

Prophylactic activities of *Olox viridis* in the liver of rats challenged with carbon tetrachloride

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Abstract

Olox viridis is a plant used for its ethnomedical properties. This experiment was focused at assessing the prophylactic activities of *O. viridis* against carbon tetrachloride (CCl₄) - induced hepatotoxicity. Twenty-five male albino rats were randomly divided into five categories. Categories 1 and 2 received distilled water, category 3 received the extract at 100 mg/kg body weight (bw), 4 received the extract at 200 mg/kg while 5 received Silymarin at 100 mg/kg. The route of administration was *per os*, 12 hourly for five days. One hour after the last treatment, groups 2-5 were challenged orally with 0.15 ml/kg of CCl₄ in olive oil. Eighteen hours later, blood samples were collected for serum assay of aspartate amino-transferase, alanine amino-transferase, alkaline phosphatase, total bilirubin and total protein. The effect of the extract on CCl₄ alteration of pentobarbitone sleeping time was assessed using another 5 groups of five rats each. The animals were treated as described for the first experiment. There was no significant difference ($P < 0.05$) between the serum biochemical markers of the animals given 100mg/kg bw of the extract, those given 100mg/kg bw of silymarin and those of the control group but they were significantly different ($P > 0.05$) from those of the category treated with 200mg/kg of the extract and the category given only CCl₄. Similar trend was observed in the pentobarbitone-induced sleeping time experiment. This study reveals that the methanolic root extract of *O. viridis* can protect the liver against CCl₄ induced hepatotoxicity.

Keywords: Hepatotoxicity, Carbon tetrachloride, Herbal medicine, *Olox viridis*, Albino rats

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INTRODUCTION

Plants had been used for medicinal purposes since antiquity. For many centuries ago, the pursuit of remedies for various illnesses has led man into discovering medicinal potentials of various parts of plants. Ancient Chinese and Egyptian papyrus writings described medicinal uses for plants (Petrovska, 2012). Indigenous cultures such as African and Native Americans used herbs in their healing rituals, while others developed traditional medical systems (such as Ayurveda and traditional Chinese medicine). As scientific investigations have discovered, people in different parts of the world use the same kind of plant to cure the same kind of ailments (Kraft, 2009). With recent advancements in scientific investigations, the reasons for the effectiveness of different medicinal plants in management of specific ailments are being discovered (Petrovska, 2012). However, a lot is yet to be done to fully establish the biochemical and pharmacological properties of the numerous plants being used for medicinal purposes (Bhat, 2022).

Olax viridis is called “Atu Ndiomu oha” by the Igbo speaking people of Eastern, Nigeria, and it is a tropical small sized woody plant with multiple stems. The bark root is used in phytomedicine and is known to contain alkaloids, glycosides, flavonoids, saponins, tannins and steroids (Okonkwo and Elechi, 2011; Anderson *et al.*, 2023). Nwaigwe *et al.* (2012) reported the LD₅₀ of *O. viridis* as 1585 miligram per kilogram body weight, their studies also indicated that animals treated with up to 400 mg/kg bw no sign of toxicity was observed. The antimicrobial, ant-venom as well as anti-inflammatory activities of the plant have been scientifically demonstrated (Ajali and Okoye, 2009; Okonkwo and Elechi, 2011; Omale *et al.*, 2013). Nwaigwe *et al.* (2012) demonstrated that the methanolic root extract of *Olax viridis* protects the liver of rats from the damages caused by acetaminophen toxicity.

This experiment was targeted towards assessing this ability of *O. viridis* to protect the liver from toxicity with a different toxic agent – carbontetrachloride (CCl₄). Both acetaminophen and carbon tetrachloride have been shown to induce liver damage through oxidative stress caused by their metabolites. However, In the case of acetaminophen toxicity, the dose-dependent depletion of glutathione results in oxidative stress that induces opening of mitochondrial permeability

transition pores resulting in the destruction of the membrane potential and inhibiting the production of ATP. The resultant effect is damages to cell membranes and DNA, induction of apoptosis and cellular necrosis (Rotundo and Pysopoulos, 2020). In CCl₄ toxicity, CCl₃[•] radical is formed under conditions of low partial oxygen pressure, this forms covalent bonds with cell components and initiates the inhibition of lipoprotein secretion and thus steatosis. However in the presence of oxygen, CCl₃[•] –OO[•] radical is formed which initiates lipid peroxidation resulting in apoptosis and necrosis (Boll *et al.*, 2001). In addition, CCl₄ have been shown to cause fibrosis in the liver, which is very helpful in differentiating the liver damage caused by each of the drugs (Vatakuti *et al.*, 2015). This study therefore, was also aimed at determining if the plant’s mechanism of protection from hepatotoxins is specific on the model of hepatotoxin involved.

