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Effects of aqueous extract of Glycine Max on Mercury Chloride -Induced Kidney Damage in Adult Wistar rats

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Abstract

Mercury chloride has a nephrotoxic activity, while Soya bean (Glycine max) on its part has antioxidant property. This present study evaluates the effect of aqueous extract of Soya bean on mercury chloride induced renal damage in Wistar rats. Thirty six (36) adult Wistar rats were divided into six groups of six rats each as follows; Group A (control); Group B (5mg/kg body weight of mercury chloride); Group C (200mg/kg body weight of Soya bean); Group D (400mg/kg body weight of Soya bean); Group E (5mg/kg body weight of mercury chloride and 200mg/kg body weight of Soya bean) ; Group F (5mg/kg body weight of mercury chloride and 400mg/kg body weight of Soya bean). Mercury chloride was administered once every day for 28 days subcutaneously. The extract was given over 28 days through orogastric feeding method. After day 28, biochemical and histopathological studies were conducted to evaluate nephrotoxicity. Blood levels of creatinine and urea were quantified. Animals treated alone with mercury chloride (Group B), showed significant ($p < 0.05$) increase in serum urea and serum creatinine with significant ($p < 0.05$) reduction in body weight of rat. Treatment of rats with Soya bean alongside mercury chloride (Groups E and F) showed a statistical significance ($p < 0.05$) improvement in renal function and body weight. Histopathological findings confirm these conservations. Administration of Soya bean aqueous extract has protective effect on mercury chloride -induced renal damage in adult Wistar rats which may be attributed to the extract isoflavones component.

Keywords: Soya bean, Kidney, Urea, Wistar Rats, mercury chloride

Introduction

Mercury is a potent neurotoxin that can cause serious health issues such as neurological disorders and kidney damage (Clarkson *et al*., 2006 and Flora *et al*., 2012). It is a toxicant found in the environment that can cause life-threatening illnesses (Barbier *et al*., 2005). It is thought to be due to its ability to bind to sulfhydryl groups of enzymes and proteins, leading to oxidative stress, lipid peroxidation, and DNA damage (Clarkson and Magos, 2006).

Soybean seeds or Glycine max (L) are members of the family Fabaceae (Milind *et al*., 2014). Soybeans contain very small amounts of saturated fatty acids. (Milind *et al*., 2014). Omega-6 and omega-3 fatty acids are present in soybeans, which are also rich in iron, phosphorus, vitamin B12 and folate (Milind *et al*., 2014). Moreover, the dried bean contains vegetable protein (40%), complex carbohydrates (35%), fat (18-22%), dietary fiber, oligosaccharides, minerals and other phytochemicals like saponin (Fern, 2000). It mobilizes complex food substances such as protein and concentrated starch into simpler free amino acids and carbohydrates, respectively (Randhir *et al*., 2004). Soybeans (Glycine max) are a legume rich in antioxidants especially isoflavones, phenolic acids and flavonoids (Lampe, 2003). Several studies have reported the potential health benefits of soybeans and its extracts, including antioxidant, anti-inflammatory, and anti-cancer properties (Mandel, 2000 and Yan, 2009). The plants bear their fruit in pods, which are casted with two halves (Okwu and Orji, 2007). The aim of the study is to investigate effects of aqueous extract of Soya beans on mercury chloride -induced kidney damage in adult Wistar rats

Materials and Methods

Collection of Plant

Soybeans (Glycine max) were purchased from Ikhin main market in Ikhin, Edo state, samples were taken to plant taxonomist at the Herbarium Unit of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, the submitted sample was identified, authenticated and a herbarium number issued (vouchers number UBH-G470).

Extraction of Plant

Soybean seed extraction was performed according to the method described by Aiyegoro and Oko (2010) with some modifications. Soybeans are first dried in an oven at 60°C for about 4 hours. The dried soybeans are then grounded into powder form. Powdered soybeans (1.5 kg) were soaked three times in distilled water at room temperature in a shaker for 48 hrs. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate of the aqueous extract was obtained and rapidly frozen at - 4°C and dried for 48 h using a freeze dryer. The resulting extract was reconstituted with distilled water to achieve the desired concentration for use during the study.

