

Catalase and Superoxide Dismutase (SOD) Activity in Albino Rats Treated with Aqueous Leaf Extract of *Ageratum conyzoides*

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Abstract: The leaves of *Ageratum conyzoides* have been found vital in treatment and management of several clinical disorders. Many of the applications have not been investigated. Therefore this research was designed to study the antioxidant ability of the aqueous extract of fresh leaves of *Ageratum conyzoides* in albino rats. A total of twenty (20) adult male albino rats were used. The rats were placed into five groups: A, B, C, D and E. Each of the groups contained four rats. Groups A, B, C and D were treated orally with doses of 200, 300, 400 and 500mg/kg body weight of the aqueous extract respectively for seven (7) consecutive days. Group E was the control and was fed only distilled water and feed. The average body weight of the treated animals decreased, while that of control increased. It was observed that there was a decrease in the feed and water intake and also decrease in physical activity of the treated animals, but for that of the control (group E), there was increase in feed and water intake. Increase in physical activity was also observed. Total protein concentrations recorded in the serum of the test animals increased significantly ($P < 0.05$) than that of the control. The catalase and superoxide dismutase (SOD) activity obtained in the serum of the test group was non-significantly higher ($P > 0.05$) than that of the control rats. The observations recorded from this research were dose dependent. The findings in this research may be used to boost the antioxidant potential of the body and may be implicated in the various therapeutic applications of the leaves.

Key words: *Ageratum conyzoides* • Antioxidant • Catalase and superoxide dismutase

INTRODUCTION

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, fungi and herbivorous mammals [1]. Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects [2].

Ageratum conyzoides is tropical plant with a long history of traditional medicinal uses in several countries of the world and also has bioactivity with insecticidal and

nematocidal activities. This herbaceous plant has for long been presented as a potentially valuable plant for agricultural resources and medical uses [3-4]. Medicinal uses of *Ageratum conyzoides* include: use as a purgative, colic treatment of ulcer, wound dressing, treatment of intestinal pain (enteralgia), treatment of acute fever, etc. Other pharmacological properties of *Ageratum conyzoides* which have been reported in recent studies include: analgesic property, anticoccidial property, schistosomicidal activity, insecticidal effect and anti-inflammatory property [5]. *Ageratum conyzoides* is an angiosperm, of the order *asterales* and of the *magnoliopsida* class and generally belong to the family *asteraceae*.

Superoxide dismutase (SOD) (EC: 1.15.1.1) and catalase (EC: 1.11.1.6) are powerful antioxidant enzymes. They play crucial roles in promoting health by forming part of our bodies' primary system of defense against free radical damage. SOD and catalase are classified as

“primary antioxidants,” and they work synergistically with the well-known “secondary antioxidants” such as vitamins C, A and E [6].

Iron and copper/zinc availability is the major factor affecting catalase and SOD expression respectively. The deficiency of the electron carrier, NADPH, has also been shown to greatly affect catalase activity. However, several factors such as concentration of substrate (hydrogen peroxide in the case of catalase), temperature, pH, salt concentration and the presence of inhibitors or activators, influence the rate at which the enzyme work, including diseases like Alzheimer’s and Parkinson’s disease, which result to decreased SOD activity [7].

Aims and Objectives: In this research, the effects of the aqueous extract of fresh leaf extract of *Ageratum conyzoides* on the catalase and superoxide dismutase (SOD) activity in albino rats were studied.

MATERIALS AND METHODS

Collection of *Ageratum conyzoides* Leaves: The *Ageratum conyzoides* leaves were collected in Abakaliki, Ebonyi State and authenticated by Prof. S. S. C. Onyekwelu of Applied Biology Department, Ebonyi State University Abakaliki.

Collection of Animals (Rats): Twenty adult male albino rats were obtained from the Department of Zoology, University of Nigeria, Nsukka and transported in steal cages to the animal house of Biochemistry Department, Ebonyi State University, Abakaliki.

