

SJMLS - 9(2) - 032

Effects of Aqueous Extract of *Chromolaena odorata* Leaves on Arsenic Acid-Induced Kidney Damage in Adult Wistar Rats

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<https://dx.doi.org/10.4314/sokjmls.v9i2.32>**Abstract**

In African folk medicine, *Chromolaena odorata* have been used for treating various ailments such as dysentery, malaria, toothache, skin diseases and diabetes. The aim of this study was to investigate effects of the aqueous extract of *Chromolaena odorata* on the kidneys of rats damaged with arsenic acid. Thirty (30) Wistar rats were randomly assigned into a Control group (A) and five treatment groups (B, C, D, E, F) containing 5 rats each. Group B was administered with 4mg/kg of Arsenic only. Rats in Group C were given 400mg/kg body weight of aqueous extract of *Chromolaena odorata* only. Group D was administered 1000mg/kg body weight of aqueous extract of *Chromolaena odorata* only. Group E was given 400mg/kg body weight of *Chromolaena odorata* aqueous extract and 4mg/kg of Arsenic acid and Group F was given 1000mg/kg body weight of *Chromolaena odorata* aqueous extract and 4mg/kg of Arsenic acid. The result showed that arsenic acid caused an increase in the urea level, decrease in SOD level and tubular necrosis of the kidney. While aqueous extract of *Chromolaena odorata* caused decrease in the level of urea, increase in SOD level across the groups plus the combination group with arsenic acid. Histological examination of the kidneys treated with arsenic acid and low and high doses of the extract showed amelioration with the lower dose having a more potent effect. It can be concluded that aqueous leaf extract of *C. odorata* was able to ameliorate arsenic acid induced kidney damage in adult Wistar rats.

Keywords: *Chromolaena odorata*, Histology, SOD and Renal tissue

Introduction

Throughout history, humans have depended on plants as valuable sources of remedies for treating illnesses and fulfilling diverse healthcare requirements (Frimpong *et al.*, 2021). *Chromolaena odorata* Linn (L.) the plant, *C. odorata*, is a perennial shrub belonging to the Asteraceae family, native to South and Central America (Akinmoladun, Akinloye, 2007). The plant is known by many names including Siam weed, Christmas bush, devil weed, camfhur grass, and common floss flower (Gunasekera, 2009). In Nigeria, *Chromolaena odorata* is commonly known as Ewe Awolowo, Siam weed, Elizabeth weed, Obirakara, Olorohuru, and independent weed (Ngozi and Osuji, 2014). It is a prolific weed that thrives in the majority of soil types, is found in abundance on open wasteland and along roadsides and prevents the establishment of other flora (Bani 2002; Warea, 2004). It can be poisonous to lives as it has exceptionally high levels of nitrates (5-6 times above the toxic level) in the dead and young shoots, the cattle feeding on this die of tissue anoxia (Sajise *et al.*, 2008).

Chromolaena odorata has been documented to possess various pharmacological properties, which encompass its potential as natural medicinal plants for wound healing, cancer, diabetes, hepatotoxicity, inflammation, antimicrobials, antioxidants (Sirinthipaporn 2017) as well as immunomodulatory activity (Taleb-Contini 2006).

The key chemical constituents in *C. odorata* included volatile oil, phytosterols, terpenoids and flavonoids, among which flavonoids possess

the largest variety (Omotuyi et al.,2018, Chuyong et al.,2019). The leaves of this plant have been found to be a rich source of flavonoids including quercetin, sinensetin, sakuranetin, padmatin, kaempferol and salvagenin (Akinmoladun et al., 2007, Torrenegra and Rodriguez,2011).

Of the many naturally occurring elements found abundantly distributed in the earth's crust, arsenic has found its way into prominence as a toxicant of significant public health risk (Tchounwou et al.,2012).

In general populations, arsenic exposure occurs mainly through drinking water and food (Hughes et al., 2002, Smith et al., 2011), cardiovascular disease (Navas-Acien, Sharrett, Silbergeld et al., 2005, Moon et al., 2013), diabetes (Maull et al., 2012; Kuo et al., 2013), respiratory outcomes (Naujokas et al., 2013), and neurodevelopmental and reproductive abnormalities (Sohel et al., 2010). Acute arsenic exposure produces toxicity of liver, kidney, intestine and brain (Klaassen 1996; NRC 1999; Liu et al., 2000). Recent epidemiologic studies also suggest that arsenic is associated with chronic kidney disease (CKD) (Hsueh et al., 2009; Chen et al., 2011; Zheng et al., 2013).