Animal and Management

The rats were raised at the University of Benin's animal house in the city of Benin. There, they were housed in plastic cages at room temperature and fed Grower's mash—a product of Premier Feed Mills Co LTD—every day along with water. Using a digital weighing scale calibrated in grams and recorded to the nearest whole number, they were weighed every day of the experiment. The guidelines for the use and care of laboratory animals were followed in the protocol of this investigation.

Animal grouping and treatment

Thirty-six (36) adult Wistar rats in total were utilized in this investigation. After two weeks of acclimatization, the rats were randomly assigned to Six (6) groups of six (6) rats each, with free access to feed and water. Then, for twenty-eight (28) days, the extract was administered orally by the use of an orogastric tube.

Group A served as the control group and received only 1 ml of distilled water per day, while rats in group B received 5mg/kg body weight of mercuric chloride, group C received 250 mg /kg body weight of soybeans while group D received 500mg/kg body weight of soybeans. Group E received 250mg/kg body weight of Glycine max water extract and 5mg/kg of mercury chloride while Group F received 500mg/kg body weight of soybean and 5mg/kg of mercury chloride after one hour.

Sacrifice of the animals

A midline abdominal incision was made through the ventral wall of the rats under mild anesthesia using chloroform. Blood was taken from the inferior vena cava and kept in blood (heparin) bottles for renal function test analysis. The kidney was harvested and fixed in 10 % formal saline for histological analysis. The tissues were dehydrated in ascending grades of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. The de-paraffinized sections were stained routinely with Haematoxylin and Eosin (Drury and Wallington, 1980). Kidney function assessment was carried out as previously described; urea (Chaney and Marbach, 1962) and creatinine (Bartels and Bohmer, 1972).

Photomicrography

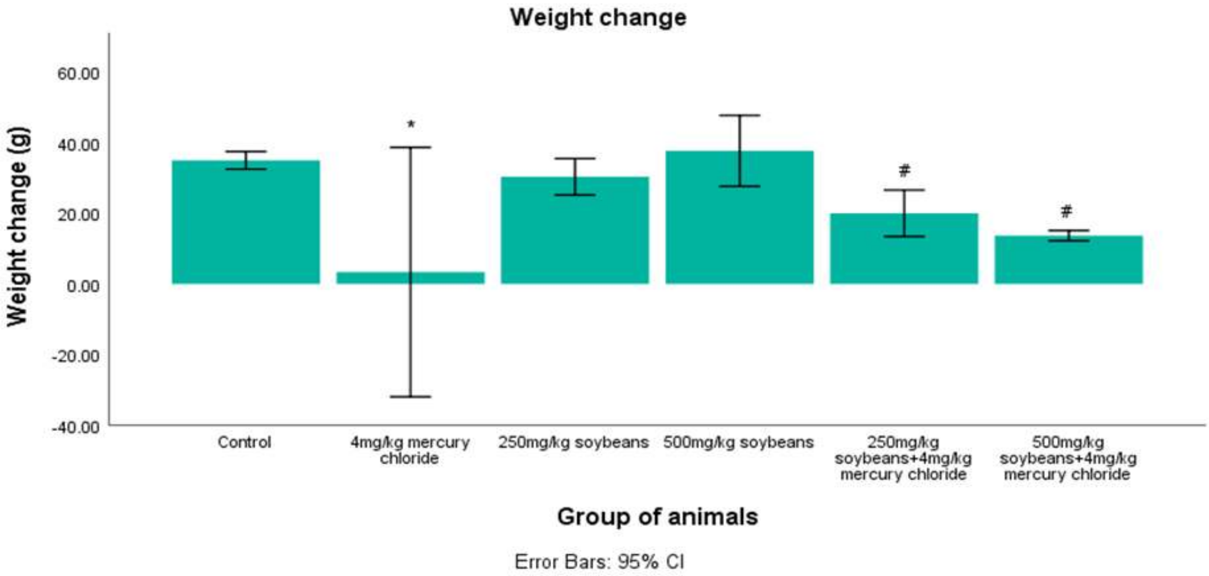
The stained slides were viewed using an optical photomicroscope (Leica MC170 HD, Leica Biosystems, Germany) and photomicrographs were taken at x100 magnification using an attached Eakins 14MP digital microscopic camera, model 2307su, manufactured by Eakins Microscope Store, UK.

