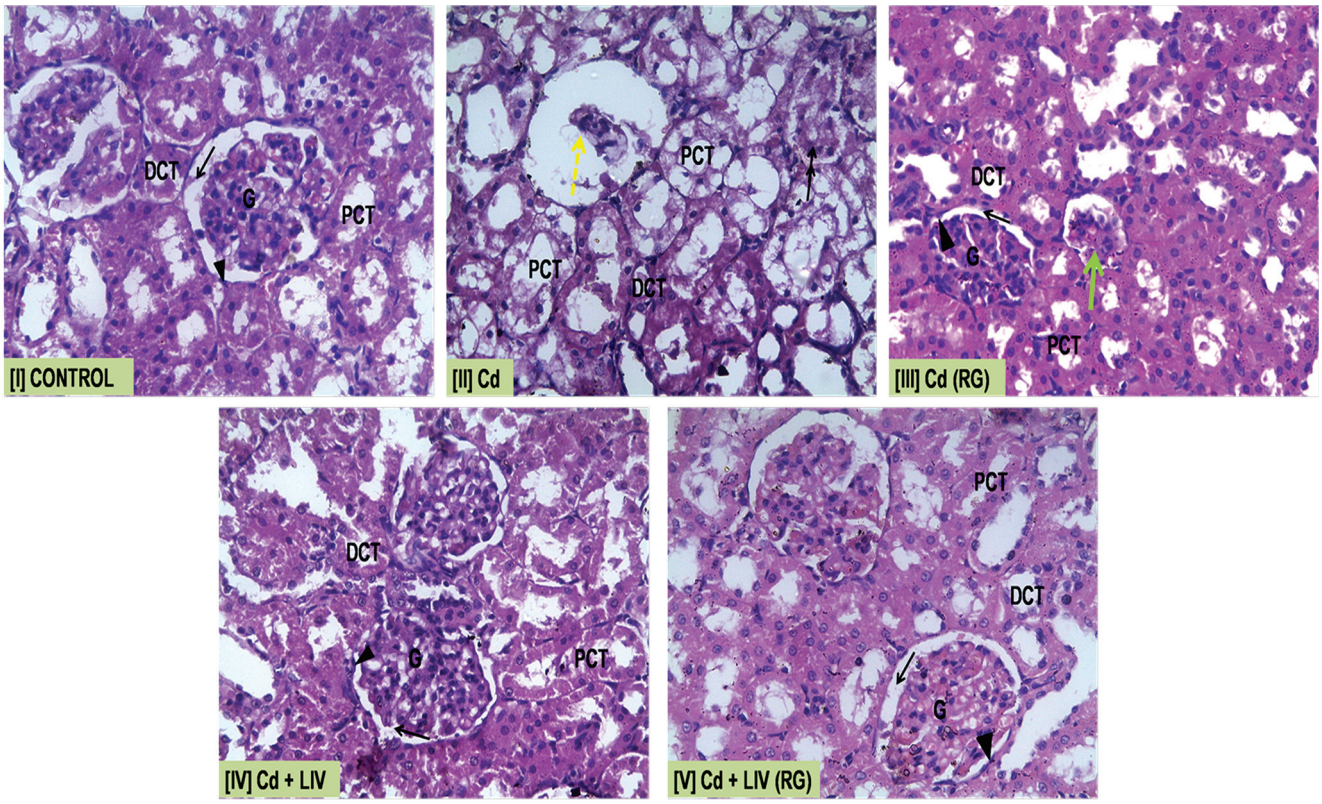

Figure 9. Photomicrographs of the kidneys. Control showed intact renal corpuscles with normal appearing glomeruli (G) and tubules (T); including the proximal convoluted tubules (PCT) and distal convoluted tubules (DCT), as well as intact Bowman's space (black arrow) and epithelial lining of Bowman's capsule (arrow head). Cadmium (Cd) showed distorted renal corpuscles with atrophic glomerulus (yellow arrow) and severe cloudy swelling of PCT (double arrow). Cd (RG) showed improvement in histoarchitecture, although with occasional atrophy of some glomeruli (yellow arrows) and shrinking of renal corpuscles (green arrows). There appear to be improvement in the histology of the tubules (T); including the PCT and DCT. Cd + LIV showed histoarchitectural improvement of the renal cortex. There appear to be no evidence of atrophic or shrunken glomerulus, Bowman's space is intact with intact epithelial linings of Bowman's capsule. Cd + LIV (RG) showed features that are similar to CN.

In the brain, neurons that produce ghrelin have been identified in the pituitary, hypothalamus, and the neuron group in the dorsal, ventral, paraventricular, and hypothalamic arcuate nucleus. Ghrelin strongly increases food intake and decreases GI motility and the secretion of insulin (23). If ghrelin production becomes inadequate as a result of the binding of cadmium to these organs, a decrease in appetite and malnutrition may occur, which shows that Cd has the capability of decreasing food intake by reducing appetite and caloric intake.

