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RESEARCH ARTICLE

THE PROTECTIVE EFFECT OF *Cleome gynandra* LEAF EXTRACT AGAINST MERCURY CHLORIDE-MEDIATED KIDNEY DAMAGE IN MALE WISTAR ALBINO RATS

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ABSTRACT

Mercury can cause toxic effects in animals and impair some organs' function, the kidney included. A promising approach of protecting tissue damage due to mercury is the use of phytochemicals. In this study, the effect of *Cleome gynandra* (CG) leaf extract against mercuric chloride ($HgCl_2$) - induced nephrotoxicity was determined. Four groups of Wistar rats were used: vehicle control, positive control (5mg $HgCl_2$/kg bwt), treatment groups I (250mg CG extract/kg bwt + 5mg $HgCl_2$/kg bwt), and II (500mg CG extract/kg bwt + 5mg $HgCl_2$/kg bwt). The variations among the groups were significant for creatinine (p=0.0002), urea (p=0.0002), and albumin (p=0.0082) levels. $HgCl_2$ significantly increased urea (p = 0.021) and creatinine (p = 0.032) levels in the positive control group compared to the vehicle control group. The CG extract did not prevent these changes. The difference between the treatment groups was insignificant for creatinine (p=0.972) and urea (p=0.341). Histologically, the vehicle control group showed normal renal morphology, while the rest showed degenerative changes on the Bowman's capsule, glomeruli, and tubules. Albumin level was significantly lower in the treatment group 2 (p=0.005). Pre-treatment with CG extract was ineffective in nephroprotection against damage induced by the high dose of mercury.

Keywords: Albumin, creatinine, mercury, morphology, nephroprotection, renal, and urea

INTRODUCTION

Mercury (Hg) is a hazardous environmental pollutant that is toxic to human health if not adequately managed (ATSDR, 1999; WHO, 2007, 2013). Exposure to Hg leads to its increased presence in the body (Steckling *et al.*, 2014). After exposure, mercury deposit is majorly found in the kidney, though deposition also occurs primarily in the brain (Park & Zheng, 2012). It can induce toxic effects impairing functions of organs like the kidney, liver, and developing brain. The impairment depends on mercuric ions' ability to bind to sulfhydryl and selenohydryl groups of peptides due to their electrophilic nature. As a result, their structure is altered and their function is inhibited (Bernhoft, 2012; Spiller, 2018).

Among the approaches that have been proposed and applied in managing the toxic effects of divalent mercury, boosting the body's defence system with exogenous phytochemicals is a promising approach to managing tissue damage (Kasote et al., 2015). In this connection, non-enzymatic compounds (Agarwal *et al*., 2010a; Agarwal *et al*., 2010b; García-Niño & Pedraza-Chaverrí, 2014; Pereira *et al*., 2018) and plant extracts (Abarikwu *et al*., 2017; Alam, 2007;

Oda & EL-Ashmawy, 2012) have been shown to have beneficial effects against mercury-related toxicities.

Cleome gynandra L. (CG), commonly known as the Spider plant, is an indigenous African and Asian leafy vegetable of the family Cleomaceae (Omondi *et al*., 2017). Increasing evidence from experimental and epidemiological studies of CG have shown some beneficial nutritional aspects and identified compounds of potent therapeutic importance (Adhikari & Paul, 2018). Hence, its increased recognition among leafy vegetables as a potential source of nutrients. Some therapeutic investigations by various scholars exist. Some studies have shown that it has anti-carcinogenic (Bala *et al.*, 2010), anti-oxidizing (Muchuweti et al., 2007), and anti-inflammatory properties (Narendhirakannan *et al*., 2007). CG is rich in nutrients like vitamins, proteins, and lipids (Ekpong, 2009). Vitamins observed include carotenoids, vitamins C, and E (Gowele *et al.*, 2019). Additionally, other authors have indicated the presence of kaempferol (Lingegowda *et al.*, 2012), saponins, iridoids, gallotannins, free gallic acid (Moyo *et al.*, 2013), alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenols, sugars, and triterpenes (Adhikari & Paul, 2018). Also, it is rich in minerals, both macronutrients and micronutrients. The notable macronutrients include sulphur, potassium, phosphorous, magnesium, and calcium, while the main micronutrients are manganese, zinc, and iron.