Statistical Analysis

In this study, all values were presented as mean ± standard error of the mean for all groups. The significance of differences in the means of all parameters was determined using one way analysis of variance (ANOVA). All statistical analysis were carried out using Statistical Package For Social Sciences (SPSS) product of the International Business Machine Corporation (IBM) in Armonk, New York

Results

Figure A



*Significantly different from the control group
#significantly different from group B
There were statistically significant weight changes ($p<0.05$) in Group B when compared to the control group

Table 1: Changes in renal function test across the groups

Groups	Urea(mg/dl)	Creatinine(mg/dl)
A	24.54±0.113	0.6±0.127
B	71.22±0.172*	6.1±0.154*
C	26.78±0.174	0.7±0.181
D	27.61±0.190	0.6±0.133
E	28.62±0.131#	0.9±0.164#
F	30.48±0.157#	1.2±0.182#

*Significantly different from the control group
#significantly different from group B

There were statistically significant increase ($p<0.05$) of urea and creatinine values in Group B when compared to the control group.

Histological Results

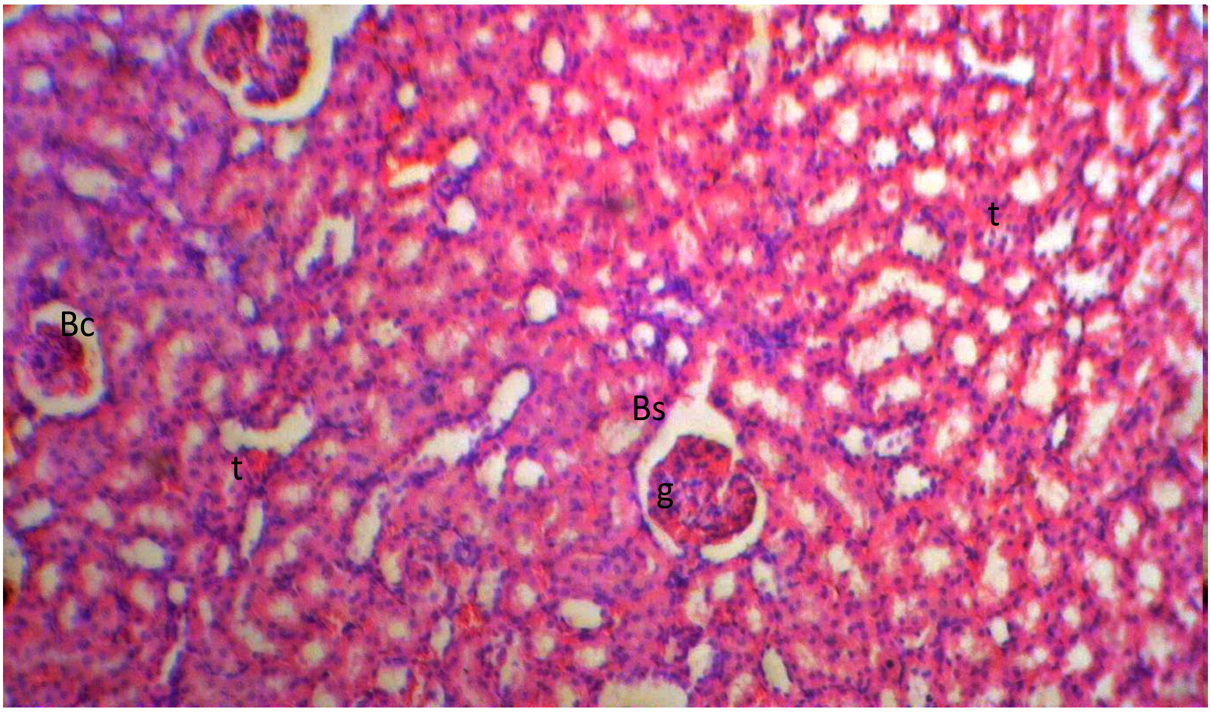


Plate 1: Representative photomicrograph of a section of the kidney of the control group capsule (Bc), Bowman's space (Bs) (H&E; 100×).