The aim of the study is to investigate effects of aqueous extract of *Chromolaena odorata* leaf on arsenic acid-induced kidney damage in adult Wistar rats.

Materials and Methods

Collection and identification of plants

Leaves of *Chromolaena odorata* were harvested from a farm in a town called Ikhin in Owan east

L.G.A of Edo state. It was identified as *Chromolaena odorata* in Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria. It was air-dried in the Department of Anatomy laboratory section for about 2 weeks, before grinded with British electric grinder into fine powder form. The fine powder was weighed and soaked with distilled water at the ratio of 1gram to 10ml of the distilled water for about 24 hours. After soaking for about 24 hours, it was filtered using a filter and funnel. Thereafter, the residues were discarded, and the filtrates were concentrated using water bath at 45°C till the filtrates appeared in paste form. Then the paste extract of the *Chromolaena odorata* was preserved in a refrigerator.

Experimental animals

Thirty (30) adult Wistar rats weighing between 180-210g were acquired from the Animal house in the Department of Human Anatomy at University of Benin. For a period of two weeks, the rats were housed and fed for the sole purpose of acclimatization. They were kept in clean, well-ventilated cages at room temperature under natural lighting and food and water were made available at all times.

Experimental design

The experimental animals were assigned into six groups: A, B, C, D, E and F consisting of 5 rats each. The experimental period lasted for 30 days. The rats were administered with Arsenic acid or/and aqueous *Chromolaena odorata* as shown in the table below. The administration was done on a daily basis for 30 days.

Table 1: Subgroups of the Experimental Rats

Group	Description
Group A:	Control group: Rats were given no extract or toxicant
Group B:	Rats were given aqueous extract of 4mg/kg of Arsenic only
Group C:	Rats were given low dose(400mg/kg) of <i>Chromolaena odorata</i> only
Group D:	Rats were given high dose(1000mg/kg) of <i>Chromolaena odorata</i> only
Group E:	Rats were given low dose(400mg/kg) of <i>Chromolaena odorata</i> + 4mg/kg of Arsenic acid
Group F:	Rats were given high dose(1000mg/kg) of <i>Chromolaena odorata</i> + 4mg/kg of Arsenic acid

Collection of samples

During sacrifice, the final weights of the rats were taken using compact electric weighing scale calibrated in grams. Cotton wool was soaked with chloroform of about 50ml in an enclosed container. The rat was put into an enclosed container with chloroform for about 2-5sec for anaesthetization. After anaesthetizing the rat was placed in a supine position on the dissection table (trolley). Abdominal-thoracic incision was made on the rat to expose the abdominal viscera. Thereafter blood samples were collected through inferior vena cava and through the heart by the process of venous and cardiac puncture respectively using 5mls syringes. The blood samples were turned into appropriate bottles for urea and SOD analysis. After, kidney was harvested at the retroperitoneal region of the abdomen, weighed and fixed with 10% formal saline in a universal bottle for histological analysis.

Kidney function assessment was carried out as previously described; Urea (Chaney and Marbach, 1962). Evaluation of Oxidative Stress Markers: They were carried out as previously described; Superoxide dismutase (SOD) (Misra and Fridovich, 1972).

Histological Assessment: The tissues were processed according to the method of Drury and Wallington (1980) for Haematoxylin and Eosin staining.

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

All data were subjected to statistical analysis using the IBM SPSS statistics software (Statistical Package for Social Science) Version 25 (SPSS, Inc., Chicago, Illinois, USA) and relevant statistical values were obtained. The values of the treated groups were compared with those of non-treated group using the one-way analysis of variance (ANOVA) and the T-test method. Values of $P < 0.05$ were considered significant. LSD was used as the post-hoc test

Results

Table 2: showing urea and SOD levels in the Kidney of the experimental animals

Groups	Urea (mg/dl)	SOD (unit/mg protein)
A	22.01±0.108	67.41±0.133
B	80.46±0.141*	15.11±0.112*
C	28.79±0.138	60.23±0.158
D	24.60±0.121	64.65±0.111
E	24.66±0.156 [#]	58.44±0.123 [#]
F	29.46±0.118 [#]	52.16±0.167 [#]

