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Original Research

# Effects of *Newboldia laevis* (ogirisi) on mercury chloride – induced kidney damage in adult male Wistar rats

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## ABSTRACT

Drug-induced injury is a key cause of kidney disease. Mercuric chloride, an antineoplastic agent used for treating various malignancies can cause kidney injury and induce nephrotoxicity. This study was to determine the effects of *Newbouldia laevis* on mercury chloride- induced kidney damage in adult male Wistar rats. Twenty-five adult male Wistar rats weighing between 110 – 200g were used for this study. The animals were divided into five groups: A to E with five rats per group. Group A served as the control group and was administered 1ml of distilled water daily. Group B received a low dose of *Newbouldia laevis* (200mg/kg body weight) for 28 days along with Mercuric chloride (10mg/kg body weight). Group C received an intermediate dose of *Newbouldia laevis* (400mg/kg) for 28 days with Mercuric chloride (10mg/kg). Group D received a high dose of *Newbouldia laevis* (800mg/kg) for 28 days with Mercuric chloride (10mg/kg). Group E received only Mercuric chloride (10mg/kg) daily for 28 days. Rats in each group were allowed unrestricted access to water and feed during the experiment. Intracardial collection of blood from the Wistar rats was carried out in each group and the kidney function were assessed. The kidney tissues were processed for light microscopy study. Group A kidney tissues showed normal tubules, glomeruli, and interstitial spaces. Group E showed mercuric chloride-induced kidney injury: perivascular infiltrates of inflammatory cells; patchy tubular necrosis. Group D showed amelioration of the damages caused by Mercuric chloride. It can be concluded that *Newbouldia laevis* ameliorated Mercuric chloride – induced kidney damage in dose-dependent-pattern.

**Keywords:** Ameliorate, Kidney, *Newbouldia laevis*, Mercuric chloride, Wistar rats

## INTRODUCTION

Plants have been reported to have wide variety of chemical compounds that are of medicinal importance (Seidel, 2020). Herbal medicine has been used for centuries in very many cultures throughout the world (Petrovska, 2012). Scientists and medical professionals have shown increased interest in herbal medicine because many drugs are derived from plants. The health benefits of these medicinal plants in the production of drugs are now appreciated (Petrovska, 2012). The role of herbal plants in primary health care in the developing countries and need for policies that encourages its development, and usage in manufacturing of drugs cannot be overemphasized (Sofowora *et al*., 2013). One of such medicinal plants is the *Newbouldia laevis.* The plant is commonly known as Boundary Tree. The Boundary Tree is a tropical plant and distributed in West and Central Africa. It is an average-sized angiosperm in the Bignoniaceae family that grows to the length of about 10 - 15m. Also, it is used as ornamental plant (Burkill, 1985).

Although, there are reports on the phytochemical constituents of many tropical medicinal plants and their efficacy in treatment of various ailment (Kang, 2021), however, report on phytochemical constituents of *Newbouldia laevis* is scanty (Dermane *et al.*, 2020). The preclinical investigations on *Newbouldia laevis* using laboratory animals revealed its protective property against toxicity produced by heavy metals (Ogbe *et al*., 2020). Heavy metals are abundant in the environment, and this includes Mercury (II) chloride. Mercury dichloride ($HgCl_{2)}$ is a water-soluble substance. It is an odourless white crystalline solid, used as laboratory reagent. It is a highly toxic compound that volatizes slightly at room temperature



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and appreciably at 150°C (Patra &Sharma, 2000). Mercuric chloride is highly toxic, both acutely and as a cumulative poison. Mercuric chloride ability to produce free radicals makes it toxic. Its bioaccumulation in the kidneys can lead to acute kidney failure (Branco *et al*., 2017). There has been report of sudden death due to mercuric toxicity (Azevedo *et al.*, 2012) even though mercuric compounds have been used in the treatment of diseases (Bernhoft, 2012). When patients are treated with Mercuric compounds, they experience severe side effects, therefore limiting the dose that can be administered. Thus, the ability to manage this induced toxicity is crucial for its applications as drug for therapy (Baum, 1999).