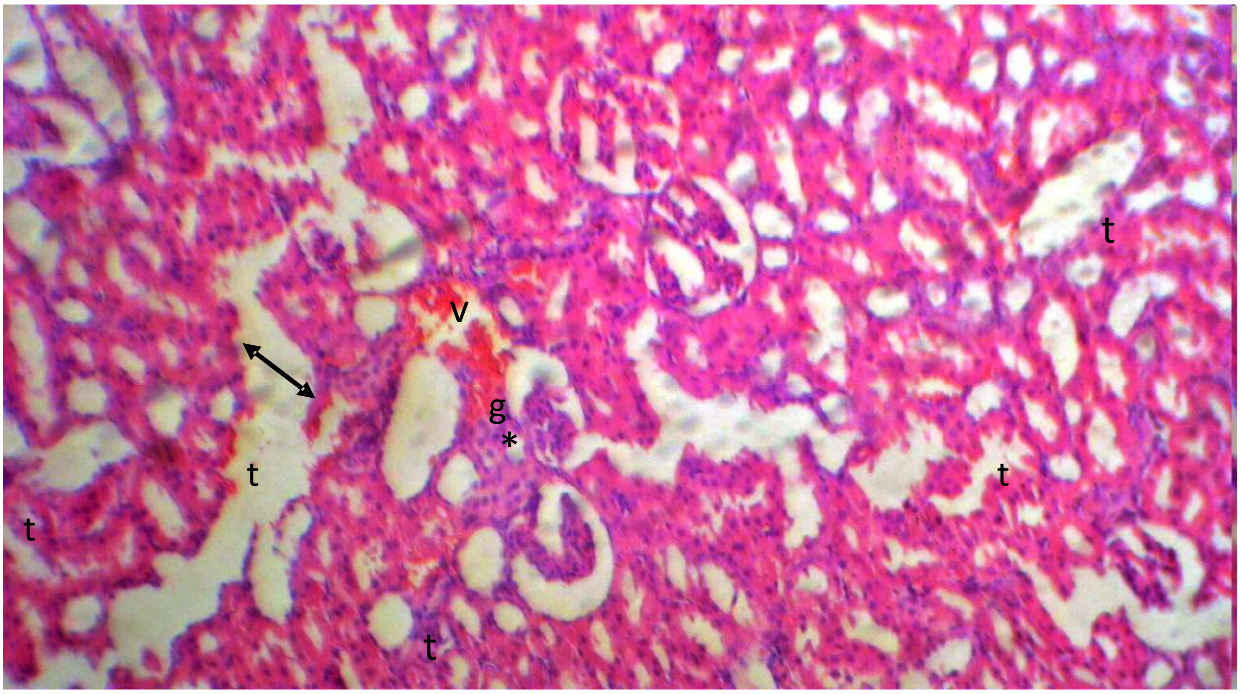


Plate 2: Representative photomicrograph of a section of the kidney of the group treated with $HgCl_2$ only

Key: Plate show non-proliferative glomerular atrophy (g) with increased urinary space (double-head arrow), focal tubular necrosis (t), severe vascular dilatation and congestion (V), severe infiltrates of inflammatory cells (*) (H&E; 100×).