Since Hg's toxic health effects involve mechanisms such as affinity for sulfhydryl functional group, oxidative stress induction, and lipid peroxidation, the chemical diversity of plants with antioxidant properties and metal binding molecules can be beneficial in managing the toxic effects of Hg on the kidney. In an *in vitro* antioxidant screening, CG exhibited an inhibitory high impact (Muchuweti et al., 2007). Also, polyphenolic phytochemicals such as flavonoids have an affinity for heavy metals (Hider et al., 2001) like mercury due to the presence of hydroxyl groups. Hence, the body burden of mercury can be reduced (Mężyńska & Brzóska, 2019), reducing tissue injury. Since Hg is toxic, an animal model of male Wistar albino rats is used to investigate the protective effects of CG on the kidney against mercury exposure.

METHODOLOGY

A. Plant materials, sample preparation, and extraction

The *Cleome gynandra* (CG) plant materials were obtained from Kibuye and Kiboswa markets in Kisumu, Kenya, and cleaned using clean water. The leaves were shed-dried in a well-ventilated room and, then ground using a pestle and motor for homogeneity. The extraction technique was cold maceration (Pisoschi et al., 2016) using absolute methanol, with frequent stirring, for three days at room temperature. The ratio of the plant material to the solvent was 1:3. The damp mixture was pressed and the liquid was filtered using Whatman® filter papers. A concentrate of the filtered extract was then obtained by rotary evaporation at 40°C. Afterwards, further drying was done in an oven at 37°C producing a solid extract that was then dissolved in 10% DMSO (dimethyl sulphoxide) before oral administration.

B. Sample size determination

The Resource Equation method was applied in the determination of the sample size (Charan & Kantharia, 2013) due to the multiple variables measured.

$$E = \text{Total number of animals} - \text{Total number of groups}$$

where here E is the degree of freedom of ANOVA. A value of E between 10 and 20, indicates adequacy in sample size. This study had an E of 16.

C. Experimental animals and housing conditions

A total of twenty male albino Wistar rats, 180-220 g in weight, were procured from the Department of Zoology animal house, University of Eldoret, Kenya. They were housed in wire cages with wood chips and soft grass laid at the base of the cage and allowed to acclimatize to the environment for one week. Throughout the study, the animals were allowed to freely access food (standard rat pellets) and water. They were maintained in a well-ventilated and clean environment, at room temperature.

D. The scheme of the experiment

The rats were randomly divided into groups of five as detailed in Table 1.

Table 1: *Experimental Animal Groups*

	Groups	Treatment
Group 1 (n=5)	Vehicle control group	- Oral administration of 1 ml of 10% dimethyl sulfoxide (DMSO) solution daily - Subcutaneous injection of 0.5 ml normal saline on the 7th day 2 hours after the last dose of the DMSO solution.
Group 2 (n=5)	$HgCl_2$-treated group	- Daily oral administration of 1 ml 10% DMSO solution - Subcutaneous injection of 5 mg of mercuric chloride ($HgCl_2$) per kg body weight (bwt) in 0.5 ml normal saline solution on the 7th day 2 hours after the last dose of the DMSO solution.
Group 3 (n=5)	CG+$HgCl_2$-treated group I (D1)	- Daily oral administration of 250 mg of the extract per kg bwt in 1 ml 10% DMSO solution - Subcutaneous injection of 5 mg $HgCl_2$/kg bwt in 0.5 ml normal saline on the 7th day 2 hours after the last dose of extract in the DMSO solution
Group 4 (n=5)	The CG+$HgCl_2$-treated group II (D2)	- Daily oral administration of 500 mg of the extract per kg bwt in 1 ml 10% DMSO solution. - Subcutaneous injection of 5 mg $HgCl_2$/kg bwt in 0.5 ml normal saline on the 7th day, 2 hours after the last dose of extract in the DMSO solution.

F. Blood and kidney sample collection and processing

24 hours after the last $HgCl_2$ administration the rats were euthanized. 5 ml of blood was collected through the cardiac puncture into ethylenediaminetetraacetic acid (EDTA) laced vacutainers and mixed gently. Blood was then centrifuged at 3,500 rpm for 15 minutes to obtain

plasma and transferred into cryogenic vials in preparation for the biochemical analyses. Using sterilized surgical blades, kidney pairs were harvested and pat dried using dry Whatman® filter papers. The kidney pairs were then submerged into 10% formalin in polypropiolactone transparent plastic tissue containers for preservation awaiting histopathological examinations.

