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Studies on the Toxicity of *Ageratum conyzoides*

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Abstract: The mean lethal dose of ethanolic extract of *Ageratum conyzoides* was evaluated. Serum levels of some enzymes and biomolecules were also evaluated after a daily oral administration of 500 and 1000 mg kg^{-1} of the extract for 28 days. The enzymes and biomolecules evaluated were: Alanine and Aspartate aminotransferases, alkaline phosphatase, amylose, glucose, total proteins, creatinine, cholesterol, high density, low density-and very low density-lipoproteins. The result showed that the LD50 of *Ageratum conyzoides* was 10,100 mg kg^{-1}. the extract did not significantly affect serum levels of alanine and aspartate transaminases, alkaline phosphatase, amylase, creatinine, glucose and total proteins at any of the two dose levels. the extract however reduced significantly the serum level of cholesterol and high density lipoproteins. One thousand milligram per kilogram of the extract also enhanced growth of the animals as observed in the weight increase of the rats. The results of this work showed that the extract of *A. conyzoides* at the dose of 500 and 1000 mg kg^{-1} administered orally and daily for a one month period did not show any toxic effect in rats. This, coupled with the high LD50 value confirm that *A. conyzoides* is safe for use in ethnomedicine.

Key words: Toxicity, *Ageratum conyzoides*, lethal dose, biomolecules

INTRODUCTION

Ageratum conyzoides L. Asteraceae is an annual herbaceous plant with a long history of traditional medicinal uses in several countries of the world.

Other synonymns of the plant include *A. album* stend, *A. caeruleum* Hort *ex*; *A. cordifolium* Roxb. The plant originated from Central America and is now found in all American countries (Jaccoud, 1961).

A. conyzoides contain flavonoids, alkaloids, coumarins, tannins and essential oils. Fifty one terpenoid compounds including precocene I and precoceneII have been identified. (Ekundayo *et al*., 1998; Borthakar *et al*., 1987; Mensah *et al*., 1993). The essential oil includes cromene, 6-angeloyky-7-methoxy-2,2-dimethleromen, cariophylene and eugeno (Gonzanles *et al*., 1991) Flavonoids such as agecony flavones A, B and C and hexametoxy Flavone have been isolated from the plant as well as cumarinic compounds which include 1,2-benzopirone (Vyas *et al*., 1984; Horie *et al*., 1993). 1,2-desifropirrolizidinic acid and licopsamin are alkaloids which have also been isolated from the plant).

A. conyzoides is widely used in traditional medicine by various cultures worldwide, although application vary from one region to another. In Africa, it is used to treat pneumonia, wounds, burns, rheumatism, headache and colic (Durodola, 1977; Bioka *et al*., 1993). It is used as a bacteriocide, antidysenteric and antilithic in India (Borthakar *et al*., 1987). The plant is recommended by Brazilian drugs central as an antirheumatic. The plant is also extensively used on trado-medicine in china, Haiwai, Nepal, Venezuela, Philipines and several other countries (Kamal and Mehra, 1991; Saxena *et al*., 1992; Almagboul *et al*., 1985).

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This study was carried out to evaluate the toxicity of such a popular and widely used medicinal plant.

MATERIALS AND METHODS

Plant Collection

Whole plant of *Ageratum conyzoides* was collected in November 2004 at the University Of Uyo Campus Nigeria. They were authenticated by the consultant herbalist of the Department of Pharmacognosy, Faculty of Pharmacy, University of Uyo, Nigeria.

Extraction

Fresh whole plant was washed with water to remove traces of sand and then set aside for one hour for the water to drain off. The plant was then homogenized using a warming blender. The plant homogenized plant material was soaked in 96% ethanol for 7 days. The extract obtained was filtered and concentrated in vacuo. It was then dried in a desiccator.

Animals

Wilstar albino rats of either sex obtained from the animal house of Biological sciences department, University of Nigeria, Nsukka, Nigeria were used for this work. The animals were kept under standard conditions in the animal house of the University of Uyo, Nigeria under the care of experienced animal technicians. They had free access to water and food (growers pelletized feed).

Determination of Lethal Dose

Thirty overnight fasted rats were divided into 6 groups (5 rats each). Groups I, II, III, IV, V and VI received 1, 2, 4, 8, 12 and 16 g kg^{-1} and 19 L kg^{-1} body weight of the extract, respectively.