*Represent $p < 0.05$ when compared to control

Represent $p < 0.05$ when compared to rats exposed to arsenic acid

Histological Result

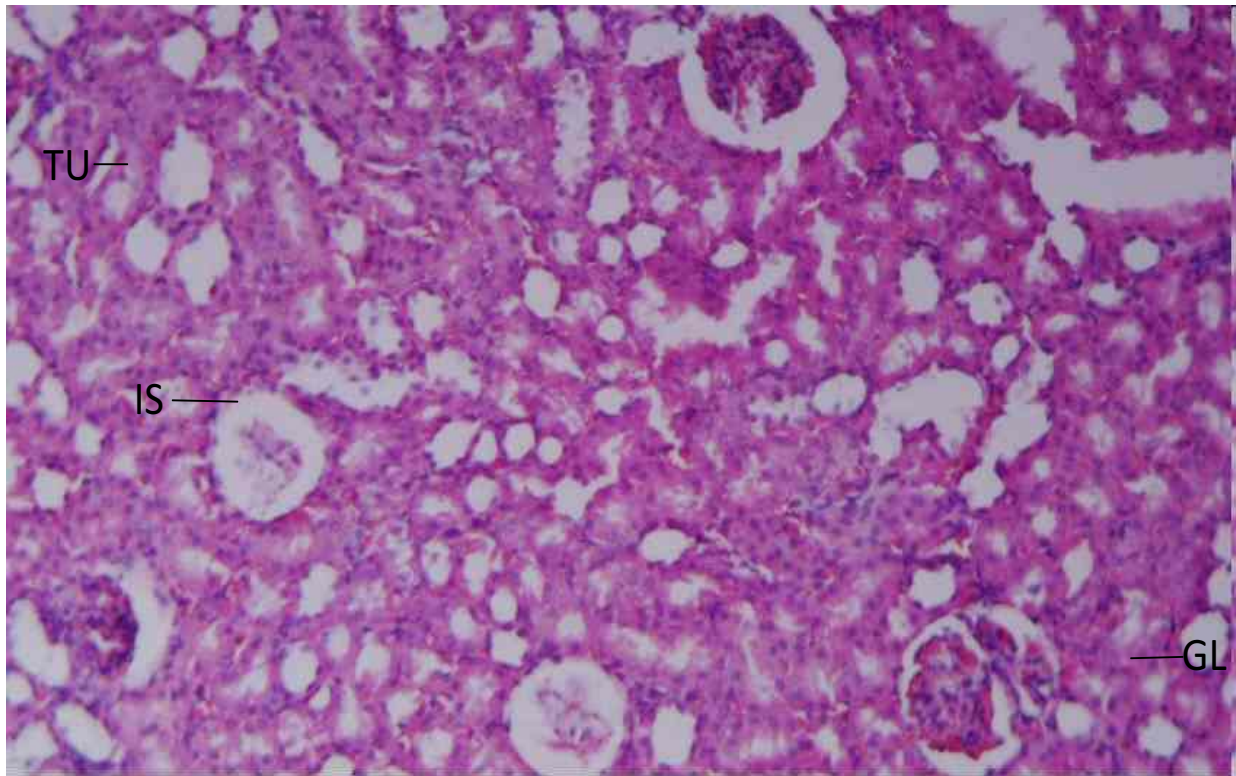


Plate 1: Rat kidney: Control. Composed of normal architecture: tubules (TU), interstitial space (IS), glomerulus (GL) : H and E x 100).