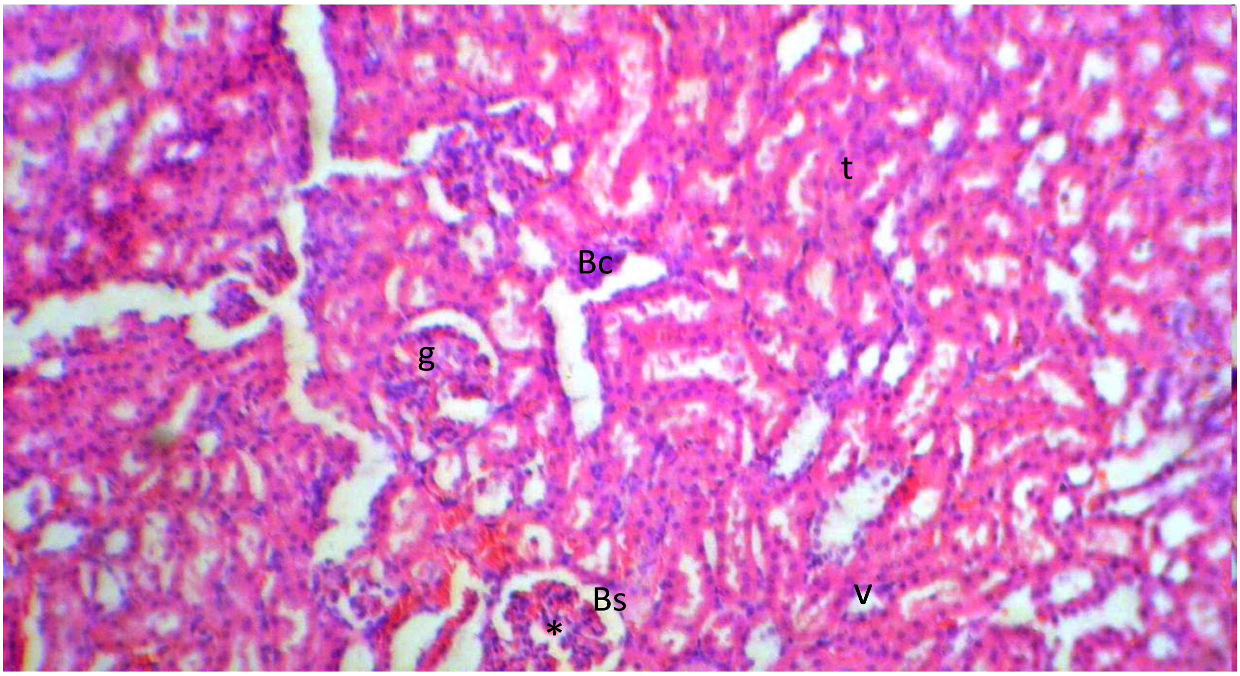


Plate 3: Representative photomicrograph of a section of the kidney of the group treated 250 mg/kg body weight of Soy beans

Key: Plate shows apparently normal histomorphology; glomerulus (g), tubules (t), Bowman's capsule (Bc), Bowman's space (Bs) (H&E; 100×).

