AMELIORATIVE EFFECT OF NATURAL SPICES MIXTURE (ginger, garlic, and turmeric) ON CADMIUM INDUCED NEPHROTOXICITY

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ABSTRACT

Cadmium (Cd), a known ubiquitous naturally occurring toxic element has been recognized to have deleterious effect in biological systems, affecting mostly the testis, liver, pancreas, kidneys, and bone. Cd accumulates mainly in the kidney and liver which are critical targets for its acute toxicity. Evaluation of toxic potentials of this metal is important for the risk assessment of those ordinarily exposed to it. This study investigated the effects of natural spices mixture on renal toxicity in rats exposed to cadmium. Graded doses (200 mg, 300 mg, and 400 mg/Kg body weight) of natural spices mixture were administered orally to the rats. After forty two days, significant increases in serum urea, and cratinine concentrations were observed in cadmium exposed rats compared to control group ($P < 0.05$). Natural spices mixture treatment of cadmium exposed rats significantly loweredurea and creatinine concentrations, compared to cadmiumalone group ($P < 0.05$).Natural spices mixture had significantly decreased all renal function parameters and protected kidney cells against Cd induced toxicity. The histology evaluation of kidney tissues of the experimental rats showed that the induction of Cd toxicity without treatment caused significant glomerular necrosis, while the administration of natural spices mixture protected the kidney tissues from necrosis and other pathological changes. The results of this study supported the potential ameliorative effects of natural spices mixture on rat kidney tissues against cadmium induced nephrotoxicity.

KEY WORDS: Cadmium chloride; natural spices mixture (ginger, garlic, and turmeric); kidney; urea; creatinine; rat.

INTRODUCTION

Background of study

In the present era, environmental contamination is one of the significant constraints of modern human society (Ali and Khan 2019; Afzal *et al.,* 2019). Among environmental contaminants, heavy metals (HMs) are the most toxic due to their persistent and bioaccumulative nature; thus, creating a deleterious risk to biological substances (Ali *et al.,* 2008). Globally, the rate of HM mobilization and transport in the ecological system has been extraordinarily expanded since the 1940s due to rapid growth rate of industrialization (Anyanwu *et al.,* 2018). Although, these metals naturally exist in the soil in a very minute concentration through characteristic lithogenic as well as pedogenic means (Wuana and Okieimen, 2011). However, various anthropogenic practices including mining, improper industrial as well as urban waste disposal, combustion of non-renewable energy sources, metallurgical industries, chemical fertilizers, and improper handling of industrial effluents are fundamental contributors to aggregate these metals in soil (Tchounwou *et al.,* 2012; Yuan *et al.*, 2019). Non-essential HMs including lead (Pb), cadmium (Cd), and silver (Ag) have no or very little biological activity and their exposure above permissible limit poses a hazard to biological systems by interfering with their physiological and metabolic processes, contaminating food chain, causing ecological imbalances and resulting in lethal health issues due to their toxic nature (Haider *et al.,* 2021). Cadmium is a highly noxious HM that is deleterious for biological systems through its uptake and accumulation in phototrophs and consequent trophic transportation. Cadmium is released into the environment via both natural and anthropogenic systems. Among natural systems, volcanic emissions, forest fires, weathering of Cd-containing rocks, and waste water are the principal means of mobilizing it from the lithosphere (Choppala *et al.,* 2014). Anthropogenic activities such as application of phosphate-based fertilizers (Roberts 2014), manufacturing of Ni–Cd batteries, Zn mining, agricultural practices such as waste water irrigation, electroplating, application of urban compost as well as metal-based pesticides, and industrial emission as a byproduct (Zhao *et al.,* 2015) are mainly responsible for Cd aggregation in soil. Human exposure to cadmium and cadmium compounds may occur in both occupational and environmental settings, the latter primarily via the diet and drinking water (ATSDR, 1989). A variety of industrial activities can lead to Cd exposure. Examples includes mining and metallurgical processing, combustion of fossil fuel (Genchi *et al.*, 2020), textile printing, production of phosphate fertilizers, recycling of ferrous scrap, electronic waste, motor oils, disposal and incineration of Cd-containing products.The increasing industrial uses of cadmium causes soil, air and water contaminations. However, human exposure to cadmium occurs chiefly through inhalation and ingestion of contaminated food and water (Ige *et al.,* 2011). Tobacco in all its forms contains appreciable amounts of Cd and tobacco smoke is one of the largest single sources of Cd exposure in humans (Ibiam and Uwakwe (2009). Foods derived from plants, depending on the level of soil contamination, generally contain higher concentrations of Cd than meat, egg, milk, and dairy products. Among these, rice and wheat, green leafy vegetables,potatoes, carrot, and celery can contain higher concentrations of the metal than other food from plants. Vegetarians and shellfish consumers may be exposed to a higher cadmium intake than omnivores (Sirot *et al.,* 2008).

