

EFFECTS OF REPEATED ADMINISTRATION OF *CHROMOLAENA ODORATA* ON SELECTED LIVER FUNCTION PARAMETERS OF APPARENTLY HEALTHY WISTAR RATS.

*Nwachukwu F C¹, Odo Christian Emeka², Ihegboro G. O¹.

¹Department of Biochemistry/Forensic Science, Faculty of Science, Nigeria Police Academy, Wudil-Kano Kano State.

²Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

ABSTRACT: *This work examines the effect of oral daily administration of a dose of 20mg/kg body weight of Chromolaena odorata leaves extract on the liver. The proper functioning of the eyes, the heart, the brain, the gonads, the joints, and the kidneys, are all dependent on good liver activity. THE insufficiency of liver from constructing thousands of enzyme systems which the body requires will cause impairment in overall body function and a resultant greater metabolic stress on the individual. The results of this study on liver function parameters estimated, four days after daily administration for four times, shows that, Total Protein decreased as days progress which was significant on day five after administration through day thirteen after administration. There was no significant increase in albumin concentration, however liver function enzymes show remarkable increase and were significantly different at $p < 0.05$ on day thirteen after administration. Then again, Bilirubin fractions remarkably increased in concentration on day thirteen after administration and were significantly different at $p > 0.05$. Additionally, Unconjugated Bilirubin and Alanine Aminotransferase was significantly difference ($p < 0.05$) on all the estimated days after administration. This work has showcased, that oral daily administration of the aqueous leaves extract of Chromolaena odorata, for thirteen days at 20mg/kg body weight has secondary medical effect on the liver. The wide therapeutic window of Chromolaena odorata must not be compromised on a longer than necessary usage.*

KEYWORDS: *Chromolaena odorata: Aminotransferases: Alkaline phosphatase: Protein: Bilirubin:*

INTRODUCTION

The liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body (Rajib *et al.*, 2009). It is uniquely different among internal organs in its natural capacity to regenerating lost tissues into whole liver (Dieter 2011). This feat is predominantly due to the hepatocytes re-entering the cell cycle. These hepatocytes go from the quiescent G₀ phase to the G₁ phase and undergo mitosis. This process is activated by the P₇₅ receptors (Suzuki *et al.*, 2008). The liver is a giant chemical factory with central and critical role in metabolism, digestion, detoxification, and the elimination of substances from the body (Robert 2008). In addition, the liver makes antibodies, and protein that stops cuts from bleeding for a long time. The liver tissues

regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Maton *et al.*, 1993). Summarily, liver has multisystemic functions of over 500 that are vital to human health yet it has no duplicate. This scores the liver as chemical factory of the body. It is unequivocally that mammals cannot live long with badly diseased liver.

The liver is located under the ribs on the right side. It is smooth, reddish-brown, and made up of two different unequal parts called “lobes.” It is a triangular organ. The liver weighs about 1.4 kg (Cotran *et al.*, 2005). It has dual blood supply.

This organ is continuously exposed to toxic substances with long adverse effect, if unnecessarily longer may adversely hamper its function through lose of functional cells (cell derangement). One of the routes of entrance of toxins is the skin, either intentional or unintentional. As the costs of healthcare and prescription drugs rapidly increase each year, policy makers worldwide, are now focusing their attention on the cost of various therapies and providers in the face of a finite healthcare budget and limited healthcare resources, cost-effectiveness research is necessary to determine the best values for limited healthcare dollars. It is estimated that 92% of Nigerians live under \$2 a day (Population Reference Bureau, 2008). In 2000 the World Health Organization published a workbook on the economic evaluations of healthcare services (World Health Organization 2012) and the cost is on the high side. These have resulted in dramatic search into herbs for treatments of varieties of diseases afflicting man and livestock. Some herbs may pose disadvantage when misused, which is a possible tendency in country with its 95% of the population living under \$2 a day (Population Reference Bureau, 2008). An innumerable array of herbs is currently in use for treatment of many diseases, among these is *Chromolaena odorata*

