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Research Article

Curative and Protective Effect of Ethanolic Leaf Extract of Cassia occidentalis on Carbon Tetrachloride Induced Kidney Damaged of Albino Rats

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Abstract

Cassia occidentalis is one of many medicinal plants used in treatment of many diseases locally, and is used as diuretics, laxative, anti-bacteria, anti-inflammatory, anti-fungal, used in treatment of liver diseases and used externally applied on healing wounds, sores, itch, skin diseases, bone fracture, ringworm and throat infection. Therefore, the needs for ascertaining the efficacy of the extract in management of kidney damage become imperative. The research was aimed at evaluating the reno-curative and reno-protective effects of ethanolic leaf extract of *Cassia occidentalis* in carbon tetrachloride induced kidney damaged of albino rats. The albino rats were grouped into six (6) groups, group I and II served as normal control which received food and fluid only and negative control which received carbon tetrachloride, feed and water respectively, group III and IV served as the hepato-protective groups and group V and VI as hepato-curative groups. The rats at the end of 6 weeks were anaesthetised by chloroform in a close jar and blood samples were collected for biochemical study, and the albino rats were sacrificed by cervical dislocation. The kidneys were harvested for histopathological analysis and the relative organ weight was determined. The result revealed that administration of the carbon tetra chloride to albino rats induced renal toxicity, observed from the study the reno-protective and reno-curative ability of the ethanolic leaf extract was seen across the test group which lead to the significant differences observed in the level of urea and creatinine when compared to negative test control (-ve control) kidney function parameters. Conclusively, the treatment of kidney related diseases may be protected and cured following the administration of Cassia occidentalis leaf extract.

Keywords: *Cassio occidentalis; Urea; Creatinine; Protective; Curative; Carbon Tetrachloride*

Introduction

Traditional medicine is considered as the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses [1]. Natural medicinal products are increasingly gaining popularity and used worldwide as complementary alternative therapies [2].

Cassia occidentalis have been reported to have many pharmacological effects including antimicrobial, anthelmintic, insecticidal, antioxidant, antianxiety, antidepressant, antimutagenic antidiabetic and wound healing, hepato-protective, anti-inflammatory, analgesic, antipyretic and other effects [3]. The plant is a shrub in nature and was reported to be a native of America and mainly used for landscape flowering purposes [4]. *Cassia occidentalis* (Coffee senna) is also reported to be used as a substitute to coffee. The seeds of the shrub are brewed into the coffee-like beverage and used for the treatment of asthma [5]. The plant is widely used by the local people of Hausa-Fulani and igbo tribe in northern and southern Nigeria for the prevention and treatment of various diseases (liver and kidney diseases inclusive) [3]. It was also been used for the treatment of stomach disorders, rheumatism, and some liver diseases [6,7]. The leaves are commonly used as a leaf vegetable and are eaten either raw or in a mixture with coconut, chilli, and onion [8,9].

Kidneys are the major organs in eliminating toxic compound metabolized by the liver, it receives about 1200ml of blood per minute [10], containing a lot of chemical compounds. Therefore, damage to the kidneys can be determined by measuring the level of urea, electrolyte and creatinine in blood as an indicator of kidney damage. Urea is a byproduct from protein breakdown. About 90% of urea produced is excreted through the kidney [11]. Meanwhile, the creatinine is a waste product from a muscle creatinine, which is used during muscle contraction. Creatinine is commonly measured as an index of glomerular function [12].

Carbon tetrachloride is a solvent that has been used in the past as a cleaning fluid or degreasing agent, as a grain fumigant, and industrially in the synthesis of refrigeration fluid and propellants for aerosol cans [13]. Although most of these uses have been discontinued, the possibility still exists for carbon tetrachloride to be released to the environment, primarily through industrial processes or old bottles of cleaning agents containing carbon tetrachloride that may still be in the home [13]. Renal injury is observed in animal studies, but usually at higher doses with lesser severity than in humans [13]. In oral exposure studies, the effect levels for kidney toxicity are generally higher than for hepatic toxicity [13]. Some intermediate-duration inhalation bioassays in rats reported the adverse effect level for the kidney to be the same as or higher than that for the liver. In a 2-year inhalation study in F344 rats, exposure to carbon tetrachloride at hepatotoxic levels increased the severity of chronic progressive nephropathy compared to the control group [13].