Studies have proposed various mechanisms for Cd toxicity which include, modification of biomolecules, modulation of DNA repairs and genotoxic effects, antagonism and displacement of bivalent metal ions (zinc, selenium, calcium, and copper from their binding sites), induction of oxidative stress and impairment of p53 protein involved in suppression of cancer (Anetor, 2012; El-Boshy *et al.,* 2015; Gong and Wang, 2019). Cadmium toxicity in liver seems to be mediated by the production of reactive oxygen spices (ROS) known to induce necrosis in various rat organs (Razinger *et al.,* 2008*,* Hsu *et al.,* 2007), lipids peroxidation (Borges *et al.,* 2008) and decrease in antioxidant enzymes (El-Sharaky *et al.,* 2007).

Condiments such as ginger, garlic, and turmeric which have been generally used as spices, flavouring agent, food preservative and medicine, have been revealed to play important role in the amelioration of heavy metal toxicity. This may be due to the presence of biologically active polyphenols and antioxidants present in them (El-Sayed *et al*., 2015). For example, concurrent intake of garlic, ginger and nutmeg at culinary dose in diet has been found to have both therapeutic and prophylactic effect at mitigating Cd toxicity (Ugwuja *et al.,* 2016). This reaffirmed the safety of spices combinations as being currently practiced. Some agents like curcumin, caffeic acid, phenylethyl ester found in spices like turmeric have been shown to be effective in preventing cadmium toxicity (Gong *et al.,* 2012 Gong *et al.,* 2014). Similarly, studies have shown that ginger (*Zingiber officinale*) has ameliorative effect against cadmium-induced liver and kidney injury in rat model (Egwurugu *et al.,* 2007; Mohammad *et al.,* 2013). Eteng *et* *al.* (2014) have also demonstrated the reversal of cadmium induced toxicity after dietary supplementation with garlic, ginger and cabbage in male albino rats.

Statement of problem

Cadmium toxicity occurs even at a very low level in the blood and toxicity is usually without obvious signs and symptoms. With increase in industrialization/urbanization, heavy metals, including cadmium are released into the ecosystem, which will ultimately find its way into human systems. Accumulation of cadmium in human systems, if left untreated may lead to adverse health effects, especially in pregnant women and children, who have been found to be most vulnerable (Gehan and Ayman, 2010). Nanoparticle based antidote is very expensive and not readily accessible. Many chelating agents and antagonists have been found to reduce Cd toxicity, but part of them reveals undesirable side effects, intrinsic limitations and variability in the efficacy (Gonick, 2008; Jalilehvand *et al.,* 2009). Reports have been inconsistent on the roles of spices in protecting against cadmium toxicity. Considering the rise in industrial activities with exponential emission of toxic metals, including cadmium, which has been classified as a category one carcinogen (IARC, 1994), coupled with reports of beneficial effects of nutrients and natural spices at ameliorating heavy metal toxicity, there is need to explore the potential beneficial effect of spices mixture at mitigating Cd toxicity.

AIM AND OBJECTIVES

This study was carried out to investigate the effects of natural spices mixture (ginger, garlic, and turmeric) on Cd-induced nephrotoxicity in male albino rats, and therefore sought to:

1. Determine the levels of serum urea and creatinine in order to assess the effects of administration of natural spices mixture on renal function of cadmium-induced nephrotoxicity in albino rats.
2. Examine thekidney cell histology of the rats for morphological changes due to administration of natural spices mixture on cadmium-induced nephrotoxicity in albino rats.
3. Compare the levels of these chemical parameters, and histological changes in the treated rats in relation to the control rats.

Significance of the study

The data generated from this study may have policy relevance in designing mitigating strategy at reducing/preventing Cd toxicity, especially in cadmium polluted settings for the improvement of health and wellbeing of residents.

