

Toxicological Evaluation of Aqueous Leaf Extract of *Chromolaena Odorata* in Male Wistar Albino Rats

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ABSTRACT

To evaluate the toxicological implications of the administration of aqueous leaf extract of *Chromolaena odorata*. The aqueous leaf extract was administered three times per week, for 90 days at doses of 161.5mg/kg, 32 3mg/kg, 538,5mg/kg and 1077mg/kg body weight, respectively. The control animals received 0.5ml of deionised water alone. The animals were sacrificed at the end of 90days. Blood samples were collected for biochemical analysis, and the heart, testes and kidney harvested for histological analysis. Histopathological examination of the heart, lungs, testis and the kidneys did not show any observable morphological alterations. The biochemical parameters; amylase, albumin and total serum protein, and Na⁺ were found to be decreased at doses of 538.5mg/kg and 1077mg/kg, while the serum levels of creatine kinase, AST, K⁺, glucose, uric acid, urea and creatinine were increased at the same dose levels. The absence of exhibition of observable toxicity below 538.5mg/kg body weight suggests that the extract may be safe and non-toxic only at very low doses.

INTRODUCTION

Chromolaena odorata has been used in various parts of the world for medicinal and nutritional purposes. The fresh leaves and extracts of the plant *Chromolaena odorata* are used as traditional herbal treatment in developing countries for burns, soft tissue wounds and skin infections (Phan *et al.*, 2000).

Traditionally, the aqueous extract or decoction is used in Vietnam and other tropical countries for the treatment of soft tissue wounds, burns and infections (Nghiem 1992, Truong 1989). Medicinally, the seeds are used to treat cough and skin diseases prepared through decoction and can also be used as a purgative (Tabuldo, 1996). Medicinal plants are often considered to be safe without considering the potential toxicities that may be associated with the plant. The role of medicinal plant in the treatment of diseases does not assure its safety to the public.

This is because although plants generally contain some bioactive principles believed to be responsible for their therapeutic effects, they also contain some phytotoxins and other heavy metal contaminants whose toxicological actions have always been ignored. Elufioye *et al.* (2009) investigated the toxicity of ethanolic extracts of aerial parts of *Tithonia diversifolia* A. Gray (Asteracea) (which is commonly used in Nigeria for the treatment of chronic malaria), at doses of 400, 800, and 1600mg/kg in rats and observed that the plant showed acute toxic effects on the liver and kidney; and concluded that the display of toxicity at the lowest dose tested raises concern over its safety.

Since reports on the sub-chronic toxicity of *Chromolaena odorata* aqueous and ethanolic extract are scanty, the toxicological evaluation of the leaf extract could justify its ethno botanical use and also educate on the uncontrolled use by an oblivious public. This study was therefore designed to evaluate the toxicity of the plant species collected from Nnewi, Nigeria in relation to the effects of its chemical constituents on biochemical and hematological parameters and any possible histopathological changes.

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MATERIAL AND METHODS

Collection of Plant Material

Leaves of *Chromolaena odorata* were collected between the months of June and August from the Local farms in Otolu Nnewi, Anambra State, Nigeria. Voucher specimen has been deposited at the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Umuahia.

Identification of the Plant

The botanical identification of the plant was confirmed by Prof J. C. Okafor of Tree Crops and Tropical Ecology Centre, No. 7 Dona Drive, Off Ihiala Street, Independence Layout, Enugu, Nigeria.

Preparation of Plant Material

Ground sample of about 10g was extracted with 100ml of deionized water by boiling. The boiled mixture was shaken vigorously for 10-15 seconds and allowed to stand for about 30 minutes and then filtered through a 150 μ m aperture sieve to obtain the aqueous extract. The aqueous extract was thereafter freeze-dried and then lyophilized to give a yield of 10.9%. The sample was then placed in air-tight containers and refrigerated.