Weight gain depends on the availability and absorption of nutrients. Previous studies have shown that cadmium decreased nutrient digestion and absorption through its direct effect on the intestinal mucosal cells (24). Moreover, exposure to low levels of heavy metals has been reported to impair the glucocorticoid system (25). The glucocorticoid hormones play a vital role in glucose regulation as well as carbohydrate, lipid, and protein metabolism. Dysfunction in the glucocorticoid system has been linked to weight loss and weight gain. Therefore, the observed reduction in body weight of the experimental rats may have resulted from a decrease in food intake, an increase in the degeneration of lipids and proteins as a result of cadmium toxicity (26), or an impaired glucocorticoid system. A gradual increase in food consumption and body weight was observed in the treated groups. The gain in body weight of the rats could be attributed to the phosphatidylcholine that is present in LIV, which would correspond to the general effect of phospholipids as growth promoters (27).

Cadmium is actively transported into brain cells. By interfering with the normal function of many cellular processes, cadmium may induce sustained release of some neurotransmitters in the brain. In contrast, in different circumstances, the metal may inhibit the proper release of brain neurochemicals. As a consequence of these biochemical events, cadmium disturbs the normal function of central cholinergic, GABAergic, dopaminergic, serotonergic, glutamatergic, and opiatergic pathways (28). The water intake of the experimental groups dropped significantly during the 5 days of treatment with cadmium compared to their pre-treatment values. This finding could suggest that cadmium acted on the central nervous system, stimulating pathways that exert an inhibitory drive on water intake or, alternatively, that the metal blocked thirst-inducing pathways. Evidently, both may occur simultaneously.

A significant increase in urine output without a corresponding increase in the water intake was observed in the experimental rats during cadmium intoxication compared

to their pre-treatment values. This finding is in consonance with the report of Horiguchi *et al* (21), who observed a significant increase in the 24-h urine volume at the end of 6 and 9 months of cadmium exposure. However, this is in contrast to the findings of (29). The observed discrepancies may be due to the dose and duration of exposure in their study, which might not be enough to cause significant alteration in urine output. The significant increase in urine output suggests that cadmium may have had an adverse effect on the juxtaglomerular apparatus, which caused renin secretion to decrease. Additionally, cadmium may have caused a probable disturbance in the renin-angiotensinogen pathway resulting in a reduction in aldosterone secretion (30). A decrease in aldosterone secretion impairs the ability of the kidneys to reabsorb greater amounts of water leading to the passage of large volumes of urine. This result may lead to dehydration and a severe depletion of the major electrolytes in the body fluids of the rats. Attenuation of the cadmium–induced alteration in the urine output of rats that were treated with LIV could suggest that this drug enhanced the ability of the renal tubules to concentrate urine by facilitating the restoration of renal tissue as revealed in the photomicrographs of the kidneys.

Kidney injury due to Cd intoxication could be assessed by measuring the plasma and urinary markers of renal function, which are the biochemical hallmarks of renal tissue damage. Changes in these biochemical indices are consistent with renal impairment. Plasma creatinine and urea are used for assessing renal glomerular function. Their concentrations in the plasma depend largely on glomerular function. In renal disease, reduction in filtration rate results in elevated plasma concentrations of excretory products (31). Thus, the plasma concentrations of urea, creatinine, and uric acid increase as the filtration rate declines. In contrast, the urine concentration of excretory products depends almost entirely on tubular function. A decrease in the urine concentration of these substances is an indication of renal tubular damage (32).

In this study, the urine concentrations of creatinine and urea decreased significantly in rats that were administered Cd alone and in rats who were left for a 2-week recovery period compared to the control rats. The plasma creatinine and urea levels in these groups were significantly higher than the control rats. This finding indicates that the administration of Cd altered the glomeruli and tubular function in the rats that were administered cadmium and did not receive treatment. A similar finding was made by Goncalves et al. (33), who reported that urea and creatinine levels were increased in the serum of Cd-intoxicated rats. Furthermore, Preet and Dua (34) reported that rats exposed to cadmium experienced a significant increase in serum urea and creatinine concentrations. However, this finding is not in agreement with Horiguchi *et al.* (21), who reported that administration of Cd in rats did not alter the blood creatinine and urea levels. The lack of consistency is likely attributable to the route of exposure and dose used in their study.

Hyperuricaemia is a renal prognostic factor. Thus, the elevated plasma uric acid concentration that was observed in this study may reflect the bodily response to an increased production of endogenous reactive oxygen species because uric acid is a potent scavenger of peroxynitrite (35). The decrease in urine excretion of uric acid further confirms that the administration of Cd induced progressive tubular damage.

