IJORP
International Journal of Research and Publications

# Biochemical Effects Of Methanol Leaf Extract Of Chromolaena Odorata On Cypermethrin- Induced Oxidative Stress In Wistar Albino Rats.

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***Abstract***

*This work investigated the biochemical effects of methanol leaf extract of C. odorata on cypermethrin-induced oxidative stress on Wistar albino rats. C. odorata is used as a medicinal plant in a traditional way by the people who lived in the tropic and subtropic areas, for treatment of bloodsucker nibbles, soft tissue injuries, skin infections, malaria, and diabetes. A methodical quest for valuable bioactivities from medicinal plants is currently considered to be a rational methodology in nutraceutical and drug research. Manufactured pyrethroid, cypermethrin is utilized broadly for the management of bugs in crops and as an ectoparasiticide in man and creatures. For this work, cypermethrin was used to induce oxidative stress in wistar albino rats. The biochemical analyses were carried out following the standard procedures. The result obtained from the biochemical analysis shows that there is highest activity of catalase (1.60±0.20 U/mg protein) for the rats fed with cypermethrin when compared to group 1, fed with chromolena odorata (0.74±0.12U/mg protein) while comparing group 3 (1.09±0.23U/mg protein) and group 4 (0.92±0.13U/mg protein) which are statistically significant at P≤ 0.05 similar to SOD and MDA result. The effects of C. Odorata on cypermethrin-induced oxidative stress in Wistar albino rats on the biochemical parameters such as the Liver marker enzymes (ALT, AST, ALP), serum protein and kidney function (urea, creatinine levels) indicated that C. Odorata methanol leaf extract helped relieve stress in Wistar albino rats and furthermore, helped stabilize liver functions.*

**Keywords**: Rats, Liver, Chromolaena, Methanol

## INTRODUCTION

During the most recent decade, a ton of antioxidant items are consumed by individuals all throughout the planet as the engineered medications, enhancements, or home grown medicines. The home grown medicines have been taken by individuals on the planet got from the natural sources like medicinal plants according to World Health Organization (WHO) [23] information. One of the natural sources that has been utilized as a medicinal plant is Chromolaena odorata. C.odorata (Asteraceae) is a species of Chromolaena sort that has been recognized by King and Robinson in 1970. C. odorata is perceived as siam weed. It is one of the invasive species with a fast development forming the shrubberies as high as around two meters. Furthermore, it spreads quickly on the open regions like fields, side of the road, backwoods, nature stores, and untamed life safe-havens [1]. As a matter of

fact, C.odorata is utilized as a medicinal plant in a traditional manner by individuals who lived in the jungle and subtropic zones. For instance, in Vietnam, this plant is utilized as a treatment of bloodsucker nibbles, soft tissue injuries, consumes, and skin infections [2]. Furthermore, a leaf water extract is generally utilized as a loose bowels, malaria, and diabetes drug [3]. Additionally, this leaf is likewise utilized as the treatment of wounds in light of the fact that the leaf's contents are protein, sugar, and fiber source [4].

A methodical quest for valuable bioactivities from medicinal plants is currently considered to be a rational methodology in nutraceutical and drug research. Manufactured pyrethroid, cypermethrin is utilized broadly for the management of bugs in crops and as an ectoparasiticide in man and creatures [5]. Environmental contamination and increased concentrations in various food items, helpful application and coincidental/occupational openness to pyrethroids are responsible for increasing oxidative stress in warm blooded animals [6]. Pyrethroids defer the Na+ channel conclusion which prompts spontaneous monotonous nerve firing, resulting in anxious issues on chronic openness. Openness to pyrethroids on Paramecium tetraurelia increases intracellular concentration of Ca++ ions [7] which may happen because of the immediate impact of pyrethroids on the Ca++ channels [8] or because of energy deficiencies resulting in the inability of cells to eliminate cytosolic Ca++ ion [9]. Cypermethrin and other pyrethroids are used in the liver through hydrolytic ester cleavage and oxidative pathways by the CYP-450 compounds yields receptive oxygen species (ROS), which might be responsible for oxidative stress in warm blooded animals [10]. Increase in ROS/free extremists interceded lipid peroxidation and increased cytosolic Ca++ concentration may prompt cytotoxicity and genotoxicity in higher vertebrates during openness [8, 11, 12]. Nature delivers a variety of antioxidants to forestall free extreme formation or to restrict their damaging effects in the cell. Vitamins E and C, selenium, carotene, and so forth are the naturally occurring antioxidants of organic frameworks.