One of the major functions of the kidney is to detoxify and eliminates toxins via filtration through the glomeruli, passive diffusion from distal tubules and active process through transportation in the blood and urine. Hence, the kidney is a potential organ susceptible to any form of drug toxicity. The kidney is retroperitoneal in the posterior abdominal region. They lie in the extra peritoneal connective tissue immediately lateral to the vertebral column. In the supine position, the kidneys extend from approximately vertebra $T_{12}$ superiorly to vertebra $L_3$ inferiorly. The right kidney is lower than the left because of its relationship with the liver (Drake *et al*., 2015). Each kidney has a smooth anterior and posterior surface covered by renal capsule. On the medial margin of each kidney is the hilum of kidney, which is a deep vertical slit through which renal vessels, lymphatics and nerves enter and leave the substance of the kidney. Internally, the hilum continuous with the renal sinus, occupied by the renal pelvis, calices, vessels and nerves and variable amount of fat. Perinephric fat continues into the hilum and sinus (Moore *et al*., 2014).

Plants extracts has been used to reduce the effect of toxicity generated from treatment using synthetic drugs (Karimi *et al*., 2015). The use of plant in treatment of diseases has been widely accepted in medicine. Plant has antioxidants or modulates gene expression and signal transduction pathways (Dandjesso *et al.*, 2012). Hence, the objective of this study is to evaluate the effect of *Newbouldia laevis* on Mercuric chloride induced toxicity to the kidney.

## MATERIALS AND METHODS

### COLLECTION AND PREPARATION OF PLANT MATERIALS

*Newbouldia laevis* were gotten from the Department of Botany, identified, and authenticated in the Department of Plant and Biological Biotechnology, University of Benin, Benin City. The seeds were pulverized using British mining machine and then macerated in a jar with distilled water. The solution was preserved in a bottle inside a refrigerator at temperature of 4°C.

### EXPERIMENTAL ANIMALS

Ethical approval for the study was obtained from the research ethics committee College of Medicine, University of Benin, whose guideline, and principles were strictly followed and complied for the proper management and utilization of laboratory animals used for research. (CMR/REC/REC/2014/57). Twenty-five adult male Wistar rats with an average weight of 142.30±5.05g were used for this experiment. They were obtained and acclimatized for two weeks in the animal house, using ventilated plastic cages. They were fed with grower's mash feed (Premier Feeds limited, Nigeria) and were given potable water *ad libitum*.

The rats were grouped into five: Group A (control) were fed with normal commercial feed and received 1ml of distilled water only. Group B were administered 10mg/kg of $HgCl_2$ (Mercuric Chloride, Country: Udpaiput, Rajasthan 31001, India) with low dose extract of *Newbouldia laevis* (200mg/kg). Group C were administered 10mg/kg of, $HgCl_2$ with intermediate dose extract of *Newbouldia laevis* (400mg/kg). Group D were administered 10mg/kg of $HgCl_2$ with high dose extract of *Newbouldia laevis* (800mg/kg). Group E were administered only $HgCl_2$ (10mg/kg). All the administration and treatments were carried out daily for 28 days.

### KIDNEY FUNCTION TEST

The blood was collected through intracardial puncture for kidney function test. The serum urea, and creatinine concentration were measured and calculated with the aid of spectrophotometer and diagnostic laboratory tests (POCh) according to Brzoska *et al.* (2023). The electrolytes: $Na^+$, $K^+$, $HCO_3$- , $Cl^-$ were measured using ion selective electrodes manufactured by SFRI Medical Diagnostics, France, model (ISE 4000).

### STATISTICAL ANALYSIS

All collected data were subjected to statistical analysis using IBM SPSS statistics software (Statistical package for social science) (version 25) and relevant statistical values were obtained. One way analysis of variance (ANOVA) was carried out and data presented as MEAN ±SEM. LSD post-hoc test was used. Values of $P<0.05$ were considered significant. The statistical values obtained were converted into graphical representations in form of bar charts.

## RESULTS

### HISTOLOGICAL OBSERVATIONS

The photomicrograph of the kidney of the control group shows normal histological features of the kidney with tubules, glomeruli, interstitial space being intact. The photomicrograph of the kidney treated with Mercuric chloride, $HCl_2$ only shows perivascular infiltrates of

inflammatory cells, and patchy tubular necrosis. The photomicrograph of the kidney treated with low dose extract of Newbouldia laevis with Mercuric chloride ($HgCl_2$) only shows interstitial congestion and interstitial infiltrates of inflammatory cells. The photomicrograph of the kidney treated with medium dose extract of Newbouldia laevis with Mercuric chloride ($HgCl_2$) only shows interstitial congestion and interstitial infiltrates of inflammatory cells. The photomicrograph of the kidney treated with high dose extract of Newbouldia laevis and Mercuric chloride ($HgCl_2$) only shows normal architecture of kidney tissues with tubules, glomerulus, and active vascular congestion (Figure 1: A, B, C, D and E).