G. Kidney function indices analyses

Photometric quantitative analysis of serum albumin, urea, and creatinine was done using COBAS INTEGRA® 400 plus auto-analyser from Roche Company. The units for albumin, urea, and creatinine concentrations used were g/dL, mg/dL, and mg/dL, respectively, as commonly used in most literature.

H. Histological examination

Tissues from the samples were fixed for 72 hours in 10% formalin (in phosphate buffer – pH 7). Afterwards, they were embedded in paraffin wax. 5-micrometer sections were cut using a rotary microtome, transferred to microscope slides, and stained using hematoxylin-eosin for microscopy. The observations were recorded primarily by estimating the tissue alterations. For consistency, observations were made on the glomerulus, Bowman's capsule, and proximal tubule. Being that this process involves estimations rather than precise measurements, it is considered semi-quantitative.

I. Statistical analysis and data representation

The quantitative data were statistically analysed with the GraphPad Prism software. The data were expressed as mean ± standard deviation. Using a one-way analysis of variance with the Tukey Post Hoc test, the statistical comparisons for all the parameters were done. The statistical significance was set at $p \leq 0.05$. The data from the biochemical studies were presented in tables. For histopathological studies, the visual data were in the form of photomicrographs.

J. Ethical consideration

The study was approved by the University of East Africa Baraton Ethics Committee (UEAB/REC/04/05/2021). Guidelines in the guide for laboratory animals' care were followed during the study (NRC, 2010). There was no conflict of interest.

RESULTS

A. Biochemical parameters

Table 2: Levels of Urea, Albumin, and Creatinine Levels in the Blood at the End of the Experiment presented as the Mean ± SD (n=5).

Groups	Mean creatinine levels (mg/dL)	Mean urea levels (mg/dL)	Mean albumin levels (g/dL)
Vehicle Control	0.294 ± 0.020	15.172±3.299	3.951 ± 0.422
$HgCl_2$-treated	1.907 ± 1.238[a]	78.482±49.595[a]	3.401 ± 0.232
CG+$HgCl_2$-treated(D1)	3.120 ± 0.291[a]	130.434±13.568[a]	3.011 ± 0.354
CG+$HgCl_2$-treated(D2)	2.893 ± 1.056[a]	97.149 ± 32.299[a]	2.117 ± 1.334[a]

Key:

[a] - Significantly different from the vehicle control, *p≤0.05*

Vehicle control (n=5) – 10% DMSO + normal saline

$HgCl_2$-treated (n=5) – 10% DMSO + 5mg $HgCl_2$/kg bwt in 0.9%NaCl
CG+$HgCl_2$-treated(D1) (n=5) – 250mg CG extract/kg bwt + 5mg $HgCl_2$/kg bwt in 0.9% NaCl
CG+$HgCl_2$-treated(D2) (n=5) – 500mg CG extract/kg bwt + 5mg $HgCl_2$/kg bwt in 0.9%NaCl

Table 2 depicts the levels of the biochemical parameters of kidney function. A single mercuric chloride dose (5 mg/kg body weight) administered subcutaneously brought about renal toxicity, exhibited by the significant biochemical increase in plasma blood urea ($p=0.021$) and creatinine ($p=0.032$) levels. In comparison to the vehicle control, creatinine and urea levels were significantly increased in CG+$HgCl_2$-treated(D1) and CG+$HgCl_2$-treated(D2) groups as indicated in the table above. Unlike other blood biochemical parameters, albumin concentrations were lower in the CG extract-treated groups compared to the controls. Significance ($p=0.02$) was in the group receiving the highest concentration of CG extract (500 mg/kg body weight) compared to the vehicle control group. Though there were differences between CG+$HgCl_2$-treated(D1) and CG+$HgCl_2$-treated(D2), the differences were insignificant for creatinine ($p=0.972$), urea ($p=0.341$), and albumin ($p=0.254$). A check for outliers in the measurements indicated no outliers.