Chromolaena odorata is a tropical species of flowering shrub in the sunflower family, Asteraceae. It is native to North America, from Florida and Texas to Mexico and the Caribbean and has been introduced to tropical Asia, West Africa, and parts of Australia. Common names include Siam Weed, Christmas Bush, Devil Weed, Camfhur Grass and Common Floss Flower (Lalith, 2009). *Chromolaena odorata* is a rapidly growing perennial herb. It is a multi-stemmed shrub, 2.5 m tall in open areas. It has soft stems but the base of the shrub is woody. In shady areas it becomes etiolated and behaves as a creeper, growing on other vegetation. It can then become up to 10 m tall. The plant is hairy and glandular and the leaves give off a pungent, aromatic odour when crushed. The leaves are opposite, triangular to elliptical with serrated edges. Leaves are 4–10 cm long by 1–5 cm wide. Leaf petioles are 1–4 cm long. The white to pale pink tubular flowers are in panicles of 10 to 35 flowers that form at the ends of branches. The seeds are achenes and are somewhat hairy. They are mostly spread by the wind, but can also cling to fur, clothes and machinery, enabling long distance dispersal. Seed production is about 80000 to 90000 per plant. Seeds need light to germinate. The plant can regenerate from the roots. In favorable conditions the plant can grow more than 3 cm a day (Lalith, 2009; King and Robinson 1997).

The plant is known locally in Nigeria through many common names as ewe Awolowo, siam weed, Elizabeth weed, obirakara, olorohuru and independence weed (Ngozi and Theresa, 2014). The plant was popularized by its effective wound healing property. The anti-microbial properties have made it a popular choice in disinfecting and treating open wounds (Odugbemi 2006). The

anthelmintic properties of the aqueous extracts of *Cromolaena odorata* have also been widely known among the rural population of Africa. The wider use as an effective therapy against diarrhoea, malaria fever, tooth ache, diabetes, skin diseases, dysentery and colitis has been severally documented (Odugbemi, 2006; Akinmoladun and Akinloye 2007).

Chromolaena odorata is considered an invasive weed of field crops and natural environments in its introduced range (King and Robinson 1997). It has been reported to be the most problematic invasive species within protected rainforests in Africa (Struhsaker *et al.*, 2005). In Western Africa, it prevents regeneration of tree species in areas of shifting cultivation. It affects species diversity in southern Africa. The plants flammability affects forest edges (King and Robinson 1997). Biological control with a defoliating artiid was started in the 1970s but without success except for Sri Lanka and Guam. In Australia a systematic eradication programme with herbicide has been initiated (King and Robinson 1997).

The toxic effect in use of *Cromolaena odorata* has not gone unnoticed. The astringent property of *Cromolaena odorata* on grazing animals has caused the development of phobia for it in pasture. The avoidance of *Cromolaena odorata* by grazing animals might not be unconnected with the offensive odour that emanates from the leaves whenever they are bruised by the grazing animals in their attempt to consume them (Aro, 1990). The plant has exhibited allelopathic effects and has been reported to cause livestock death (Prasad *et al.*, 2005). The alkaloids in the flowers are said to have killed goats which ate the flowers (McFadyen, 2004).

Furthermore, the high nitrate content of the leaves has been implicated in the resultant death that ensued from their consumption by animals in the raw form (Sajise *et al* 1974). This death was probably caused by the conversion of the nitrate either in the feedstuff or in the alimentary canal of the animals to nitrite. The nitrite then combines with the haemoglobin of the blood thus converting it to methaemoglobin, which is unable to act as oxygen carrier and this might possibly lead to the death of the animals through tissue anoxia (Sajise *et al* 1974). Another plausible mechanism is, in the implication of pyrrolizidine alkaloids, the pyrrolizidine alkaloids have been associated with a severe type of liver disorder known as veno-occlusive disease. In this disease, the hepatic vein becomes clogged, blocking off the blood supply to the liver.

Death from *Cromolaena odorata* leaves might have been caused by tissue anoxia. Although unverified, the death of some cattle in Benue state may be connected to this phenomenon of eating *Cromolaena odorata*. These shortcomings in the use of *Cromolaena odorata* as a feed resource for livestock may require much scientific research to maximise the advantages in the inherent good qualities of *Cromolaena odorata*.