Preparation of Aqueous Sample (Leaf Extract): The preparation of extract was done using the method as described by Agbafor (2004) [8]. Fresh leaves of *Ageratum conyzoides* were washed and 140g of the leaves were ground with mortar and pestle into a paste. The paste was collected with the aid of hand gloves to exclude contamination. A volume of 350ml of distilled water was mixed with the sample paste and allowed to soak for one hour in a conical flask. After soaking, a green solution was squeezed out using a muslin cloth to completely separate the aqueous extract from the chaff. The aqueous extract was subsequently evaporated using a rotor evaporator to get a gel-like residue. After evaporation, 20g of the residue was mixed with 100ml of distilled water to make a concentrated solution of 0.2g/ml of the aqueous extract, which was stored at room temperature in a refrigerator.

Animal Handling and Treatment

Measurement of Weight of Animals: The animals were allowed to acclimatize for seven (7) days, before administration. The weights of the rats were taken on a daily basis to determine the actual volume of extract (*A. conyzoides* leaves) to be administered.

Animal Grouping: The animals were placed in five groups (A-E) with four animals in each group and the fifth group (E) was used as the control. They were allowed free access to feed (growers mash) and distilled water before and throughout the period of the experiment.

Administration of Sample (Extract) to the Rats: The route of administration adopted was oral. Doses of 200mg/kg, 300mg/kg, 400mg/kg and 500mg/kg body weight were given to groups A, B, C and D respectively for seven (7) consecutive days. Group E served as the control and was given distilled water and growers mash only. The animals were allowed free access to feed and water throughout the period of the experiment.

Collection of Samples from the Animals: After treatment, the animals were starved overnight and sacrificed under mild anesthesia using chloroform and the blood samples were collected by cardiac puncture into sterile specimen bottles free from anticoagulants and the bottles were well labeled for each animal.

Preparation of Serum: The blood samples collected from the rats were allowed to clot after which they were centrifuged for ten (10) minutes at 3000xg and allowed to stand. The supernatant (serum) was collected with the aid of a micropipette.

Preparation of Reagents

Adrenaline Solution (0.059%): A standard adrenaline solution (0.059%) was prepared by dissolving 0.2g of adrenaline in 34ml of distilled water.

Phosphate Buffer (0.05M): A phosphate buffer of 0.05M was prepared by dissolving 6.76g of K_2HPO_4 and 1.36g of KH_2PO_4 in distilled water and made up to 100ml with distilled water. The pH was adjusted to 7.8.

Hydrogen Peroxide (30mM) Standard Solution: Hydrogen peroxide (30mM) standard solution was prepared by dissolving 0.34ml of 30% hydrogen peroxide (8.82M) and made up to 100ml with phosphate buffer.

Determination of Catalase Activity

Principle: In the U.V. range, hydrogen peroxide shows a continual increase in absorption with decreasing wavelength. The method is based on the reaction of the enzyme with methanol in the presence of optimal concentration of H_2O_2 . The formaldehyde produced is measured colorimetrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole as the chromogen. The decomposition of hydrogen peroxide can be followed directly by the decrease in extinction at 750nm. The difference in extinction (ΔE_{750}) per unit time is a measure of the catalase activity

Procedure: The catalase activity was determined according to U.V. assay method of Aebi (1974) [9]. In this method, 0.1ml of hemolysate was added to 5ml of water, mixed and allowed to stand for 5minutes. In duplicate, 1ml of phosphate buffer was added to 2ml of hemolyte, 1ml of hydrogen peroxide (30mM) solution was added beside the spectrophotometer and the absorbance was monitored at 750nm for 3minutes.

Calculation of Results: The catalase activity was calculated using the equation;

$$K = \frac{2.3}{\Delta t} \times \frac{\text{Log } W1}{E2}$$

E1= Absorbance after 15seconds

E2= absorbance after 180seconds

Δt = Change in time

The catalase content of the sample is expressed in activity of catalase per second (K)

$$\text{Specific enzyme activity} = \frac{\text{Enzyme activity}}{\text{Total protein concentration}}$$

Determination of Suderoxide Dismutase (SOD) Activity

Principle: The ability of superoxide dismutase to inhibit auto oxidation of adrenaline is the basis of the SOD assay. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction.

