Protective Effect of Ethanolic Extract of Zingiber Officinale Against Mercuric Chloride Induced Renal Toxicity In Rats

Sawsan A. Ali[1], Mariam A. Kadhem[2], Hameed A. Ali[3]

[1]Lecturer in Histology, Department of Anatomy and Histology, College of Veterinary Medicine, University of Basra, Basra, Iraq, [2]Assistant professor of Pharmacology and Toxicology, Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Basra, Basra, Iraq, [3]Assistant Professor of Medical Microbiology Department of Microbiology, Alzahra College of Medicine, University of Basra, Iraq

Abstract

The current study was designed to investigate the possible protective effects of ethanolic extract of *Zingiber officinale* on renal damage induced by mercuric chloride intoxicate in rats. Forty albino adult rats (180±10g, n=10 per group) were administered $HgCl_2$ (3mg/kg, Ip,3times weekly). The treatment of *Z officinale* extract (200 mg orally for 8 weeks. $HgCl_2$ administration altered the body weight, kidney weight, chemical parameters of creatinine, urea, and MDA. Body, kidney weight the biochemical and histopathological findings of this study significantly($P \leq 0.05$) showed a protective effect of a *Z officinale* extract against $HgCl_2$ intoxication. In conclusion, *Z officinale* extracts may be an ideal choice against kidney damage induced by $HgCl_2$.

Keywords: *mercuric chloride, Zingiber extract, histopathology, kidney.*

Introduction

Mercuric chloride accumulates mostly in rat liver and kidney as these organs involved in the detoxification and excretion of foreign materials[1]. Mercury contamination of the environment continues to be a concern and a major source of this contamination is from human activities by industrial waste which releases mercury into the environment[2]. The renal cortex and liver are considered to be the most susceptible organs affected which leads to functional impairment (3). Several studies were focused on the role of free radicals and oxidants due to mercuric toxicity affect the proximal convoluted tubules injury, which suggests that the nephron plays an important role in the active transport of this heavy metal[3,4,5,6,7,8,9]

Corresponding Author:
Hameed Abbas Ali
Lecturer in Histology, Department of Anatomy and Histology, College of Veterinary Medicine, University of Basra, Basra, Iraq
e-mail: haalhasson@yahoo.com

Natural herbaceous plant *Zingiber officinale* Roscoe is one of the most common food-flavoring spices used worldwide[7]. For many years, several pharmacological properties of ginger, such as anti-inflammatory, analgesic, gastrointestinal regulating agent, antioxidant and antimicrobial properties have been identified[8,9,10]. Efforts are now being directed in obtaining drugs with different chemical features since many modern drugs originated from plants the investigation of the chemical composition of traditional medicinal plants could lead to the development of new drugs. The study therefore, investigated the possibility of utilizing the ethanolic extract of *Z officinale* in the treatment of $HgCl_2$ induced nephrotoxicity.

Method

Experimental Design: Forty healthy adult albino rats 180±19g, were separated into four groups of ten rats for each group. eight weeks the animals kept in suitable cages and was feeder standard diet and water ad libitum. The control group received distilled water (10ml/kg/ intraperitoneal (Ip) the second group-administered three times per week (ip) injection of $HgCl_2$ at a dose of 3mg/

kg. Third group, treated with [$HgCl_2$(3mg/kg) plus (200mg/daily orally)] of Z officinale extract, and the fourth group treated with Z officinale extract (200mg/daily orally).

Sample Preparation: At the end of 8 weeks, all animals were scarificed the body and kidney weight is measured. Clear sera were collected and stored at -20°C for biochemical assay. The kidneys was rapidly excised and stored in 10% formalin solution and processed for histological examination.

Determination of biochemical and histomorphometeric parameters: Serum creatinine, urea and lipid peroxide contents were measured in all rats involved in this study using standard laboratory method.

Body and kidney weights were estimated at the end of 8 weeks. Under light microscopy the number and the diameter of (pct) were measured beside the histological examination.

Statistical Analysis: The data were expressed as means ± standard deviation (X ± SD). ANOVA (analysis of variance) to compare between more than two groups of numerical (parametric) data followed by post hoc Tukey test. A P value <0.05 was considered statistically significant.

