The Effect of The Ethanolic Extract of The Leaf of Chromolaena odorata on The Liver of Wistar Albino Rats.

Okafor, C.S 1*; Amuluche1, C.S; Ezinwa1, L.L; Nnabuenyi2, O.H and Okafor3, N.C.
1 Dept. of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Nigeria.
2 Dept. of Science Lab. Technology, Federal Polytechnic, Oko.
3 Dept. of Medical Lab. Science, Nnamdi Azikiwe University, Awka, Nigeria
*Corresponding author’s E-mail: chikeoka@gmail.com.

Abstract
The study investigated the hepatotoxic effect of the ethanolic extract of Chromolaena odorata leaf on groups of male wistar albino rats. The rats were divided into 3 groups of 5 rats each. Group A and B which were the treated groups received 10mg/kg and 20mg/kg body weight of the extract. Group C (control) was given 5% solution of tween 80 only. The administration of the extract was done orally for a total period of 21 days. Blood samples were collected at the end of the study and the results of the blood samples analysis showed that group A, B, and C (control) had mean ALT levels of 10.4±3.32U/L,12.0±1.7U/L and 8.4±2.24U/L respectively while the mean AST levels were 20.2±4.66U/L, 23.8±4.65U/L and 24.0±5.59U/L respectively. There was no significant difference (P>0.05) in the levels of ALT and AST among the 3 groups at 10mg/kg body weight dose level while there was significant increase (p<0.05) in the ALT but no significant difference in the AST levels at 20mg/kg body weight dose level between the treated and non-treated groups. The results of the study showed that the ethanolic extract of Chromolaena odorata leaf at 10mg/kg body weight dose level had no adverse effect on the liver of the experimental rats while at 20mg/kg body weight it might be toxic to the liver. So the dose for human should be limited to less than 20mg/kg body weight dose level.

Key words: Chromolaena odorata, hepatotoxicity, blood samples, ethanolic extract.

Introduction
A systemic research for useful bioactive compounds from medicinal plants is now considered to be a rational approach in drug research. Medicinal plants can make important contribution to the World Health Organization (WHO) goal to ensure that all people world wide lead a sustainable socio-economic productive lives. Chromolaena odorata is a fast growing perennial and invasive weed growing mainly in the south and central America and distributed throughout Africa and tropical Asia (Muniappa and Muratani, 2006). Chromolaena odorata can be poisonous to lives as it has exceptional high levels of nitrates (5-6 times above the toxic level) in the dead and young shoots, the cattle feeding on this dies of tissue anoxia (Sajise et al, 2008).

Despite these negative effects, Chromolaena odorata is still being used by medical practitioners of traditional medicine because of its anti-spasmodic, anti protozoa, anti-trypanosomes, anti-bacterial, anti-hypertensive, anti-inflammatory, astringent, diuretic and hepatotrophic effects (Feng et al, 1994). The leaves could be ground and the extracted juice taken to alleviate fever or the treatment of diabetes (Chung and Yun, 2001). The medical values of Chromolaena odorata are due to some specific phenolic compounds that have been isolated from it (Metwall and Ekejimba, 1981). In Southern part of
Nigeria, the leaves are used for wound dressing, skin infection and to stop bleeding.

The extract of *Chromolaena odorata* at 50-200mg/kg inhibits paw oedema in rats. It also has anti-motility and anti-diarrhoeal effects (Oludare *et al.*, 2000). The ethanolic extract of *Chromolaena odorata* has LD$_{50}$ of 16.50/kg body weight (Ogbonnia *et al.*, 2010).

The liver plays a central role in biotransforming and clearing chemicals and it is therefore, susceptible to the toxicity from agents. Natural chemicals and herbal remedies can induce hepatotoxicity. More than 900 drugs have been implicated in causing liver injury (Friedman, 2003).

This study is aimed at investigating the effects of ethanolic extract of *Chromolaena odorata* on the liver of Wistar albino rats.

**Materials and Methods**

**Collection of Sample.**

The *Chromolaena odorata* leaves were collected within the locality of Science Village, Nnamdi Azikiwe University, Awka, Nigeria and was identified by a Taxonomist, in Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria.

**Procurement of Rats:**

Fifteen male albino rats 8 weeks old weighing 100-125g were randomly divided into 3 groups of five rats each. They were acclimatized for one week. Groups A and B received 10 and 20mg/kg body weight of the extract respectively while group C (control) received only 0.2ml of 5% tween 80. The extract was administered orally for 21 days using cannular. The animals received water and food *ad libitum* and had 12 hours light/12 hours darkness. They were handled in line with the guidelines laid down by the ethical committee on animal handling.