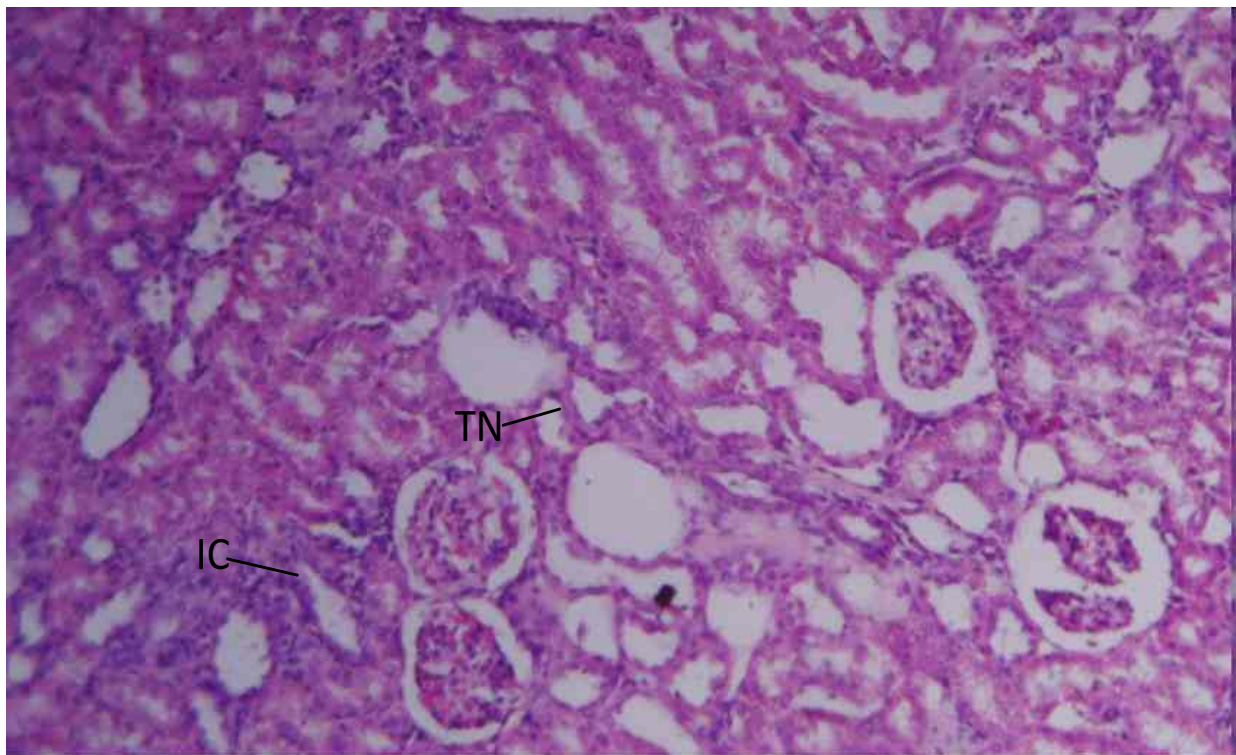


Plate 2: Rat kidney given 4mg/kg body weight of Arsenic only showing: patchy tubular necrosis (TN), heavy interstitial infiltrates of inflammatory cells (IC): H and E x 100).

