Possible Reversal of Sodium Arsenate-induced Liver Toxicity by Hexane Leaf Extract of Alchornea laxiflora

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Abstract: This research was aimed at evaluating the anti-toxicity potentials or properties of A. laxiflora. Thus, the possible reversing effect of sodium arsenate-induced liver toxicity by hexane leaf extract of Alchornea laxiflora was investigated using adult wistar albino rats weighing between 150-200 g as experimental model. Oral administration of the extract at varying doses (0.5, 1.0, 5.0 and 10.0 mg/kg body weight) was shown to significantly (p<0.05) decrease the effect of sodium arsenate-induced liver damage by reducing the activities of AST, ALT, ALP, GGT and TB, both in the serum and liver homogenate. Pre-treatment with the extract prior to the intra-peritoneal administration of the toxicant (sodium arsenate) at a dose of 2 mg/kg body weight was shown to be more effective at reversing the effect of the toxicant than post-treatment with the extract after intoxication. Thus, the ability of Alchornea laxiflora leaf extract to protect against sodium arsenate induced liver damage by possible reversal of arsenic toxicity suggests that this plant extract might be suitable for the treatment of sodium arsenate toxicity.

Keywords: Alchornea laxiflora, hexane, liver homogenate, serum enzymes, sodium arsenate, toxicity

INTRODUCTION

Arsenic, as an environmental agent, is considered to be a very high priority toxic substance due to its carcinogenic potentials in humans (Bishayi, 2000). Inorganic arsenic exists in a pentavalent form, arsenate and a trivalent form arsenite, the latter being more toxic (Bishayi, 2000). On a molecular level, arsenate acts as a sulphhydryl reagent which binds to free thiol (-SH) groups of proteins (Snow, 1992). The primary biochemical mechanism of arsenic toxicity is binding of the metal to the sulphhydryl groups of proteins, resulting in the inhibition of numerous cellular enzyme systems (Sjöbäck and Fowlar, 1983). The principal mechanism of arsenic intoxication is disruption of the thiol groups of proteins. Enhanced cellular destruction of damaged thiol proteins may produce toxic oxygen radicals (Lee and Ho, 1994).

Medicinal plants are plants that possess substance(s) that may be of therapeutic purposes or that are precursors for the synthesis of drugs. The folklore knowledge of medicinal plants has significantly contributed in discovering many important drugs of the modern system of medicine (Olaleye et al., 2006). Medicinal plants have various effects on living systems. Some are sedatives, analgesics, antipyretics, cardioprotectives, antibacterials, antivirals and antiprotozoals.

Alchornea laxiflora (A. laxiflora) is of the family of plants, Euphorbiaceae (Burkhill, 1994). It is commonly called the “Three-veined bead string”. The leaves play important role in the preservation of kolanut widely eaten in Nigeria. The stem and branchlets are used in Nigeria as chewing sticks (Kayode and Omotoyinbo, 2008). In Ekiti state, Nigeria, the stems of A. laxiflora is been used in the treatment of Sexually Transmitted Diseases (STD), with alkaloid as the principal ingredient (Kayode and Omotoyinbo, 2008). Decoctions of the leaves are used in the treatment and management of inflammatory and infectious diseases, as well as an important component of herbal anti-malaria formulations (Adewale, 1993). Phytochemical screening of the powdered leaf sample of Alchornea laxiflora revealed the presence of alkaloids, cardiac glycosides, saponins and phenolic compounds (Ogundipe et al., 2001). The presence of terpenoid compound was recently discovered in the root samples of A. laxiflora (Farombi et al., 2003). Despite the popular uses of this plant in traditional medicine, there is dearth of information on its anti-toxicity potentials. In continued search for medicinal plants with anti-toxicity or protective potentials, this research was aimed at evaluating the anti-toxicity potentials or properties of A. laxiflora.

MATERIALS AND METHODS

Plant extraction: Fresh leaves of Alchornea laxiflora were obtained from local gardens in Benin City, and
authenticated at the department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State Nigeria. The leaves were air-dried and ground into fine powder. Hundred grams of the powdered leaf was extracted with 500 cm$^3$ of hexane, continuously at 80°C for 40 min, using a soxhlet apparatus. The crude extract was collected and concentrated to low volume with a rotary evaporator, finally dried and weighed.

**Experimental animals:** Fifty (50) adult albino rats of Wistar strain weighing between 150-200 g were obtained from the animal house in department of Pharmacognosy, University of Benin. The animals were kept in clean disinfected cages and made to adjust to laboratory conditions. They were fed on standard rat pellets from Pfizer feeds, Nigeria and allowed free access to water. The animals were randomized into two major groups (groups A and B), with each group having five subgroups (subgroups 1, 2, 3, 4 and 5) of five (5) animals each.

