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Research Article

Investigation of the Hepatoprotective Activity of Leaf Extract of Clerodendrum Polycephalum

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ABSTRACT

The hepatoprotective effect of leaf extract of clerodendrum polycephalum was investigated using albino wistar rats. Carbon tetrachloride (CCl_4) was used to induce liver damage. Preliminary phytochemical analysis revealed the presence of Resins, Saponins, Tannins, Terprenoids and Steroids. Acute toxicity study showed an oral LD_{50} greater than 400mg/Kg in rats. The rats were divided into five groups (A- E) of 5 rats each. Group A served as the normal control group and received no treatment; Groups B-D served as the experimental treatment groups and received 100,200, and 400mg/Kg daily of the extract while group E was the control treatment and was administered 50mg/Kg of silymarin daily. Treatment lasted for two weeks before CCl_4 intoxication. Blood was collected via retro-orbital puncture for liver marker enzyme studies. Total protein and serum albumin levels were also estimated. Results showed a significant decrease ($p<0.001$) in the levels of alkaline phosphatese(ALP), alanine transaminase (ALT) and aspartate aminotransfarase (AST) in the treatment groups which appears to be dose-dependent but total protein and serum albumin levels increased. Methanol extract of clerodendrum polycephalum demonstrated significant hepatoprotective activity and improved liver function.

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INTRODUCTION

Man has sought to combat and control disease and pain with assistance, inspiration and guidance from his natural environment. Several plant materials from roots, stems, barks, leaves, flowers, and so on, by instincts, intuition or trial and error have been used to combat various ailments. In Europe (1400-1500), use of medicinal plants was based on doctrine of signatures developed by Paracelcus (1490-1514) which stated that healing herbs have features made by God for identifying which part of the body they can treat. Plants with heart shaped leaves were used for treatment of heart diseases; those with liver shaped parts were prescribed for bilious diseases [1].

In Nigeria, many indigenous plants are used as food spices or medicinal plants [2]. Many of the plants possess bioactive compounds that inhibit physiological activity against bacteria and micro-organisms.

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These plants are traditionally used in the treatment of rheumatism, diarrhea, dysentery, cough, asthma, diabetes, malaria, cold, and so on [3, 4].

The liver performs many functions essential for life. It receives processes and stores amino acids, carbohydrates, lipids, vitamins and minerals. Plasma proteins are also produced in the liver. The liver is the site of detoxification of exogenous compounds such as drugs and toxins. Hepatic injury is associated with distortion of metabolic functions [5]. Liver diseases remain one of the most serious health problems which modern medicine has little for their alleviation. Thus, there has been a lot of dependency on plant extracts for treatment of liver disorders.

Clerodendrum species are widely distributed in the tropical and subtropical regions and there are about 500 species with some having medicinal properties [6]. It is used in different countries including India, China, and Africa for treatment of diseases such as syphilis, typhoid, cancer, jaundice and hypertension.

Carbon tetrachloride is very toxic and we get exposed to it via inhalation, ingestion and absorption. On entry into the body, it causes a lot of injury to body organs including lungs, heart, liver, kidney, central nervous system and GIT [7]. Ingestion of CCl_4 can lead to marked hepatotoxicity [5].

Thus, the present study is designed to investigate the hepatoprotective effect of methanolic extract of leaf of clerodendrum polycephalum in rats exposed to CCl_4 intoxication.

MATERIALS AND METHODS

Plant Materials:

Fresh leaves of cleodendrum polycephalum were obtained in Sango Ota in Ota local Government Area of Ogun State,South- West, Nigeria. The leaves were authenticated at the herbarium unit of Botany Department, Univeristy of Lagos, Nigeria by Prof Dele Olowokudejo and a voucher specimen (ULAGH/104B) deposited for future references.

Methanol Extraction:

The leaves were dried under shade and powdered with a mechanical grinder and stored in an air-tight container. 500g of the powdered material was soaked in 2.5L of 80% methanol for 48 hours with intermittent agitation. The solution was filtered using Whatmann no.1 filter paper, evaporated to dryness using rotary evaporator (Model 349LZ, Corning, England). The extract yielded 11.6% (w/w). 10g of the residue was dissolved in normal saline and made up to 100ml with distilled water.

Experimental Animals:

Thirty-Five Albino Wistar rats weighing 140-200g were used for this study. They were obtained from the animal house of the College of Medicine, University of Nigeria, Enugu Campus. They were housed in stainless cages and maintained under standard conditions (Temperature: 25±5^{0}C) with 12:12hr light/dark cycle. The rats were allowed to acclimatize for 2 weeks, they were fed with standard rat feed (Super Starter-Guinea feed® Nig. PLC) and water was provided ad libitum. All animals were handled according to international guideline for handling experimental animals [8].