Animal husbandry

All animal experiments in this study followed the principles of laboratory Animal Care (NIH publication 1985). Adult male Wistar albino rats, weighing between 80-150g were obtained from University of Jos, Animal House and allowed to acclimatize for fourteen days at the experimental site (National Institute of Veterinary Research Vom), housed in steel cages under standard conditions of temperature, 22 \pm 3 $^{\circ}$ C under light period of 16hr and 8hr darkness. Standard laboratory fresh pellets were collected weekly from Dangwon farm of National Veterinary Research Institute (NVRI), Vom, Plateau state, Nigeria, and the animals were allowed access to feed and deionized water ad-libitum before the commencement of the experiment.

Sub-Chronic Toxicity Study

Grouping of the animals

Fifty five male albino rats weighing (80-150) g were used for the sub chronic studies. The animals were divided into four dose treatment groups (2, 3, 4&5,) and control group (1) of eleven rats- each.

Administration of the Extract

The animals in the control group were administered with 0.5ml of deionised water only. While the second, third, fourth and fifth groups were given single doses of 161.5, 383, 538.5 and 1077mg/kg body weight of the aqueous extract of *Chromolaena odorata* three times a week by gavage. Gavage dosing was

performed using curved, ball tipped intubation metal needle fixed to a 1ml syringe. All solutions were constituted just before use and left over were discarded.

Monitor of fluid, feed intake and body weight of animals

The fluid and feed intake of the animal were monitored and recorded daily while the weights of the animals were taken weekly.

Necropsy

After 90 days of exposure the final body weights of the Wistar albino rats were taken, and thereafter, sacrificed.

Blood was collected for hematological, biochemical evaluation, while the kidney, testis and heart were harvested and weighed immediately.

Thereafter, the organs were fixed in 10% buffered formal saline and processed for routine histopathological studies, and the slides analyzed.

Biochemical Studies:

The blood was collected for the following biochemical tests: Na⁺ and K⁺ were estimated using Flame photometer, serum creatinine determined by the alkaline picrate method (Slot, 1965; Heinegard and Tinderstrom, 1973). Creatine kinase was determined photometrically by coupled enzyme method of (Gerhardt and Wulff 1983). Determination of serum Lactate Dehydrogenase was done by (UV Method), while LDH was assayed using methods of Vassault, (1983). Serum Urea concentration was by diaacetylmonoxime method described by Natelson, *et al.*, (1951). Serum Glucose was determined using oxidase method by Trinder (1969). Determination of serum amylase activity was carried out by amyloclastic methods by Somogyi (1938), Van loon *et al.*, (1952). Phosphate was assayed by colorimetric method of Fiske and Subbarow, (1925). Uric acid was determined colorimetrically by Henry *et al.*, (1957).

RESULTS

Statistical analysis

Fluid and feed consumption, animal body weights, heart, testis and kidney weights (relative), hematological parameters and biochemical parameters were evaluated using the statistical package of social sciences (SPSS) software version 13.0 (SPSS Inc. Chicago and Microsoft).

Effect of the extract on organ relative weight, body weight changes, mortality and fluid/feed intake

The extract did not show any observable effect on the weight of the testis but significant differences in weight ($p < 0.05$) were seen at dose levels of 538.5 and 1077mg/kg for the kidney and heart respectively. A significant ($p < 0.05$) increase in body weight change (at the 13th week) and 43% mortality was observed after the oral administration of the extract at the dose level of 1077mg/kg in rats (Table 1).

Table. 1: The Effect of Aqueous Leaf Extract of *Chromolaena odorata* on mean relative organ changes mortality, bodyweight changes and feed/ fluid intake Control and Treated Rats after 90 days of Sub-chronic toxicity studies.

Parameter	Group 1 control (0.5ml deionized water)	Group 2 (161.5mg/kg b.wt.)	Group 332mg/kg b.wt.	Group 4 5385mg/kg b.wt.	Group 5 1077mg/kg b.wt.
Kidney Relative Weight	0.30±0.04	0.32±0.04	0.33±0.05	0.36±0.11**	0.34±0.03
Heart Relative Weight	0.31±0.03	0.31±0.05	0.33±0.06	0.36±0.13	0.48±0.10**
Testis Relative Weight	0.46±0.12	0.51±0.08	0.52±0.10	0.51±0.16	0.49±0.05
Mortality(%)	nil	nil	nil	Nil	43
Body weight change %(13th wk)	149.21±36.17	181.15±47.14	146.28±38.62	124.44±44.98	65.25±18.54**
Feed intake g/animal/day	21.02±1.73*	20.00±1.84*	19.07±1.24*	18.35±1.45*	18.05±1.95*
Fluid intake ml/animal/day	30.16±4.39	28.42±3.08*	27.55±4.16*	27.47±4.16*	27.55±3.43*