**Experimental design:**

**Group A:** (Post-treatment group) was administered with the toxicant for the first 2 days and then treated with hexane leave extract of *Alchornea laxiflora* through the rest of the experimental days.

**Group B:** (Pre-treatment group) was administered with the extract for 14 days, but was given the toxicant for the first 2 days and then treated with the toxicant for the rest of the experimental days.

The toxicant (Sodium arsenate) was administered (intra-peritoneal) at a dose of 2 mg/kg body weight, and the plant extract (orally) at varying dosages (0.5-10.0 mg/kg body weight) to all the groups, except the control.

Thus, the subgroups were treated as follows:

- Subgroup (SBG) Control (no extract and no toxicant)
- 0.5 mg/kg of plant extract and toxicant
- 1.0 mg/kg of plant extract and toxicant
- 5.0 mg/kg of plant extract and toxicant
- 10.0 mg/kg of plant extract and toxicant

**Sample collection:** The animals were sacrificed 24 h after the last administration by cervical decapitation. The livers were removed and blotted with 1.5% KCl and kept in a sterile container in a phosphate buffer solution of pH 7.4. The liver was homogenized in ice-cold isotonic sucrose solution, centrifuged, and the supernatant (homogenate) separated from the pellets. The blood, obtained from the heart, was also centrifuged with the clear supernatant (serum) separated from the pellets. The liver homogenate and the serum were then used for the estimation of the liver function parameters.

**Biochemical analysis:** Serum and liver homogenates were analyzed for the following parameters: Total Bilirubin (TB) (Jendrassik and Grof, 1938), L-$\gamma$-Glutamyltransferase (GGT) (Szasz, 1974), Aspartate Aminotransferase (AST) (Reitman and Frankel, 1957), Alanine Aminotransferase (ALT) (Reitman and Frankel, 1957) and Alkaline Phosphatase (ALP) (Kochmar and Moss, 1976). These were carried out using standard reagent kits from Randox Laboratory Limited, U.K.

**Statistical analysis:** All analyses were performed in triplicates. The data were recorded as means±standard deviation and analyzed using Microsoft excel. Student t-test was used to analyze the significant differences between their means. Differences between means at 5% significant level (p-value <0.05) were considered.

**RESULTS**

As shown in this study, (Table 1) shows the dose dependent effect of hexane leaf extract of *Alchornea laxiflora* on some liver biochemical indices of the experimental animals administered 2 mg/kg body weight sodium arsenate (post-treatment group). The various doses of the extract showed AST and GGT values that are significantly (**p<0.05**) higher than that of the control, except for SBG 5 (10.0 mg/kg dose). The increase in AST and GGT values of the SBGs far higher than that of the control may be due to the toxic effect of sodium arsenate, with the 10.0 mg/kg dose having the most significant effect.

Table 2: Effects of varying doses of hexane leaf extract of Alchornea laxiflora on some liver biochemical indices of rats given 2 mg/kg sodium arsenate (pre-treatment group)

<table>
<thead>
<tr>
<th>SBG</th>
<th>Treatment</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (IU/L)</th>
<th>GGT (U/L)</th>
<th>TB (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>27.0±0.5**</td>
<td>33.7±0.6</td>
<td>234.6±3.3**</td>
<td>11.6±1.7**</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.5 mg/dL</td>
<td>52.0±0.7**</td>
<td>35.2±0.7</td>
<td>151.8±4.7*</td>
<td>17.4±1.7**</td>
<td>0.77±0.05*</td>
</tr>
<tr>
<td>3</td>
<td>1.0 mg/dL</td>
<td>89.5±0.6**</td>
<td>28.2±0.5</td>
<td>138.0±1.4*</td>
<td>34.7±2.5**</td>
<td>0.43±0.07*</td>
</tr>
<tr>
<td>4</td>
<td>5.0 mg/dL</td>
<td>23.0±0.9*</td>
<td>22.2±0.8</td>
<td>165.6±4.1*</td>
<td>9.3±0.8</td>
<td>0.89±0.23*</td>
</tr>
<tr>
<td>5</td>
<td>10.0 mg/dL</td>
<td>17.8±0.4*</td>
<td>26.9±2.1</td>
<td>193.2±3.4*</td>
<td>34.7±3.3**</td>
<td>0.22±0.06*</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±S.D.; n: 5; AST: *p<0.05 (SBG 3, 4 and 5 compared to control); ALT: *p<0.05 (SBG 3 compared to control); ALP: **p<0.05 (SBG 2, 3, 4 and 5 compared to control); GGT: *p<0.05 (SBG 2, 3 and 5 compared to control); TB: *p<0.05 (SBG 2, 3 and 5 compared to control)