Significance of Study: We must learn to recognize that herbs are two-edged swords, with great potentialities for both benefit and harm, and that rigorous attention must be paid to matters of toxicity. Herbal remedies always carry some uncertainty, since their strength, ingredients and dosing is unregulated. Without prejudice, this in anyway does not undermine the effort NAFDAC (National Agency for Food and Drug Administration and Control) in ensuring safety of drugs in Nigeria. Current mechanisms to track adverse effects of herbal medicines are inadequate (Sheikh *et al.*, 1997). Consumers generally consider herbal medicines to be safe and view them as natural.

The mere fact that herbs are natural does not mean that they are harmless. In fact, there have been reports of people suffering serious health problems or even dying as a result of their use of herbal remedies. Although not frequent, adverse reactions have been reported for herbs in widespread use (Pinn, 2001).

On occasion, serious untoward outcomes have been linked to herb consumption. Since everything that enters the mouth is metabolized through the liver, the liver is a prime target for the toxic effects of some herbs. People with normal functioning livers and no history of prior liver disease have suffered adverse consequences to the liver as a result of taking certain herbs.

Studies have shown that only 40% of people who use herbal medicines informed their primary care physicians (Eisenberg *et al.*, 1998) Therefore, cases of herbal medicine toxicity may go unrecognized. Establishing a diagnosis of herbal hepatotoxicity can be difficult. Even when herbal-related toxicity is suspected, a definitive diagnosis is difficult to establish without proper analysis of the product or plant material. This work shall reveal the effects of *Cromolaena odorata* on the liver. In addition, urges Health care providers and consumers to report any suspected adverse effect of an herbal product to NAFDAC (Nigerian MEDWATCH). It therefore may play a lead role in awareness creation in Nigerian populace.



Obirakara Plant: The picture of the plant leaves (*C. odorata*)

Justification of the study

In Nigeria, it is commonplace for radio and television programs to be interrupted not for advert but to solicit fund for liver treatment abroad. As we are moving towards the expiration of the MDGs in 2015 and the Nigeria's vision 2020, this situation not only represent a serious danger for the affected but also impede economic growth as individuals may not be able to shoulder the responsibility alone. Hence the risk of death from liver disease remains even more a serious challenge to achieving these objectives and our integrity as the largest black nation. Liver disease is among the major humiliating disease of this century and the next century in Nigeria. Liver failure

is in part due to toxins from plants. *Chromolaena odorata* contains carcinogenic pyrrolizidine alkaloids (Fu *et al.*, 2002). This can cause liver toxicity, cancer and failure. Pyrrolizidine alkaloids are the responsible agents for liver injury.

In general, a key point to keep in mind is that any herb containing pyrrolizidine alkaloids is potentially hepatotoxic (toxic to the liver). Hepatotoxicity due to pyrrolizidine-containing herbs can result from either small amounts ingested over long periods of time or from large amounts ingested over a short period of time. Considering the innumerable functions of the liver and as it relates to the good performances of other organs, it is essential that we look after the health of the liver through infinitesimal exposure to injurious substances.

MATERIALS AND METHODS

Animals and Assay Kits

A total of thirty white albino rats (*Rattus norvegicus*) weighing between 120-140g were obtained from the laboratory Animal Unit of the Department of Science laboratory Technology of Dorben Polytechnic Abuja FCT.

The Total Protein, albumin, urea and bilirubin assay kits were obtained from Randox Laboratories, Ltd., United Kingdom while enzyme assay were obtained from ELITECH SYSTEMS. Other reagents used were of analytical grade and were prepared in all glass-distilled water.

Plant material

The plant *Chromolaena odorata* materials used in this study were collected from the premises of Dorben polytechnic Abuja FCT, Nigeria in February 2012. It was identified and authenticated in the Department of science laboratory technology.