## OXIDATIVE STRESS

Oxidative stress is found in the manner a sickness creates. Sickness, for example, diabetes mellitus, hypertension, ischemic infection, atherosclerosis, malignancies and so on is identified with oxidative stress. Oxidative stress can additionally cause danger in the body like lipid peroxidation and oxidative DNA harm. It has been named "hurtful" on account of the assault on organic particles by oxygen free revolutionaries. Oxidative stress can be defined as a state where oxidative powers surpass the antioxidant frameworks because of loss of the harmony between them [13]. Biomarkers, that can be utilized to survey oxidative stress in vivo is attracting interest because of its precision during estimation of such stress and further vital for investigation of its part in way of life infections just as to help assess the viability of treatment. Many markers of oxidative stress have been proposed, including lipid hydroperoxides, 4-hydroxynonenal, isoprostan, 8-hydroxyguanine, and ubiquinol-10.

When an exact biomarker that ponders the oxidative stress are accessible, such markers would be exceptionally crucial for the specialists in request to gain information on the highlights of the illnesses brought about by the oxidative stress and furthermore relate with the achievement of the medication administered.

## CYPERMETHRIN

Cypermethrin is a pyrethroid insecticide. Cypermethrin was initially synthesized in 1974 and first marketed in 1977 as a highly active synthetic pyrethroid insecticide, effective against a wide range of pests in agriculture, public health, and animal husbandry [5]. In agriculture, its main use is against foliage pests and certain surface soil pests, such as cutworms, but because of its rapid breakdown in soil, it is not recommended for use against soil-borne pests below the surface. In 1980, 92.5% of all the cypermethrin produced in the world was used on cotton; in 1982, world production was 340 tonnes of the active material. It is mainly used in the form of an emulsifiable concentrate, but ultra low volume concentrates, wettable powders, and combined formulations with other pesticides are also available [5]. Chemically, cypermethrin is the alpha-cyano-3-phenoxybenzyl ester of the dichloro analogue of chrysanthemic acid, 2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropanecarboxylic acid. The molecule embodies three chiral centres, two in the cyclopropane ring and one on the alpha cyano carbon. These isomers are commonly grouped into four cis- and four trans-isomers, the cis-group being the more powerful insecticide. The rati~f cis-to trans-isomers varies from *50:50* to 40:60. Cypermethrin is theruemic mixture of all eight isomers and, in this appraisal; cypermethrin refers exclusively to the racemic mixture (ratio *50:50)* unless otherwise stated.

## BIOCHEMICAL INDICES

### Liver Function Tests

The liver is a significant organ which helps in the maintenance of good wellbeing. They play out various job like detoxification, union including proteins, cholesterols and fatty oils, clothing factors. They likewise help in the production of carb and is the organ responsible for converting glucose to glycogen which are put away in the liver. They liver likewise stores vitamins and synthetics that the body needs as building blocks. These includes the fat solvent vitamins A, D and K. It additionally stores folic acids, iron, and copper.

Liver function tests (LFTs or LFs) are gatherings of blood tests that give information about the condition of a patient's liver [14]. These tests include tests for liver transaminases (AST or SGOT and ALT or SGPT), albumin, bilirubin (immediate and indirect). These tests are valuable biomarkers of liver injury in a patient with some level of intact liver function [14, 15].

## MATERIALS AND METHODS

### MATERIALS

#### Plant Materials

Leaf of *C. odoarata* was collected from a farmland in Ekwulobia in Aguata Local Government Area of Anambra state, Nigeria and identified by a Taxonomist, Dr. Ogbuozobe, G.O. They were dried under room temperature, packed in paper bags and stored.

#### Animals

Twenty four (24) Male Wistar albino rats weighing between 150-200g were used for the study. The rats were obtained from the Animal House of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The rats were fed with rat pellets and water *ad libitum* for a week to acclimatize.

