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Assessment of toxicologic and radical scavenging potentials of methanol extract of *ageratum conyzoides*

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Abstract

Aims: *Ageratum conyzoides* is an annual herbaceous plant commonly used in African traditional medicine as a purgative, antipyretic and wound dressing agent. The objective of this study was to investigate the free radical scavenging and toxicologic potentials of methanol extract of *A.conyzoides* leaves in wistar rats.

Methods: The ground plant leaves were macerated to prepare a methanol extract and the ability of the extract to scavenge DPPH and reduce Fe^{3+} to Fe^{2+} was assayed following standard methods. The acute toxicity test was done using eighteen (18) male albino mice. The limit test dose of 5000 mg/kg body weight (b.w.) was administered to the albino mice and then observed individually 24 hrs post-dosing. Sub-chronic toxicity was evaluated after administering daily oral doses of 100, 200 and 400 mg/kg (b.w.), for 28 days using thirty two (32) rats. The effect of the extract on liver and kidney functions were assessed following standard methods.

Results: The extract scavenged DPPH in a dose dependent manner with EC_{50} of 3.88 μ g/ml compared to ascorbic acid EC_{50} of 2.37 μ g/ml also reduced Fe^{3+} to Fe^{2+} in a concentration dependent form. The limit dose of 5000 mg/kg did not cause any mortality or signs of acute toxicity in the rats tested during the observation period. In the sub-chronic tests, the results did not show any treatment-related abnormalities in biochemical parameters. However, urea was significantly ($p < 0.05$) lower in the group treated with 500 mg/kg of *A. conyzoides* extract.

Conclusion: The results suggest that the methanol extract of *A. conyzoides* is relatively safe when administered orally in rats and possesses free radical scavenging properties.

Keywords: *A. conyzoides*, DPPH, acute and sub-chronic toxicity, biochemical parameters and wistar rats

1. Introduction

Toxicity refers to the ability of a substance to produce adverse effects. Toxicology is a discipline, overlapping with biology, chemistry, pharmacology, and medicine, which involves the study of the adverse effects of chemical substances on living organisms [1]. The relationship between dose and its effects on the exposed organism is of high significance in toxicology. Factors that influence chemical toxicity include the dosage (chronic and acute), route of administration (inhalation, ingestion or dermal), species, age, sex, individual characteristics and environment [2]. The aim of toxicity determination is to identify adverse effects of a substance [3]. Adverse effects depend on two main factors; routes of exposure and dose. To explore dose, substances are tested in both chronic and acute models.

Goatweed (*A. conyzoides*) also known traditionally as 'Ewu eri Okuko atu' is an erect, herbaceous annual of height about 30 to 80 cm [4]. It has an erect stem and a strong, unpleasant smell. Its stems are covered with fine white hairs, leaves are opposite, pubescent with long petioles and include glandular trichomes [5]. In some countries the species is considered a weed, and control is often difficult [6, 7, 8, 9]. It grows sometimes as an ornamental plant [10] and is commonly used as a traditional medicine [11, 12]. It is native to Southeastern North America and Central America, but the essential origin is in Central America and the Caribbean. *A. conyzoides* is found in several countries in tropical and sub-tropical regions, including Brazil [13]. The plant is classified into two subspecies, *latifolium* and *conyzoides*. Subspecies *latifolium* is found in all the Americas and subsp. *conyzoides* has a pantropical distribution [14]. There is high variability in the secondary metabolites of *A. conyzoides* which include flavonoids, alkaloids, coumarins, essential oils, and tannins [1].

A. conyzoides is widely utilized in traditional medicine by various cultures worldwide, although applications vary by region. In Central Africa, it is used to treat pneumonia, but the most common use is to cure wounds and burns^[15]. Traditional communities in India use this species as a bacteriocide, antidiarrheal, and antilithic^[16], and in Asia, South America, and Africa, aqueous extract of this plant is used as a bacteriocide^[23]. In Cameroon and Congo, traditional use is to treat fever, rheumatism, headache, and colic^[17, 18]. In Reunion, the whole plant is used as an antidiarrheal^[19]. Aqueous extracts of leaves or whole plants have been used to treat colic, colds and fevers, diarrhea, rheumatism, spasms, or as a tonic^[20]. *A. conyzoides* has quick and effective action in burn wounds and is recommended by Brazilian Drugs Central as an antirheumatic^[4]. Effective analgesic action was reported in rats using aqueous extract of *A. conyzoides* leaves (100 to 400 mg/kg)^[21].