Results

Effect of Zingiber officinale extract treatment on body and kidney weights: Table 1 showed a significant decrease in (b.w) and a significant increase in (k.w) of the rats among the $HgCl_2$ group(P≤0.05). While the (k.w) of the rats treated with extract of *Z officinale* showed a significant decrease when compared with rats among the $HgCl_2$ group(P≤0.05). No significant difference in the body and kidney weight between (*Z officinale* + $HgCl_2$) group and the control group.

Effect of Zingiber officinale extract treatment on the diameter and number of (pct): Means diameter of (pct) shows a significant increase in $HgCl_2$ treated group compared with other groups (P≤0.05), while the number of (pct) showed a significant decrease in $HgCl_2$ compared with other treated groups (P≤0.05). Table 3 showed a significant decrease in the diameter of (pct) among rats treated with *Z officinale* + $HgCl_2$ group (P≤0.05), also showed a significant increase in the number of the (pct) of *Z officinale* + Hgcl2 group(P≤0.05). No significant difference in the diameter of (pct), and the number of (pct) of the *Z officinale* extract and control group.

Table 1: Effect of Zingiber officinale extract treatment on body and kidney weights

Groups	Bodyweight (gm)	Kidney Weight (gm)
Control	190.50±6.123 (A)	1.397±0.013 (C)
$HgCl_2$	157.83±2.041 (C)	4.055±0.181 (A)
$HgCl_2$ +Z officinale	171.50±5.205 (B)	2.220±0.216 (B)
Z officinale	189.67±5.887 (A)	1.406±0.019 (C)

Values are expressed as mean± SD of 10 rats in each group. Significantly different from Group $HgCl_2$ (P≤ 0.05). Capital letters denote differences between the groups.

Table 3: Effect of Zingiber officinale extract treatment on the diameter and number of (pct)

Groups	The Diameter of (pct)	No. of (pct)
Control	41.373±0.329 (C)	17.728±0.746 (A)
$HgCl_2$	53.353±0.654 (A)	14.725±0.502 (C)
Z officinale + $HgCl_2$	44.893±1.0731 (B)	16.415±0.521 (B)
Z officinale	41.165±0.245 (C)	18.036±0.934 (A)

Values are expressed as mean± SD of 10 rats in each group. Significantly different in Group $Hgcl_2$, (P≤ 0.05). Capital letters denote differences between the groups, P≤0.05 vs. control.

Effect of Zingiber officinale extract treatment on creatinine, urea and MDA levels: Creatinine, urea and MDA levels shows significant elevate in the $HgCl_2$ group of rats compared with the control group (p < 0.05). While the *Z officinale* + $HgCl_2$ group rats shows a significant reduction in the serum creatinine and urea levels (P≤ 0.05) (table 2).

Table 2: Effect of Zingiber officinale extract treatment on creatinine, urea and MDA levels

Groups	Creatinine	Urea	MDA
Control	127.96±0.076 (C)	58.045±1.413 (C)	4.365±0.199 (C)
$HgCl_2$	437.77±24.013 (A)	149.08±1.652 (A)	7.908±0.263 (A)
$HgCl_2$ +Z officinale	244.67±14.684 (B)	87.848±1.889 (B)	5.063±0.398 (B)
Z officinale	123.77±0.537 (C)	55.400±0.530 (C)	4.410±0.197 (C)

Values are expressed as mean± SD of 10 rats in each group. Significantly different from Group $HgCl_2$ (P≤ 0.05). Capital letters denote differences between groups.

Morphological Examination: The kidneys of rats of the *Z officinale* extract, *Z officinale* + $HgCl_2$ groups showed bean-shaped, reddish-brown in color with smooth and possess convex and concave borders nearly normal (fig. 1a,b & d), while the kidney of rats of the $HgCl_2$ group was pale, swollen and enlarged (fig.1c).