Procedure: Superoxide dismutase activity was determined by Epinephrine method of Pajovic *et al.*, (2003) [10]. In this method, 0.1ml of serum was added to 2.5ml of 0.05M of phosphate buffer (pH 7.8). Beside the spectrophotometer, 0.3ml of adrenaline solution (0.059%) was added and the absorbance was read at 750nm for 1minute 30 seconds at 15seconds.

Calculation of Result:

$$\text{Increase in absorbance for substrate} = \frac{A_3 - A_0}{3}$$

A_0 = Absorbance after 15seconds

A_3 = absorbance after 90seconds

$$\% \text{ inhibition} = 100 - \left[\frac{\text{Increase in absorbance of substrate}}{\text{Increase in absorbance of blank}} \right] \times 100$$

Increase in Absorbance of Blank: One unit SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline. The unit of SOD activity is in international unit (U/L).

$$\text{Specific enzyme activity} = \frac{\text{Enzyme activity}}{\text{Total Protein concentration}}$$

Determination of Total Protein: The method described by Lowry (1951) [11] was used in the determination of the total protein.

Principle: Under alkaline condition, divalent copper ion forms a complex with peptide bond and it is reduced to a monovalent ion. Monovalent copper and the radical groups of tyrosine, tryptophan and cysteine react with phenol reagent to produce an unstable product that becomes reduced to molybdenum or tungsten blue. The absorbance of the coloured compound was measured at 750nm.

Procedure: The protein concentration was determined according to Lowry (1951) [11] using bovine serum albumin as standard. 0.1ml of serum sample was mixed with 5ml of incubation mixture and mixed after 10minutes. 0.5ml of Folin reagent was added and mixed after 30minutes. The absorbance was read at 750nm against the reagent using spectrophotometer and the protein concentrations were obtained from the standard curve.

RESULTS

Physical Observation: During the period of administration, the rats in cages A, B, C and D which were treated with the aqueous extract showed slight decrease in their feed and water intake and also a decrease in physical activity, but for that of the control (group E), there was an increase in feed and water intake and increase in physical activity was also observed.

Changes in Average Body Weight (g) of the Test Animals During Seven Days of Treatment: Table 1 shows the changes in the average body weight of animals during the seven days of treatment. There was a decrease in the average weight of the treated rats and an increase in the average weight of the control group.

The increase in the body weight of the control is significantly higher ($P < 0.05$) than in the treated animals.

Average Total Protein, Enzyme Activity and Specific Enzyme Activity Levels of the Albino Rats after Seven (7) Days of Treatment: After the seven days of administration of the aqueous extract as shown in table 2 it was observed that catalase and SOD activity and specific enzyme activity in the test animals differ significantly ($P < 0.05$) from the control.

From the table, the protein concentration (mg/dl) increased significantly ($P < 0.05$) from that of the control while the enzyme activity and specific enzyme activity of the test groups differ significantly ($P < 0.05$) from that of the control.

DISCUSSION

Catalase and SOD activity was studied in albino rats treated with aqueous extract of fresh leaves of *Ageratum conyzoides*. There was decrease in physical activity, feed and water intake in the treated animals. Physical activity, feed and water intake was however found to increase in the control. The exact biochemical mechanism underlying the observed decrease in physical activity, feed and water intake is a recommendation for further studies. Though the reason behind this is not yet clearly understood, it may be that the aqueous fresh leaf extract decreased their metabolic rate by inactivating cellular digestive enzymes as was observed by Tygat (2005) [12].

There was a general decrease in the average body weight of the animals in the treated groups. However, the increase was significantly ($P < 0.05$) higher in the control group (Table 2). This is in line with the information stated by Victoria (2010) [13] that *Ageratum conyzoides* could decrease the rate of feed intake.