Extraction of plant materials

Fresh leaves of *Chromolaena odorata* were air-dried at room temperature for twenty two days, in controlled environmental conditions. The dried leaves were pulverised to a uniform powder and sieved through 1mm sieve. The powdered plant materials was soaked in cold distilled water (25% w/v) for a period of 48 H, after which it was filtered using a piece of clean, sterile, white Muslin cloth to remove debris. 6ml of the filtrate was evaporated in a drying cabinet, and used in calculating equivalent amount present in milligram.

Animal Groupings and Herb Administration

The rats which had been maintained on growers (Grand Cereals LTD Nigeria PLC) and water ad libitum, were allowed to acclimatize for seven days after which they were randomly grouped into two: (i) Group A- which consisted of 5 rats, received orally, 5ml sterile distilled water on daily basis. This served as the control. (ii) Group B- which consisted of 25 rats received orally appropriate volume corresponding to the therapeutic dose of 20mg/kg body weight of *Chromolaena odorata* preparation on daily basis. This served as the test group. Five rats each in group A were sacrificed 24hours after 1, 5, 9 and 13 daily doses of *Chromolaena odorata* while the remaining five rats in the control group were sacrificed 24hours after the 13 daily doses of sterile distilled water.

Serum Preparation

The rats were anaesthetized using cotton wool soaked in chloroform vapour. When they became unconscious, they were quickly brought out of the jar. The neck area was cleared of fur and skin to expose the jugular veins. These veins were then cut sharply with sterile scalpel blade and the rats were held head downwards and allowed to bleed into clean dry corked test tubes, allowed to clot and left for 10mins at room temperature for serum formation (Akanji and Ngaha 1989). The serum was collected using Pasteur pipette after centrifugation at 3000rpm for 5minutes, kept frozen and used for the selected liver function analyses within 14hours of collection.

Equipment: Stat Fax®, 4500 Chemistry Analyzer and Colorimeter

Biochemical Assays

Serum Liver enzymes activities were assayed according to the method of Rec of 1972 (Alkaline phosphatase) and Reitman and Frankel of 1957 (Aspartate aminotransferase and Alanine aminotransferase).

Serum albumin concentration was determined based on its quantitative binding to the indicator 3, 3', 5, 5'-tetrabromo-m-cresol sulphonaphthalein (bromocresol green, BCG), according to the method of Doumas, *et al.*, 1971 while Bilirubin is by the colorimetric method based on that described by Jendrassik and Grof (1938). Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid. Serum Protein concentration was determined by Biuret reaction, which involves a reagent containing copper (cupric) ions in alkaline solution. Globulin was calculated by the difference between concentration of Total protein and Albumin while Albumin –Globulin Ratio was calculated by simple ratio formula.

Statistical analysis

Data were expressed as mean \pm Standard deviation. The results were analyzed using One way ANOVA. Post hoc test was also conducted to determine level of significance between the treated and control groups using Tukey-Kramer Multiple Comparisons Test LSD. Statistical significance was considered at $P < 0.05$.

Results analysis

The results were presented in tables 1, 2 and 3. In Table 1, the Total Protein concentration decreased from control (day zero) throughout the period of treatment but not in definite pattern. The extract caused significant difference ($p < 0.05$), in protein concentration from control on the days after administration except on day one after administration (Table 1). Conversely, Albumin concentration increased from the control in all the days after treatment yet not in definite pattern and there was no significant difference from the control (Table 1). Globulin also decreased from control in line with protein concentration and was significantly different on the days after administration except on day one after administration (Table 1). Albumin-Globulin ratio decreased from control only on day 9 after administration (Table 1).

On liver function enzymes (Table 2), there was increased in the enzymes activities from the control in all the measured liver enzymes, on the days after administration but definite fashion shown only in Aspartate aminotransferase activities (AST). The day thirteen after administration showed a remarkable increase in all the liver function enzymes. There was significant difference from control ($p < 0.05$), in Alanine aminotransferase (ALT) activity, on all the days after administration. Then again Alkaline phosphatase (ALT) activity was significantly different ($P < 0.05$) from the Control on days nine and thirteen after administration. AST activity was only significantly different from control on day thirteen after treatment.

