Phytomedical Potentials of *Chromolaena Odorata* Against Arsenic-Induced Testicular Toxicity In Wistar Rats

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**ABSTRACT**

The testicular, sperm and endocrine protective properties of *Chromolaena odorata* (CA) in arsenic treated rats were investigated using forty male wistar rats (190-200g) grouped into 4 (A to D) of ten rats each. Oral administrations for 2 weeks of 0.2ml com oil (A), 2.5mg/kg of sodium arsenite (B), 200mg/kg ethanol leaf extract (ELE) of CA (C), 200mg/kg ELE of CA and 2.5mg/kg sodium arsenite given at 1 hour interval (D) were done. Twenty-four hours after final administrations, semen, blood biochemical and hormonal analyses were carried out after sacrifice of the rats. Results revealed that Group C’s mean scrotal circumference, Left and Right testicular weights were highest across groups and significant compared to group B values (p<0.05). Group C had significantly (P<0.05) highest mean value of sperm motility across the groups just as group A was significantly (p<0.05) higher than those of groups B and D. Significantly low Testosterone and high luteinizing hormone concentrations were observed in group B. It was concluded in this study that ethanol leaf extract of *Chromolaena odorata* had a profound scrotal, testicular, sperm and endocrine protective properties in arsenic-treated wistar strain albino rats.

**Keywords:** *Chromolaena odorata*, Reproductive toxicity, Arsenic, Testicular, Wistar rats

**INTRODUCTION**

Arsenic is among the most toxic heavy metals in the environment (ATSDR, 2005). Many systems within the body are affected by inorganic arsenic exposure. Some of these toxic effects range from skin lesions, cardiovascular, haematological, hepatic, reproductive and renal defects (Abernathy et al., 2003). Urothelial cytotoxicity, increased cell proliferation and ultimately tumour generation have been reported in rats that were exposed to arsenic. (Suzuki et al., 2008). Arsenic poisoning is one of the greatest risk factors contributing to reproductive failure in animals and humans (Jaishankar et al., 2014). Sources are largely from herbicides, insecticides and food preservatives (ATSDR, 2005). Arsenic in ground water is the major source of arsenic intoxication (Argos et al., 2012). The intoxicative effect could be through its thiol-reactive property in which arsenic compounds inhibit enzymes by altering proteins through its reaction with proteinaceous thiol groups (Sharma et al. 2014). Phytotherapeutic products from medicinal plants have become universally popular in the control of arsenic poisoning especially in developing countries (Leonardo et al., 2000). The plant, *Chromolaena odorata*, is known for its wide range therapeutic potentials. It is a perennial semi woody shrub, which belong to the family Asteraceae. The plant is native to central and South America and is now distributed through Africa and Tropical Asia (Burkill, 1997). In Nigeria, It is called *Akintola-ta-ku*, *Awo-lowo* and *Obiarakara* in Yoruba, *Igbo* and *Hausa Languages respectively*. It is a deep-rooted plant and can survive harsh climatic condition. Traditionally, the plant's leaf is used as a coagulant to stop bleeding (vasoconstriction) in fresh wounds and treatment of stomach ache (Sofowora, 1982). It has also been found to be an effective anti diarrhoea, astringent, antihypertensive, anti-inflammatory and diuretic (Iwu, 1993). The flowers of the plant contain flavanones (5, 7 – dihydroxy – 7 4 –dime-thoxy-flavanone, 4-tetramethoxy flavanone, and 4-hydroxy-5,6,7-trimethoxy flavanone), chalcones (2-hydroxy-4, 4,5,6,–tetramethoxylcalcone and 4, 2-dihydroxy-4,5,6-trimethoxylcalcone) and
flavones (acacetin and luteolin) (Arene et al., 1985). Notable phenolic acids such as protocatechuic acid, p-hydroxy benzoic acid, P-Coumaric acid, ferulic acid and vanillic acids have been isolated from the leaves and identified (Phan et al., 2001) together with essential oils (Bamba et al., 1993; Chowdhury, 2002), Steroids (Ahmed et al; 1995) triterpenes and flavonoids (Arene et al; 1985). It has been generally accepted that plant’s actions are traceable to the chemical components of the plant extracts (Phan et al., 2001). This plant, known for its vast therapeutic potentials has been used in humans and animals as treatment for various conditions, however, there is paucity of information on its sperm protective potential in rats exposed to sodium arsenite toxicity. This work sought to investigate the plant’s sperm, testicular and endocrine protective properties in arsenic-exposed rats.