The critical process underlying CCl_4 hepatotoxicity is the combining effect of both lipid peroxidation and the covalent binding of CCl_4 reactive metabolites to lipids and proteins (Masuda and Nakamura, 1990). It has been shown that CCl_4 induced lipid peroxidation can be obstructed by natural antioxidants [14]. The identification of naturally occurring inhibitors of peroxidation resulting in cell damage could therefore lead to important new strategies for disease prevention [14]. Carbon tetrachloride and its metabolites are excreted primarily in exhaled air and to a lesser extent in the urine and faeces (IPCS, 1999). Studies on the uptake, tissue distribution and elimination of carbon tetrachloride by mice, rats and hamsters have shown that rats are the least sensitive to the hepatotoxic effects of repeated inhaled carbon tetrachloride (32 - 770 mg/m^3) and are notably less sensitive than mice to the hepatocarcinogenic effects [15] and that this species sensitivity correlates with carbon tetrachloride equivalent dose to liver and with the ability to metabolize carbon tetrachloride [15,16]. Predictions obtained from physiologically based pharmacokinetic models suggest that metabolism of carbon tetrachloride in the average rat is greater than that in the average human (Delic., *et al.* 2000). Calore., *et al.* in their studies demonstrated that oral treatment of pregnant rats with hydroalcoholic extracts of *Cassia occidentalis* stem and leaf in doses of 0.25 and 0.5 g/kg did not change most of the reproductive parameters [17]. it was observed that there an index of dead foetuses from pregnant rats treated with *Cassia occidentalis* 0.25 and 0.5 g/kg, suggesting a possible abortive effect of this extract [18]. However, the study aimed at evaluating the Curative and protective effects of ethanolic leaf extract of *Cassia occidentalis* in carbon tetrachloride induced kidney damaged of albino rats.

Materials and Methods

Ethical approval

Necessary approval was sought and obtained from the Ethical Committee, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus.

Collection and authentication of the plant material

Fresh mature leaf samples of *Cassia occidentalis* were collected in Nnewi, Anambra State, and botanical identification of the plant was done by Mr. Egboka Tochukwu of the Department of Botany, Nnamdi Azikiwe University, Awka.

Plant preparation

All the samples of *Cassia occidentalis were* thoroughly rinsed with running tap water and distilled water before being air-dried at room temperature for 30 days. Then, the plant sample was pulverized to dry powder using an electric grinder into minute pieces and the extract was soaked in absolute ethanol for 4 days with frequent agitation at room temperature. The extract was filtered with Whatman paper No. 1 and the residue of fine powder was then re-soaked with a fresh portion of ethanol twice for four days each time at room temperature. The filtrate was concentrated under reduced pressure in vacuum at 45ºC and evaporated to dryness on a rotary evaporator (Model 342/7, Corning Ltd). The yield of the extract was 17.1% based on dry weight.

Animal care

All experimental investigations were done in compliance with "humane animal" as stated in the "Guide to the care and use of Laboratory Animals Resources" (NRC, 2011). A total of 30 male albino wistar rats were used for this study. Animals were acclimatized for two (2) weeks in the animal holding unit of the Anatomy Department, Faculty of Basic Medical Sciences, Nnamdi Azikwe University, Nnewi Campus. The animals were maintained under standard and good laboratory conditions of light (12 hours), temperature (23 ± 2°C), humidity (60% - 70%) and ventilation. They were given standard rat diet purchased from the same farm to avoid changes in dietary compositions and weight variability and adequate water ad libitum was given.

Experimental protocol and animal grouping

After acclimatization, animals were divided into six groups; A, B, C, D, E and F (n = 5). Group A was the control group with animals receiving feeds (Grower's Mesh produced by flour mills Nigeria) and distilled water only. Considering the lethal dose which was reported in a study by Nnama., *et al.* [19]*;* the animals were grouped and treated as follows;

- Group B: Carbon tetrachloride 0.2 mls, diluted with 'olive oil/kg daily for 2 weeks.
- Group C: Carbon tetrachloride 0.2 mls, diluted with 'olive oil/kg for 2 weeks then carbon tetrachloride 0.2 mls diluted with 'olive oil /kg + *Cassia occidentalis* 1000 mg/kg for 4 weeks.
- Group D: Carbon tetrachloride 0.2 mls, diluted with 'olive oil/kg for 2 weeks then carbon tetrachloride 0.2 mls diluted with 'olive oil /kg + *Cassia occidentalis* 2000 mg/kg for 4 weeks.
- Group E: *Cassia occidentalis* 1000 mg/kg for 2 weeks then carbon tetrachloride 0.2 mls, diluted with 'olive oil /kg + *Cassia occidentalis* for 4weeks.
- Group F: *Cassia occidentalis* 2000 mg/kg for 2 weeks then carbon tetrachloride 0.2mls, diluted with 'olive oil + *Cassia occidentalis* for 4weeks.