Scope of the study

This is a sub-acute study, and is limited to animal model – adult male albino rats of age 120 – 150 days.

MATERIALS AND METHODS

Subjects – rats

Twenty adult male albino rats, weighing between 150 and 205g were used for this study. The rats were obtained from the Animal House of Enugu State University of Science and Technology, College of Medicine (ESUCOM), Enugu. They were arranged into five groups (A, B, C, D, and E) of four rats each and were kept for five days to acclimatize before initiating the study. They were allowed free access to clean drinking water, and were fed on standard growers mash rat pellets (Grand Cereals LTD, Enugu) throughout the period of the study.

The rats in group A were not induced with cadmium chloride, received no treatment with natural spices mixture, and served as normal control, while group B was administered 25 mg/kg body weight of cadmium chloride ($CdCl_2$) without any treatment with natural spices mixture, and served as positive control. Those rats in groups C, D, and E were induced with 25 mg $CdCl_2$/kg body weight and treated with graded concentrations 200 mg/kg, 300 mg/kg, and 400 mg/kg body weights respectively for forty two days. All administrations were through oral route. Two rats from each group were sacrificed at the end of the treatment period and their kidneys harvested.

Reagents and chemicals

Analytical grade of Cadmium Chloride ($CdCl_2$) in the form of pure crystals were obtained from Riedel-De HaenAGSeelze-Hannover Germany through the Analar grade Laboratory reagents and chemical dealer at Ogbete main market, Enugu State. The reagents used for the biochemical assays were commercial test kits and products of Randox (USA).

Natural Spices Mixture

Ginger rhizomes, garlic bulbs and turmeric rhizomes were purchased from Ogbete main market Enugu, Enugu State. The ginger, turmeric and garlic were peeled and all the three spices were washed with water. Exactly 5.0g of each spice were homogenized and soaked overnight in distilled water. It was filtered and the filtrate was concentrated at 60^0C using electric oven. It was carefully evapourated to dryness in water bath at 40^0C (Sofowora, 1977).

Collection of blood samples and preparation of serum

At the end of the experiment (42 days), the animals were fasted overnight for sample collection and harvest of organs. The specimens (blood samples) were collected through the retro-bulbar plexus of the nasal canthus, into plain bottles, for the estimation of the chemical parameters. Serum samples were prepared from the whole blood as follows:

Three milliliters (3ml) of whole blood was collected into clean plain test tube. This was allowed to clot and retract, and then centrifuged at 3,000 rpm for five minutes. The supernatant (serum) was separated from the sediment (red cells) and transferred into another clean plain test tube, for the chemical analyses. All serum samples were refrigerated (-4^OC) until they were analysed. Two rats from each group were sacrificed by euthanasia, with chloroform, their kidney dissected out and fixed in 10% formol saline solution, for histological studies.

Serum Urea and Creatinine Estimation

Serum urea concentration was estimated by the modified diacetylmonoxime method of Wybenga *et al*., 1971; while serum creatinine concentration was determined using alkaline picrate method of Fabiny and Ertingshaysen, 1971.

Principles

In acid solution, diacetylmonoxime is hydrolysed to diacetyl, which reacts with urea to form a pink condensation product, which is measured at 480nm. The concentration of urea in each sample was determined by interpolation from urea standard curve. Creatinine in the sample reacts with picrate in alkaline medium, forming a red-orange coloured complex. The intensity of the colour read at 500nm is directly proportional to the concentration of creatinine present in the sample. The complex formation rate is measured in a short period to avoid interference from non-creatinine chromogens and creatinine present in the samples was interpolated from the creatinine standard curve.

Histopathological examination

The histological evaluation was carried out using the method described by Drury *et al.,* (1967). Tissue sections of the kidney from all the experimental groups were fixed in 10% formol saline for a minimum of 48 hours. The tissues were subsequently processed in an automatic tissue processor (trimmed, dehydrated in graded concentrations of alcohol (70%, 80%, 90%, and absolute alcohol), cleared in three grades of xylene and embedded in molten paraffin wax). On solidifying, thin sections (about 4-5 microns thick) were cut using rotary microtome, and stained by heamatoxylin and eosin (H & E) method, permanently mounted on degreased glass slides using DPX mountant. The prepared slides were examined using a light Olympus microscope (magnification X100). The photomicrographs were taken using microscope camera at x400 magnification.