Table 1: Changes in Average Weight (g) of Animals during Seven Days of Treatment

S/N	Group a	Group B	Group C	Group D	Group E
1	93.13±5.54	95.63±5.91	103.13±3.59	103.13±6.25	103.13±6.12
2	93.75±4.79	92.50±8.66	98.88±2.25	94.38±5.15	109.38±6.25
3	93.75±4.47	92.25±3.84	97.75±2.30	92.50±5.14	115.63±6.26
4	86.25±4.78	91.88±3.75	96.38±2.34	90.94±4.72	117.5±6.32
5	76.25±6.29	87.5±8.66	91.88±3.75	91.25±4.33	121.88±6.37
6	88.75±2.50	81.88±5.54	86.38±1.60	89.69±3.59	146.88±6.47
7	83.13±3.37	85.75±1.19	86.25±1.44	88.56±2.02	153.13±7.47

Values are mean ± Standard deviation; n=4

LEGEND

GROUP A = 200mg/kg; GROUP B = 300mg/kg; GROUP C = 400mg/kg;
GROUP D = 500mg/kg; GROUP E = Distilled water (Control group).

Table 2: Average Protein, Average Enzyme Activity and Average Specific Enzyme Activity

Group	Average Catalase Activity (U/L)	Average SOD Activity (U/L)	Average Protein Concentration (Mg/dl)	Average Specific Catalase Activity (U/L)	Average Specific SOD Activity (U/L)
A	124.03±2.07 ^a	5.14±0.7 ^a	0.66±0.04 ^a	188.39±2.57 ^a	7.86±1.51 ^a
B	142.80±2.76 ^a	7.92±0.53 ^a	0.61±0.04 ^a	233.66±3.49 ^b	12.99±1.36 ^a
C	161.44±7.72 ^b	9.32±0.90 ^b	0.56±0.09 ^a	293.90±7.25 ^c	16.87±1.42 ^a
D	180.15±7.09 ^b	12.24±0.33 ^b	0.58±0.10 ^a	323.17±8.25 ^c	21.77±3.66 ^b
E	105.12±6.28 ^c	3.38±0.45 ^c	0.69±0.04 ^a	152.85±3.77 ^b	4.89±0.49 ^c

Values are mean ± standard deviation; n = 4. Table 2: showing average catalase activity, average SOD activity, average total protein concentration and specific average enzymes activity. Values with the same superscript are not significant at $P < 0.05$; CAT= catalase; SOD= superoxide dismutase.

Chemical constituents of the extract administered to the rats may also be a possible reason for this. Phytochemicals have been reported to influence various body processes such as: appetite, relaxation, muscle contraction and overall body metabolism of the organism. This influence may be as a result of activation or inhibition of metabolic enzymes of cells. Vital components of cells such as membranes, have also been reported to be affected by plant components [14].

According to Agbafor (2004) [8], treatment of Guinea pigs with leaf extracts of *Baphian nitida* produced decreased physical activities, feed and water intake. Reason behind the decrease in average body weight of the treated animals is still partially not understood, it may be partially related to the reported decrease in feed and water intake caused by the introduction of the extract into the animals.

Further, presence of caffeic acid, fumaric acid, spinasterol, saponins and tannins, have been reported to have anti-nutritional and toxic effects including reduced feed intake, growth and body weight. This observation has also been made by Becker (2007) [15].

The inability of the aqueous leaves extract to produce a significant difference ($P < 0.05$) (Table 2) in the total protein levels from the test and control groups suggest that the chemical constituent of the fresh leaves extract at the doses administered may not influence the rate of protein synthesis and degradation significantly. The fact that total protein concentration increased non-significantly ($P > 0.05$) (Table 2) from the control and the treated groups, is similar to the result obtained by Becker (2007) with the leaf of *Gongronema latifolium*. The mechanism behind this is not yet known, but it may be as a result of the toxic effect of the plant.

The significant ($P < 0.05$) increase in catalase and SOD activity obtained in the animals administered with aqueous extract of *Ageratum conyzoides* suggests that the extract possesses antioxidant property. The exact mechanism involved in this observed and recorded increase in catalase activity is still under investigation; however, several phytochemicals which includes flavonoids, are known to enhance the antioxidant activities of the body system of an organism. This is in line with the observation made by Jagetia (2003) [16].

CONCLUSION

The findings of this research suggest that the leaf extract of *Ageratum conyzoides* may be useful in the management and treatment of free radical related diseases like oxidative stress.

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