Carbon tetrachloride and ethanolic leaf extract of *Cassia occidentalis* was done orally using 2 mls syringe and a cannula.

Animal sacrifice, organ harvesting and collection of blood samples

At the end of the experiment, animals were anesthetize *with diethyl ether in a close jar,* blood samples were collected directly from the apex of the heart (at the thoracic region) using 2 ml syringes and *put into plain* serum bottle, and then serum were separated by centrifugation and was stored in a refrigerator of temperature -18°C for biochemical analysis. Thereafter the animals were sacrificed with overdose of anesthetic (diethyl ether). Each animal was placed on the dissecting board, pinned to the board and dissecting set (sharp scalpel on scalpel holder for making incision; scissors for cutting and dissecting forceps for harvesting) were used to harvest the kidney which was immediately weighed before transferring into 10% formal saline for proper fixing for histological sectioning and histochemical analysis.

Biochemical analysis

Blood samples collected and centrifuged were used for kidney function assessment i.e. analysing the level of urea and creatinine

Statistical analysis

Data were expressed *as* Mean ± Standard Deviation (SD) using tables. The differences between the groups were compared for statistical significance by using ANOVA and student '' test. $P < 0.05$ was taken as the significant level.

Results

Table 1 showed organ weight for test and control groups, data showed mean and standard deviation. Analysis of variance (ANOVA) between groups showed significant difference in weights of the left kidney ($p < 0.05$) when compared with the negative control group B.

Organ Wt	Group A(g)	Group C(g)	Group D(g)	Group E (g)	Group F (g)	Group B -(-ve Control)	Sig
Right Kidney	0.85 ± 0.13*	0.70 ± 0.00	0.73 ± 0.05	0.73 ± 0.13	0.73 ± 0.13	0.55 ± 0.21	0.075
Left Kidney	0.78 ± 0.05*	0.88 ± 0.05*	0.70 ± 0.00*	0.70 ± 0.08*	0.70 ± 0.08*	0.55 ± 0.21	0.004

Table 1: *Organ weight for test and control groups.*

Table 2 showed relative organ weight for test and control group, data showed means and standard deviation. Analysis of variance (ANOVA) between groups showed significant difference in % relative weight of the left kidney ($p < 0.05$). Post-hoc analysis further depicted significant increase in % relative weight between normal control and test groups with the - Ve control group.

% of Organ Weight in Relation to Body Weight	Group A	Group C	Group D	Group E	Group F	Control Group B	Sig
Right Kidney	0.43 ± 0.07*	0.34 ± 0.01	0.36 ± 0.02	0.37 ± 0.07*	0.36 ± 0.15	0.25 ± 0.10	0.056
Left Kidney	0.39 ± 0.01*	0.42 ± 0.03*	0.34 ± 0.01*	0.36 ± 0.04*	0.46 ± 0.10	0.25 ± 0.10	0.003

Table 2: *Percentage of Organ Weight Relative to Body Weight of the albino rats.*

Table 3 showed kidney function test for all groups data showed, mean and standard deviation. Analysis of variance (ANOVA) showed significant difference in creatinine ($p < 0.005$), urea ($p < 0.05$).

Parameter	Group A	Group C	Group D	Group E	Group F	Group B (control)	Sig
Creatinine(mmol/l)	27.30 ± 2.15*	57.73 ± 6.23*	58.03 ± 7.10*	29.57 ± 0.47*	65.07 ± 3.36*	9.15 ± 1.34	0.000
Urea(mmol/l)	3.20 ± .40*	3.03 ± .71*	3.67 ± .12*	1.90 ± 0.00*	3.80 ± .26*	1.45 ± .35	0.002