MATERIALS AND METHODS

Ethical Approval

This research was conducted with ethical approval received from the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, University of Nigeria, Nsukka with the Approval Reference Number as FVM-UNN-IACUC-2023-03101 and was conducted following standard approved procedures for handling research animals.

Animals

Male wister albino rats (*Rattus norvegicus*) weighing between 100 – 160 g. were used for the research. They were obtained from Veterinary Obstetrics and Reproductive Disease Department of the University, hygienically housed with stainless steel carriages and maintained following the recommended (DDHS, 1985). The rats fed on commercial poultry grower ration, the provision of adequate feed and water for unrestricted feeding was ensured and they acclimatized for a period of two weeks before the experiment commenced.

Plant Material

Fresh roots of the plant were collected from Obollo-Eke in Udenu L.G.A. of Enugu State, Nigeria and were identified in the Department of Plant Science and Biotechnology. A voucher specimen (06/056) was deposited in the Pharmacognosy Herbarium of the same institution.

Preparation of Extracts from Roots of *Oxalis viridis*

The Plant root was cleaned, dried under room temperature and pulverized. The pulverized root was weighed and defatted with absolute N – hexane using Soxhlet apparatus. Extraction was done with 70% methanol over a period of 72 hours; afterwards, concentration to dryness was carried out by means of a vacuum rotary evaporator, then the extract after weighing was subsequently preserved at 4°C before its use.

Analysis of the Hepatoprotective Effects of the Extract on some Serum Biochemical Indices of Rats

This was done following the pattern earlier described (Nwaigwe *et al.*, 2012). The research was done with 25 rats divided into 5 categories. Category 1 was treated with just normal saline 12 hourly, category 2 did not receive any treatment, category 3 was treated with 100 mg/kg of the extract, category 4 200 mg/kg of the extract while category 5 received 100 mg/kg silymarin. All treatments were given per os, 12 hourly for 5 days. An hour following the second treatment on day 5, rats in category 2, 3, 4, and 5 received single dose of carbon tetrachloride in olive oil at 0.15 ml/kg bw per os.

After 18 hours, blood samples were collected from the animals through the retro-orbital sinus using sterile plain tubes, the blood was allowed for 30 minutes under room temperature for coagulation to take effect. Subsequently, separation of the serum was achieved by centrifugation at 3000 revolutions per minutes for 10 minutes. The separated serum was harvested with the aid of syringe and needle and was used for serum biochemical assay of the targeted biomarkers of hepatic injury with the aid of Commercial test kits manufactured by Quimica Clinica Aplicada (QCA), Amposta, Spain. The following biomarkers were assayed for:

1. Aspartate amino – transferase (AST) following the technique of Reitman and Frankel (1979)
2. Alanine amino – transferase (ALT) with the technique of Reitman and Frankel (1979)
3. Alkaline phosphatase (ALP) with the phenolphthalein monophosphate method (Babson *et al.*, 1966).
4. Total bilirubin with the technique of Jendrassik-Grof (Doumas *et al.*, 1973)

5. Total protein level by the Direct Biuret technique (Lubran, 1978).

Hepatoprotective Effect of *O. viridis* on CCl₄ Alteration of Pentobarbitone Sleeping Time

This second aspect of the experiment was done using another set of 25 rats, divided into 5 categories. Each category of animals was treated exactly as in the first stage of the experiment. However, 18 hours after the rats got challenged with CCl₄, all the animals were given injection Pentobarbitone – Na at the dose of 35 mg/kg bw, intraperitoneally. The time anesthesia took place with the loss of righting reflex and the time the animals recovered from anesthesia with the restoration of righting reflex was recorded and mean of the time of sleep for each category of rats was determined

Statistical Analysis

One-Way Analysis of Variance (ANOVA) followed by Duncan's test was used to determine if there are significant differences between the mean serum levels of the biochemical parameters and the mean sleeping time of the different treatment groups. The p values ≤ 0.05 were taken to be statistically significant.