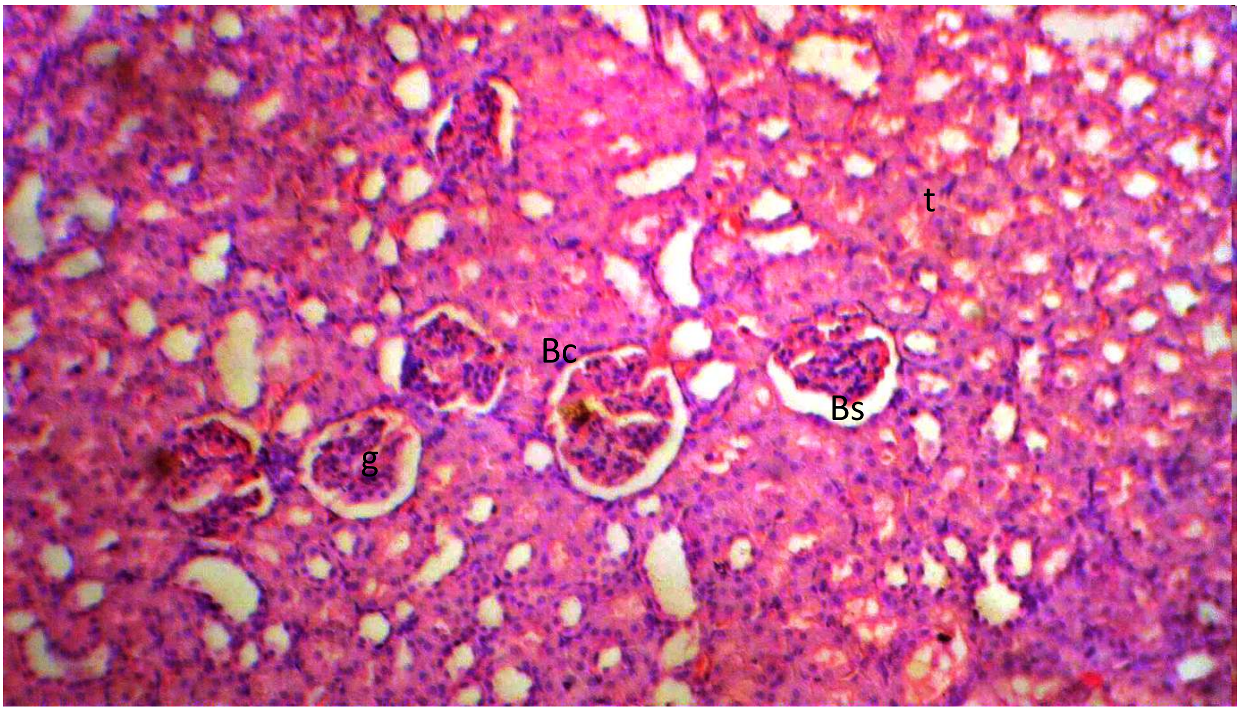


Plate 4: Representative photomicrograph of a section of the kidney of the group treated 500 mg/kg body weight of Soybeans.

Key: Plate shows apparently normal histomorphology; glomerulus (g), tubules (t), Bowman's capsule (Bc), Bowman's space (Bs) (H&E; 100×).

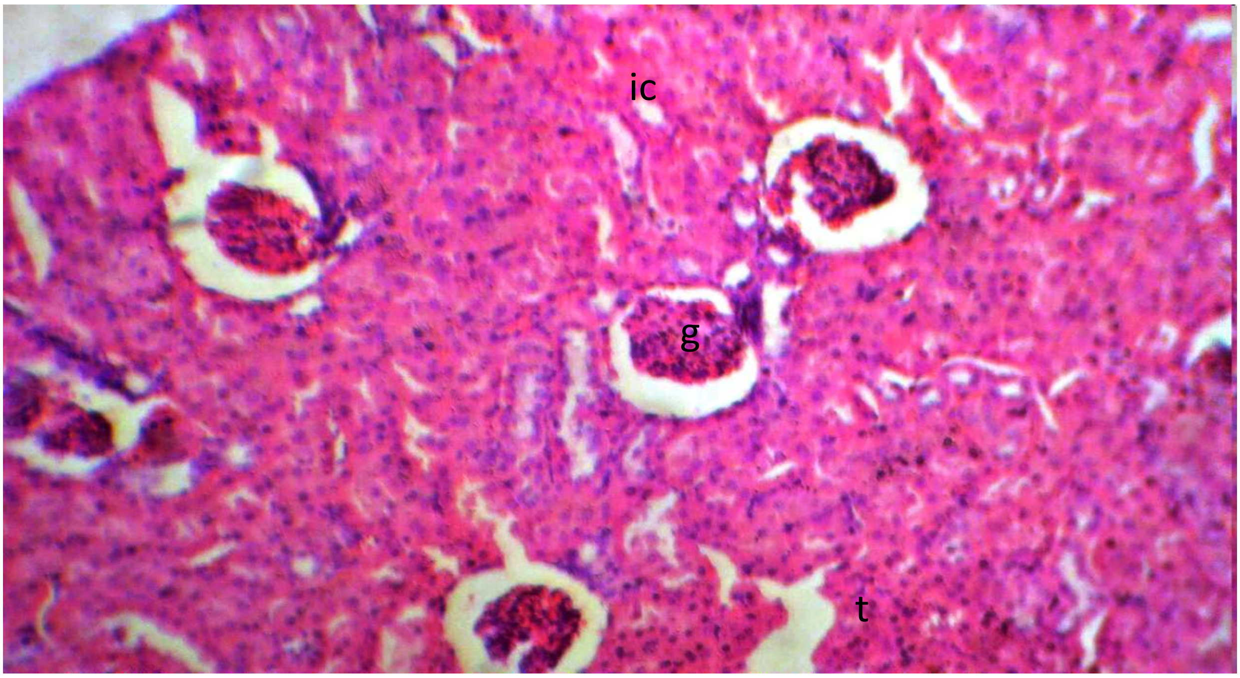


Plate 5: Representative photomicrograph of a section of the kidney of the group treated with $HgCL_2$ and 250 mg/kg body weight of Soybeans

Key: Plate shows normal glomerulus (g), focal tubular swelling (t), mild interstitial congestion (ic) (H&E; 100×).