Significant decreases in plasma creatinine, urea, and uric acid were observed in groups treated with LIV. The restorative effect may be due to the phosphatidylcholine (PC) and vitamin E present in the drug. Vitamin E and PC have been reported to possess a membrane fluidizing effect, thereby restoring the structural integrity of the cell membrane.

The plasma glucose concentrations in rats that were administered Cd alone and rats that were left for a 2-week recovery period were significantly elevated compared to the control group. Blood glucose is commonly elevated in heavy metal toxicity and is usually linked to the inhibition of insulin release from the islets of Langerhans (36). Additionally, this finding can be linked with a reduction in glucose utilization by cells even in the presence of an elevated concentration of insulin (37) or due to disruption in glucagon secretion resulting in high glycogen breakdown (38). As the plasma concentration of glucose is increased above the renal plasma threshold, glucose appears in the urine. The higher the plasma concentration of glucose is, the greater the quantity excreted in the urine. Thus, the significant increase in urine glucose concentration that was observed in these groups may be due to higher plasma concentrations of glucose. The decrease in the plasma total protein in Cd-intoxicated rats could have resulted from changes in protein synthesis and/or metabolism. Furthermore, the increase in the total protein concentration in the urine indicates the impairment of renal function. Treatment with LIV significantly reversed the alteration caused by cadmium in plasma and urine levels of protein and glucose, suggesting its potential in restoring the structural integrity of the glomeruli and renal tubules.

Creatinine clearance is a useful measure of the glomerular filtration rate. A decrease in creatinine clearance is an indication of a marked reduction in the glomerular filtration rate and renal blood flow, resulting from an increase in the constriction of renal blood vessels or damage to the glomerular capillary endothelium. The significant decrease in creatinine clearance that was observed in the Cd-intoxicated rats seems to reflect a disruption in glomerular function. Cadmium has been reported to induce mesangial glomerular cell contraction that was evidenced by a decrease in mesangial cell surface (39). It is conceivable that even a minor reduction in the mesangial cell area considerably affects the filtering surface of the glomeruli and could explain the decreased glomerular filtration rate observed *in vivo* after toxic exposure (40). Similarly, a significant increase in urea clearance was observed in this group. However, urea clearance is not an accurate measure of the filtration rate; it varies roughly in proportion to the filtration rate in renal disease

(31). The creatinine and urea clearance in the groups treated with LIV increased significantly compared to rats that were administered Cd only. This finding is an indication of significant repair of the renal tissue as well as improved renal blood flow to the kidneys, a fact that was also corroborated by the photomicrographs of the renal tissue and the reduced level of creatinine and urea in their plasma.

Lipid peroxidation is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity of cadmium (41). Cadmium induces oxidative stress by producing hydroxyl radicals, superoxide anions, nitric oxide and hydrogen peroxide (42). A significant increase in the level of TBARS in rats that were administered cadmium only could be related to the excessive formation of free radicals, which lead to the degradation of biological macromolecules (43). In this study, Cd-intoxicated rats post-treated with LIV showed a marked decrease in the level of TBARS. This finding may be due to the presence of vitamin E in LIV. Vitamin E is an excellent scavenger of free radicals, thereby inhibiting lipid peroxidation and protein carbonylation (44). Glutathione (GSH) is a tripeptide that participates in the maintenance of cytoplasmic and membrane thiol status. It is an antioxidant, a powerful nucleophile and is critical for cellular protection by aiding in detoxification of reactive oxygen species (ROS), conjugation and excretion of toxic molecules, and control of the inflammatory cytokine cascade (45). The significant decrease in GSH activity by cadmium could be due to either increased use of GSH in the scavenging of free radicals that were produced by Cd or increased utilization of GSH for the activity of GPx forming oxidized GSH (GSSG) due to increased generation of ROS (46). Chronic pre-treatment with phosphatidylcholine partially inhibits GSH depletion in the forebrains of aged rats (47), suggesting that alterations in the phospholipid composition of the mitochondrial inner membrane and/or cytochrome oxidase activity might play a role in oxygen free radical production. Additionally, vitamin E neutralizes lipid peroxidation and unsaturated membrane lipids because of its free radical scavenging activity (48). Therefore, the significant increase in GSH activity that was observed in rats treated with LIV may be due to the ability of phosphatidylcholine and vitamin E to improve the integrity of the cell membrane by literally carrying dangerous oxidative species through the body's detoxification system, thus preventing damage to membranes.

From the results of this study, it is concluded that LIV significantly ameliorated Cd-induced kidney injury in rats. The anti-inflammatory, antioxidant, and membrane-stabilizing properties can be considered as the key factors responsible for the nephron-restorative effect of LIV. Thus, LIV represents a prospective therapeutic choice to prevent kidney injury inflicted by Cd exposure.

Conflict of Interest Statement

The authors declare that there were no conflicts of interest for this study.

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