RESULTS

Extract Yield

The methanol extract obtained from the *Oxalis viridis* root had a colour which was reddish brown and a smell that was slightly aromatic. It weighed 115 g showing a yield of 11.5 % w/w.

Analysis of the Hepatoprotective Effects of the Extract on Serum Biochemical Indices of Rats

Administration of carbon tetrachloride resulted in hepatotoxicity in challenged rats as increase in the level of the serum enzymes and total bilirubin indicated, as well as the decrease in level of total protein with reference to those in category 1. The difference between the level of ALT and total bilirubin in serum of the animals in category 1 and those of the category that were pre-administered 100 mg/kg extract and 100 mg/kg Silymarin was not statistically significant ($p \leq 0.05$). While the difference between the levels of AST, ALP and total protein in serum of the category that was pretreated with 100 mg/kg of the extract and that of those that

received 100 mg/kg Silymarin was not statistically significant ($p \leq 0.05$), with reference to the Category 1 animals, the serum levels of the two enzymes significantly increased ($p \leq 0.05$) for both categories while total protein level significantly reduced ($p \leq 0.05$).

The difference between the levels of AST, ALT and total protein of those pre-administered the extract at 200 mg/kg bw and those of the category given at 100 mg/kg and those given Silymarin was not statistically significant ($p \leq 0.05$), however, the ALP level and total bilirubin level increased significantly ($p \leq 0.05$) in the category given 200 mg/kg extract more than in those given 100 mg/kg extract and Silymarin. Furthermore, the AST, ALP and total bilirubin levels of the animals pre-administered the extract at 200 mg/kg increased significantly ($p \leq 0.05$) while the level of their total protein reduced significantly ($p \leq 0.05$) in reference to category 1 animals. Serum enzymes as well as level of total bilirubin of the category that received only carbon tetrachloride were significantly higher ($p \leq 0.05$).

while their total protein significantly reduced ($p \leq 0.05$) with reference to the animals in the categories given 100 mg/kg extract, Silymarin and the category 1 animals (Table 1).

Effect of Methanolic Extract of *O. viridis* on CCl₄ Alteration of Pentobarbitone-Induced Sleeping Time

There was significant reduction ($p \leq 0.05$) in the delay of time of awakening from anesthesia caused by pentobarbitone in animal categories given extract at 100 mg/kg and Silymarin at 100 mg/kg before CCl₄ intoxication as compared to those of the categories that received carbontetrachloride alone and those pretreated with 200 mg/kg extract before intoxication. The difference in sleeping time duration of the animals given just CCl₄ and those of the category of animals pre-administered the extract at 200 mg/kg was not statistically significant (Table 2).

Table 1: Result of the Analysis of the Hepatoprotective Effects of the Extract on Serum Biochemical Indices of Rats

Treatment	AST (I μ /L)	ALT (I μ /L)	ALP (I μ /L)	Total Bilirubin (mg/dl)	Total Protein (mg/dl)
Distilled Water	99.89 \pm 2.97 ^a	33.71 \pm 6.72 ^a	168.95 \pm 9.39 ^a	3.02 \pm 0.21 ^a	6.59 \pm 0.29 ^a
Carbon tetrachloride only	119.65 \pm 1.89 ^{ab}	55.55 \pm 3.34 ^{ab}	220.53 \pm 4.35 ^b	4.75 \pm 0.41 ^{ab}	4.82 \pm 0.27 ^c
100mg/kg Extract + CCl ₄	109.77 \pm 2.79 ^c	38.26 \pm 1.76 ^a	195.79 \pm 3.81 ^{ab}	3.03 \pm 0.27 ^a	6.00 \pm 0.25 ^b
200mg/kg Extract + CCl ₄	111.74 \pm 0.95 ^c	40.08 \pm 2.33 ^a	203.68 \pm 8.03 ^c	3.24 \pm 0.28 ^b	5.86 \pm 0.20 ^b
100mg/kg Silymarin + CCl ₄	107.79 \pm 4.73 ^c	36.90 \pm 9.28 ^a	192.63 \pm 6.11 ^{ab}	3.03 \pm 0.37 ^a	6.18 \pm 0.22 ^b

a, ab, c, = Different superscripts indicates that the means of the animal categories differed significantly ($p \leq 0.05$)