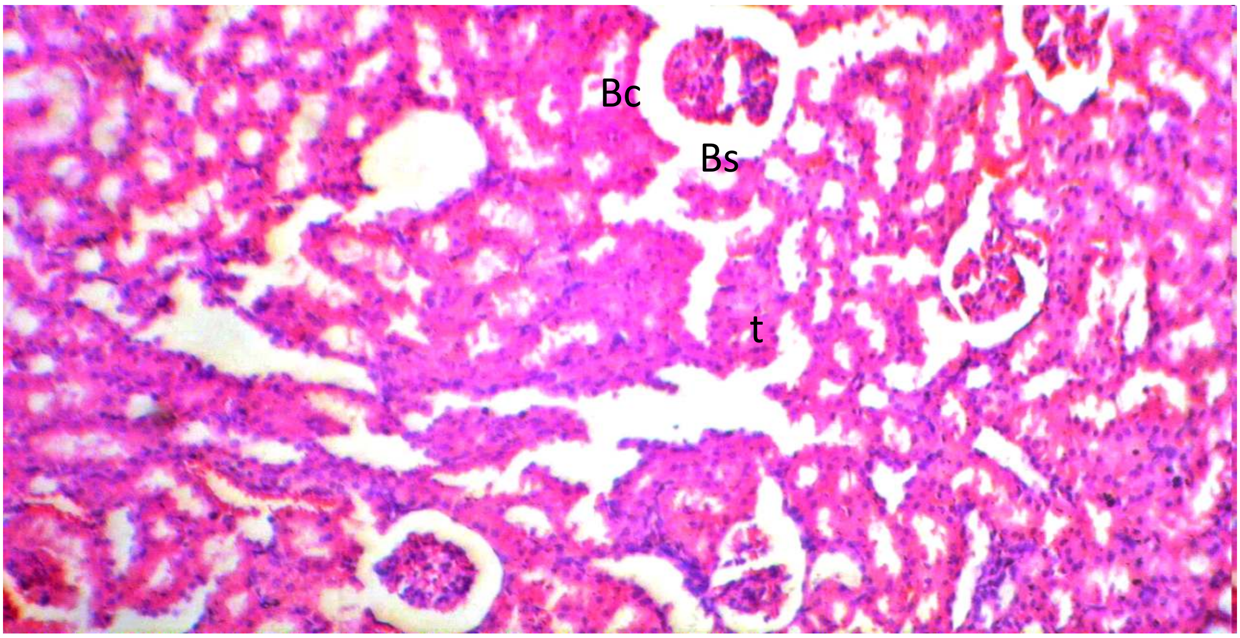


Plate 6: Representative photomicrograph of a section of the kidney of the group treated with $HgCL_2$ and 500 mg/kg body weight of Soybeans

Key: Plate shows apparently normal histomorphology; glomerulus (g), tubules (t), Bowman's capsule (Bc), mildly dilated Bowman's space (Bs) (H&E; 100×).

Discussion

Herbal leaves are widely accepted to have a curative effect among the people of the third world countries (Ehimigbai, 2015). In our study, we reported a significant decrease in body weight of rats due to administration of 5mg/kg body weight of mercury chloride. The observed decrease in the body weight in the mercury chloride group indicates the toxicity effect of mercury chloride on overall health. This result is consistent with the study conducted by (Hussain *et al*., 2018) who also reported a significant decrease in body weight in rats treated with doses of mercury chloride. While group E (treated with 250mg/kg body weight of soybeans and 5mg/kg body weight of mercury chloride) and group F (treated with 500mg/kg body weight of soybeans and 5mg/kg body weight of mercury chloride) showed a significant increase in body weight. This result is consistent with (Masoumeh *et al.,* 2017) who reported that soy isoflavones may have an increasing impact on weight under certain circumstances. This study suggested that Soybean may contain a lot of protein, which may be supportive in gaining weight.