2. Materials and methods

2.1. Chemicals and drugs

All chemicals and drugs used in this study were of analytical grade and products of Sigma Aldrich, Germany.

2.2 Plant Materials

The plant *A. conyzoides* were collected from Uli and Egbuoma of Oguta local government area of Imo state, Nigeria. They were authenticated by Dr. C. J. Ukpaka, a botanist, of the Biological sciences department of Chukwuemeka Odumegwu Ojukwu University, Uli campus Anambra State.

The leaves were plucked from the plants and then dried at room temperature (29-35 °C) for four weeks, after which they were blended into fine powder. The ground leaves were macerated for 92 hours in 80% methanol. This was filtered through muslin cloth on a plug of glass wool in a glass column. The resulting methanol extract was finely filtered using Whatman No 1 qualitative filter paper and concentrated using rotary evaporator (IKA, Germany) at an optimum temperature of 40 – 50 °C (to avoid denaturation of the active ingredients).

2.3. Animals used

Male albino rats of wistar strain weighing (139 -2 15) g obtained from the animal unit of the department of Zoology and Environmental Biology, University of Nigeria, Nsukka were used for the study. They were housed in ventilated metal steel cages with sufficient space to ease their movement. The rats were fed with growers mash (Niger feeds, Nigeria) purchased from the Local market and water. They were allowed to acclimatize in the laboratory for seven days before the experiment and were given free access to water.

All the animals were carefully monitored and maintained in accordance with accepted principles for laboratory animal use and care by National Institute of Health Guide for care and use of laboratory animal (Pub No. 85-23, revised 1985).

2.4. Lethal Toxicity Studies (LD₅₀)

Lethal toxicity study was determined using method of Lorke^[22]. The animals were divided into two phases i and ii with each phase subdivided into three groups made up of three mice each. The mice were given the extract at doses of 10, 100, 1000 mg/kg b.w respectively for phase i while 1600, 2900 and 5000 mg/kg b.w of the extract was given in phase ii, they were monitored closely for 24 hours for signs of toxicity and lethality.

2.5. In vitro Antioxidant Studies of Methanol Extract of *A. conyzoides*

2.5.1. Quantitative 2, 2- Diphenyl-picrylhydrazyl (DPPH) radical scavenging assay of the extract

Scavenging activity of DPPH free radicals by the extract was determined according to the method of Gyamfi *et al.* (1999)^[23].

Procedure

At different dilutions ranging from 15.62 - 250 µg/ml, 2.0 ml of the extract in methanol was mixed with 1.0 ml of 3 mM DPPH in methanol, the mixture was then shaken vigorously and allowed to stand in the dark at room temperature for 25 minutes. One millilitre (1.0 ml) of methanol and 2.0 ml of the test sample were used to prepare the blank solution while the negative control was prepared with 1.0 ml of 3 mM DPPH in 2.0 ml of methanol. L-Ascorbic acid was used as the standard after which the absorbance of the assay mixture was read at 518 nm wavelength against the blank. Percentage inhibition of DPPH was calculated using the equation.

$$\% \text{ Inhibition} = \frac{Ac - As}{Ac} \times 100$$

Where Ac is Absorbance of the control and As is absorbance of the test sample

2.6. Ferric Reducing Antioxidant Power Assay (FRAP)

The reducing power of *A. conyzoides* methanol extract was determined as described by Oyaizu^[24].

Procedure

Two millilitres (2.0 ml) of the extract at different dilutions ranging from 15.62- 250 µg/ml were differently mixed with 2.0 ml of 10 mg/l potassium ferricyanide (0.1% w/v). The mixtures were incubated in a water bath at 50°C for 20 minutes. Following this, 2.0 ml of 100 mg/l trichloroacetic acid solution (10% w/v) was added. Two millilitres (2.0 ml) of distilled water was then added to an aliquot of 2.0 ml of the mixture then 0.4 ml of 0.1% ferric chloride (FeCl₂. 6H₂O) solution was added to the solution and mixed thoroughly. The absorbance of the reaction mixture was then read at 700 nm wavelength after 10 minutes of reaction. The ferric reducing antioxidant power of the extract was calculated with the following equation.

2.7. Toxicity studies on Liver Function Markers

Assay method

Activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) was assayed using the method of Reitman and Frankle^[25] (1957) as described in randox kit. While, serum urea and creatinine concentration was determined following the colorimetric method of Bartels and Bohmer^[26].