Table 2: Activities of Methanolic Extract of *O. viridis* on CCl₄ Alteration of the Duration of Sleep Caused by Pentobarbitone-Na

GROUPS	DURATION OF SLEEP (MIN)
Distilled Water	90.50 \pm 12.50 ^a
Carbon Tetrachloride only	175.50 \pm 21.92 ^b
100mg/kg Extract + CCl ₄	105.50 \pm 3.54 ^a
200mg/kg Extract + CCl ₄	162.50 \pm 17.68 ^b
100mg/kg Silymarin	101.00 \pm 9.90 ^a

a, b = Different superscripts indicates that the means of the animal categories differed significantly ($p \leq 0.05$)

DISCUSSION

Carbon tetrachloride has been known as a potent liver toxin commonly applied in research works involving the liver (Toriumi *et al.*, 2013). It induces oxidative damage, inflammation, fatty degeneration and hepatic fibrosis. Following the ingestion of CCl₄, the liver metabolizes it to form the reactive trichloromethyl radical by the catalytic action of CYP2E1 – a cytochrome P-450 enzyme. Further oxidizing action leads to the formation of trichloromethyl peroxy radical which is more reactive and tend to bind covalently to macromolecules, leading to lipid peroxidation (Unsal *et al.*, 2021). The lipid peroxidation plays a major role in the toxic effect of CCl₄ observed in the liver by degradation of biomembranes (Al Amin and Menezes, 2023).

The cells of the liver and bile contains the enzymes; alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase (Kalas *et al.*, 2021). When there is an injury or inflammatory conditions in the liver or bile as could result from drug toxicity or xenobiotics, the enzymes tend to be released from the cells of these organs into the blood stream leading to the elevation of their serum levels. Alkaline phosphatase level is mostly increased when the biliary cells are affected (Enoh *et al.*, 2020; Al Amin and Menezes, 2023). The degree of alterations of the enzyme levels is usually determined by the nature of the toxicity, degree of proximity to the toxin and how long the toxicity has lasted (Shi *et al.*, 2003; Song *et al.*, 2003). In conditions of hepatic disease, enzymes within the cells such as ALT seep into the blood stream causing its serum level to increase more than that of AST. However, during conditions of generalised necrosis of cells which could be involving other organs such as the myocardium or skeletal muscles commonly resulting from carbon tetrachloride or paracetamol poisoning, AST tend to increase more than ALT (Enoh *et al.*, 2020; Kalas *et al.*, 2021). Therefore ALT serves as a more effective biomarker for detecting damages to the liver.

Serum ALP, Bilirubin and total protein level are parameters that reflects hepatic cell functions. For instance, when there is increased pressure in the bile ducts, it will lead to higher synthesis of ALP which is then elevated in the serum (Levick, 2017). Elevated total Bilirubin can be associated with pre-hepatic, hepatic, or post-hepatic conditions.

Increase in total Bilirubin of hepatic origin is caused by impaired conjugation and secretion of Bilirubin by hepatocytes consequent upon injury to the hepatocytes. However, it can only be assumed as the situation in this study as the direct Bilirubin assay known to confirm elevated bilirubin of hepatic origin was not carried out (Xu *et al.*, 2019). It has been shown that the products of CCl₄ metabolism can disintegrate the endoplasmic reticulum and break down ribosomes into subunits, separating the 40S subunit from the messenger RNA. Since the major component of the total protein, albumin, is synthesized on an endoplasmic reticulum attached polysome, its disintegration makes the liver incapable of synthesizing albumin leading to reduction in the serum total protein level (Levitt and Levitt, 2016).

In the present study, the statistically significant increase ($P < 0.05$) in the level of serum enzymes and total bilirubin, as well as reduction in the level of the total protein after CCl₄ was administered indicates possible injury to the liver, in contrast to the control categories. It was observed that serum level of AST increased more than that of ALT and this is likely because CCl₄ may have caused damages not only to the liver but other organs also leading to more AST released into the blood stream. However, in animal groups treated with 100mg/kg silymarin, 100mg/kg of the extract and slightly in the groups treated with 200mg/kg extract, it could be seen that there was inhibition of the rise in serum enzymes and total bilirubin, as well as inhibition of total protein level in contrast to those of the group given CCl₄ only, indicating potent hepatoprotection in this groups. According to Gupta and Dixit (2009), the indication that a drug can protect the liver from a toxic agent is that the drug is able to minimize the outcome of the damage caused to the liver or maintain the integrity of the hepatic anatomy and physiology after being exposed to the toxin.