**Sacrifice and sample collection:**

The animals were starved overnight prior to sacrifice. They were sedated with cotton soaked in chloroform and then blood samples were collected by heart puncture from each of the rats. The blood samples were dispensed into non-anticoagulant sample tubes and sera obtained after centrifuging the blood samples at 4000rpm for 15 minutes were subjected to analyses.

**Methods:**

Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) activities were determined using a blood chemistry analyzer (Vitros DT 6011, USA). Ten microlitres of sera were pipette into the slides which already contained the substrates for the enzymes. The results were printed out after a short while.

in 300ml of 70% ethanol. This was properly mixed and allowed to stand for 48 hours. It was filtered using Whatmann no.1 filter paper. The filtrate was concentrated by heating in a water bath at 40ºC and the remaining solvent was removed in a rotary evaporator. The extract was collected and weighed and then used for the study.

**Sample Preparation:**

Five percent tween 80 in distilled water was prepared. Two grams of the extract was properly dissolved in 85mls of 5 % tween 80.

**Treatment of Animals:**

Fifteen male wistar albino rats 8 weeks old weighing 100-125g were randomly divided into 3 groups of five rats each. They were acclimatized for one week. Groups A and B received 10 and 20mg/kg body weight of the extract respectively while group C (control) received only 0.2ml of 5% tween 80. The extract was administered orally for 21 days using cannular. The animals received water and food *ad libitum* and had 12 hours light/12 hours darkness. They were handled in line with the guidelines laid down by the ethical committee on animal handling.

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Statistical Analysis:
Mean values of the treated and control groups were compared using Analysis Of Variance (ANOVA) as described by Whitely and Ball (2002). Results were significant at p<0.05.

Results:
The results of the ALT and AST activities for the 3 groups are expressed as mean±standard deviation(S.D) in Tables 1 and 2 respectively.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (u/l)</th>
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<tbody>
<tr>
<td>A(10mg/kg b.w)</td>
<td>10.4±3.32</td>
</tr>
<tr>
<td>B(20mg/kg b.w)</td>
<td>12±1.70</td>
</tr>
<tr>
<td>C(Control)</td>
<td>8.4±2.24</td>
</tr>
</tbody>
</table>

Table 2: AST Activities of both the Control Rats and Rats Administered the extract.

<table>
<thead>
<tr>
<th>Group</th>
<th>AST (u/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(10mg/kg b.w)</td>
<td>20.2±4.66</td>
</tr>
<tr>
<td>B(20mg/kg b.w)</td>
<td>23.8±4.65</td>
</tr>
<tr>
<td>C(Control)</td>
<td>24.0±5.59</td>
</tr>
</tbody>
</table>

Discussion:
There was no significant difference(p>0.05) in the activities of Alanine Amino Transferase and Aspartate Amino Transferase between the treated and control groups at the 10mg/kg body weight dose level, while there was significant increase(p<0.05) in the Alanine Amino Transferase activity of the group treated with 20mg/kg body weight dose level compared to the control. At the same time there was no significant difference(p>0.05) in the activities of Aspartate Amino Transferase of the group treated with 20mg/kg body weight dose level compared to the control. Significant increases in liver enzymes suggest liver damage.

Histological examination of the liver after the administration of Chromolaena odorata on rats and mice showed the presence of necrosis, oedema and inflammatory infiltrations (Taziebou et al.,2008). Iwu (1993) and Feng et al (1994) observed that Chromolaena odorata has hepatotrophic activities.

At the dose of 10mg/kg body weight the ethanolic extract of Chromolaena odorata leaf showed no evidence of liver toxicity while at 20mg/kg body weight the hepatotoxicity of the extract was not clear since only one of the two liver enzymes investigated showed significant elevation.

Conclusion:
The result obtained from this study shows that ethanolic extract of Chromolaena odorata leaf may be very safe for consumers at the dose level of 10mg/kg body weight. Restricting the use of the leaf extract of Chromolaena odorata at less than 20mg/kg body weight may be wise and advisable since the results of this study showed that one of the two liver enzymes investigated showed significant elevation at 20mg/kg body weight.
Acknowledgement:
We recognize and appreciate the immense contribution of Prof. Okeke, C.U., a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria, who identified the Chromolaena odorata leaf used in this study.

References