B. Histological Changes

The histological data supplemented the biochemical parameters quantitative data. The alterations varied in the animal groups ranging from normal morphology to extensive alterations at the end of the experiment period among the groups. As shown in figure 1, the vehicle control group exhibited normal morphology of the parenchyma tissue of the kidney. There were no visible lesions and the glomeruli and tubules had well-defined structures.

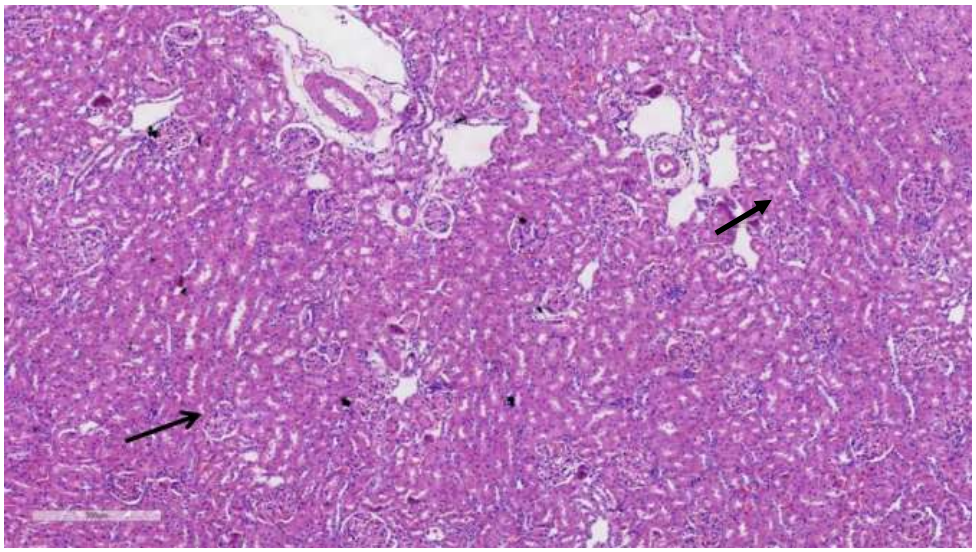

Figure 1: A Photomicrograph of a Kidney Section showing the Renal Cortex of a Vehicle Control Rat. The Arrows indicate Normal Tubule and Glomeruli

Unlike the vehicle control group, the positive control group showed extensive tubular and glomerular damage (figure 2). The Bowman capsule was dilated and the glomerulus exhibited atrophy and instances of swelling. Also, a report of tubular necrosis in the damaged areas accompanied the observation of glomerular degeneration.

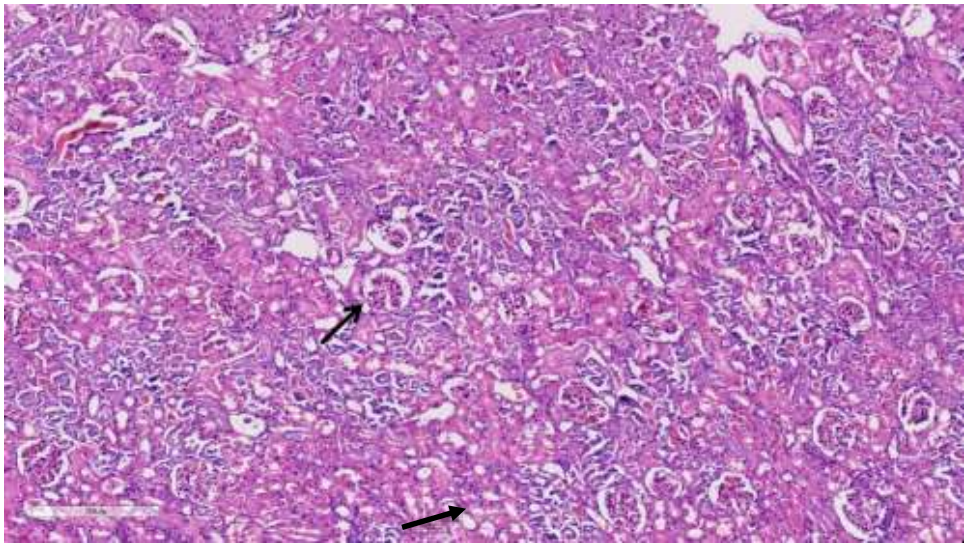

Figure 2: A Photomicrograph of a Kidney Section of Mercuric Chloride-Treated Rats. The Arrows show Degenerative Changes

In the CG+$HgCl_2$-treated(D1) group, damage on the glomeruli was moderate while the tubular damage was extensive (figure 3). Also, dilation of the Bowman's capsule and tubular necrosis was observed. However, the glomeruli were swollen.