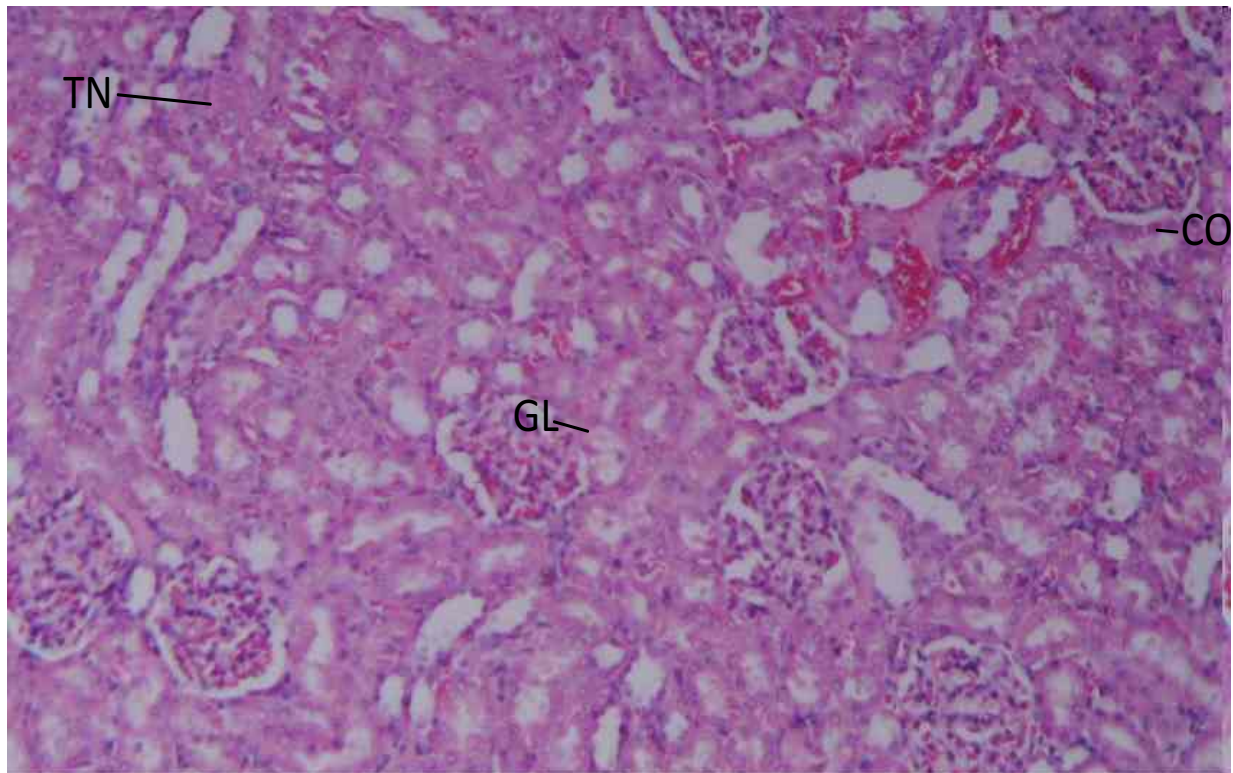


Plate 3. Rat kidney given 400mg/kg of aqueous extract of *Chromolaena odorata* only showing normal architecture: tubules (TU), glomeruli (GL), active interstitial congestion (CO): H and E x 100

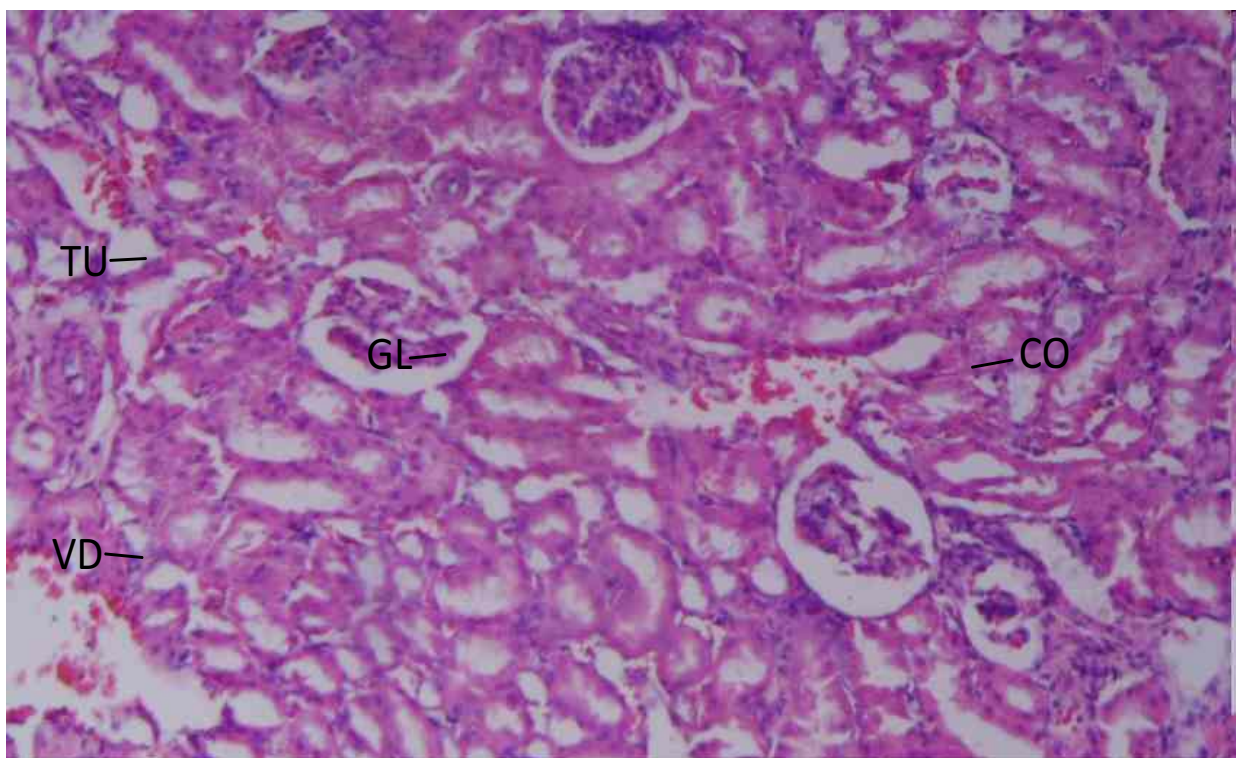


Plate 4. Rat kidney given 1000mg/kg of aqueous extract of *Chromolaena odorata* only showing normal architecture: tubules (TU), glomeruli (GL), interstitial congestion (CO) vasodilatation (VD): H and E x 100.