Staining Principle

Haematoxylin was used as a nuclear stain preceding staining of cytoplasm and connective tissues with eosin. Sections were stained with haematoxylin stain of sufficient power and for long enough to ensure some overstraining of nuclei, the superfluous and obscurative coloration of structures being removed by treatment with acid alcohol. Eosin stains connective tissues and cytoplasm in varying intensity and shades of primary colour giving a most useful differential stain with haematoxylin.

ANALYSIS OF RESLTS

Statistical Analysis of results was carried out using Statistical Product and Service Solutions (SPSS) version 20. Results were expressed as mean ± standard error. Statistical differences were evaluated using One way Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test to detect the significant differences among the mean values of the different groups at p-values < 0.05.

RESULTS

After forty two days, significant increase in serum urea and creatinine were observed in cadmium-exposed rats without treatment, compared to the normal control group (P< 0.05), while treatment with natural spices mixture had significant effects on these renal function parameters. Natural spices mixture treatment of cadmium exposed rats significantly lowered serum urea and creatinine concentrations compared to the cadmium alone group (P < 0.05). The concentrations of urea (2.48±0.10 mmol/L), and creatinine (50.25±1.18 µmol/L) in group D, which were treated with 300 mg/kg body weight of natural spices mixture were comparable with their levels in the normal control (2.74±0.05 mmol/L; 49.50±1.32 µmol/L), showing better ameliorative effect at mid dose. Group E rats which were treated with high dose of natural spices mixture recorded a non significant decrease (P > 0.05) when compared to cadmium alone group (Table 1).

The histological evaluation of kidney tissues of the experimental rats showed that the normal control rats had normal histological architecture characterized by intact convoluted tubules (CT) and glomeruli (G) as shown in plate 1. However, the induction of Cd toxicity without treatment caused a significant glomerular necrosis (plate 2), while the administration of natural spices mixture 300 mg/kg, protected the kidney tissues from necrosis and other pathological changes (Plate 3). Morphological changes in those groups treated with low dose of SM (200 mg/kg) and high dose of same (400 mg/kg) manifested as mild capsular hypertrophy, brush border loss, and tubular atrophy (Plates 4, and 5).

TABLE 1: Effect of spices mixture on the indices of renal function of cadmium induced toxicity in albino rats

Parameters	Group A	Group B	Group C	Group D	Group E
Urea (mmol/L)	2.74±0.05	5.88±0.14	3.80±0.08	2.48±0.10	5.63±0.15
Creatinine (µmol/L)	49.50±1.32	106.75±1.18	65.50±1.32	50.25±1.18	81.75±3.93

Results are expressed as Mean ± Standard Error of Mean (n = 4).