**KIDNEY FUNCTION TESTS**

There was statistically significant increase in the weights of all the treated groups of Wistar rat against the control group. The kidney function test revealed no statistically significant differences in the in the urea, $Na^+$, $K^+$, $HCO_3$-. Also, there was no statistically significant differences in the levels $Cl^-$ and of creatinine concentrations across the groups. However, there were statistically in the significant differences in the renal weights of the Wistar rats and their Renosomatic index (Figure 2). The initial and final weights of the Wistar rats showed statistically significant differences. The Renosomatic index of the control group was significantly different from the treated groups (Figure1, Figure 2 & Figure 3) and (Tables 1& Table 2).

**DISCUSSION**

This work was carried out to investigate the amelioratory effect of *Newbouldia laevis* at low, intermediate, and high doses, on mercuric induced damage in the kidney of Wistar rats. The herbal plant used in this investigation displayed ameliorative potential, an indication of its antioxidant and anti-inflammatory properties which play a protective role against the mercuric induced kidney damage. The protective effect of *Newbouldia laevis* against mercuric induced nephrotoxicity in the Wistar rat because of its antioxidant and anti-inflammatory properties is similar to previous study where *Carica Papaya* also displayed protective role against mercuric induced kidney damage (Francis *et al*., 2023). Our finding showed statistically significant increase in body weights in all the groups when the initial body weights were compared to the final body weights. However, the administration of $H_gCl_2$ retarded the rate of body weight gain in the Wistar rat. The rate of body weight gain was improved in the $H_gCl_2$ and high dose of *Newbouldia laevis* treated group when compared to the control group. The kidney weight of the $H_gCl_2$ treated groups increased compared to the control, suggesting the bioaccumulation of $H_gCl_2$ with its attendant free radicals, therefore inducing the renal toxicity, inflammatory processes, and accumulation of fluid in the kidney of the Wistar rats. Our observation is similar to the findings of Kellum et al (2021) but not consistent with the observation in the work of Yadav et al (2019) where mercuric induced renal injury caused kidney weight loss. The renosomatic indices increased when the $H_gCl_2$ and *Newbouldia laevis* and control groups were compared. The changes noticed in the renosomatic indices in the treated groups were significantly different from the control renosomatic index but suggest *N. laevis* attenuation of the damage cause by mercury induced renal toxicity. This corresponds to the findings in the work of (Gargour *et al*., 2020).

The renal function test showed that the urea concentration increased when the treated $H_gCl_2$ only, $H_gCl_2$ and *Newbouldia laevis* groups (except the $H_gCl_2$ and intermediate

**Table 1. The Initial and Final Body Weights of the Control and Treated Groups of Wistar Rats**

| Groups | Initial Body Weight (Unit) | Final Body Weight | P-Value |
|---|---|---|---|
| **Control** | 110.60±2.29 | 174.00±7.80* | 0.001 |
| **$HgCl_2$ + Low dose of N. laevis** | 126.80±7.12 | 169.80±12.48* | 0.011 |
| **$HgCl_2$ + Intermediate dose of N. laevis** | 132.00±9.73 | 153.60±5.55* | 0.049 |
| **$HgCl_2$ + High dose of N. laevis** | 143.80±8.35 | 200.40±9.98* | 0.012 |
| **$HgCl_2$ Only** | 134.67±11.57 | 182.00±7.81* | 0.009 |

**Table II. The Kidney Weights of the Control and Treated Groups of Wistar Rats and Their Renosomatic Indices**

| | Control | $HgCl_2$ + Low dose of *N. laevis* | $HgCl_2$ + Intermediate dose of *N. laevis* | $HgCl_2$ + High dose of *N. laevis* | $HgCl_2$ Only | P- value |
|---|---|---|---|---|---|---|
| **Renal weight (g)** | 0.39±0.03 | 0.51±0.02* | 0.55±0.05* | 0.58±0.05* | 0.58±0.02* | 0.006 |
| **Renosomatic index (%)** | 0.22±0.01 | 0.30±0.02* | 0.36±0.03* | 0.29±0.02* | 0.32±0.02* | 0.004 |

