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SUPEROXIDE DISMUTASE AND CATALASE ACTIVITY IN ALBINO RATS TREATED WITH AQUEOUS EXTRACT OF FRESH LEAVES OF *CORCHORIUS OLITORIUS.*

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ABSTRACT

Leaves, roots and other parts of *Corchorus olitorius* are used by traditional medicine practitioners in different parts of Nigeria in management and treatment of several disorders. The present investigation was designed to determine possible antioxidant potential of an aqueous extract of fresh leaves of *Corchorus olitorius.* The study was conducted with twenty-five adult male albino rats distributed into five groups A, B, C, D and E), five rats in each group. Groups B, C, D and E were treated orally with 100, 200, 400 and 600mg/kg respectively of the extract for seven consecutive days. Group A was the control. After treatment, a decrease in average body weight was recorded in the treated groups, while the control gained weight. The catalase and superoxide dismutase activities in the groups administered the extracts were significantly higher ($P<0.05$) than those of the control. This increase in catalase and superoxide dismutase activities by the extract was found to be linearly dose-dependent. The ability of the extract to increase the activity of these enzymes suggests that the extract has antioxidant property. This antioxidant property may be due to the chemical constituents of the extract which could be responsible for some of the medicinal applications of the leaves of *Corchorus olitorius*.

Keywords: Superoxide dismutase, Catalase, Albino rats, Aqueous extract and C*orchorius olitorius.*

INTRODUCTION

Plants have been used from ancient times to attempt cures for diseases and to relieve physical suffering. Ancient peoples all had acquired some knowledge of medicinal plants. Oftentimes these primitive attempts at medicine were based on superstition and speculation. Evil spirits in the body were thought to be the cause of medical problems. They could be driven out of the body through the

use of poisonous or disagreeable plant substances that rendered the body a disagreeable habitat. Medicine men or women of a tribe were usually charged with knowledge of such plants. The progress of medicine has often been guided by the earlier observations and beliefs[1].

As early as 5,000 B.C. many drugs were in use in China. Sanskrit writings testify to methods of gathering and preparing drugs in these early times [2]. Pharmacology and pharmacognosy owe their beginnings to the earlier beliefs and knowledge about medicinal plants. The interest in medicinal plants was especially pronounced among the early botanists who were often physicians [3].

Medicinal plants since the dark ages have been of so much importance to man, for example, the dried leaves of the shrubs *Barosma betulina*, *B. serratifolia* and *B. crenulata* contain the drug buchu. The active ingredient is an essential oil that is used to disinfect and to stimulate excretion and also in the treatment of indigestion and urinary disorders.

Foxglove, *Digitalis purpurea* is native to Southern and Central Europe and has been used to treat disorders of the heart. The dried leaves are dried for use. It contains a glucoside, digitoxin. Its action improves the tone and rhythm of the heart beats thereby making contractions more powerful and complete. As a result, more blood is sent from the heart, which aids circulation and improves body nutrition and hastens waste elimination[4].

Corchorus olitorius, is commonly known as bush okra. It is a species of shrub in the family Malvaceae. It is the primary source of jute fibre. Corchorus olitorius is found in Africa and Asia. Authorities consider that it originated from the Indo-Burmese area or from India, along with several other related species. Others point out that there is a greater genetic variation in Africa and a larger number of wild species in the genus Corchorus [5]. Corchorus is most widely spread in the central senatorial region of Ebonyi state (Abakaliki region) seen amongst other shrubs in bushes.*Corchorus olitorius* is an erect annual herb of about 2 to 4m tall, usually strongly branched with tough, fibrous, reddish stems and alternate leaves [6].

Superoxide dismutase enzymes (SODs) act as antioxidants and protect cellular components from being oxidised by reactive oxygen species (ROS) [7]. ROS can form as a result of stress, injury, metabolic activity, nutrient deficiencies, photo-inhibition, temperature above and below ground, toxic metals, and UV or gamma rays [8]. To be specific, molecular O2 is reduced to O2− (an ROS called superoxide) when it absorbs an excited electron released from compounds of the electron transport chain SODs catalyse the production of O_2 and H_2O_2 from superoxide (O2−), which results in less harmful reactants [9].

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants, and animals). It catalyses the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). Catalase can also catalyze the oxidation, by hydrogen peroxide, of various

metabolites and toxins, including formaldehyde, formic acid, phenols, acetaldehyde and alcohols. It does so according to the following reaction:

Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert approximately 5 million molecule of hydrogen peroxide to water and oxygen each minute. Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide.

Decomposition reaction of hydrogen peroxide in tissue:

$2H_2O_2 \rightarrow 2H_2O + O_2$

The presence of catalase in a microbial or tissue sample can be tested by adding a volume of hydrogen peroxide and observing the reaction. The formation of bubbles, oxygen, indicates a positive result. This easy assay, which can be seen with the naked eye, without the aid of instruments, is possible because catalase has a very high specific activity, which produces a detectable response. Alternative splicing may result in different protein variants.

AIM/ OBJECTIVE

The aim of the experiment was to determine the superoxide dismutase, catalase and total protein activity in albino rat treated with aqueous leaf extract of *Corchorus olitorius*.

MATERIAL AND METHODS

METHODS

Collection of *Corchorus Olitortius*

The *Corchorus olitorius* leaves were got from Izzi in Abakaliki local government, Ebonyi State and authenticated by Dr Mrs Nnamani in the Department of Applied Biology, Ebonyi State University.