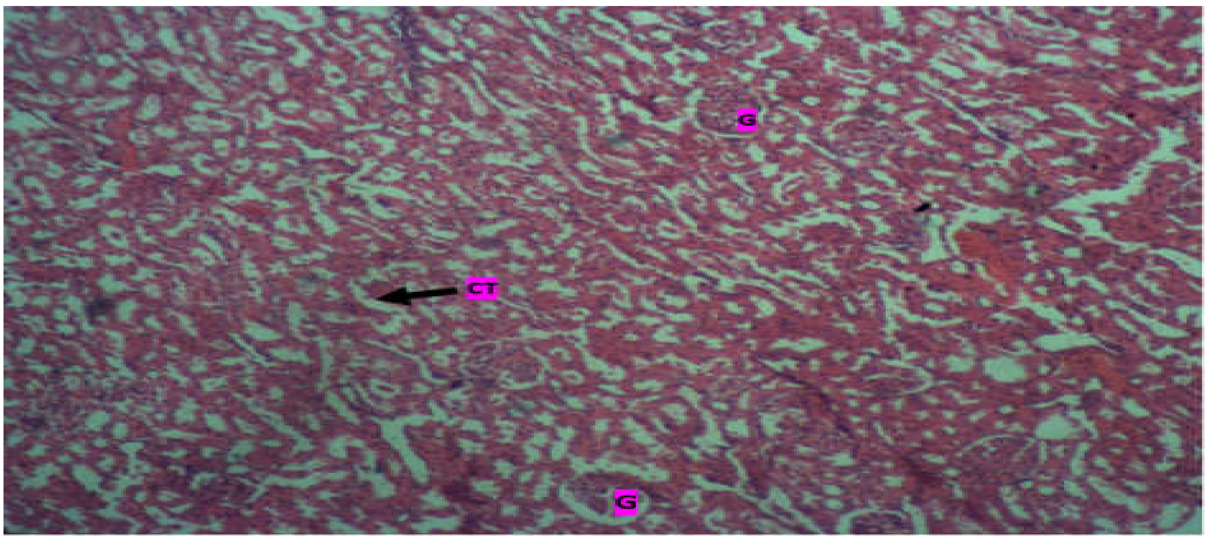

Plate 1. Photomicrograph of normal control kidney.
The kidney tissues showed intact convoluted tubules (CT) and glomeruli (G).
H & E. magnification 100 X

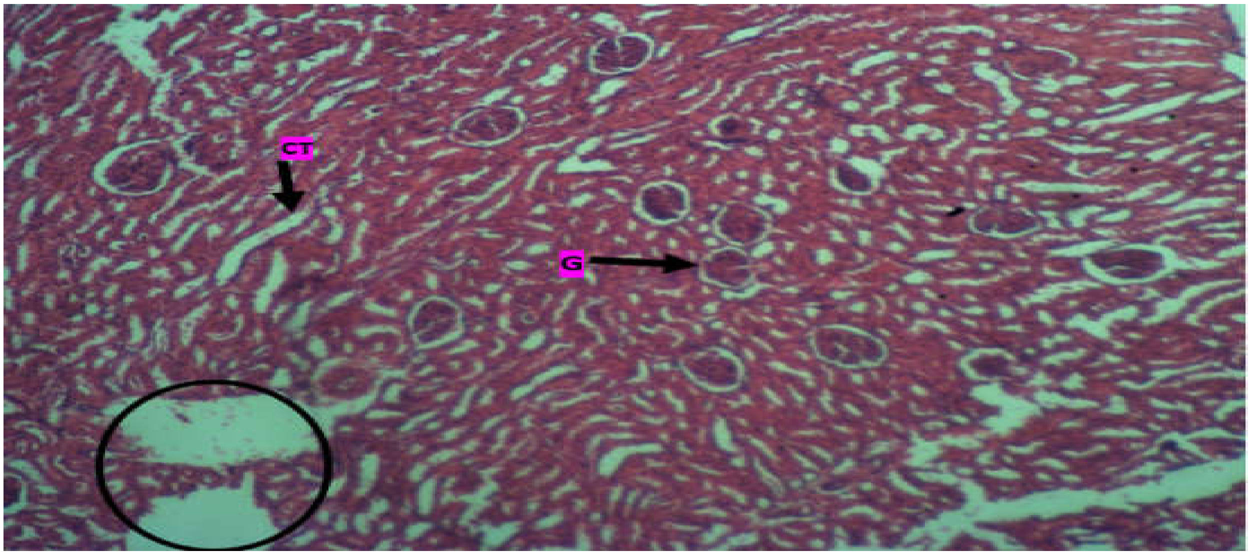

Plate 2. Photomicrograph of cadmium exposed but untreated kidney.
The kidney tissues showed glomerular necrosis (circled area), and hypertrophy of capsular space.
H & E. magnification 100 X