Experimental Design

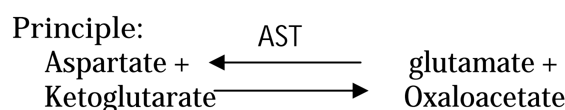
Acute toxicity study was carried out using the method of Lorke [9] and 10 rats were used. The remaining 25 rats were divided into five groups of 5 rats each. Group A served as the normal control and were fed with normal rat feed only. Rats in groups B-D were given 100, 200, and 400mg/Kg of the extract daily respectively for 2 weeks. Group E was the control experimental group and were given 50mg/Kg of Silymarin daily for 2 weeks. On day 15, 2mg/Kg of CCl_4 was injected subcutaneously to all the rats in every group after an initial 12 hour fasting. 24 hours after CCl_4 administration, blood samples were collected by retro-orbital puncture and analyzed for liver marker enzymes. The samples were left to clot, centrifuged at 4000rpm for 10minutes to serum. Sera were stored in a refrigerator at 4^{0}C and analyzed the next day.

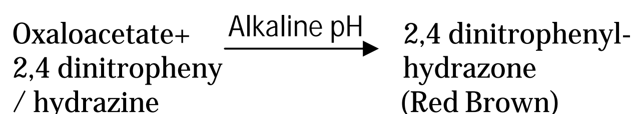
Determination of Liver Marker Enzymes

Measurement of serum AST

The colorimetric method of Reitman and Frankel [10] was used.

Principle:





Absorbance read at 505 nm.

Materials: Buffered substrate, Serum, Dinitrophenlhydrazine (DNPH), 0.4N sodium hydroxide (NaOH) (Table 1)

Table 1: Procedure for determination of Liver Marker Enzymes Measurement of serum AST

Procedure	Test	Control
Buffered substrate (Incubate at 37^{0}c for 3 minutes)	0.5ml	0.5ml
Serum (Incubate at 37^{0}c for 60 minutes)	0.1ml	-
Serum	-	0.1ml
DNPH (Incubate at room temperature for 20 minutes)	0.5ml	0.5ml
0.4N N90H	5.0ml	5.0ml

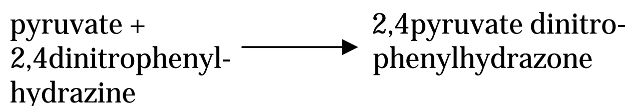
Allowed to stand at room temperature for 5- 10 minutes and absorbance read at 505nm.

Enzymes activity: Absorbance of Test – control
Enzyme activity determined from the calibration curve.

Estimation of Serum Alt

Method: The colorimetric method of Reitman and Frankel [10] was used.

Principle:



The absorbance of the brown hydrazone colour produced was measured at 505nm.

Procedure: ALT was estimated in the same way as the AST except that it uses alanine as the substrate and the incubation period is 30 minutes instead of 60 minutes.

Enzymes activity:

The enzyme activity was determined from the calibration curve.

ESTIMATION OF SERUM ALKALINE PHOSPHATASE

Principle: Serum level was determined using the method of Roy [11], provided by Teco diagnostics, USA. The enzymes alkaline phosphate hydrolyses the substrate, disodium phenylphosphate to release phenol. The quantity of phenol released under standardized conditions of time, temperature and pH, is measured by the absorbance of red colour it assumes in alkaline solution.

The phenol reacts with 4 – aminophenazone in the presence of alkaline potassium ferricyanide to produce red colour. Absorbance read at 520nm (Table 2).

Statistical Analysis

Data were analyzed using student's t-test and results expressed as mean±SEM. Differences between means were determined by ANOVA. Values of $p<0.05$ were considered significant.

RESULTS

Preliminary phytochemical analysis revealed the presence of large amount of Resins and Saponins, moderate amount of Tannins and Terprenoids and little amount of Steroids (Table 3).

Acute toxicity study showed an oral LD_{50} of greater than 400mg/Kg.

Biochemical analysis of sera showed that the levels of ALT, AST and ALP were significantly raised ($p<0.001$) in group A (normal control) compared to those in the treatment groups (Table 4). There was a reduction in the levels of ALT and AST in a dose-dependent manner in the extract treated groups, ALP level also reduced but did not follow a similar pattern. Marker enzyme levels obtained at the highest dose of the extract (400mg/Kg) were closest and similar to those of the Silymarin treated group. AST/ALT ratio was less than 1 in all the groups (Table 4).

There was increase in total protein and serum albumin levels in the treatment groups, with the highest increase seen in group D (Table 5).

DISCUSSION

Liver injury induced by CCl_4 is characterized by impairment of several cellular functions, including those of the mitochondria. This is due to the fact that CCl_4 is converted to a highly reactive and toxic free radical which initiates auto-oxidation of fatty acid present within the membrane and consequent oxidative decomposition of lipids [12]. The organic peroxides formed will lead to swelling of smooth endoplasmic reticulum and dissociation of ribosomes from rough endoplasmic reticulum. This causes accumulation of lipids due to inability of cells to synthesize lipoproteins from triglycerides and lipid acceptor proteins leading to fatty liver. Release of products of lipid peroxidation causes damage to plasma membrane owing to its increased permeability, followed by progressive swelling of cells, massive influx of Ca^{2+} and cell death [13].