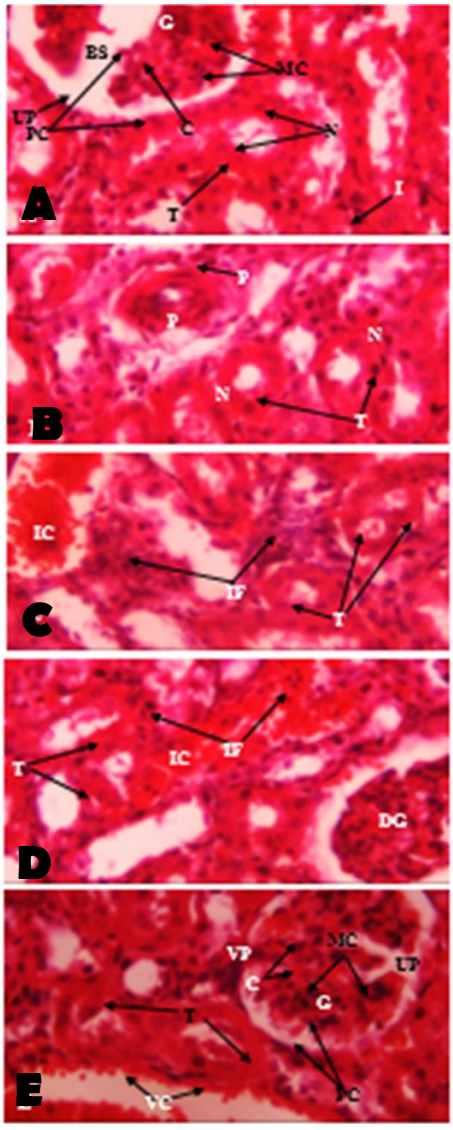


**Figure 1. Showing the photomicrography of the histology of the Wistar rat kidney**

In (A), the control group, the kidney is composed of normal tissue: (T) tubules, (G) glomeruli, (I) interstitial space. Observe the urinary pole (UP), Bowman's space (BS), Mesangial cells (MC), Podocytes (PC), capillaries (C) and the cuboidal nucleus of the tubules (N), (H&E × 400).

In (B), the Wistar rat kidney given $HgCL_2$ only shows (P) perivascular infiltrates of inflammatory cells, (N) patchy tubular (T) necrosis (H&E × 400)

In (C), the Wistar rat kidney given low dose of extract+$HgCL_2$ only shows interstitial congestion (IC), interstitial infiltrates (IF) of inflammatory cells between the renal tubules (H&E × 400)

In (D), the Wistar rat kidney given medium dose extract + HgCL2 only shows interstitial congestion (IC), interstitial infiltrates of inflammatory cells between tubules. Observe the degenerating (DG) glomerulus (H&E × 400)

In (E), the Wistar rat kidney given high dose extract + HgCL2 shows normal architecture tubules (T), glomerulus (G), active vascular congestions (VC). Observe the vascular pole (VP), capillaries (C), urinary pole (UP), Mesangial cells, and podocytes (PC) (H&E × 400).

dose of *Newbouldia laevis*) were compared to the control group. Serum urea concentration is an indicator of renal function. In previous study, the renal urea concentration of (60-80) mg/dl suggest severe renal damage (Karwasra *et al*., 2016). In this work, the intermediate dose of *Newbouldia laevis* helped to reduce the increased concentration of urea induced $H_gCl_2$ renal toxicity in the Wistar rat from (71.67±13.37) mg/dl to (54.00±8.00) mg/dl indicating the ameliorative effect of *Newbouldia laevis* at an intermediate dose because there was increase in the urea concentration at high dose of *Newbouldia laevis.* The values of the serum $Na^+$ and $K^+$ concentrations in the $H_gCl_2$ and *Newbouldia laevis* treated groups were not significantly different from the value of the control group. The reduction in value of the serum $Na^+$ and $K^+$ concentrations was indicative of renal tubular degeneration and failure to reabsorb the solutes because of disruption of the $Na^+$ and $K^+$-ATPase activity on the basolateral membrane by mercuric toxicity to the tubules of the kidney (Kramer *et al*., 1986). The severity of exposure may however be mild as reported in the investigation of Dhanapriya et al. (2016). Also, the statistically insignificant change in the serum levels of $Na^+$ and $K^+$ between the control and treated groups in this work is in congruence with the observation of João et al. (2021). The increased excretion of $HCO_3$- in treated groups, especially in the group treated with $H_gCl_2$ only is an indication that renal tubules had been compromised. Bicarbonate should help to balance the physiological pH in the Wistar rat. Our finding was in congruence with the observation made on mercuric accumulation in the kidney of Wistar rat (Yadav *et al.*, 2019). Excess retention of $Cl^-$ in the system of the Wistar rat used in this work is an indication of compromised glomeruli filtration by the administration of $H_gCl_2$ which may result in metabolic acidosis, and renal failure (Moviat *et al*., 2012).