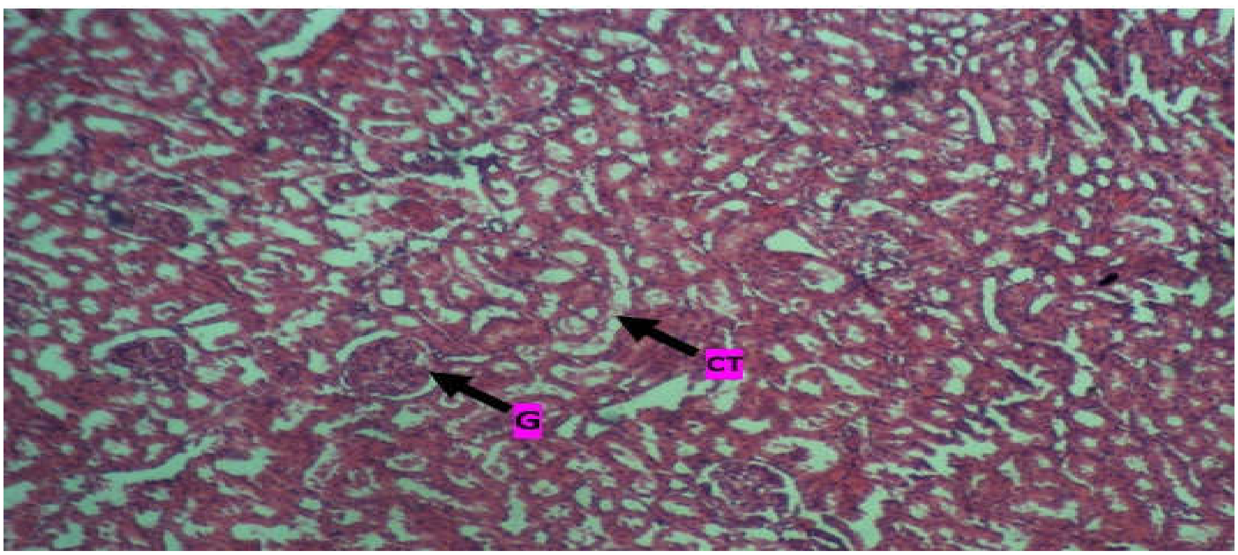

Plate 3. Photomicrograph of the group that received 300 mg SM/kg b.w.
The kidney tissues appeared normal with intact convoluted tubules (CT) and glomeruli (G).
H & E. magnification 100 X

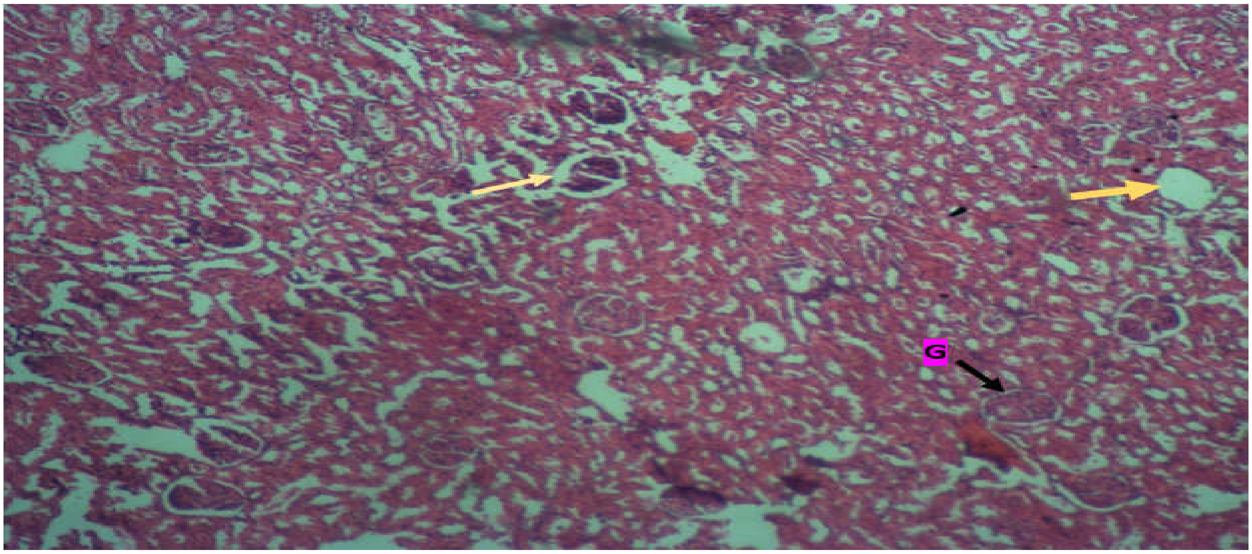

Plate 4. Photomicrograph of the group that received 200 mg SM/kg b.w.
The tissues showed mild tubular atrophy and loss of brush boarders
H & E. magnification 100 X