Collection of Animals (Rats)

Twenty-five adult male albino rats were obtained from the Department of Zoology, University of Nigeria, Nsukka and transported in steel cages to the animal house of Biochemistry department, Ebonyi State University, Abakaliki.

Preparation of Aqueous Extract

Fresh leaves of *Corchorus Olitorius* were washed and 100g of the leaves ground with mortar and pestle into a paste.

A volume of 350ml of distilled water was mixed with the sample paste and allowed to stand 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; it 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. The aqueous extract, which was stored at room temperature in a refrigerator.

Animal Groups

The animals were placed in five groups (A-E) with five animals in each group; the A group was used as the control. They were allowed free access to feed (growers mash) and 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 100, 200, 400 and 600mg/kg body weight were given to groups B, C, D and E respectively for seven (7) consecutive days. Group A served as the control and was given normal saline.

Preparation of Liver Homogenate

After an overnight fasting, the liver was removed and homogenised in KCl [10 mM] phosphate buffer (1.15%) with ethylene-diamine tetra aceticacid (EDTA:pH 7.4) and centrifuged at 12000 rpm for 20 min. The supernatant was used.

Determination of Catalase Activity

The catalase activity was determined according to U.V. assay method of [10].

Determination of Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity was determined by Epinephrine method of [11].

RESULTS

Physical Observation

During the period of administration, the rats in cages A, B and 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 body weight (g) of the animals during seven days of administration of the *Corchorus olitorius*

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, while the control group gained weight.

Days / Groups	A(g)	B(g)	C(g)	D(g)	E(g)
1	137.4±6.0	135.4±5.01	155.28±7.74	121.12±4.84	123.2. ±5.83
2	115.5 ±4.6	109.1±4.69	129. 46±6.38	102.48±4.50	105.5±5.01
3	134.72±5.49	109.1±4.69	137.12±6.11	136.3±3.70	111.7±5.02
4	129.34±4.73	100.28±4.61	137.44±7.75	91.22±5.16	119.12±5.14
5	108.94±3.95	94.24±4.26	115.4±6.76	81.52±3.78	84.16±5.11
6	138.66±5.67	134.1±3.87	155.22±6.17	119.74±4.0	121.6±5.30
7	120.72±5.36	104.38±4.16	135.06±6.80	98.98±3.75	106.44±4.90

Values are mean ± SD; N = 5.

Superoxide Dismutase and Catalase Activity of Rats After Seven Days of Treatment

The results presented in table 2 below shows the mean activity values of the superoxide dismutase and catalase, and total protein concentration in the five different groups of rats after a seven-day treatment. Statistical analysis showed that there was a significant difference ($P<0.05$) in the catalase and SOD activity between the treated groups and control group.

Table 2: Table showing the SOD and Catalase Activity of Rats After Seven Days of Treatment

Group/ Rats	Superoxide dismutase Activity (U/L)	Catalase Activity (U/L)
A	150.916 ± 6.62	7.72 ±1.73
B	229.74 ± 8.14	15.70 ± 2.92
C	176.33 ± 1.99	8.74 ± 3.12
D	188.32 ± 5.59	10.54 ± 3.45
E	235.88 ± 9.40	28.45 ± 2.82

Values are mean ± SD; N = 5.

DISCUSSION AND CONCLUSSION

The biochemical mechanism underlying the observed decreased in the physical activities and rate of feed and water intake of the rats administered with the aqueous extract is not full known. However, such decrease may be as a result of the chemical constituents of the extract administered to the animals as suggested by Agbafor [12], who observed similar effect when he treated guinea pigs with leaf extract of *Baphia nitida*. Phytochemicals such as alkaloids, tannins, saponins flavonoids, etc, have been reported to decrease the physical activity of laboratory animals [13]. This decrease in body weight may be due to the observed decrease in feed and water intake. Similar observations have been reported by Agbafor [12].

The recorded significant increase ($p<0.05$) in the activity of catalase and superoxide dismutase in the test groups relative to the untreated group (Table 2) suggests that the extract possesses antioxidant property.Free radical damage and oxidative stress are the major reasons for tissue damage. The antioxidant enzymes are the first-line defense against such damage and thus provide protection against the deteriorating outcome. It is probable that the various phytoconstituents of the extract are involved in scavenging free radicals from tissues, thus, reducing oxidative stress. For example, flavonoids and tannins are phenolic

compounds, and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Similarly, terpenoids, as vitamins, act as regulators of metabolism and play a protective role as antioxidants.C. *olitorius* have been traditionally used to treat some ailments which are caused by oxidative stress (from ROS). Studies have shown that many of these antioxidant compounds (sourced from plants) possess anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, antibacterial and antiviral activities [13 and 14].

In a study conducted by [15], the *in-vitro* anti-oxidative properties of methanolic extract of *Corchorus olitorius* were investigated. Qualitative and quantitative phytochemical screenings of the vegetable extracts were determined using the standard method and the anti-oxidative activity was assessed using I,1-diphenyl-2-picrylyhydrazyl(DPPH) and reducing power method. The results obtained showed the presence of alkaloids, saponins, tannins, total phenols and flavonoids in the vegetable extract. Corchorius olitorius extracts had the most significant amount of flavonoids of 157.38mg/g when compared to other medicinal plant extracts. Thus, *Corchorus olitorius*is a rich source of antioxidants. Specifically, Phenolic compounds have been reported to serve as antioxidants, and exhibit a wide range spectrum of medicinal properties such as anti-inflammatory and diabetes which are clinical implications of oxidative stress damage.

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