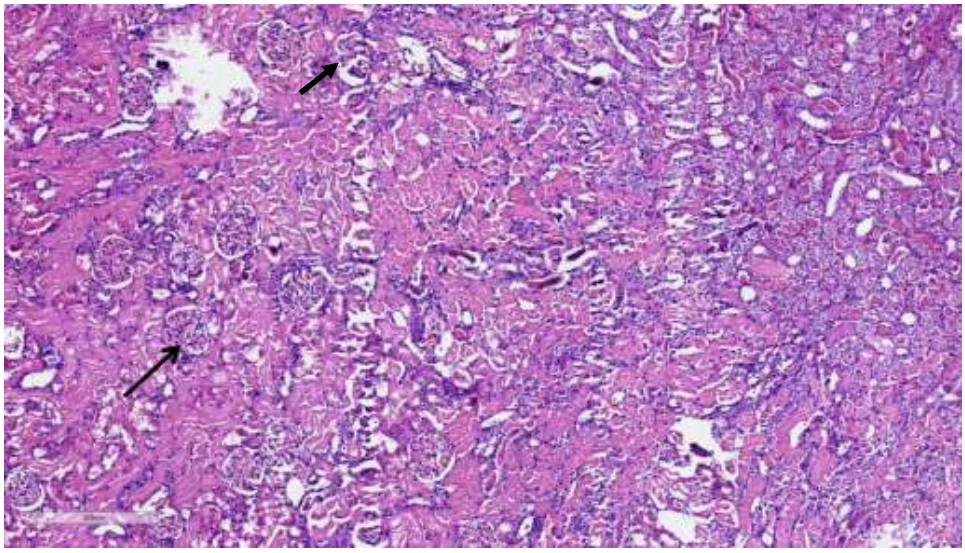

Figure 3: A Photomicrograph of a Kidney Section of CG (250 mg/kg bwt) Extract and Mercuric Chloride-Treated Rat; The Arrows Show Degenerative Alterations

In the CG+$HgCl_2$-treated(D2) group as shown in figure 4 below, both the glomeruli and the tubules were affected. The damage on the glomerular was extensive with minimal tubular protection. There was dilation of the Bowman's capsule and swelling of the glomeruli with tubular necrosis in degenerative instances of tissue alteration. Unaffected tissues showed normal physiology.

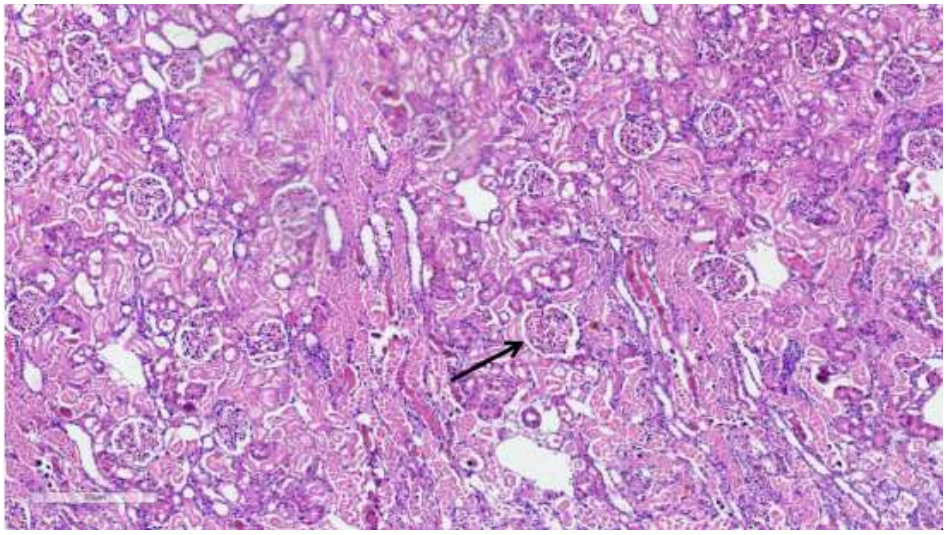

Figure 4: A Photomicrograph of a Kidney Section of CG Extract (500mg/Kg Bwt) and Mercuric Chloride Exposed Rats. The Arrows Show Evidence of Degenerative Changes