MATERIALS AND METHODS
Preparation of Sodium arsenite
Sodium arsenite (Ioba, Chemie Co. India) solution was prepared and a dose of 2.5mg/kg body weight was administered according to the guidelines for in vivo assays in rats (Preston et al., 1987). Freshly prepared stock solution was used for the experiment.

Plant Collection and extraction
The leaves of Chromolaena odorata were collected from the Postgraduate College Environs, University of Ibadan and authenticated at the Department of Botany, Faculty of Science, University of Ibadan, Ibadan, Nigeria with voucher number UIH-22425. Freshly obtained Chromolaena odorata leaves were dried at room temperature after which the dried leaves were ground into fine powder for cold extraction process. This was carried out by soaking the ground leaves in n-hexane for 48 h to de-fat the leaves. The ground materials were then sieved out, air-dried and then soaked in 96% ethanol for 72 h. The soaked particles were removed and the supernatant was collected. Both the n-hexane and ethanol fractions of the extract were concentrated using rotary evaporator at 40°C. The ethanol fraction was then used to dose the animals using anti-oxidant free corn oil as the vehicle.

Phytochemical screening
This was carried out using the methods previously described by Trease and Evans (1983) and Harbourne (1983). The metabolites tested for were alkaloids, anthraquinones, cardiac glycosides, tannins, flavonoids, saponins, phenols and steroids.

Experimental animals
Forty male Albino rats weighing between 190-200g were used. They were obtained from the experimental animal house of the Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. All the animals received humane care according to the criteria outlined in the Public Health Service Policy on Humane Care and the Use of Laboratory Animals US Department of Health and Humane Services.

Experimental Protocol
The animals were grouped into 4 (A to D) of ten rats each. All animals were kept in plastic laboratory cages under controlled conditions of temperature (25 ± 2°C), relative humidity (50 ± 15%) and normal photoperiod (12 h light and 12 h dark). The rats were allowed to acclimatize for a period of two weeks before the commencement of the experiment. They were fed on a standard diet (commercial pelletized diet from Grand Cereals, Jos, Plateau State, Nigeria) and provided water ad libitum. All animals were weighed weekly from 8th week to 12th week.

Preparation of plant fraction
Ethanol extract suspensions were freshly dissolved in antioxidant-free corn oil, which served as a vehicle. Suspensions were administered orally (per os) at a dose of 200mg/kg body weight of the ethanol leaf extract. Prepared suspensions were kept at room temperature.
Experimental protocol
Group A animals were administered 0.2ml of corn oil, animals in group B were given 2mg per kg body weight of sodium arsenite while group C received 200mg/kg of the ethanol leaf extract only and Group D were served 200mg/kg ethanol leaf extract and sodium arsenite at 1 h interval. All administration was carried out orally for 8 weeks and animals were sacrificed 24 h after the final administration.

Blood Collection and Biochemical assay
The rats were anaesthetized using diethylether in a desiccator and blood samples were collected via the retro-orbital venous plexus into sterile sample tubes without anticoagulant. It was allowed to settle, and separated by centrifuging at 500 rpm for 10 minutes. Supernatant serum was collected and analyzed using Randox specific kits for levels of the creatine kinase (CK), acid phosphatase, lactate dehydrogenase (LDH), using an automated analyzer, ATAC 8000 (Elan diagnostic, CA USA). Serum testosterone and luteinizing hormone concentrations were also determined using automated analyzer

Semen collection and analysis
After blood collection, a mid caudoventral abdominal incision was made on the rats with sterilized scissors, permitting instant access to the testis once pushed upward from the scrotum. The testes were then separated from the epididymides. The right and left epididymides were trimmed off the body of the testes and semen sample was collected from the tail of the epididymis through a stab incision made with a scalpel blade (Ola-Davies and Ajani 2016 a) from where semen was milked out onto a warm glass slide for evaluations.