Table. 2: Changes in some biochemical hematological Parameters of Wistar Albino Rats after 90 Days Treatment of Aqueous Leaf Extract of *Chromolaena odorata*

Parameter	Group 1 control (0.5ml deionized water)	Group 2 (161.5mg/kg b.wt.)	Group 332mg/kg b.wt.	Group 4 5385mg/kg b.wt.	Group 5 1077mg/kg b.wt.
Urea (mg/dl)	9.37±1.17	8.86±1.33	9.30±0.83	10.43±2.98	12.62±1.39**
Creatinine µmol/L	42.90±4.26	40.75±5.72	43.78±5.83	49.38±5.51**	42.73±4.92
Uric acid (mg/dl)	1.74±0.43	2.78±0.58**	2.75±0.60**	2.50±1.06**	3.38±0.68**
Phosphate(mmol/L)	3.22±0.30	2.81±0.57	3.11±0.32	4.31±0.77**	5.07±0.98**
Calcium (mmol/L)	2.35±0.21	2.21±0.25	2.36±0.17	2.36±0.14	2.32±0.12
Sodium (mmol/L)	144.36±2.20	141.73±5.68	139.55±5.32	135.27±4.56	135.45±2.73**
Potassium (mmol/L)	7.49±0.40	8.97±2.24**	9.74±1.42**	14.16±2.52**	17.40±1.16**
Glucose (mmol/L)	4.96±0.74	5.96±2.66**	5.16±0.94**	69.09±9.21**	6.83±0.40**
GOT (mmol/L)	234.96±71.72	281.71±58.42	287.01±54.00	372.72±21.91**	337.02±51.82**
Creatine kinase (U/L)	1680.91±1012.20	1408±608.87	1742.27±615.50	2991.09±1069.95	4150.00±1010.37**
Amylase(U/L)	1999.83±338.79	2192.17±139.54	1941.70±261.85	2068.86±270.13	1570±59.52*
Total protein g/L	81.85±5.78	81.89±4.35	82.82±5.47	79.16±8.65	74.62±1.84*
Albumin mmol/L	40.66±3.47	42.32±3.13	42.46±3.29	40.67±4.52	33.55±5.13*
PCV (%)	42.00±8.68	40.25±2.22	38.25±3.40	41.75±5.68	44.50±4.04
WBC(mm ³)	6775.00±2185.37	6650.00±1676.31	6412.50±1409.71	6150.00±2188.99	8225.00±1183.57

Effect of the extract on biochemical and hematological parameters

Levels of potassium were significantly increased ($p < 0.05$) in all the treatment groups, while inorganic phosphorous was significantly increased in the 538.5mg/kg and 1077mg/kg body weight dose groups when compared with the control after the administration of aqueous leaf extract of *chromolaena odorata*. Sodium ion was significantly decreased at 538.5mg/kg and 1077mg/kg body weight dose groups while calcium ion was not significantly altered. Statistically increased serum levels of creatine kinase and aspartate transaminase ($p < 0.05$) was seen at the 538.5 and 1077mg/kg dose groups when compared to the control. Serum amylase was significantly ($p < 0.05$) decreased at 1077mg/kg body weight dose groups. The extract did not significantly alter the levels of PCV, and WBC at all the doses administered (Table 2).

DISCUSSION

The phytoconstituents namely flavonoids, saponins, protease inhibitor, glycosides, anthraquinones, terpenes, tannins, sterols, alkaloids, allicin may be responsible for the bioactivity of the plant.