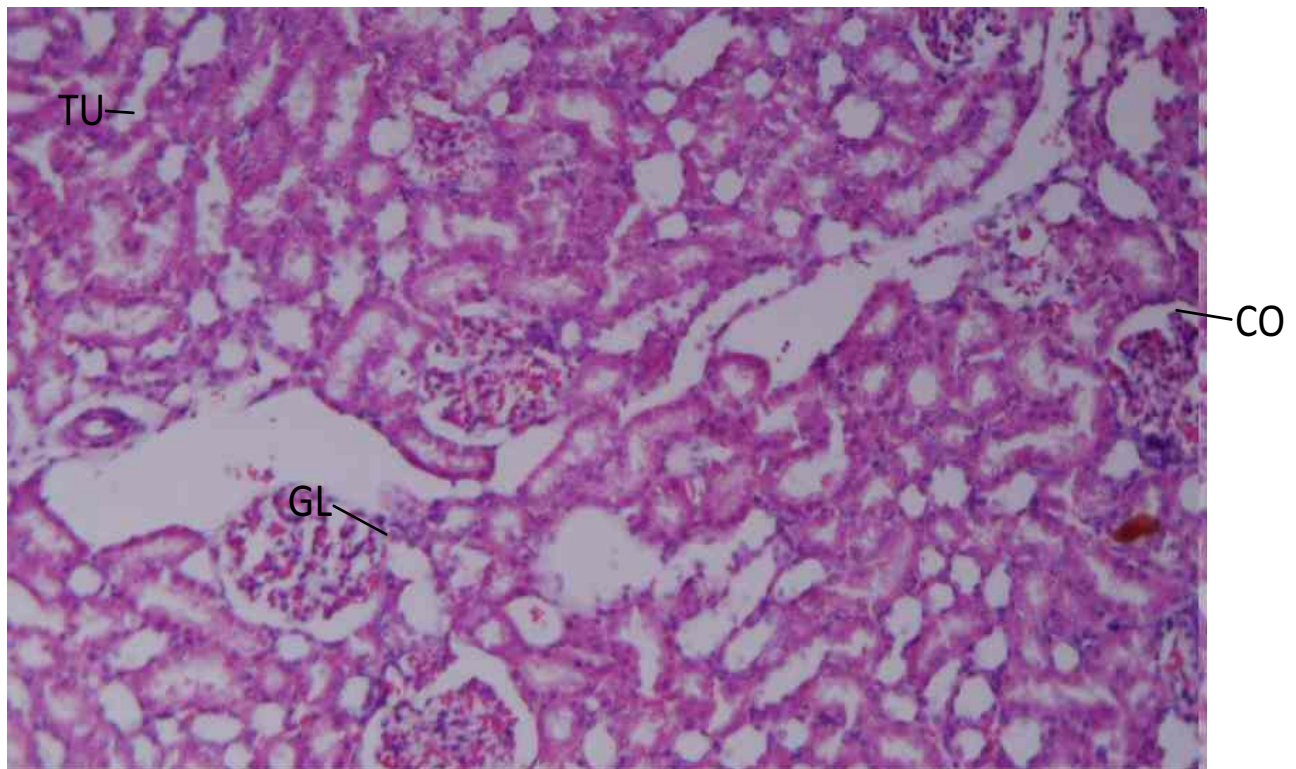


Plate 5: Rat kidney given 4mg/kg Arsenic + 400mg/kg extract showing normal architecture: tubules (TU), glomeruli (GL), active interstitial congestion (CO) :H and E x 100