Table 3: Effects of varying doses of hexane leaf extract of Alchornea laxiflora on some serum biochemical indices of rats given 2 mg/kg sodium arsenate (post-treatment group)

<table>
<thead>
<tr>
<th>SBG</th>
<th>Treatment</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (IU/L)</th>
<th>GGT (U/L)</th>
<th>TB (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>10.7±0.4*</td>
<td>8.8±0.5**</td>
<td>165.6±1.2**</td>
<td>30.8±1.0**</td>
<td>0.86±0.4**</td>
</tr>
<tr>
<td>2</td>
<td>0.5 mg/dL</td>
<td>13.0±0.3</td>
<td>15.3±0.7</td>
<td>552.0±1.4**</td>
<td>23.1±1.2*</td>
<td>0.54±0.07**</td>
</tr>
<tr>
<td>3</td>
<td>1.0 mg/dL</td>
<td>27.0±0.6*</td>
<td>19.2±1.0*</td>
<td>938.4±11.7**</td>
<td>34.7±1.2*</td>
<td>0.43±0.06**</td>
</tr>
<tr>
<td>4</td>
<td>5.0 mg/dL</td>
<td>18.4±0.5*</td>
<td>6.4±0.5</td>
<td>441.6±6.9**</td>
<td>57.9±0.2*</td>
<td>0.27±0.1**</td>
</tr>
<tr>
<td>5</td>
<td>10.0 mg/dL</td>
<td>10.0±0.4</td>
<td>17.8±1.0</td>
<td>1794.0±11.7</td>
<td>86.8±1.1*</td>
<td>0.22±0.03**</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±S.D.; n: 5; AST: *p<0.05 (SBG 3, 4 and 5 compared to control); ALT: *p<0.05 (SBG 3 compared to control); ALP: **p<0.05 (SBG 2, 3, 4 and 5 compared to control); GGT: *p<0.05 (SBG 2 compared to control); TB: *p<0.05 (SBG 2, 3, 4 and 5 compared to control)

In Table 2, the dose dependent effects of the extract on some liver biochemical indices was also observed on rats given 2 mg/kg body weight sodium arsenate (pre-treatment group). AST levels were shown to be significantly (**p<0.05) higher in SBGs 2 and 3 compared to the control. Reverse was the case in SBGs 4 and 5 as they show AST values significantly (**p<0.05) lower as compared with the control. ALP levels for SBGs 3, 4 and 5 were shown to be significantly (p<0.05) lower than that of the control. However, SBG 2 showed no significant difference compared to the control. Thus, for liver AST and ALT, higher doses of the extract seem to have better reducing effect. The values of ALP were shown to be significantly (p<0.05) lower in all the SBGs compared to the control, while TB values were shown to be significantly (p<0.05) higher in all the SBGs compared to the control. But, GGT values were shown to be significantly (**p<0.05) higher in SBGs 2 and 3 that of control. There is also a steady dose related increase in the values of serum TB. These increases are however not steady with respect to the doses of the extract, with that of SBG 2 being significantly (*p<0.05) lower and that of SBGs 4 and 5, significantly (**p<0.05) higher than that of the control SBG. There is also a steady dose related decrease in the values of serum TB. These decreases are however significant (++p<0.05) when compared to the control.

Table 4 also shows the dose dependent effects of the extract on some serum biochemical indices of the
experimental animals administered with the toxicant (pre-treatment group). No significant difference was observed in SBGs 3 and 4 when compared with the control, for the activity of AST. But SBGs 2 and 5 showed a significant (*p<0.05) increase as compared to the control SBG. Thus, 1.0 and 5.0 mg/kg doses of the extract tend to be more effective at reducing the values of serum AST than the other doses. This trend is also observed in the activity of ALT, with those of SBGs 3 and 4 significantly (*p<0.05) lower than those of the control. ALT values for SBG 2 can be seen to be higher and that of SBG 5 lower than that of the control, but not significantly different. SBGs 2, 3 and 4 showed ALP activities significantly (***p<0.05) higher than that of the control, while SBG 5 showed ALP activity significantly (*p<0.05) lower than that of the control. This actually portends the 10.0 mg/kg dose to be more effective at reducing the activity of ALP. For the activity of GGT, SBG 2 showed a significantly (*p<0.05) lower value compared with the control, while SBG 3 showed a significantly (*p<0.05) higher value compared with the control. SBGs 4 and 5 however, showed no significant difference as compared with the control. For TB, there is a decrease in values in all the SBGs as compared with the control. This trend is however not steady in the SBGs, with SBGs 2, 4 and 5 showing significant (*p<0.05) decreases and SBG 3 showing no significant decrease as compared with the control.