For the Bilirubin concentrations on Table 3, Total and Unconjugated Bilirubin increased from the control in definite pattern and significantly different at $P < 0.05$ from the control, on days five and one after administration through day thirteen after administration respectively. On the other hand, Direct Bilirubin concentration did not increase on day one after administration however, was significantly different ($p < 0.05$), from control on day five after administration through day thirteen after administration. In the Unconjugated Bilirubin, there was patterned increased and all are significantly different from Control at $P < 0.05$. The Bilirubin concentration was remarkably high on day thirteen after administration.

Table 1: Effects of repeated administration of *Chromolaena odorata* on serum Protein levels (g/d) of apparently Healthy Rats

Days after administration	0	1	5	9	13
Total Protein	76.60±3.5	76.58±5.78	68.78±6.34*	66.68±3.57*	69.58±5.41*
Albumin	41.00±4.47	45.4±5.18	43.8±7.69	44.6±7.73	44.40±3.28
Globulin	35.60±7.13	31.80±0.88	25.97±0.68*	22.08±0.40*	25.16±7.84*
Albumin-Globulin Ratio	1.32	1.69	1.69	2.01	1.76

Table 2: Effects of repeated administration of *Chromolaena odorata* on serum liver enzyme activities (u/l) of apparently Healthy Rats

Days after administration	0	1	5	9	13
Alkaline Phosphatase	46.20±7.73	55.60±7.44	53.00±8.43	69.20±7.95*	134.40±5.94*
Alanine aminotransferase	32.00±6.44	49.00±6.44*	46.00±6.16*	45.80±6.72*	137.80±6.30*
Aspartate Aminotransferase	58.00±10.37	55.60±5.50	56.00±5.48	65.00±5.48	167.60±9.32*

Table 3: Effects of repeated administration of *Chromolaena odorata* on serum Bilirubin concentration (umol/l) of apparently Healthy Rats .

Days after administration	0	1	5	9	13
Total Bilirubin	4.26±0.71	4.48±0.52	10.03±0.70*	9.03±0.70*	29.65±4.48*
Direct Bilirubin	3.18±0.41	2.7±0.59	5.53±0.54*	4.30±0.55*	20.54±0.62*
Unconjugated Bilirubin	0.90±0.4	1.59±0.54*	4.59±0.57*	4.84±0.54*	7.37±0.50*

DISCUSSION

Liver disease is often first suspected based on increased liver enzymes activity which was noted in this research, exclusive in ALT and ALP activities but partial in AST activity as days after administrations progressed (Table 2). Hepatic injury is associated with distortion of metabolic function (Mohd *et al.*, 2013). Though abnormally increased liver enzyme activity is considerably more common than the prevalence of liver disease, a wide spectrum of non hepatic disorder may influence liver enzyme activity. This is premised on the activity of liver enzyme in other tissues. Liver test reflect hepatocyte membrane integrity, hepatocyte or biliary epithelial necrosis, cholestasis or induction phenomenon. The concentrations of transaminases in the serum are normally low. Nevertheless, if the liver is damaged, the liver cell (hepatocyte) membrane becomes more permeable and some of the enzymes leak out into the blood circulation. In general, any damage to the liver will cause medium elevations in blood of transaminases. However, the presence of elevated transaminases, commonly the ALT and AST, may be an indicator of liver damage (Giboney, 2005). ALP is marker enzyme for the plasma membrane and endoplasmic reticulum (Wright and Plummer 1974), it is occasionally employed to assess the integrity of plasma membrane of liver (Akanji *et al.*, 1993). The significant increased in ALP activity (Table 2) on day nine and thirteen after administration of extract may be due to disruption of liver plasma membrane. Again in the study (Table 2), there was elevation of ALT and ALP activity from control on all the days after administration, which may be an indication of liver damage (Giboney, 2005) and hepatobiliary obstruction respectively. The elevation of AST activity started on day nine after administration through day thirteen after administration, all the measured liver enzymes were stupendously high in activities which further indicate strong evidence of enzymes leakage from cytosol of the hepatocyte as it is found abundantly in the cytosol of the hepatocyte (Kim, *et al.*, 2008). The use of many medications has been associated with elevated liver enzymes levels (33 Green *et al.*, 2002). Over-the-counter medications and herbal preparations are also implicated; by extension *Chromolaena Odorata* as revealed in the study on Table 2 may be importantly injurious on the thirteen day after administration in accordance with the administered dose and dose duration, to the rats. The raised level of ALT from control in all the days after administration and the raised level of AST activity on day nine after administration through thirteen day after administration, may relate to the plasma half-lives of the two enzymes. ALT has longer plasma half-life than AST (Kim, *et al.*, 2008). Hence AST may easily be cleared from the plasma. Because catabolism of transaminases occurs by absorptive endocytosis at the hepatocyte sinusoidal border, slow enzyme clearance may sustain plasma enzyme activity in hepatic insufficiency. In view of this, liver enzyme has high diagnostic for liver lesions, in clinical reference laboratory, liver enzyme are reported to measure liver homeostasis (Robber, 1999), fortunately indiscriminate release leakage limits their diagnostic utility, and this is our opinion on the liver enzymes, for what it's worth.