2.8. Statistical Analysis

Results obtained were subjected to statistical tests using Statistical Package for Social Sciences (SPSS). All values were expressed as mean ± SEM. Data were analysed by one way ANOVA and difference between means will be assessed by two- tailed students' t-test. *P*<0.05 was considered statistically significant.

3. Results

3.1. Acute toxicity study

Result of acute toxicity showed that the oral administration of the methanol leaf extract of *A. conyzoides* to albino mice up to the dose of 5000 mg/kg b.w. did not record any mortality. However, a mild clinical sign of writhing and tremors in mice treated with the dosage of 5000 mg/kg b.w. was observed. This indicated that the extract could be safe at 5000 mg/kg b.w.

3.2. In -vitro antioxidant properties of *A. conyzoides* extract

3.2.1. 2, 2- Diphenyl-picrylhydrazyl (DPPH) Radical Scavenging Assay

The result of scavenging activity of DPPH free radicals by the extract showed an increase with increasing concentration of the extract (Figure 1). The scavenging activity of DPPH free radicals by the extract was significantly higher ($p \leq 0.05$) at higher concentrations with EC_{50} of 3.88 $\mu\text{g/ml}$.

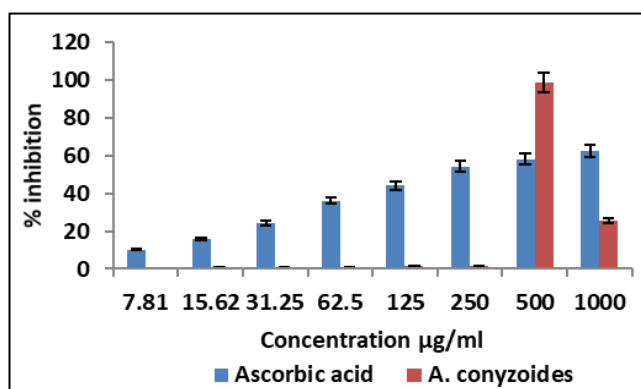


Fig 1: DPPH scavenging ability of methanol extract of *A. conyzoides*

3.2.2. Ferric Reducing Antioxidant Power (FRAP) Assay

The result of ferric reducing antioxidant power (FRAP) assay showed that *A. conyzoides* methanol extract has a high ferric reducing power (Figure 2). The antioxidant activity increased significantly with increase in concentration of the extract (7.82 – 1000 $\mu\text{g/ml}$). The results indicate that methanol extract of *A. conyzoides* had a lower ferric reducing antioxidant capacity than gallic acid.

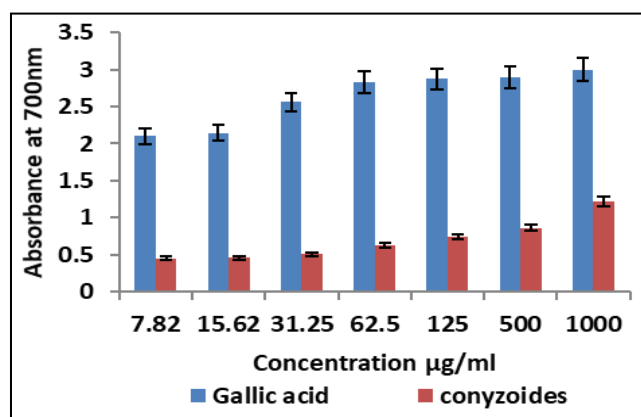


Fig 2: Ferric reducing antioxidant power capacities of *A. conyzoides* methanol extract at different concentrations of 7.81-1000 $\mu\text{g/ml}$.

3.3 Effect of the extract on serum liver biomarker enzymes

3.3.1 Effect of the Extract on Aspartate Aminotransferase (AST)

From the result (Figure 3), Groups 3 and 4 showed

insignificant ($p > 0.05$) increased AST activity when compared with that of the control. At the third week, Group 3 had elevated levels of AST activity when compared with that of the control (group 1) which was significantly higher ($p < 0.05$) than that of the control (grp 1). At other weeks, there was no significant difference ($p < 0.05$) within the other groups when compared with that of the control.