Percentage motility
This was evaluated using a drop of semen with drop of 2.9% buffered sodium citrate on a warm glass slide covered with a glass slip and viewed at a magnification of ×40. Only sperm cells moving in unidirectional motion were included in the motility rating, while sperm cells moving in circles, in backward direction or pendulating movement were excluded (Zemjanis, 1977).

Morphological abnormalities
Semen dropped on warm glass slide was mixed with a drop of warm Wells and Awa stains. A thin smear was then made of the mixture for morphological studies (Zemjanis, 1977).

Percentage liveability
Mixture of a drop of semen and one drop of warm Eosin-Nigrosin stain on a warm slide was used to make a thin smear. The smear was air dried and observed under the microscope. The ratio of the in vitro dead sperm cells was observed and it is based upon the principle of Eosin penetrating and staining the dead autolysing sperm cells whereas viable sperm cells repel the stain.

Data analysis
Data was presented as the mean ± standard error of the mean (SEM). Statistical analysis was by analysis of variance (ANOVA) using least significant difference model Statistical package used was Graphpad prism (version 6.0). P<0.05 was considered significant

RESULTS
Phytochemical screening of ethanol leaf extract of Chromolaena odorata revealed the presence of alkaloids, anthraquinones, cardiac glycosides, tannins, flavonoids, saponins, phenols and steroids (Table 1).

Scrotal and Testicular Biometry
The mean scrotal circumference, left testicular weight (LTW) and right testicular weight (RTW) in group B (arsenite-exposed) were lower than groups A (Control) treated Corn-oil, C (treated 200mg/kg extract) and D (treated 200mg +
Sodium arsenite (SA) but were only significant when compared with group A (p<0.05). There were consistent higher mean values of other scrotal and testicular parameters obtained in group C (treated 200mg/kg extract) compared to groups A (Control), B (Arsenic group) and D (treated 200mg + SA) although the changes were not significant (P>0.05) (Table II).

The Arsenic group B showed consistent decreased mean values in almost all the parameters measured when compared across the groups.

There were observable slight increases in the mean values of all the parameters (LTL being significant at p<0.05) in Group D (treated 200mg + SA) when compared with group B (treated arsenite only) (Table 2).

**Percentage sperm motility and Livability**
The mean percentage sperm motility in Group A (treated with corn-oil) was significantly (p<0.05) higher than those in groups B (Arsenic group), and D (treated with 200mg + SA). However group C (treated with 200mg/kg extract) had the highest mean value when compared across the groups. The Animals exposed to sodium arsenite (group B) had a significantly decreased sperm percentage motility compared to groups A and C; whereas, there was a compensatory and significant increase (p<0.05) in sperm percentage motility of group D rats (treated 200mg + SA) when compared to group B (Arsenic exposed). The sperm percentage livability of group B rats (Arsenic group) was the lowest although there were no significant changes (p>0.05) in the mean values obtained across the groups (Figure 1).

**Table 1:** Phytochemical screening of Ethanol leaf extract of *Chromolaena odorata*

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Qualitative test</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendoff’s</td>
<td>+ve</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Borntragers</td>
<td>-ve</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>Killer-killanins</td>
<td>+ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Ferric chloride</td>
<td>+ve</td>
</tr>
<tr>
<td>Phenols</td>
<td>Sodium hydroxide</td>
<td>+ve</td>
</tr>
<tr>
<td>Steroids</td>
<td>Salkoski’s</td>
<td>+ve</td>
</tr>
</tbody>
</table>