Furthermore, results obtained from our study showed that there was a significant increase ($p<0.05$) in the urea and creatinine level in 5mg/Kg mercury chloride group when compared with the control. Increase in urea means kidney injury. This agrees with research conducted by Boroushaki *et al*. (2014) and Mesquita *et al*.,2016) who reported that mercury administration was associated with a significant increase in urea level. This suggested that mercury ion serve as nephrotoxic agents. This also agrees with research conducted by (Ehimigbai, 2024) who also reported elevated amounts of urea in the $Hgcl_2$ group owing to renal damage. This suggested that urea and creatinine are strongly associated with kidney injury. Plasma levels of urea and creatinine are the main indicators of kidney function. While group D, group E and group F showed a significant decrease in urea and creatinine level when compared to group B. In the present study, groups D, E, and F showed significantly reduced urea and creatinine concentrations compared with the mercury-treated group. Specifically, group D showed a significant decrease in urea and creatinine concentration, suggesting a potential protective effect of soybeans on kidney function. Groups E and F also showed a significant decrease in urea and creatinine concentrations, suggesting that soybeans may have an effect used synergistically when combined with mercury chloride. These results are consistent with previous research conducted by (Uchendu *et al*.,2017) who reported that glycerol and soybean group had significantly reduced urea levels. The results showed a protective effect of soybeans. This is also consistent with the statement of Anderson (2008), who stated that soy has protective effects in animal models of kidney disease. While Wisudanti *et al., (*2020) evaluated the nephroprotective effect of soy flour on diazinon-induced nephrotoxicity in rats and concluded that administration of soy flour had a nephroprotective effect in preventing increases in blood urea and creatinine levels of diazinon-induced nephrotoxic male Wistar rats. Additionally, soy contains the greatest concentration of potent antioxidants called isoflavones which are a class of phytoestrogens (Cederroth and Nef, 2009). This kidney-protective ability may be due to properties reported in soybeans.

Histologically, rats administered mercury chloride showed significant alterations in the kidney structure. It showed non-proliferative glomerular atrophy with increased urinary space and focal tubular necrosis. It also showed severe vascular dilatation and congestion and severe infiltration of inflammatory cells. These observations is in line with a study by Wei *et al*. (2012) who studied the effects of mercury exposure on renal histopathology in rats and reported similar findings of tubular necrosis, glomerular atrophy, and inflammatory cell infiltration. This study demonstrates that mercury exposure leads to oxidative stress and kidney damage, which may contribute to the histological changes observed. Additionally, studies by (Zalups *et al*., 1995) elucidated the molecular mechanisms of mercury-induced nephrotoxicity, including interference with the ion transport system and direct cellular damage. These mechanisms may contribute to the observed vascular dilation and congestion.

Rats administered Soybean and mercury chloride showed improvements compared to rats administered mercury chloride alone. Although the section of the kidney of the group treated with $HgCL_2$ and 250 mg/kg body weight of soybeans showed normal glomerulus. It also showed focal tubular swelling with mild interstitial congestion. Although soybean contains bioactive compounds with potential regenerative protective effects, such as isoflavones, it also contains other components that may have varying effects on kidney function. For example, a study by Jing *et al*. (2016) studied the effects of soy protein consumption on kidney function in rats with diabetic nephropathy. The results showed that soy protein supplementation improved kidney function and reduced tubular damage and interstitial fibrosis. Therefore, the pattern changes observed in our study at 250 mg/kg of extract may be influenced by the combined effects of $HgCl_2$ toxicity and soybean components. Normal glomeruli may show a protective effect of soy ingredients against $HgCl_2$-induced glomerular damage, whereas tubular necrosis and interstitial congestion may reflect a nephrotoxic effect of $HgCl_2$. Mercury chloride and 500 mg/kg body weight of soybeans showed apparently normal histomorphology with mildly dilated Bowman's space. This observation is in line with a study that showed soy isoflavones reduced kidney damage in rats with chronic kidney illness by reducing oxidative stress and inflammation (Wei *et al*., 2012). Another study found that soy isoflavones reduced oxidative stress and improved kidney function in rats with chronic kidney disease (Hou *et al*., 2017). While Ramasamy *et al*., (2018) investigated the nephroprotective effect of Glycine seed extract against gentamicin- and rifampicin-induced nephrotoxicity in Sprague-Dawley rats and they concluded that Soybean demonstrated good nephroprotective activity with good antioxidant.

Conclusion

It can be concluded that aqueous extract of Glycine max have a protective role against the toxic effects of mercury chloride in the kidney of adult Wistar rat, and it can act as a good source of edible variety for mankind.

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