Sub acute Toxicity Test

Three groups of animals (5 rats per group) were used. Groups A, B and C received orally distilled water (control), 0.5 g kg^{-1} of the plant extract, respectively once daily for 4 weeks. The animals ere given free access to water and food throughout this period.

Collection of Blood

On the 28th day of the experiment the animals were fasted overnight. And on the 29th day they were sacrificed under chloroform anaesthesia. Blood was collected through cardiac puncture into centrifuge tubes, allowed to clot and centrifuged at 3000 rpm for 5 min to obtain the serum. The serum obtained was stored in a refrigerator at -4°C until it was used for analyses.

Estimation of Biochemical Parameters

Appropriate commercial kits (Randox Laboratories, United Kingdom) were used to estimate serum levels of the following enzymes and biomolecules: Alanine and Aspartate aminotransferases (ALAT and ASAT), alkaline phosphatase, amylase, creatinine, total proteins, glucose, cholesterol, Free Fatty Acid (FFA) Low Density Lipoproteins (LDL), High Density Lipoproteins (HDL) and Very Low Density Lipoproteins (VLDL).

- **Alanine Transaminase (ALAT):** The method involves the monitoring of the concentration of pyruvate hydrazone formed with 2,4-einitrophenyl hydrazine
- **Aspartate Aminotransferase (ASAT):** The principle of the method used involved monitoring the concentration of oxaloacetate hydrazone formed with 2,4,-dinitrophenyl hydrazine

- **Alkaline Phosphatase (Phenolphthalein Monophoshate Method):** This method is based on the principle that serum alkaline phosphatase hydrolyzes a colourless substrate of phenolphthalein that results in phosphoric acid and phenolphthalein at alkaline pH values. The pinkly coloured product is measured colorimetrically at 550 nm.
- **Triglycerides:** This involves the enzymatic colorimetric test of glycerol phosphate oxidase method.
- **Total Cholesterol:** This was carried out by the enzymatic colorimetric chod-PAP method.
- **HDL-Cholesterol:** High Density Lipoprotein (HDL) separated from chylomicrons. Very Low Density Lipoproteins (VLDL) and Low Density Lipoproteins (LDL) by the addition of a phosphotungstic and magnesium chloride (precipitating reagent) to the serum. After centrifugation, the cholesterol content was determined by the enzymatic colorimetric method.
- **Total Protein:** This was done using the Biuret method.
- **HDL-Cholesterol:** High Density Lipoprotein (HDL) separated from chylomicrons. Very Low Density Lipoproteins (VLDL) and Low Density Lipoproteins (LDL) by the addition of a phosphotungstic and magnesium chloride (precipitating reagent) to the serum. After centrifugation, the cholesterol content was determined by the enzymatic colorimetric method.
- **LDL and VLDL Cholesterol:** These were calculated as recommended by Tietz (1999).
- **Creatinine:** Modified Jaffe's method was used. Creatinine which is a hydride of creatine reacts with alkaline sodium picrate to form a red complex which can be determined photometrically.

Weight of Animals

The weight of all the animals used in the sub-acute test was taken on a weekly basis.

Statistical Analysis

Data were expressed as mean±SEM, n = 5. Students' test was used to check the level of significance. $p<0.05$ indicates significant reduction as compared with control group.

RESULTS AND DISCUSSION

The mean lethal dose that killed 50% of the animals (LD50) as obtained from probit analysis was 10 and 100 mg kg^{-1} (or 10.1 g kg^{-1}) (Table 1). There was no significant difference in the serum levels of alanine and aspartate aminotransferases, alkaline phosphatases, amylase, creatinine, total proteins and glucose.

There was however a significant difference in the lipid profile at the two dose levels compared to that of control. The plant extract significantly reduced the level of cholesterol, high density lipoproteins and low density lipoproteins at the two dose levels of 500 and 1000 mg kg^{-1} (Table 2). The serum levels of triglycerides and very low density lipoproteins were however not significantly different at any of the two dose level from that of the control. Administration of 1000 mg kg^{-1} of the plant extract produced a significant increase in the weight of the rats from the first to the last week of the experiment. However, 500 mg kg^{-1} dose did not show any significant improvement on the weight of the rats.