Table 3: *Kidney function test for all groups.*

** Significant increase ($p < 0.05$) when compared to the control.*

Histological findings

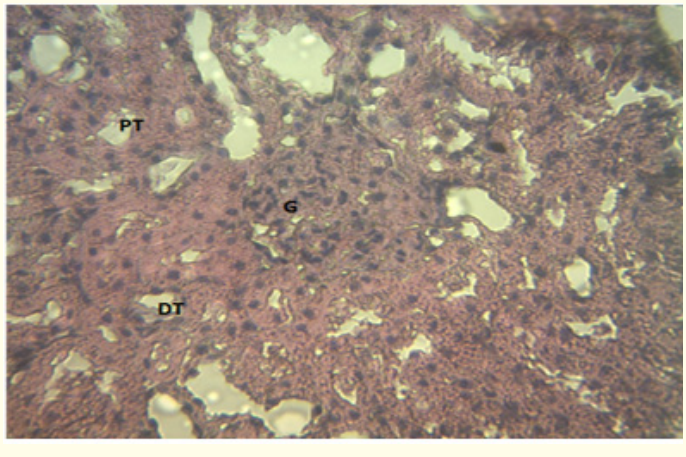

Plate 1: *Photomicrograph of the kidney of group A (control) of albino rat, with a normal kidney histo-architectural features with no visible lesion, showing proximal convoluted tubule (PCT), distal convoluted tubule (DCT) and glomerulus (G) of normal histology [H&E ×400].*

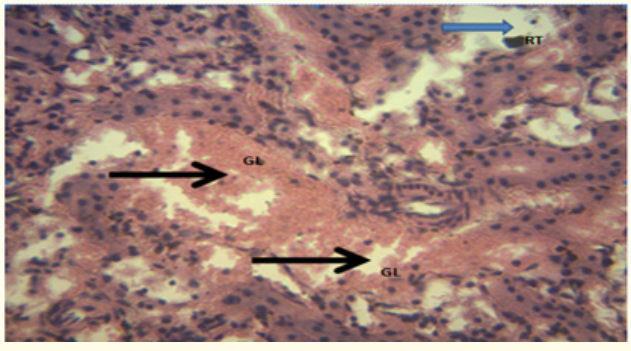

Plate 2: *Photomicrograph of the kidney of group B (-Ve control) of albino rat, showing very severe diffuse interstitial congestion and haemorrhage, glomerulus (GL). Many tubules are necrotic and have protein casts. Renal tubules (RT) [H&E×400].*

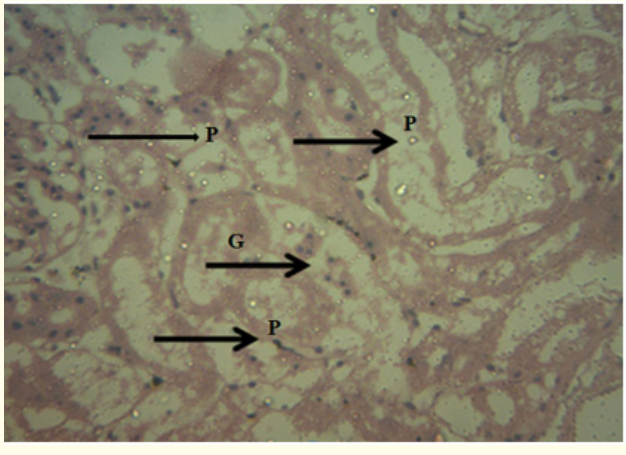

Plate 3: *Showed the Photomicrograph of kidney of group C of albino rat showing the renal glomeruli (G) and the renal tubules (P) entirely disrupted [H&E×400].*

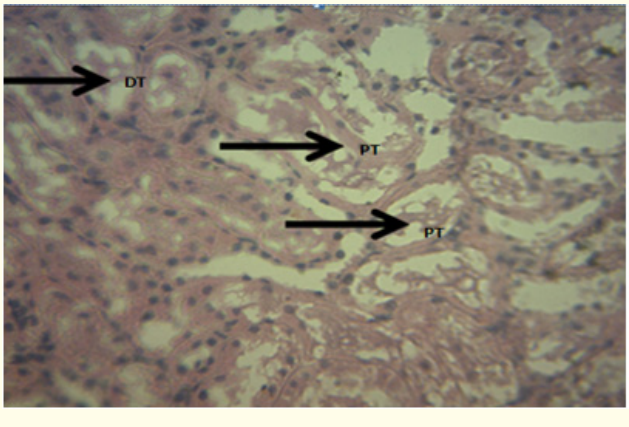

Plate 4: *Photomicrograph of the kidney of group D of albino rat showing kidney tubules are degenerated and have protein casts. Distal tubules (DT), proximal tubules (PT) [H&E×400].*