### METHODOLOGY

#### Preparation of Plant Extracts

The *Chromolaena odorata* leaf were dried under room temperature and grounded. Known weights (200g) of fresh leaf of *Chromolaena odorata* separated from the stem, were washed with clean water to remove dirt and sand, drained, and chopped. They were macerated in 500 ml of water and then filtered to obtain homogenous aqueous extracts. The same procedures were carried using methanol.

Twenty four (24) male Wistar albino rats were used for the study. They were acclimatized for seven days with free access to feed and water. After acclimatization, they were randomly distributed into four (4) groups of 6 rats each. The rats were fed for 3 weeks (21 days) in which analyses will be done on the last day. The experimental design were; Group 1: 0.5ml/kg normal saline, Group 2: Cypermethrin-induced rats + No intervention, Group 3: Cypermethrin-induced rats + 200 mg/kg b.w. of methanol leaf extract of *C. Odorata*, Group 4: Cypermethrin-induced rats + 400 mg/kg b.w. of methanol leaf extract of *C. odorata*.

#### Animal Sacrifice and Sample Collection

The blood from the rats were collected through cardiac puncture, centrifuged and the serum separated from the cells. All rats was sacrificed on day 28**.**

#### Blood Sample Collection for Haematology and Clinical Biochemistry

**Name of the Method**: Orbital technique [16].

**Procedure**: Blood sample for haematological and clinical biochemistry determinations was collected from the retro-bulbar plexus of the medial canthus of the eye of the rats. Blood samples meant for haematology, a microcapillary tube was carefully inserted into the medial canthus of the eye to puncture the retro-bulbar plexus and thus enable outflow of blood into a sample bottle containing ethylene-diamine-tetra-acetic acid (EDTA). The blood was gently mixed well with the EDTA by shaking to prevent clotting.

In order to collect blood sample for clinical biochemistry determinations, a microcapillary tube was carefully inserted into the medial canthus of the eye to puncture the retro-bulbar plexus and thus enable outflow of about 3ml of blood into a clean glass test tube. The blood sample

was kept at room temperature for 30 minutes to clot. Afterwards, the test tube containing the clotted blood sample was centrifuged at 3,000 revolutions per minute for ten minutes using a table centrifuge, to enable a complete separation of the serum from the clotted blood. The clear serum supernatant was then carefully aspirated with syringe and needle and stored in a clean sample bottle for the clinical chemistry determinations.

**Liver Function Tests Assays**

**Determination of Alanine aminotransferase (ALT)/Serum glutamic pyruvic transaminase (GPT)**

ALT was determined by the Reitman-Frankel colorimetric method for *in vitro* determination of GPT/ALT in serum or plasma [17] using a Quimica Clinica Applicada (QCA) test kit (Quimica Clinica Applicada, Spain).

**Principle:** ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine. The shading intensity is estimated against the clear at 540nm.

**Determination of aspartate aminotransferase (AST)/Serum glutamic oxaloacetic transaminase (GOT)**

AST determination by the Reitman-Frankel colorimetric method for *in vitro* determination of GOT/AST in serum or plasma [17], using a Quimica Clinica Applicada (QCA) test kit (Quimica Clinica Applicada, Spain).

**Principle:** AST or SGOT is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine. The colour intensity is measured against the blank at 546nm.

**Determination of serum alkaline phosphatase**

Phenolphthalein monophosphate method for the *in vitro* determination of alkaline phosphatase in serum or plasma [18, 19], using Quimica Clinica Applicada (QCA) test kit. (QCA, Spain).

**Principle:** ALP catalyses the hydrolysis of p-nitrophenyl phosphate (PNPP) (substrate) at pH 10.4, liberating p-nitrophenol and phosphoric acid, according to the reaction below

P-Nitrophenylphosphate + $H_2O$ $\xleftrightarrow{ALP}$ P- Nitrophenol + Phosphoric Acid

The rate of p-Nitrophenol formation, measured photometrically at the wavelength of 505nm is proportional to the catalytic concentration of alkaline phosphatase present in the sample. PNPP is colourless in acid or alkaline medium while p- nitophenol (PNP) is yellow in colour in alkaline medium.