DISCUSSION

In the current study, a significant rise in blood creatinine levels was noted in the $HgCl_2$-treated group. A similar study reported a similar finding using mercuric chloride administered subcutaneously (Alam *et al.*, 2007; Gado *et al.,* 2014). A rise in the level of creatinine often signifies cases of renal injury, primarily, a decline in the functionality of the glomerular filtration (Zalups, 2000), which in this study is evidenced by degenerative damages to the glomeruli including Bowman's capsule dilation and glomerular atrophy in figure 2. Also, since the proximal tubules excrete creatinine, the concentration of the creatinine can, to an extent, indicate damage to the proximal tubules (Zalups, 2000) as shown by the tubular damage with tubular necrosis in the $HgCl_2$-treated group of the current study. As a biomarker, creatinine is relatively insensitive to minor injuries to the kidney (Waikar & Bonventre, 2009). Since the reported results in the current study show a significant increase in the creatinine concentration, the pathological injury to the kidney tissue structure due to $HgCl_2$ exposure cannot be considered minor, as evidenced by the extensive tubular and glomerular damage (figure 2). The increase in creatinine level was 1.613 mg/dL, which is considered acute since the creatinine increase was more than 0.3 mg/dL within two days (Waikar & Bonventre, 2009). An increase of more than 0.3 mg/dL was also indicated by Abarikwu *et al.* (2017), that is, 2.018 mg/dL.

In the CG+$HgCl_2$-treated (D1) group 1 and (D2) group 2, the creatinine levels were insignificantly different from the $HgCl_2$-treated group. In comparison to the vehicle control group, the differences were significant compared to the intervention groups (Table 2). Ezeugwunne *et al*. (2017) using a different plant, showed similar findings indicating kidney dysfunction based on the significant increase in serum creatinine and urea levels after *Sida corymbosa* leaf extract treatment. Histologically, degenerative structures supported the above-mentioned biochemical observations, indicating decreased kidney function. Similar studies using extracts such as *Moringa oleifera* oil (Abarikwu *et al.,* 2017) and *Juglans sinensis* Dode extract (30) have reported a decline in creatinine concentration in the intervention groups and a less evident difference compared to the positive and the vehicle control group, respectively. Abarikwu *et al.* (2017) related the protection to the anti-peroxidative property of the *Moringa oleifera* oil to the kidney and Beohm *et al*. (2002) further associated kidney dysfunction to the

mitochondria of the renal proximal tubule cell. Unlike the CG leaf extract, silymarin limited the necrotic and degenerative alterations localized at the corticomedullary junction and the renal cortex (Oda & El-Ashmawy, 2012). Also, in a pre-treatment case in rats, Alam *et al*. (2007) showed protection against HgCl2-induced injury. However, the tissues presented instances of congested glomeruli with reduced oedema. These results show that prophylaxis has the potential to protect against mercury-induced nephrotoxicity. The higher creatinine levels in the treatment groups compared to the control groups could depict a possible harmful effect of CG extract in combination with mercury on the kidney which is inconsistent with other similar studies focusing on nephroprotection against mercury-induced renal damage (Abarikwu *et al.*, 2017; Oda & El-Ashmawy 2012). However, the CG methanolic extract has been associated with nephroprotective (Narsimhulu *et al*., 2019) and is non-toxic up to concentrations of 2000 mg per kg body weight in healthy adult albino rats based on the OECD guidelines 423 (Kalpana *et al.*, 2018). The observations might be due to the extract's component(s) that promote mercury bioavailability in circulation, such as vitamin C, whose content in CG ranges from 214.31 to 319.12 mg per 100 g dry weight (Jinazali *et al.*, 2017). It is an essential nutrient to the body known to maintain the flexibility of blood vessels and improve blood circulation (Emmanuel *et al.*, 2016).