Histological Examination: Figure 2a: show histopathological findings from H & E stained kidney sections. In the control group rats, no glomerular or pathological abnormalities. However, $HgCl_2$ group rats showed multiple pathological changes, which include dilated of Bowman's capsules, atrophy glomerulus tuft, vacuolated of epithelial cells and cast formation with the presence of interstitial exudates. Also, a glomerular cell cast of renal tubules proximal dense nuclear of epithelial cell, hyper atrophy of other cells, a hemorrhagic area with a mild aggregation of some inflammatory cells beside a swelling of epithelial cells as shown in (fig. 2b). Rat kidneys treated with *Z officinale* + $HgCl_2$ group showed normal renal tubules and nearly normal epithelial cells, with very mild degeneration, while normal kidney tissues appear in (fig. 2c) rats.

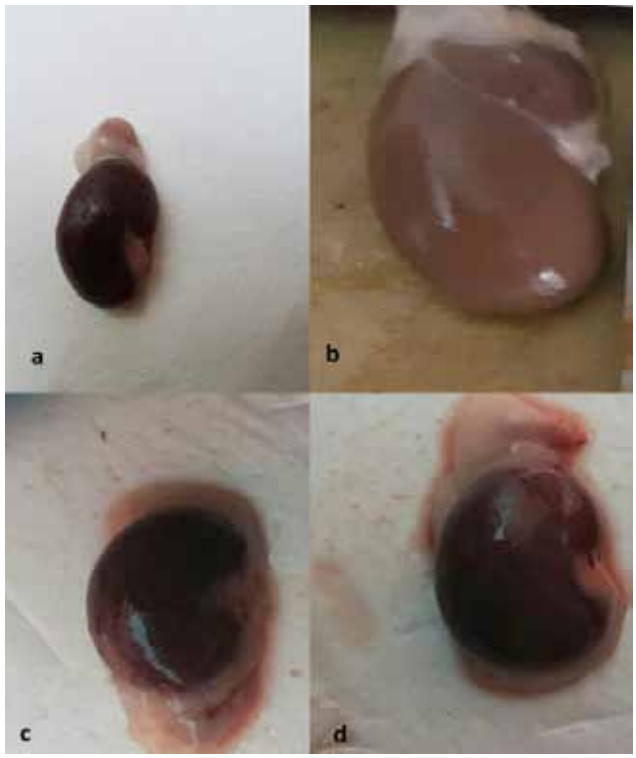

Fig. 1: a, c & d. (normal, Z officinale & HgCl2 + Z officinale extract groups) showed normal gross appearance kidney. b, HgCl2 group showing pale and swollen kidney.

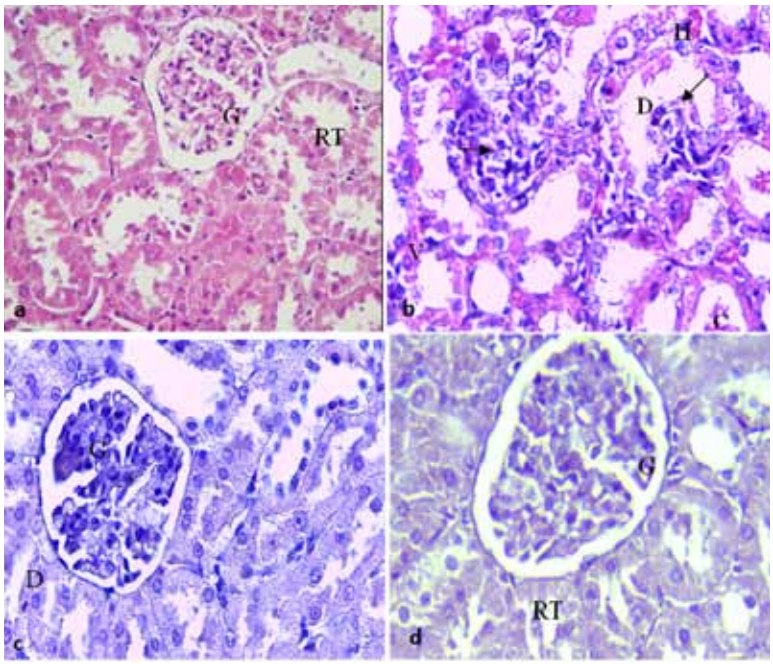

Fig. 2:a. Control group, showing normal glomeruli (G) and normal renal tubules (RT). b, $HgCl_2$ group showing dilated of Bowman's capsules (D), glomeruli tuft atrophy (→) vacuolated of epithelial cells (V) and glomerulus cells cast of renal tubules (C), hemorrhagic area (H). c, Zingiber officinale + $HgCl_2$ group showed nearly normal renal tubules with normal epithelial cells, and very mild degeneration(D). d, Zingiber officinale group showed normal glomerulus tuft, normal renal tubules and normal glomeruli of Bowman capsule H & E400X.