Table 1: Lethal dose of the ethanolic extract of *Ageratum conyzoides* in rats

Dose (mg kg^{-1})	No. of dead in 48 h	Death (%)	Probit	LD50 (mg kg^{-1})
1,000	0/5	0.0	3.04	
2,000	0/5	0.0	3.04	
4,000	0/5	0.0	3.04	10,100
8,000	1/5	20.0	3.72	
12,000	2/5	40.0	4.16	
16,000	5/5	100.0	6.96	

LD50 = 10,100±1612.75 mg kg^{-1}

The very high LD50 of the ethanolic extract of *Ageratum conyzoides* which was observed to be 10.1 g kg^{-1} shows that the extract was not toxic (Table 3). When a substance is administered to animals orally and it results to greater than 90% survival, the substance is classified as non-toxic (Anonymous, 1984). This result confirms the safety of the plant.

Enzyme levels in the intracellular fluids especially blood plasma and serum forms an integral part of diagnosis. An increase in the activities of enzymes in the serum is indicative of cell damage or over production in a tissue rich in the enzyme. Some disease states also decrease enzyme synthesis leading to a reduction in the serum concentration of such enzyme. Elevated serum level of alkaline phosphatase is associated with a variety of bone and liver disorder such as hyperparathyroidism, tumoral bone tissues, rickets, viral hepatitis, jaundice, biliary cirrhosis and bile duct obstruction. The major diagnostic value of aspartate aminotransferase (ASAT) is in myocardial infarction while alanine aminotransfransferase (ALAT) is in viral hepatitis. Amylase level is used to diagnose acute pancreatitis and inflammatory conditions (Sroev, 1989; Murray *et al*., 2000). The extract of *A. Conyzoides* did not significantly alter the serum levels of any of these enzymes implying that the extract did not have any detrimental effect on the liver, bone and pancreas at any of the dose levels after 4 weeks of daily oral administration.

Creatinine level in serum is used to assess kidney function. Lowering of the serum concentration indicates low clearance rate of creatinine which signifies kidney dysfunction (Ukoha, 1998; Murray *et al*., 2000). The extract of *A. conyzoides* also did not significantly affect the serum level of creatinine, showing that the extract had no detrimental effect on the kidney. The extract of *A. conyzoides* also did not affect the metabolism of glucose and proteins since the serum levels of these biomolecules were not different from that of control.

Serum lipid profile serve as vital diagnostic index in clinical conditions like chronic obstructive jaundice, hepatitis coronary heart disease, atherosclerosis and others. Cholestsol fraction is the major lipid constituent of atherosclerotic plague. Clinical signs daemia carrisk factor for coronary heart disease of are an increase in fasting serum cholesterol level, or triglyceride level, or both (Stroev and Makarova, 1989; Murray *et al*., 2000). The extract of *A. conyzoides* reduced the serum levels of cholesterol and high density lipoproteins indicating that it had hypolipidaemic effect and suggesting that it could be useful in the ethnotherapy of atherosclerosis.

Table 2: Effect of the sub-acute administration of ethanolic extract of *Ageratum conyzoides* on biochemical parameters in rat

Parameters	500 (mg kg^{-1})	1000 (mg kg^{-1})	Control
Aspartate aminotransferase (IU L^{-1})	9.500±4.730	7.000±1.410	9.400±3.510
Alanine aminotransferase (IU L^{-1})	2.500±0.580	3.500±1.000	4.200±1.700
Alkaline Phosphatase (IU L^{-1})	103.750±11.77	109.750±17.00	114.200±21.06
Amylase (IU L^{-1})	306.250±72.15	315.000±75.61	180.000±42.87
Creatinine (mmol L^{-1})	50.200±17.92	47.500±12.01	48.000±16.49
Glucose (mmol L^{-1})	2.350±0.656	1.550±0.252	4.100±0.980
Total Proteins (g L^{-1})	35.000±7.260	30.750±0.620	38.800±4.080
Cholesterol (mmol L^{-1})	1.550±0.192*	1.075±0.171*	2.540±0.393
Triglycerides (mmol L^{-1})	0.833±0.174	0.590±0.137	1.452±0.783
Low Density Lipoproteins (mmol L^{-1})	1.337±0.208*	0.871±0.140*	2.228±0.211
High density lipoproteins (mmol L^{-1})	0.380±0.052*	0.323±0.072*	0.550±0.081
Very low density lipoproteins (mmol L^{-1})	0.167±0.034	0.118±0.027	0.238±0.115

Mean±SEM, n = 5; *: Significantly different at $p<0.05$

Table 3: Effect of ethanolic extract of *Ageratum conyzoides* on the weight (g) of rats