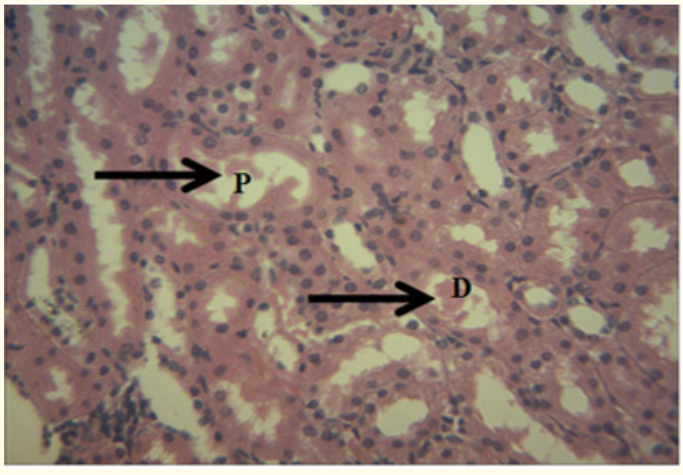

Plate 5: *Showed the Photomicrograph of kidney of group E of albino rat showing proximal tubules (P) distal tubules (D) with protein cast in their lumina [H&E×400].*

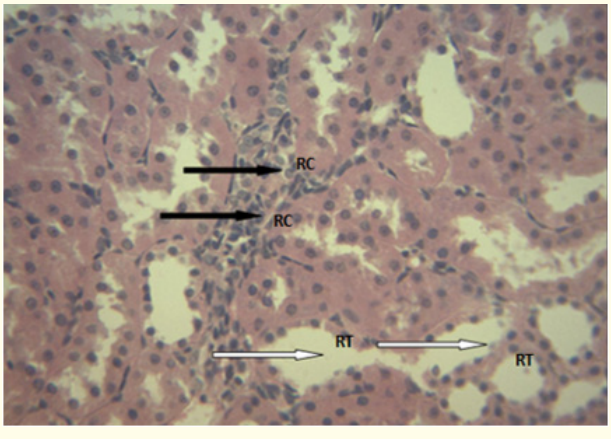

Plate 6: *Photomicrograph of the kidney of group F of albino rat showing the tubules having enlarged Lumina (RT) and a mild congestion of the renal cortex (RC) [H&E×400].*

Discussion and Conclusion

Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a drug or toxin. Many medicinal plants have been reported to exhibit protective effect and curative effects of renal tissues against injuries.

The organ body weight ratio is defined as the ratio of the organ weight to that of the body weight of the organism. This ratio gives an idea of whether the extract administered interacts with the various organs favorably or unfavorably. The result of the organ body ratio revealed a gradual significant difference in the ratio with increase in the organ ration of the left kidney which agreed with the study by Möell 1956, that it is known that the left kidney is larger than the right one, regardless of gender [20]. Equally, the result of organ weight (Table 1) showed significant difference in weights of the left kidney ($p < 0.05$) when compared with the negative control group B.

Administration of the carbon tetra chloride to rats induced renal toxicity, observed from the study the reno protective and curative ability of the ethanolic leaf extract was seen across the test group which lead to the significant differences observed in the level of

urea and creatinine (Table 3) when compared to negative test control (-ve control) kidney function parameters and was supported by histopathological analysis of the tissues which showed very severe diffuse interstitial congestion and haemorrhage, glomerulus (GL) to structures of the kidney just have protein casts and the tubules having enlarged Lumina (RT) and a mild congestion of the renal cortex (Plate 3-6). This may be as a result of un-favorable interaction (cellular constriction and inflammation) of the extract or its component with the respective organs. The histo pathological findings also revealed that the protective and curative efficacy of the leaf extract maybe dose dependent as such that the higher the dose, the higher the efficacy. The study agreed with a research by Alhassan., *et al.* [3] that following administration of the extract led to slight decrease in both serum urea and creatinine suggesting that the extract does possess a nephron-curative effect on the kidney. Tanimu and Wudil 2012, reported that the leave extract has a curative effect on kidney using 300, 600, and 900 mg/kg does of the extract for 14 days. The observed decrease in the serum urea and creatinine concentration may become significant at higher dosage of the extract [21].

Conclusively, following the administration of ethanolic leaf extract of *Cassia occidentalis*, that the extract possessed restorative and protective efficacy and therefore may be recommended for the treatment of kidney related diseases.

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