Discussion

The pathogenesis of nephrotoxicity of mercury is due to binding of mercuric ions with sulfur, like a thiol group of amino acids, which transfer mercury ion via sodium ion channels to the kidney tubules, causing in accumulation in the renal proximal tubular cells and increasing free radicals formation and damaging renal tubules[4,11,12]. So the current study was undertaken to determine whether Zingiber can reduce or prevent $HgCl_2$ induced renal damage by examining different biochemical and histological parameters related to kidney function of intoxicated and treated rats.

Reduce of the body weight may be resulting from being an interruption of $HgCl_2$ in the absorption and metabolism of feed nutrients essential for health[13]. Mercury is capable of damaging the organism in many ways because of its high affinity to various tissues and its tendency to accumulates[14,15,16] the result of the present study is inconsistent with other findings[12,16,17]. The biological effects ascribed to Zingiber include induction of endogenous antioxidants in rat tissue organs, scavenged free radicals by giving electron(s) to them, protecting cells from oxidative stress and increased detoxification of foreign compounds[18].

Serum Creatinine and urea are the most sensitive biochemical marker used in the assessment of renal tissue damage because the creatinine and urea are excreted through kidney therefore, in cellular damage there is retention of creatinine, urea in the blood[19]. Many previous studies reported that proteinuria from mercuric toxicity is due to disorders in the glomerular filtration barrier which resulted from damage in podocytes, and decreased reabsorption of filtered protein[16,20]. The significant increase in serum creatinine level is evidence of the reduced ability of the renal tubules to excrete and creatinine from the blood of the $HgCI_2$ group rats, a reality that was also supported by the photomicrographs of the kidney tissue. Creatinine and urea levels

return to nearly normal levels in the (*Z officinale* + $HgCl_2$) group that give an indication that Zingiber has protective and ameliorative effects on $HgCl_2$ induced kidney damage (table 2). These results are inconsistent with a previous explanation that the zingiber contains a high concentration of flavonoids and alkaloids[21,22] and acts as an antioxidant and/or free radical scavenging activity. Also the present study showed that treated of rats with the $HgCl_2$, markedly induced elevation in malondialdehyde (MDA) due to the induction of free radical generation and stimulation of lipid peroxidation. Treatment of rats with Zingiber significantly reduces MDA level, due to Ginger compounds like gingerols, shagols, and ketone they have the capacity to reduce the free radical capacity[22,23].

The epithelium of (pct) is the most sensitive part of the kidney to the toxic effects of mercuric chloride due to their enzymatic activity[4,24]. In a histomorphometric study the diameter of (pct) in the treated $HgCI_2$ group was significantly increased ($P \leq 0.05$) as compared with other experimental groups, this an agreement with previous results[4,2526]. One of the reasons for the increase in the diameter of (pct) is loss of brush border due to oxidative stress produced by $HgCl_2$[21,22]. A significant decrease in the number of (pct) of the $HgCl_2$ reflects oxidative stress-induced necrosis and apoptosis[27,28]. In the present study the maintenance of the diameter of (pct) in the (Z officinale + $HgCl_2$) group indicates the antioxidant and anti-inflammatory effects of Zingiber, as reported previously by[26,29,30].

Figure 2 shows that the $HgCI_2$ causes pathological changes and, On another side the morphological picture of Z officinale + $HgCl_2$ rat kidney group shows nearly normal tissue architecture with very few degenerative changes. These results were consistent with previously published articles[4,24]. The result of the present study confirms the role of Zingiber in improving tissue damages by reducing/inhibit the number of pct and pathological changes induced by a toxic dose of $HgCI_2$.

Conclusion

Z officinale has an antioxidant effect and able to retard the progression of renal tubular pathological changes induced by $HgCI_2$.

Conflict of Interest Statement: The authors declare that there is no conflict of interest.

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Ethical Clearance: Health Research Ethics Committee, College of Veterinary Medicine University of Basrah.

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