KEY: +ve = detected; -ve= not detected

**Table 2:** Scrotal and Testicular Biometry of wistar strain Albino rats in different treatment groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Corn oil Group (A)</th>
<th>Sodium Arsenite Group (B)</th>
<th>200mg/kg leaf extract Group (C)</th>
<th>200mg/kg leaf extract + Sodium Arsenite Group (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scrotal circumference (cm)</td>
<td>6.67±0.38</td>
<td>5.03±0.39*</td>
<td>6.92±0.70*</td>
<td>6.78±0.21</td>
</tr>
<tr>
<td>Scrotal length (cm)</td>
<td>3.57±0.18</td>
<td>3.28±1.16</td>
<td>4.08±0.45</td>
<td>3.28±0.31</td>
</tr>
<tr>
<td>Left testis weight (g)</td>
<td>1.07±0.04</td>
<td>0.76±0.04*</td>
<td>1.14±0.04*</td>
<td>0.98±0.10</td>
</tr>
<tr>
<td>Left testis diameter (cm)</td>
<td>3.30±0.06</td>
<td>3.13±0.09</td>
<td>3.35±0.08</td>
<td>3.20±0.17</td>
</tr>
<tr>
<td>Left testis length (cm)</td>
<td>2.27±0.12</td>
<td>2.30±0.06</td>
<td>2.43±0.15</td>
<td>2.48±0.95</td>
</tr>
<tr>
<td>Right testis weight (g)</td>
<td>1.06±0.06</td>
<td>0.76±0.05*</td>
<td>1.18±0.05*</td>
<td>0.98±0.11</td>
</tr>
<tr>
<td>Right testis diameter (cm)</td>
<td>3.20±0.11</td>
<td>3.00±0.09</td>
<td>3.37±0.11</td>
<td>3.00±0.82</td>
</tr>
<tr>
<td>Right testis length (cm)</td>
<td>2.27±0.12</td>
<td>2.30±0.07</td>
<td>2.40±0.14</td>
<td>2.50±0.07</td>
</tr>
</tbody>
</table>

*Refers to values along the row with significant difference (P<0.05)
Figure 1: Percentage sperm motility and Liveability of wistar strain Albino rats in different treatment groups

Biochemical assays
The mean LH concentration of group B rats was the highest and was significant compared with those of groups A, C and D just as that of group D was higher compared to that of group A. The converse was observed with mean testosterone concentration. Group B rats had significantly lowest testosterone compared with groups A, C and D. The acid phosphatase enzyme level was significantly higher in group D compared with groups A, B and C while Lactate dehydrogenase(LDH) concentration of group A was higher significantly compared to groups B, C and D. The serum Creatine kinase (CK) level of group B was higher significantly when compared with group C. (Table IV).

DISCUSSION
The mean scrotal circumference of arsenic-exposed animals (group B), was significantly (p<0.05) lower than other treatment groups. This has a negative implication on scrotal health with scrotal circumference being a significant correlate of fertility in animals (Bezerra et al., 2009), it implies that arsenite is capable of inducing scrotal pathology and hence precipitates infertility in animals (Ola-Davies et al., 2014). It is known that scrotal circumference is highly related to semen quality and reproductive soundness (Bongso et al., 1982). It is strongly correlated to body weight (Bezerra et al., 2009), testicular weight, whole epididymal sperm reserves (Ugwu, 2009). Therefore, the results obtained in the current study in which the arsenic-treated group (B) showed a consistent decreased mean values in scrotal circumference corroborate earlier report (Fouad et al., 2014) showing that arsenite could induce male reproductive toxicity, reduction of testicular weight and scrotal circumference in experimental animals.

Table 3: Hormones and Enzymes profile of wistar strain Albino rats in different treatment groups

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Corn oil Group (A)</th>
<th>Sodium Arsenite Group (B)</th>
<th>200mg/kg leaf extract (C)</th>
<th>200mg/kg leaf extract + Sodium Arsenite Group (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (ng/ml)</td>
<td>11.67±0.88&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>17.25±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.75±0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.50±0.87&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>TESTO (ng/ml)</td>
<td>1.50±0.06&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>1.03±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.28±0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.23±0.10&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>ACID PHOS (U/I)</td>
<td>1.57±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.45±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.55±0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.87±0.05&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>ACID MNOS(U/I)</td>
<td>0.60±0.06</td>
<td>0.78±0.11</td>
<td>0.65±0.06</td>
<td>0.65±0.06</td>
</tr>
<tr>
<td>LDH(U/I)</td>
<td>17.67±1.86&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>25.50±1.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.50±1.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.50±1.32&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CK(U/I)</td>
<td>15.33±0.67</td>
<td>18.75±1.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.00±0.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.00±1.73</td>
</tr>
</tbody>
</table>

Key: TESTO = Testosterone; LH = Luteinising Hormone; ACID PHOS = Acid Phosphatase; LDH = Lactate Dehydrogenase; CK = Creatine Kinase
Mean values with same superscripts along the rows are significantly different (p<0.05)
The significant increase observed in the mean scrotal circumference, left testis weight (LTW) and right testis weight (RTW) of group C (treated with 200mg/kg extract) compared to groups A (Control), B (Arsenic group) and D (treated with 200mg + SA) indicates that the extract of *Chromoleana odorata* at 200mg/kg body weight possesses a pro-fertility property.