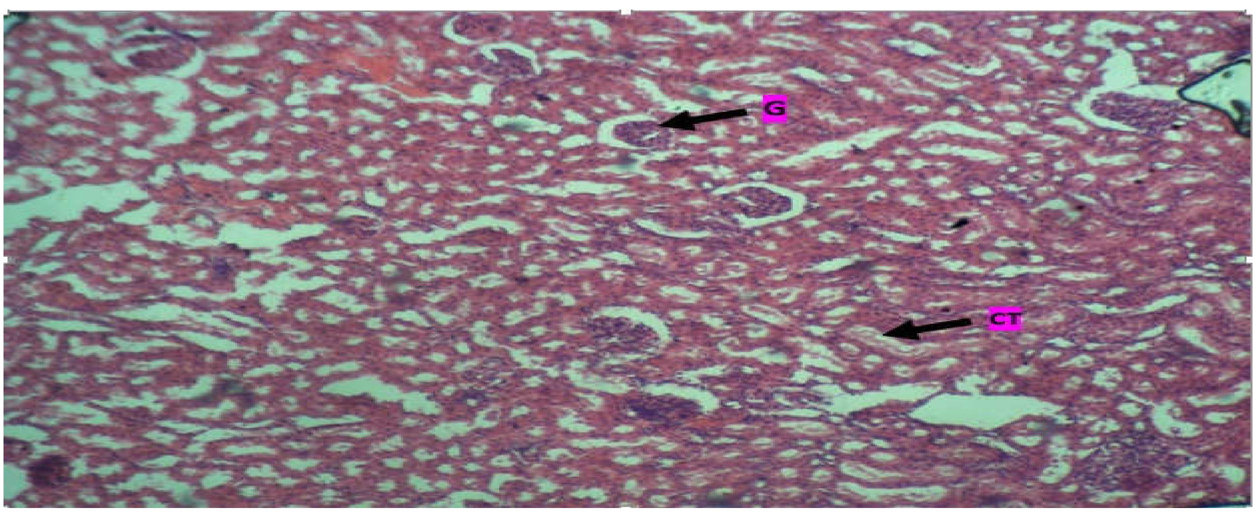

Plate 5. Photomicrograph of the group that received 400 mg SM/kg b.w.
Tissue showed mild tubular atrophy and loss of brush borders
H & E. magnification 100 X

DISCUSSIONS

The diverse deleterious health effect upon exposure to heavy metals in the environment, especially Cadmium (Cd), a heavy metal of considerable toxicity with destructive impact on most organ system, is of serious concern and a global issue. Literature survey shows different treatment methods for Cd toxicity such asNanoparticle based treatments and use of chelating agents. The main disadvantage of synthetic chelators is that the chelation process removes vital nutrients along with toxic metals. However, natural spices mixture (ginger, garlic, and turmeric) could provide a credible alternative to the presently used drugs for the amelioration of cadmium toxicity to avoid long term adverse health effects. This study evaluated the ameliorative effects of natural spices mixtures on cadmium nephrotoxicity.

In the present study, serum urea, and creatinine were estimated to assess renal glomerular function. The data obtained showed significant increase in renal function biomarkers; urea, and creatinine following six weeks of Cd exposure. This can be attributed to the impairment of the kidney, muscle wasting (weight loss), and dehydration (diarrhea) caused by the toxicant. The change in the levels of serum renal biomarkers represents renal damage caused by Cd as reported by Bekheet *et al.*, (2011). These data were supported by others who reported that cadmium toxicity induced tubular necrosis or loss of the brush border and damage in the small tubules of kidney (Wang *et al.,* 2011). Studies by Jacquillet *et al.,* (2006) suggested that in chronic Cd^{2+} contamination, Cd^{2+} has an effect in the proximal as well as in distal tubule, leading to a pronounced renal defect. This can affect the filtration as well as absorption of these substances in the renal tubules.

In addition to that, the elevated blood urea and creatinine concentrations, caused by cadmium toxicity may be related to disorder in protein catabolism as a result of increase in the synthesis of arginase enzyme involved in urea production (Tormanen, 2006), and glomerular functional disturbances such as lower filtration rate which results in poor creatinine clearance in cadmium intoxicated rats (L'Azou *et al.,* 2002).

The levels of renal function parameters, urea and creatinine, of group treated with 300 mg natural spices mixture/kg body weight, was comparable to those of normal control and achieved a statistically significant decrease ($P < 0.05$) when compared with $CdCl_2$ induced but untreated group. Groups treated with low dose (200 mg/kg), or medium dose (300 mg/kg) of natural spices mixture, were better protected from Cd nephrotoxicity. Treatment with high dose of natural spices mixture (400 mg/kg), caused a non significant ($p > 0.05$) decrease in the serum urea, and creatinine. The degree of increase in the levels of these renal parameters may be an indication of extent of renal tissue damage and impairment of kidney functions

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