It has been shown that the mechanism of action of some hepatoprotective herbs is increasing the production of protein and nucleic acid by the cells of the liver and facilitating regeneration of cells (Pradhan and Girish, 2006). The antioxidative effect of hepatoprotective herbs have been associated with their ability to scavenge free radicals and interact directly with constituents of the cell membrane in order to maintain the integrity of its lipid fraction and thereby uphold its fluidity (Pradhan and Girish, 2006). Tannins are potent antioxidants which can inhibit the devastating activities of lipid

peroxide in hepatocytes by decreasing or inhibiting their formation, it has been shown to possess the ability to counteract the toxic effect of CCl₄ by inhibiting collagen accumulation, oxidative stress, inflammation and apoptosis (Chu *et al.*, 2016). Furthermore, the ability of a plant material to offer protection to the liver cells has also been associated with their content of other phytoconstituents like flavonoids, saponins as well as alkaloids (Xiong *et al.*, 2003; Vijyan *et al.*, 2003). Therefore the hepatoprotective effect observed with the methanolic root extract of *O. viridis* against CCl₄ induced hepatotoxicity may be attributed to its content of these phytoconstituents (Okonkwo and Elechi, 2011; Anderson *et al.*, 2023) particularly tannins; since tannins are known to be potent antioxidant, and lipid peroxidation is an established mechanism of liver damage caused by CCl₄ (Al-Amin and Menezes, 2023).

The Pentobarbitone sleeping time has been used as a simple and non-invasive test of liver function. Its duration is determined by how fast P-450 cytochromes metabolize pentobarbitone in the liver. The major organ responsible for metabolism of barbiturates is the liver and the duration of sleep after its administration is determined by hepatic metabolism (Battochio *et al.*, 2008). Prolonged anaesthetic recovery can be a problem in individuals whose liver and kidney physiology has been damaged affecting the biotransformation of the drugs (Gupta and Dixit, 2009). Result of the sleeping time test showed a delayed metabolism of the pentobarbitone in the animal group given CCl₄ depicted by prolongation of the sleeping time with reference to the control animal category. However, prior treatment with the extract at 100mg/kg and 100mg/kg silymarin reduced significantly, but 200mg/kg of extract reduced slightly, the sleeping time as comparable with the group given only CCl₄. This shows the protective effects of these agents on the liver leading to more efficient metabolic capability of the liver of treated animals.

Sadek and Saleh (2014) demonstrated that fasting resulted in increased sensitivity to carbon tetrachloride. On the other hand saponins have been suggested to interact with micronutrients which make them unavailable (Sharma *et al.*, 2023). The reduced effect of the extract at 200mg/kg dose compared to that of 100mg/kg, therefore, may be related to increased saponin intake at higher dose which might have by their interaction with the micronutrients resulted in increased sensitivity to carbon tetrachloride.

Bio-Research Vol.21 No.3 pp.2150-2157 (2023)

CONCLUSION

This study has demonstrated that *Oxalys viridis* at the dose of 100 mg/kg was comparable to Silymarin in inhibiting the alteration of serum levels of the liver enzymes, total bilirubin and total protein induced by CCl₄ toxicity. It equally demonstrated similar effect in inhibiting the prolongation of pentobarbitone sleeping time as caused by CCl₄ toxicity. These findings therefore, indicate that the roots of *Oxalys viridis* have protective effects on experimental model of carbon tetrachloride induced hepatotoxicity. This confirms that the plant has the ability to protect the liver from different hepatotoxins, suggesting that its mechanism of protection is by strengthening the liver's physiological or anatomical defense against xenobiotics and not necessarily by antagonizing or neutralizing them.

The histopathological studies of the liver of the experimental animals used in this study were not carried out due to limitation of resources. However, this study will provide baseline information for more advanced studies into the molecular and cellular mechanisms by which *Oxalys viridis* confers protection against hepatotoxins.

Conflict of Interest

The authors do not have any conflict of interest

Author contribution

The study was designed by OUC, NCU and MII. OUC, NCO and MEC performed the experiments. NCU did the statistical analysis. All the authors were involved in writing and approval of the final draft of the manuscript.

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