The significantly elevated serum urea and creatinine levels observed with the 1077mg/kg and 583.5mg/kg body weight dose groups respectively compared to the control ($p < 0.05$) could probably be due to renal tissue toxicity exhibited by *Chromolaena odorata* at these dose levels. Finco (1989) observed that besides other factors, starvation or stress among could contribute to

increased protein metabolism. In the present study animals in the high dose groups showed a decrease in the rate of feed consumption from the 2nd and the 3rd weeks of the study. The stress resulting from starvation could have partly contributed to the elevated serum levels of urea and creatinine through increased protein catabolism. Renal function indices such as serum electrolyte, urea, creatinine, and uric acid could be used to evaluate the functional capacity of the nephrons of animals, elevated values being indicative of defective functional state (Yakubu *et al.*, 2003). The kidneys however did not show any obvious histopathological changes despite significant derangement seen in creatinine and urea levels observed with the middle (538.5 mg/kg) and high (1077 mg/kg) dose groups respectively. This result may indicate a functional rather than a structural derangement that is usually seen in the acute setting. In this type of setting, there may be alterations in renal functional indices without a concurrent alteration in the histopathological picture (Pfaller and Gstraunthaler, 1998). This is particularly so in a setting of a hepatic functional and histologic derangement, as seen in our study (unpublished paper): a condition that may be referred to as hepato-renal syndrome. This observation may well buttress earlier studies by Kluwe (1981) who stated that increased kidney weight (either absolute or relative), derangement of at least one serum parameter is an indicator of nephrotoxicity. Further studies may need to be conducted on the toxicity of the *Chromolaena odorata* on the kidney, seeing that this study has demonstrated its ability to alter serum levels of creatinine, uric acid, inorganic phosphate, sodium and potassium ions, and also the relative weight of the kidney. These findings may be a prelude to histopathological changes that may be observed or noted if the

duration of the study was longer.. Flavonoids, found in large amounts in the extracts of *Chromolaena odorata* have been reported to have a possible protective effect against coronary heart disease (Frankel *et al.*, 1993). Possible mechanism has been postulated by Laughton *et al.* (1991), to be probably due to the fact that flavonoids inhibit cyclo-oxygenase and 5-lipo-oxygenase which have been implicated to be involved in cardiovascular disease. In the present study the high content of flavonoid in the aqueous extract of *Chromolaena odorata* could confer a similar protective effect against coronary heart disease. However, no histopathological changes were observed in all the treatment groups. It is known that, exercise and trauma (contact sports; traffic accidents, intra muscular injections, surgery, convulsions, wasp or bee stings and burns) can elevate serum CK values (Moss *et al.*, 1994). The mode of sacrifice of the animals in this study probably may have contributed to elevated serum CK levels. Elevation in marker enzyme levels usually precede observable histopathological lesion. They therefore represent early indication of toxic lesions. No treatment related effect was observed in the mean relative testis weights expressed as the percentage of the total body weight when compared with the control. The treatment groups showed no significant difference at $p < 0.05$. This finding is also in agreement with the histopathological report which showed no visible lesions, and may be an indication that the extract has no untoward effect on the testes at the dose levels studied.

In this study, increases in serum levels of glucose, potassium ion and amylase and decreased serum total protein and albumin observed could be an indication of pancreas induced toxicity by the aqueous extract. These manifestations of toxicity were more evident with the animals administered with the 538.5 and 1077mg/kg dose levels. These laboratory manifestations of pancreas toxicity may primarily be due to insulin deficiency. It has been stated that insulin deficiency is associated with disordered glucose utilization, impaired protein synthesis, and promotes breakdown of protein. As a result of protein breakdown, glycogenolysis, and tissue hypoxia, intracellular potassium ions (K^+) escapes into the extracellular fluid and is lost in urine (Barar, 2005). Furthermore, Ngokere and Ngokere (2004) noted that an inverse relationship between blood glucose and glycogen storage reflects diabetes mellitus. Although the histology of the pancreas was not investigated, the biochemical findings suggest that the extract could predispose the rats to diabetes mellitus. It may therefore be safe to conclude that administration of aqueous extract of *Chromolaena odorata* appears relatively safe at low doses, but begin to manifest biochemical and histological evidences of toxicity at high dose levels.

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