In Protein concentration, the extract decreased Total protein concentration, these decreased in Total protein concentration from control on all the days after administration might indicate inhibition of protein synthesis (Musa *et al.*, 2005), in line with this, the extract might have inhibited the synthesis of some proteins, consequently resulting in decreased in serum Total Protein. Low proteins concentrations result, if there is large in amount of liver damage as seen in liver function test (Wada and Snell 1962). Abubakar *et. el.*, (2010) reported that albumin is 60% of Total protein

in serum. This was observed in Albumin Globulin ratio, but not strictly (Table 1). A low Albumin-globulin ratio may indicate inflammatory reaction and infection among other things, possibly due to syntheses of more positive acute phase proteins and sparing effect due to fewer syntheses of negative acute proteins. But the increase Albumin – globulin ratio may negates inflammatory and infection among other things. Serum Albumin determination is more informative on the synthetic function of the liver (Corless and Middleton, 1993). According to the result there was no significant increase in albumin concentration (Table 1).

When old Red Blood Cells and other heme containing compounds, pass through the spleen, macrophages take them up and break down the heme into unconjugated bilirubin (which is not water soluble). The unconjugated bilirubin is then sent to the liver, which conjugate the bilirubin with glucuronic acid, making it soluble in water, and subsequently excrete them. This research presents in table 3, a steady increase in Total and Unconjugated Bilirubin concentration on all the days after administration of extract (Table 3). The conjugated bilirubin, decreased in concentration only on day one after administration and has no patterned increase in the other days' after administration. Elevated bilirubin concentration could mean either because the body is making too much bilirubin (usually due to an increase in red cell breakdown) or because of the liver is having a hard time in properly removing bilirubin from the system- a situation where the bile ducts are blocked or there is underlying liver problem.

Of late, studies have indicated that in the nonexistence of liver disease, individuals with high levels of total bilirubin may confirm various health benefits exceeding those with lower levels of bilirubin (Sadlak *et al.*, 2004). Bilirubin has antioxidant activity (Stroker *et al.*, 1998). This has favoured an argument hypothesizing that the main physiological role of bilirubin is as a cellular antioxidant (Baranano *et al.*, 1998; Sedlak, *et al.*, 2009 and Liu *et al.*, 2008). Further studies have also revealed that levels of serum bilirubin are inversely related to risk of certain heart diseases (Novotny and Vetek 2003:45 Schwertner *et al.*, 2008).

The remarkable increase in Bilirubin on the day thirteen after administration as reported in this work may be attributable to excretory impairment of the liver cells.

CONCLUSION

The Nigeria populace is not only grappling with rising costs but it is also facing an undeniable shortage of primary care providers amidst of other hampering problems. There should be wise use of medicinal plant, following the results from this study; it has shown that oral daily administration of the aqueous leaves extract of *Chromolaena odorata* for long period has secondary medical effect on the liver. Therefore habitual usage of the leave extract of the *Chromolaena odorata* should be discouraged.

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