The serum creatinine levels in the treated groups were higher than the creatinine level in the control group. The intermediate dose *Newbouldia laevis* and $H_gCl_2$ had serum creatinine level that is significantly different from the control

creatine hype while $H_gCl_2$ only had the highest serum creatinine level in the treated groups. The high dose of *Newbouldia laevis* and $H_gCl_2$ had the least serum creatine level. This suggests damages to the glomerular filtration mechanism, and the increased level of creatine in our investigation is similar to the report on mercuric injured kidney in the male mice (Jalili *et al*., 2020). The histology of the treated groups of this study revealed a perivascular and interstitial infiltration of inflammatory cells, patchy tubules and tubular necrosis, interstitial congestion when compared to the normal microarchitecture of the renal tissue. This is similar to the submission of Francis et al (2023) given on mercuric nephrotoxicity in the rat. Also, the histopathology on mercuric toxicity on mice kidney corroborates our findings (Jalili *et al*., 2020).

*Newbouldia laevis* in our study was effective in protecting kidney against $HgCl_2$ induced injury. This corresponds to previous reports on herbal plant extracts having protective effect against nephrotoxicity in rats (Agarwal *et al*., 2010, Boroushaki *et al*., 2014, Gao *et al*., 2016, Hosseini et al., 2018, Francis *et al*., 2023). The low, moderate, and high doses of *Newbouldia laevis* when administered to the $HgCl_2$ treated Wistar rats showed improvement on the increasing weight of the animal which is approximately the same size as that of the control. Our finding is similar to the study carried out on rat, using *Tribulus terrestris* to prevent against mercuric chloride induced renal injury (Yadav *et al*., 2019). There was steady increase in the renal weights of the Wistar rats as all the treated groups with low, intermediate, and moderate doses of *Newbouldia laevis* retained their high values which were significantly different from the control. High dose of *Newbouldia laevis* was therapeutic to the induced renal damage showed by perivascular and interstitial infiltration of inflammatory cells, tubular necrosis and glomeruli degenerations. This indicates the antioxidant effect of the plant and suggesting its ability in the prevention of oxidative stress. The antioxidant ability of *Newbouldia laevis* is similar to other herbal plants that have been reported (Lukitaningsih, 2020). Our finding also showed that *Newbouldia laevis* had no toxic effect on the kidney, and other organs such as the intestine and liver as indicated by the food intake and its conversion into energy through body weight gain. The nontoxic effect of *Newbouldia laevis* phytochemicals on the experimental animal is similar to reports given on extracts from herbal plant (Altemimi *et al*., 2017).

Oral administration of mercuric chloride induced renal damage in Wistar rat. The administration of *Newbouldia laevis* was able to protect and ameliorate the damaging effect of the administered $HgCl_2$ on the kidney of the Wistar rat because of the antioxidant and anti-inflammatory properties of *Newbouldia laevis* and this is at par with the report on the antioxidant property of *Oxalis cernua* (Belghoul *et al*., 2020) and the therapeutic property of *Carica papaya* (Francis *et al*, 2023).

## CONCLUSION

Conclusively, *Newbouldia laevis* ameliorated mercuric chloride-induced kidney damage in the adult Wistar rats in progressive and increased-dose-pattern. Further study using immunohistochemistry to ascertain the molecular interactions during the mercuric chloride-induced kidney damage and healing using *Newbouldia laevis* is required.

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