Increased serum levels of ALP, AST, and ALT as observed in the normal control group may suggest loss of integrity of cell membrane and cellular leakage [5].

AST/ALT ratio greater than 1 has been identified as a predictor of severe liver fibrosis [14] but in all the groups studied, the ratio was less than 1 suggesting the absence of such disorder.

In the present study, the fall in the serum levels of liver marker enzymes in the extract treated groups, especially at the highest dose was similar to that produced by Silymarin, a powerful antioxidant with strong hepatoprotective effect. This suggests that the extract protected the liver from injury caused by CCl_4, more so, in a dose-dependent manner.

Table 2: Procedure for estimation of Serum Alkaline Phosphatase

Procedure	Test	Blank	Standard	Standard blank
Buffer substrate (Incubate at 37^{0}C for 2-3 minutes)	2.0ml	2.0ml	1.1ml	1.1ml
Serum (Incubate at 37^{0}c for Exactly 15 minutes)	0.1ml	-	-	-
0.5M NaOH	0.8ml	0.8ml	-	-
Standard	-	-	1.0ml	-
0.5M NaoH	-	-	0.8ml	0.8ml
0.5M $NaHCO_3$	1.2ml	1.2ml	1.2ml	1.2ml
Serum	-	0.1ml	-	-
4–aminophenzone	1.0ml	1.0ml	1.0ml	1.0ml
Potassium ferrocyanide	1.0ml	1.0ml	1.0ml	1.0ml

Absorbance read at 520nm.

Table 4: Comparison of the liver marker enzymes in the treated groups with the control group

GROUPS	ALP (iu/L)	ALT (iu/L)	AST (iu/L)	AST/ALT
A Control	96.8±6.37	78.6±5.77	65.8±3.36	0.86±0.75
B 100mg/Kg MECP	34.0±6.17 a. c	45.8±2.87 a. d	44.6±2.87 b. e	0.96±0.74
C 200mg/Kg MECP	53.4±5.11 b	40.0±2.51 a. e	38.0±2.24 a, e	0.94±0.68
D 400mg/Kg MECP	36.4±2.42 a. c	34.6±2.66 a	24.2±2.24 a	0.68±0.64 b
E 50mg/Kg Silymarin	45.2±2.60 a	26.4±2.56 a	20.8±2.42 a	0.76±0.34 b
F- Ratio	28.06	58.02	38.41	38.1
P- Value	P<0.001	P<0.001	P<0.001	P<0.01

a = P < 0.001 with respect to normal control; b = P < 0.01 with respect to normal control
c = P < 0.05 with respect to Silymarin; d = P < 0.001 with respect to Silymarin
e = P < 0.01 with respect to Silymarin

Table 3: Result of preliminary phytochemical Analysis

Sl. No.	Phytochemical Parameters	Results
1	Alkaloid	-
2	Saponins	+++
3	Tannins	++
4	Glycosides	-
5	Flavonoids	-
6	Resins	+++
7	Carbohydrates	-
8	Protein	++
9	Reducing Sugar	-
10	Oil	-
11	Steroids	+
12	pH	Neutral
13	Terprenoids	++

Key:
- \- Absent
- \+ Present in small concentration
- ++ Moderate high concentration
- +++ Very high concentration

Table 5: Total Protein and Serum Albumin levels in treated and control groups

GROUPS	T. PROTEIN (g/dl)	ALBUMIN (g/dl)
A Control	4.80±0.80	3.50±0.55
B 100mg/Kg MECP	5.40±0.81	3.86±3.84
C 200mg/Kg MECP	6.40±0.24	4.0±4.4
D 400mg/Kg MECP	6.80±0.37	4.36±3.84
E 50mg/Kg Silymarin	6.0±0.0	4.10±3.38
F- Ratio	1.240	1.00
P- Value	P >0.05	P >0.05

Clerodendrum polcephalum contains Saponins, Resins and Tannins which are well known for their antioxidant and hepatoprotective activities [15]. Antioxidants such as Silymarin has been shown to stabilize plasma membrane and repair damaged hepatic cells [16]; antioxidants are also known to interrupt the process leading to destruction of hepatic cells, restore cellular methylation or promote calcium sequestration [17]. Antioxidants can also prevent the

metabolism of CCl_4 into a toxic metabolite and consequently minimize the production of free radicals [18]. The antioxidant present in the chemical constituents of clerodendrum polycephalum may be responsible for the observed hepatoprotective activity.

Low serum albumin indicates poor liver function [19]. Liver function is reduced or impaired by agents or diseases that cause injury/damage to the liver such CCl_4 and Liver cirrhosis. The increase in total protein and serum albumin levels observed in the treatment groups may be an indication of improvement in liver function.
Thus, methanolic extract of clerodendrum polycephalum protected the liver from injury caused by CCl_4 and caused improvement in liver function.

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