In the treatment groups, the CG leaf extract did not show a dose-dependent potency in nephroprotection (*table 2*). Another study with a similar study design shows dose-dependent potency in renal protective activity (Alam *et al*., 2007). The creatinine levels were reduced to a normal level. CG extract's inability to significantly reduce the increased creatinine level is backed by degenerative changes in the glomeruli and tubules. Alam *et al*. (2007) on the other hand, showed that a higher dose improved the tubules and glomeruli degenerative changes to within normal limits when using *Eruca sativa* seed extract.

The concentration of blood urea in the positive control group was observed to be higher compared to the vehicle control (*Table 2*), a sign of nephrotoxicity in combination with the report on the creatinine levels similarly as observed by Oda & El-Ashamawy (2012). Similar to creatinine, it shows reduced functionality in the glomerular filtration process, leading to its accumulation in the blood. This observation is supported by extensive tubular and glomerular damage observed with features such as Bowman’s capsule dilation, glomerular atrophy, and tubular necrosis, compared to the vehicle control that had normal morphology. A study by Gado *et al*. (2014) also signified a disruption of normal kidney function in combination with changes observed in the creatinine concentration. An increase in urea concentration is usually accompanied by an increase in creatinine concentration, as was the case in the current study. However, Abarikwu *et al*. (2017) noted that a rise in creatinine levels was not accompanied by a noticeable increase in urea concentration in mercury-induced nephrotoxicity. Hypothetically, this observation presents a scenario where the nephrotoxicity of mercury could be a reflection of a degree of complexity, with a variety of outcomes. Instead of the CG leaf extract influencing the reduction of the blood urea concentration in the treatment groups compared to the positive control group, there was no significant difference. This observation is in line with the degenerative changes in the glomeruli and tubules in the current study. In similar studies using different plant extracts, there was a reduction in the urea concentration, showing protection (Alam *et al*., 2007; Gado *et al*., 2014). However, similar to the current study, pre-treatment with vitamin E did not reverse creatinine and BUN levels; instead, they were further enhanced (Agarwal *et al.*, 2010b). It points to mercury accumulation in kidney tissues due to mercury’s influx, promoting tissue injury.

A decrease in the albumin concentration accompanied exposure to mercuric chloride, though insignificantly, in this study. Oda & El-Ashmawy (2012) also demonstrated a decrease in the blood albumin concentration level, though the decrease in the positive control group was significant. An injury to the kidney is normally linked to a decrease in blood albumin levels (ATSDR, 1999; Lang *et al*., 2018). However, the decline can be multi-factorial, reflecting a much broader variety of abnormalities (36), such as the decline in protein synthesis (Samipillai *et al.*, 2013), increased epithelial degradation, and a systemic inflammatory response (Don & Kaysen, 2004; Haller, 2006). Unlike other blood biochemical parameters, albumin concentrations were lower in the CG extract-treated groups compared to both controls. Similar to the finding of this current study, Oda & El-Ashmawy (2012) demonstrated an insignificant decrease in the blood albumin concentration level in the treatment group. The decreased albumin levels seen in CG-treated groups are difficult to explain considering the data collected. However, it indicates a hypoalbuminemic property and the extract's inability to maintain albumin levels within a normal range on exposure to 5 mg of $HgCl_2$ per kg body weight. In other studies, blood albumin levels have been found to increase with exposure to mercury (Abarikwu *et al*., 2017; Lukačínová *et al*., 2011). It could be linked to the mercury's ionic species' propensity to bind –SH group in albumin for transportation purposes (ATSDR, 1999; Chunmei *et al.*, 2014).

CONCLUSION

The study determined that the CG extract was ineffective against nephrotoxicity brought about by a high dose of mercury (5mg/kg body weight). Therefore, it does not pose as a potent nephroprotective agent due to its inability to maintain kidney function at a normal range based on observations made on the biochemical parameters and histological changes.

RECOMMENDATIONS

In light of the findings of this study, it is recommended that the results serve as an additional information resource on the potential of *Cleome gynandra* in managing various conditions and as an advancement of knowledge about this plant. The study further suggests that pre-treatment with the methanolic extract of *C. gynandra* is inadvisable in managing high mercury concentrations in experimental rat models. However, post-treatment with the extract can be investigated to determine its effectiveness against mercury-induced renal damage. In addition, further studies should be considered to expand the search for particular extracts from other plant species that may prove more effective, while simultaneously identifying the mercury dose levels against which such extracts can offer protection.

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