Although there were no observable significant (p>0.05) changes in all the testicular indices measured across the groups except for LTW (left testis length) and RTW (right testis length), there were consistent higher mean values obtained in group C (treated 200mg/kg extract only) than groups A (Control), B (Arsenic group) and D (treated with 200mg + SA). Also, group D showed consistent higher mean values compared to arsenic treated group B. This also suggests that extract of *Chromoleana odorata* at 200mg/kg body weight may offer some ameliorative effects against arsenic toxicity in rats. This is similar to a report by Ola-Davies and Ajani (2016a) in which ethanol leaf extract of *Ageratum conyzoides* protected spermatozoa against arsenic reproductive toxicity in wistar strain albino rats.

The pro-fertility potential of *Chromoleana odorata* extract also reflected in the mean percentage motility of group C (treated 200mg/kg extract) being the highest mean value when compared across the groups. This agrees with the findings of Oyeyemi and Ajani, (2014) in which extract of *Mormodica charantia* enhanced sperm motility in wistar strain albino rats. The Arsenic treated group B had a significantly lowest sperm percentage motility across the groups; whereas, there was always a significantly increase (p<0.05) in group D rats (treated 200mg + SA). This increase might be attributed to the inclusion of 200mg/kg leaf extract of *Chromolaena odorata* having a sort of ameliorative effect against arsenic toxicity. This is similar to a report by Ola-Davies and Ajani (2016b) in which ethanol leaf extract of *Pistia stratiotes* improved sperm motility and protected spermatozoa against arsenic reproductive toxicity in wistar strain albino rats.

The phytochemical analysis (Table 1) revealed the presence of tannins in *Chromolaena odorata* and this could have interfered with the absorption of arsenic thereby minimizing the effect of arsenic toxicity. Also, flavonoids contained in the plant provided antioxidant effects against the toxicosis (Bhattacharya, 2017). In addition, the flavonoids might have inhibited actions of pro-inflammatory endogenously-produced substances thereby decreasing the adverse inflammatory degenerative effect of toxicosis induced by arsenite (Roy et al, 2014).

The arsenic-exposed group B had the lowest percentage mean value of live spermatozoa although this was not adversely affected by the treatment across the groups. This finding is similar to a report by Ola-Davies et al. (2014) in which male wistar strain albino rats were exposed to arsenite and fractions of *Spondia mombin*.

The significantly lowest testosterone concentration of arsenic-treated group could be linked to the reduced scrotal circumference which is indicative of reduction in testicular mass hence reduced capacity of the testis to perform its exocrine (spermatogenesis) and endocrine (steroidogenesis) function. Reduction in testosterone removed negatively feedback to LH production hence, compensatorily, luteinizing hormone (LH) production increased in the group to stimulate the Leydig cells to produce more testosterone. This probably explains the significantly high LH concentration in the arsenic group compared to all other groups. The increased testosterone concentration of group D compared with group B indicate ameliorative property of the extract against arsenic reproductive toxicity (Oloye et al., 2017). The testosterone levels in all the groups fell within the reported range of 2-48 nmol/l expected for the wistar rat within experimental laboratory (Heywood, 1980). However, a prolonged exposure to arsenic could further damage the testis and eventually lead to azoospermia.
The higher concentrations of creatine kinase in group B compared to other groups may be the reaction of the rat to toxicity of arsenite as observed by Ola-Davies et al. (2017). This might be made possible due to myolysis of the cardiac muscle and other skeletal muscles induced by toxicosis caused by arsenite in the exposed, untreated group (B). Also, in groups B and D (both exposed to arsenic), LDH levels were higher significantly when both were compared with the control. This might be due to the fact that hepatotoxicity, myolysis and genotoxicity triggered production and release of LDH (Garg, 2008; AlForkan et al., 2016).

CONCLUSION
The study showed that 200mg/kg extract of *Chromolea odorata* had a profound scrotal, testicular and sperm protective properties in arsenic-treated wistar strain albino rats and therefore could be incorporated into animal feeds especially in the chronically exposed population.

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