Dose	Day			
(mg kg^{-1})	1st day	8th day	15th day	28th day
500	80.00±11.55 (100)	98.50±15.76 (123.13)	111.50±19.70 (139.38)*	106.75±16.96 (133.44)*
1000	105.60±11.58 (100)	139.00±15.70 (131.63)*	142.00±8.76 (134.47)*	136.00±18.83 (128.79)
Control	152.00±11.59 (100)	107.00±4.64 (70.40)	117.60±7.09 (77.37)	115.20±6.83 (75.79)

Means±SEM, n = 5; *: Significantly different at $p<0.05$. Values in parenthesis are percent change in weight obtained as follows: Percent change in weight = weight on day × weight on day 1×100

In conclusion, the extract of *A. conyzoides* at the dose of 500 and 1000 mg kg^{-1} orally administered daily for 28 days did not have any detrimental effects on the liver, kidney, bone and pancreas of rats. The extract showed a beneficial hypolipidaemic effect. One thousand milligram per kilogram of the extract enhanced growth of the animals as observed from their weights. The extract of *A. conyzoides* therefore appear safe for its popular use in ethnomedicine.

REFERENCES

Almagboul, A.Z., A.A. Farroq and B.R. Tyagi, 1985. Antimicrobial activity of certain Sudanese plants used in folkloric medicine: Screening for antibacterial activity. Part II. Fitoterapia, 56: 103-109.

Anonymous, 1984. A new approach to the classification of substances and preparations on the basis of their acute toxicity. A Report by the British Toxicology Society Working Party on Toxicity. Hum. Toxicol., 3: 85-92.

Bioka, D., F.F. Banyikwa and M.A. Choudhuri, 1993. Analgesic effects of a crude extract of *Ageratum conzoides* in Rat. J. Hortic., 322: 176.

Borthakar, N. and A.K.S. Baruah, 1987. Search for precocenes in *Ageratum conyzoides* Linn. of North-East India. J. Indian Chem. Soc., 64: 580-581.

Durodola, J.J., 1977. Antibacterial property of crude extract from Herbal wound healing remedy *Ageratum conyzoides*. Plant Med., 32: 388-390.

Ekundayo, O., A. Sharma and E.V. Rao, 1988. Essential oil of *Ageratum conyzoides*. Planta Med., 54: 55-57.

Gonzanles, A.G., G. Thomas and P. Ran, 1991. Chromenes from *Ageratum conyzoides*. Phytochemistry, 30: 1137-1139.

Horie, T., Tominaga and Y. Kawamura, 1993. Revised structure of a Natural flavone from *Ageratum conyzoides*. Phytochemistry, 32: 1079-1077.

Jaccoud, R.J.S., 1961. Contribuicao para o estudo formacognostico do *Ageratum conyzoides* L. Rev. Bras Farm, 42: 177-197.

Kamal, R. and P. Mehra, 1991. Efficacy of Pyrethrins extracted from *Dysodia tennifolius* and *Ageratum conyzoides* against larva of Anopheles Stephensi. Indian J. Chem., 4: 7-9.

Mensah, M., E.V. Rao and S.P. Singh, 1993. The essential oil of *Ageratum conyzoides* L. from Ghana. J. Essential Oil Residues, 5: 113-115.

Murray, R.K., D.K. Grammar, P.A. Mayes and V.W. Rodwell, 2000. Harpers Biochemistry. 25th Edn., McGraw-Hill.

Saxena, R.C., O.P. Dixit and P. Sukumaran, 1992. Laboratory assessment of indigenous plant extracts (or anti-juvenile Horrnone activity in culex quinquefasciatu). Indian J. Med. Res., 95: 204-206.

Sroev, E.A. and V.G. Makarova, 1989. Laboratory Manual in Biochemistry. Moscow: Mikr Publisher, pp: 58-108.

Tietz, N.W., 1999. Textbook of Clinical Chemistry. 3rd Edn., Philadelphia; W.B. Saunders, pp: 1204-1208.

Ukoha, A.J., 1998. Modern Enzymology. FUTO Press Association with Brumine Publishers. Owerri, pp: 162-163.

Vyas, A.V. and N.B. Mulchani, 1984. Structure reinvestigation of conzorigum, a chromone from *Ageratum conyzoides*. J. Chem. Soc. Perkin, 1: 945-2947.