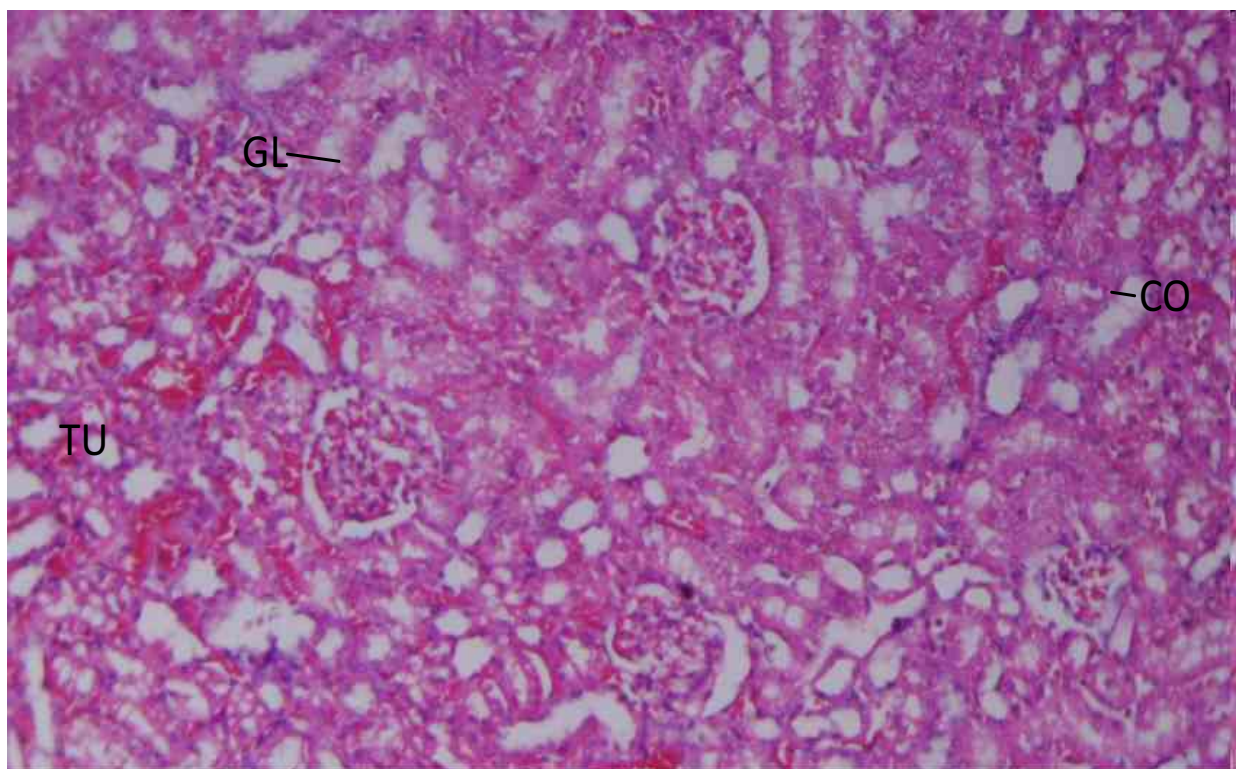


Plate 6: Rat kidney given 4mg/kg Arsenic + 1000mg/kg extract showing normal architecture: glomeruli (GL), tubules (TU), active interstitial congestion (CO): H and E x 100.

Discussion

The evaluation of nephron functional capacity in animals can be conducted by measuring renal function indices, which include serum urea and creatinine levels. Increased values of these parameters may reveal a compromised functional state of the renal micro-system. During renal impairment, the excretion of these metabolites by the kidney is altered and thus accumulates in the plasma (Lawal et al., 2016).

In this study, table 2 shows the groups of Wistar rats used in the experiment and the level of urea and SOD after treatment with arsenic acid or aqueous extract of *Chromolaena odorata* or arsenic acid and aqueous extract of *Chromolaena odorata* for 30 days. The level of urea in Group B administered 4mg/kg of Arsenic only, at 80.460.141mg/dl showed significant increase when compared to the control group (group A) with urea level of 22.010.108^{*} mg/dl indicating toxicity and renal impairment. In contrast, the level of urea in group C, D, E, and F at 28.79 ± 0.138 mg/dl, 24.60 ± 0.121 mg/dl, 24.66 ± 0.156 mg/dl and 29.46 ± 0.118 mg/dl respectively showed no significant difference when compared to the control group. This indicates that *Chromolaena odorata* in Group C which was treated with 400mg/kg of aqueous extract *Chromolaena odorata* and Group D which was treated with 1000mg/kg of aqueous extract *Chromolaena odorata* did not exhibit toxicity and enhanced the excretion of urea by the kidney. In Groups E and F treated with 400mg/kg of *Chromolaena odorata* and 4mg/kg of Arsenic acid and 1000mg/kg of *Chromolaena odorata* and 4mg/kg of Arsenic acid, the urea levels indicated that there was protective effect of *Chromolaena odorata* on the kidney preventing renal impairment caused by arsenic acid in the groups.

A prior study by Phan et al. in 2001 also reported a high concentration of phenolic compounds in *Chromolaena odorata*, which can protect against oxidative damage. *C. odorata l.* shows high antioxidant activity. Various hydrolytic complexes of aglycone flavonoids such as flavones, flavanones, chalcones, and flavonols are also present in *C. odorata l.* acting as the primary and potent antioxidants (Ukpai and

Amaechi, 2012). The body's antioxidant defense system is the first line of defense against free radicals generation and oxidative damage.