In Table 3 and 4, the serum activities of AST and ALT of the experimental animals in the pre-treatment group were significantly lower than that of the post-treatment group. This trend was opposite in the liver homogenate. Increase in the serum enzyme activity signifies damages to the liver membrane.

The serum activities of ALP of the pre-treatment animals were significantly different from that of the post-treatment group. The pre-treatment group showed reduced healing of the liver damages in Table 1, while the pre-treatment group was able to heal the liver damage by restoring them back to normal as shown in Table 2, although there were significant increases in serum activities in both groups when compared to the control as shown in Table 3 and 4.

Although GGT activity was within normal range in the post-treatment group (Table 3), it decreased with increase in dose of the extract when compared with the pre-treatment group (Table 4), which showed a 54% increase in GGT activity as compared with the normal range of 0-50 U/L in the control. Increase in the serum enzyme activities signifies damages to the liver tissues. Total Bilirubin (TB) levels were reduced in a dose-dependent manner down the post-treatment group as compared with the pre-treatment group.

**DISCUSSION**

Animal studies have shown that the liver is a major target organ for arsenic toxicity, being a vital organ for methylation of inorganic arsenite (Shen et al., 2003). Acute arsenic ingestions are associated frequently with increased liver enzyme activities and hyperbilirubinaemia, although these abnormalities usually resolve (Lee and Ho, 1994). Diagnostic enzymes are enzymes that are used in diagnosing or differentiating between certain or specific diseases (Omage et al., 2011). When enzymes are present in the blood, they are usually found in low concentrations, but when there is damage in an organ, the enzymes present in the organ (within the cells) leak out into the blood (Omage et al., 2011). From our results, the increased liver enzyme activities are most likely due to the toxic effect of sodium arsenate as against that of the control. Oral administration of the hexane leaf extract of *Alchornea laxiflora* at varying doses, from our studies, have shown a dose related effect at reversing the toxic effect of sodium arsenate. The protective effect of the plant extract may be responsible for the pharmacological activities of this plant extract.

The serum activities of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) of the experimental animals in the pre-treatment group were significantly lower than those of the post-treatment group. Increased serum enzyme activities signify damages to the liver tissues. The reducing effects of the plant extract on the activities of the enzymes indicate a
arsenate. These effects are more pronounced in the pre-treatment group. Our results showed that the enzyme activities were lower in the pre-treatment group. This may suggest that oral administration or intake of this plant extract prior to intoxication may offer a better protection against liver damage than treatment after intoxication. This may be more effective at a dose range of 1.0-5.0 mg/kg body weight.

The serum and liver Alkaline Phosphatase (ALP) and Gamma-glutamyltransfrase activities were shown to be relatively higher in the post-treatment group than the pre-treatment group. Thus, administration of the extract at varying doses showed a better anti-toxic effect against arsenate toxicity prior to intoxication. The primary biochemical mechanism of arsenic toxicity is binding of the metal to the sulphydryl groups of proteins, resulting in the inhibition of numerous cellular enzyme systems (Sjibb and Fowlar, 1983). These inhibitions may result in the increased activities of these enzymes in the serum or liver homogenate. The counter effects of the hexane leaf extract of *Alchornea laxiflora* on the activities of these enzymes may therefore be due to its ability to reverse the binding of the metal to the sulphydryl groups of proteins (or enzymes). Alternatively, it may be due to the competitive binding of certain active principles (phytochemicals or secondary metabolites) present in the plant extract, with the metal, thereby rendering the sulphydryl groups (of the enzymes) free to carry out catalysis.

Our result showed that after oral administration of the extract at varying doses to the experimental animals, total bilirubin (from liver homogenate) tends to be higher than control in both the post- and pre-treatment group, while serum total bilirubin tends to be lower than control. Comparatively, the total bilirubin levels of the pre-treatment group tend to be lower than that of the post-treatment group. These again, lay credence to the possible fact that the plant extract exhibit a better counter effect to sodium arsenate toxicity when administered prior to intoxication than after intoxication. Thus, the ability of *Alchornea laxiflora* leaf extract to protect against sodium arsenate induced liver damage by possible reversal of arsenic toxicity suggests that this plant extract might be suitable for the treatment of sodium arsenate toxicity.

**CONCLUSION**

Our research showed that the oral administration of hexane leaf extract of *Alchornea laxiflora* at varying doses (0.5-10.0 mg/kg body weight) caused reduction in the serum and liver homogenate enzyme activities of the experimental animals, intoxicated with sodium arsenate. These effects are more pronounced in the pre-treatment group than the post-treatment group. This suggests that the ability of the plant extract to reverse sodium arsenate liver toxicity is better when the extract is administered to the animals before intoxication, than after intoxication.

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