**Statistical Analysis**

Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS) version 19. Two-way and one way analyses of variance were adopted for comparison, and the results were subject to post hoc test using least square deviation (LSD). $p<0.05$ were considered significant for all the results. The data obtained were expressed as mean ± SD of triplicate determinations.

**RESULTS**

**LIVER FUNCTION TEST**

The result obtained from liver function test (ALT, AST, ALP and serum protein) were presented in Figures 1.1-1.4.

**Alanine amino transferase (ALT) activity**

The result obtained for the ALT analysis (Fig. 1.1) indicates that group 2 had the highest ALT activity of 22.93±4.11 U/L which is statistically different from group 1 (9.70±1.90 U/L) and group 3 (15.00±1.71 U/L). There was no statistical difference between group 1 and group 4 (15.22±5.61 U/L) and between group 3 and 4 at $p \leq 0.05$.

**Aspartate amino transferase (AST) activity**

The result obtained for the AST analysis (Fig. 1.2) shows that group 2 had the highest AST activity of 21.79±5.78 U/L which is statistically different from group 1 (4.14±2.11 U/L), group 3 (13.21±2.43 U/L) and group 4 group 4 (11.00±1.74 U/L). There is no statistical difference between group 3 and group 4 at $p \leq 0.05$.

**Alkaline phosphatase (ALP) activity**

The result obtained for the ALP studies (Fig. 1.3) revealed that group 2 had the highest ALP activity of 174.24±34.09 U/L which is statistically different from group 1 (100.98±16.92 U/L), group 3 (106.92±12.48 U/L) and group 4 (93.06±20.79 U/L). There is no statistical difference between group 1 and group 3 and group 4 at $p \leq 0.05$.

**Serum protein**

The result obtained from the serum protein analysis (Fig. 1.4) revealed that group 1 (normal) had the highest serum protein of 10.63±0.70 mg/ml. This was followed by group 4 (10.02±0.53 mg/ml), group 3 (9.92±0.63 mg/ml) and group 2 (8.30±0.29 mg/ml). From the statistical analysis, it was found that there is statistical difference between group 1 and group 2 at $p \leq 0.05$ while no statistical difference exist between group 1 and 3 and group 1 and 4at $p \leq 0.05$.

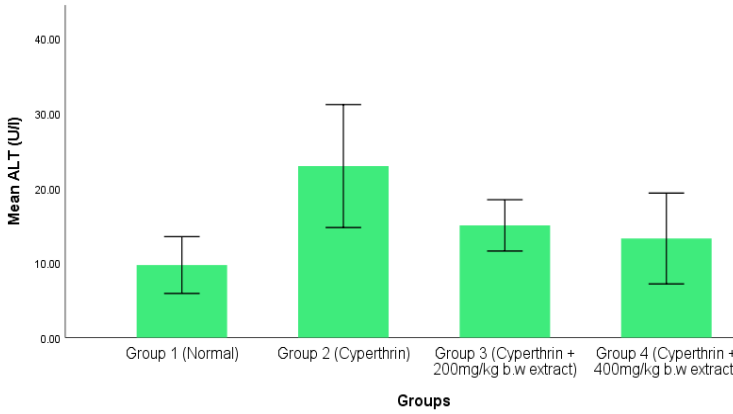


Figure 1.1: The effects of cypermethrin and methanol leaf extract on ALT activity

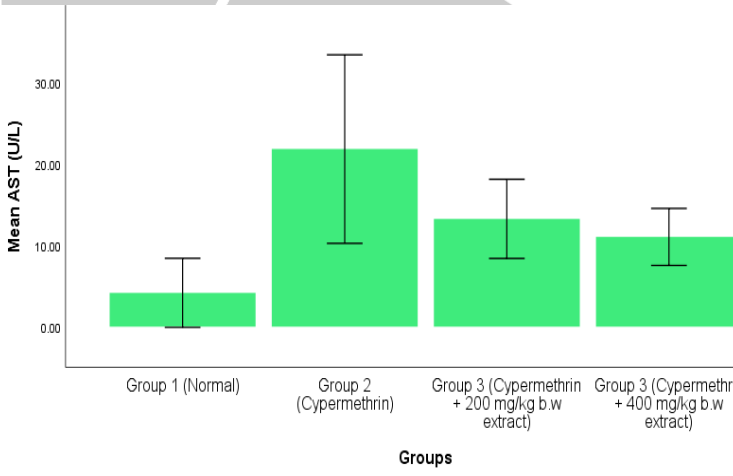


Figure 1.2: The effects of cypermethrin and methanol leaf extract on AST activity