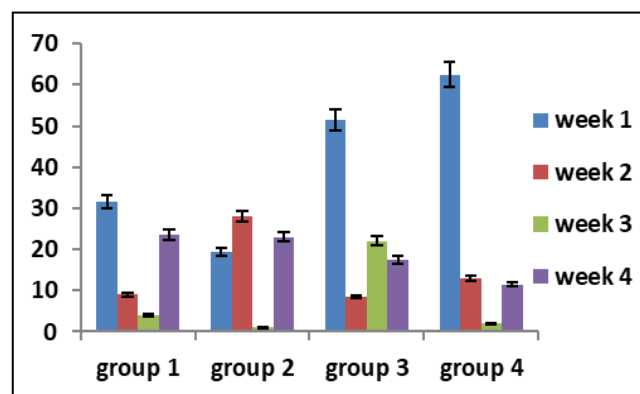


Fig 3: The effect of different concentrations of methanol extract of *A. conyzoides* on serum Aspartate Aminotransferase (AST) activity in albino rats

3.3.2 Effect of the Extract on Alanine Aminotransferase (ALT)

The result of the effect of the extract on serum alanine aminotransferase (fig,4) showed all groups in same range of increased serum ALT at the first week and the fourth week of administration when compared with that of the control (grp 1). At the second and third weeks, all groups had reduced activities of serum ALT activities with no significant difference. The ALT activity of Group 3 showed significantly higher ($p < 0.05$) activities at the fourth week when compared with that of the control. The ALT activity of group 4 seem to varied insignificantly ($p > 0.05$) when compared with that of control group.

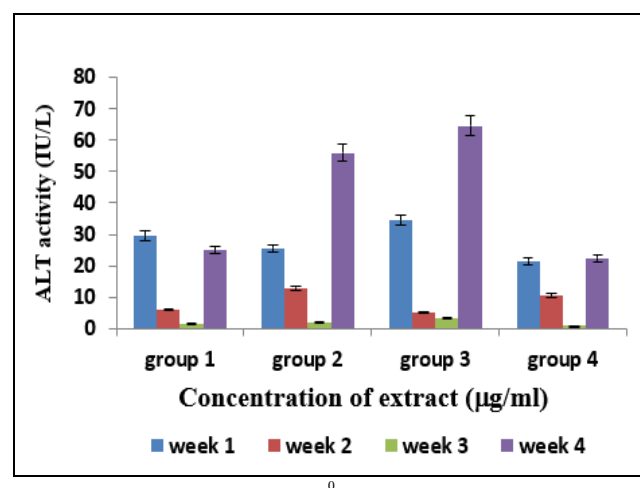


Fig 4: The effect of different concentrations of extract of *A. conyzoides* on Alanine aminotransferase (ALT) activity of albino rats

3.3.3 Effect of Extract on Serum Alkaline Phosphatase (ALP)

The results on the effect of extract on serum alkaline phosphatase (ALP) activities are shown in figure 5. The result of serum ALP showed lower activity in group 4 at the fourth week, but not significant ($p > 0.05$) compared to the control group. There was no significant difference ($p > 0.05$) within the groups at any time.

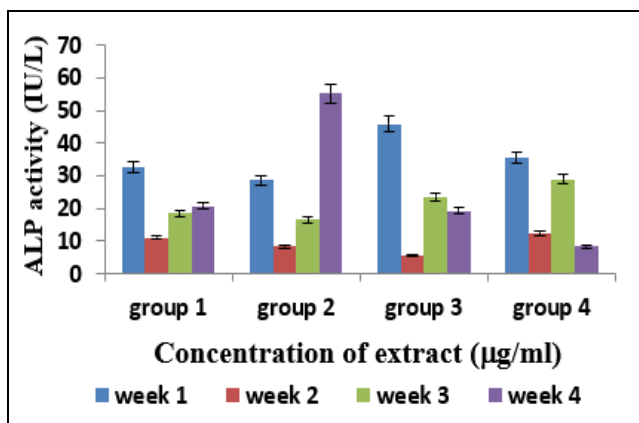


Fig 5: The effect of different concentrations of methanol extract of *A. conyzoides* on serum alkaline phosphatase (ALP) of albino rats

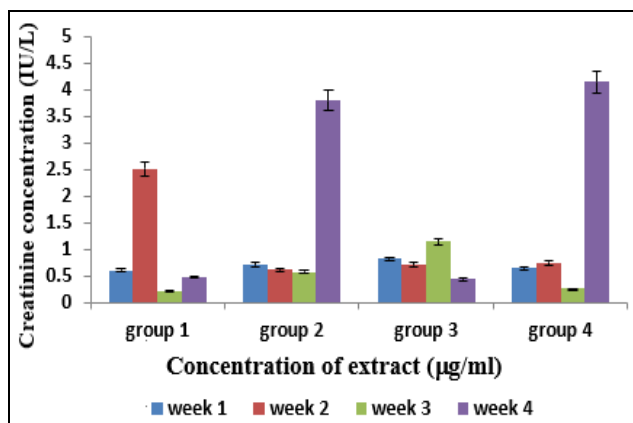


Fig 6: The effect of different concentrations of methanol extract of *A. conyzoides* on serum creatinine concentration of albino rats