In a study by Liu *et al.* (2010), the antioxidant enzymes SOD limit the effects of oxidant molecules in tissues and act in the defense against oxidative cell injury by means of their being free radical scavengers. They reported that these enzymes work together to eliminate active oxygen species. From our experiment, group A had the highest SOD level at 67.41±0.133 unit/mg protein suggesting strong primary antioxidant components. Group B had the lowest level of SOD at 15.11±0.112 unit/mg protein, in which there was a significant decrease in SOD when compared to group A, indicating a reduced antioxidant defense response due to arsenic acid toxicity. Group C at 60.23±0.158 unit/mg protein and Group D at 64.65±0.111 unit/mg protein values were not significantly different from SOD level of the control group suggesting that the extract at 400mg/kg and 1000mg/kg has the ability to counteract oxidative stress, bind to free radicals and are not toxic. Different mechanisms have been accounted for the Arsenic toxicity. It can produce a wide variety of ROS including singlet oxygen, superoxide, nitric oxide, dimethylarsinic peroxy radicals and also the dimethylarsinic radicals (Miller *et al.*, 2002). Antioxidant defense in groups treated with arsenic acid and *C. odorata* aqueous extract was higher in group E with SOD level of 58.44 ± 0.123 unit/mg protein than in group F with SOD level of 52.16 ± 0.167 unit/mg protein although there was no significant difference when compared to group A, the control group. This also indicates that *Chromolaena odorata* accelerates the dismutation of hydrogen peroxide (H₂O₂) suggesting good defense against oxidative stress at 400mg/kg and 1000mg/kg of aqueous extract *Chromolaena odorata* with administration of 4mg/kg of Arsenic acid. The radical scavenging activity and the reducing ability of the *C. odorata* leaves extracts can be attributed to the presence of redox active substances such as phenol, flavonoids, alkaloids and tannin. The flavonoids and phenolic compounds in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-carcinogenic (Lalitha et al 2012).

Upon further investigation, histopathological examination of the renal tissues treated for arsenic acid only (Group B) as seen in plate 2 showed significant histopathological changes such as presence of heavy interstitial infiltrates of inflammatory cells characterized by cloudy swelling of tubular epithelium causing the lumen to narrow, non-distinct tubules around the glomerulus and patchy tubular necrosis when compared to Group A (plate 1) where there was normal tubules, glomerulus and interstitial space. In plate 3, Group C treated with 400mg/kg of aqueous extract of *Chromolaena odorata*, the kidney showed normal histological structures when compared to Group A (plate 1), tubules with well opened lumen, increased blood flow (active interstitial congestion) and no seen damage. Group D which was treated with 1000mg/kg of aqueous extract of *Chromolaena odorata* as shown in plate 4 also looked normal with no visible damage when compared to the control group with more blood vessels indicating increase in blood flow (active interstitial congestion) causing vasodilation of blood vessels. As seen in plate 5, Group E which was treated with 4mg/kg of arsenic acid and 400mg/kg of aqueous extract of *Chromolaena odorata* when compared to Group B (plate 2) had ameliorative effect on tubular necrosis such that the tubules, glomerulus and interstitial space were restored and there was increase in blood flow (active interstitial congestion). In Group F (plate 6), Wistar rats treated with 4mg/kg arsenic acid and 1000mg/kg of *Chromolaena odorata* also revealed ameliorative effect with normal histological architecture and active interstitial congestion, although the tubules didn't appear very healthy and were characterized by narrowed lumen when compared to the group treated with 400mg/kg of aqueous extract of *Chromolaena odorata* and 400mg/kg of aqueous extract of *Chromolaena odorata* and arsenic acid respectively.

Conclusion

It can be concluded that low dose (400mg/kg body weight) of aqueous extract of *Chromolaena odorata* had more protective effect on arsenic acid induced kidney damage in adult Wistar rat.

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Citation: Ehimigbai, A.R.O., Olime, O.J. Effects of Aqueous Extract of *Chromolaena odorata* Leaves on Arsenic Acid-Induced Kidney Damage in Adult Wistar Rats. *Sokoto Journal of Medical Laboratory Science*; **9(2): 280 – 289**. <https://dx.doi.org/10.4314/sokjmls.v9i2.32>

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