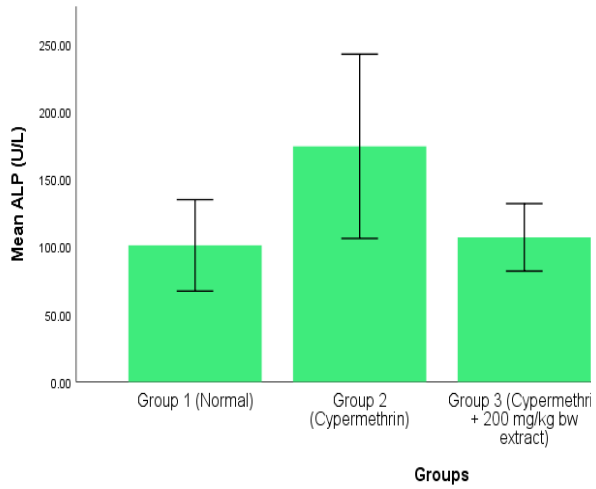


Figure 1.3: The effects of cypermethrin and methanol leaf extract on ALP activity

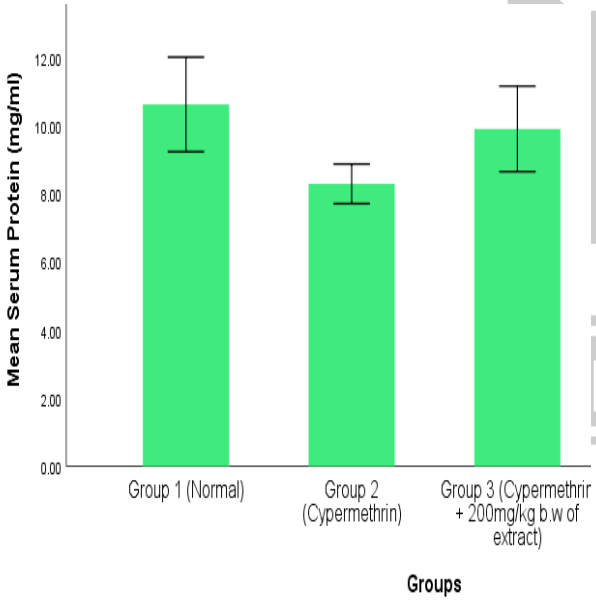


Figure 1.4: The effects of cypermethrin and methanol leaf extract on the serum protein

**LIVER FUNCTION TEST**

The liver function test was carried out to ascertain the working state of the liver. Usually, ALT is found in the liver but once found in the serum could mean that the ALT in the liver leaked into the serum maybe as a result of injury. The result obtained for the ALT analysis (Fig. 1.1) indicated that group 2 had the highest ALT activity of 22.93±4.11 U/L which is statistically different from group 1 (9.70±1.90 U/L) and group 3 (15.00±1.71 U/L). There was no statistical difference (P> 0.05) between group 1 and group 4 (15.22±5.61 U/L) and between group 3 and 4 at $p \leq 0.05$. The result indicated that *C. Odorata* methanol extract helped restoring liver function in group 3 and 4.

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exist between group 1 and 3 and group 1 and 4 at $p \leq 0.05$.

In a related development, it was found that the activities of liver function enzymes correlate with the synthesis of the enzymes in the liver and are important indicators of liver tissue derangement [20]. The authors further stated that the activities of these enzymes are a measure of liver integrity [20]. Liver damage such as necrosis and hepatic degeneration were confirmed through histopathological examinations in rats induced with cypermethrin only. However, rats given C. odorata leaves extracts and induced with cypermethrin showed less necrosis and hepatic degeneration. These showed that the C. odorata leaves extracts may help in the prevention of liver tissue damage. Similar protections by plant extracts were documented [21, 22].

## CONCLUSION

The result obtained from this study justified the medicinal value of *C. odorata*. The extract from C. *odorata* could help to neutralize oxidants caused by cypermethrin and in stabilizing the liver function.

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