3.4 Effects of Extract On Kidney Function

3.4.1 Effect of Extract on Kidney Function (Parameter – Creatinine)

The results on figure 6 showed all groups had relatively same range of concentration of serum creatinine when compared with that of control (group 1) which has no clinical significant. However, at the fourth week of administration, Groups 2 and 4 had insignificant elevated concentration of serum creatinine ($p>0.05$) when compared with that of control (group 1). The rats of groups 2 and 4 in week 4 showed significant increase in creatinine concentration ($p<0.05$) when compared with those of their respective controls indicating some effect on the kidney of the rats.

3.4.2 Effect of Extract on Kidney Function using Serum Urea concentration

The result showed all groups had relatively same range of serum Urea with the control (Figure 7). There is no significant difference ($p>0.05$) within the groups at all times. However, at the second and third weeks of administration, Groups 4 and 2 had higher concentration of serum urea. It is not significantly higher ($p<0.05$) than that of the control. There is no significant difference ($p>0.05$) within the groups at all times.

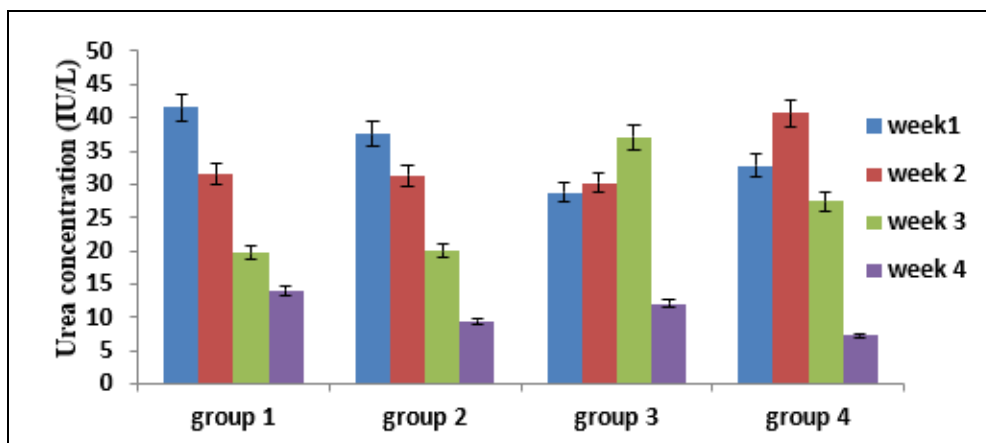


Fig 7: The effect of different concentrations of methanol extract of *A. conyzoides* on serum urea concentration of albino rats

4. Discussion

Antioxidants can be explained as reductants, and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species is reduced at the expense of the oxidation of the other [27]. The ferric reducing antioxidant capacity and DPPH scavenging properties demonstrated by the extract compared to ascorbic acid and gallic acid in this study indicates high antioxidant activity of the extract, this could be the reason for the extracts potency in oxidative stress related diseases.

The results of the acute toxicity study indicate that the LD₅₀ of the extract of *A. conyzoides* extract is more than 5000 mg/kg. This finding, therefore, suggest that the extract at the limit dose tested is essentially non-toxic and safe in oral formulation [29]. This result is in line with previous data from [30] who reported that *A. conyzoides* LD₅₀ in mice is more than 10,000 mg/kg. This study showed a high activity of ALT in the second and third groups at fourth week of

administration of the extracts, it also showed high activity of AST in the third and fourth groups of first week of administration. All these observations seem to indicate that this extract could be toxic at higher dose. Hepatocellular category of liver disease is characterized by predominantly high activity of ALT and AST, while alkaline phosphate may be normal [28].

The extract produced significant decrease in urea levels at the lower dose (100 mg/kg b.w); the reason is not exactly known but it could possibility be that the diverse active phytochemicals present in the extract are acting differently at the doses tested and moreover, some drugs are known to decrease blood urea levels [31].

Healthy kidneys remove wastes and excess fluid from the blood [32]. Creatinine is a waste product that comes from normal wear and tear of muscles in the body [33] its concentration in the blood can vary depending on age, race and body size [34].

5. Declaration of interest

Authors declare that we have no competing interest.

6. Acknowledgement

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