

Effect of Aqueous-Methanol Leaves Extract of *Cassia occidentalis* on Carbon Tetrachloride Induced Hepatotoxic Rat

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Abstract

The indiscriminate usage of different parts of *Cassia occidentalis* in the management and/or treatment of several diseases has led to several researches about the plant's safety, efficacy and probable mode of action. This research investigates the effects of aqueous-methanol leaves extract of *Cassia occidentalis* on liver function indices (ALT, AST, ALP, Total protein, Albumin, Bilirubin and Globulin) in carbon tetrachloride induced hepatotoxicity. A total of twenty five rats divided into five groups of five rats each were used. Group I served as normal control, Group II-V were induced with hepatotoxicity using CCl₄ (120mg/kg bodyweight). Group II served as test control, group III and IV were administered with the extract at a dose of 50mg/kg and 100mg/kg while group V were administered with standard drug (10mg/kg of livolin), per day for two weeks. The animals were euthanized after 24 hours of last extract administration and liver function indices (ALT, AST, ALP, total protein, Albumin, Bilirubin and Globulin) were assayed. A significant increase ($p < 0.05$) in ALT, AST, ALP, Total protein and Globulin was observed in test control group compared to normal control. Administration of the extract led to a significant decrease ($p < 0.05$) in ALT, AST, ALP, total protein and Globulin in a dose dependent manner compared to test control group. The observed hepatocurative effect of the plant may be due to the presence of phytochemicals.

Keywords: *Cassia occidentalis*; leaves; carbon tetrachloride and hepatocurative.

1. Introduction

Liver is the largest organ of the human body weighing approximately 1.5kg, and is located in the upper right corner of the abdomen on top of the stomach, right kidney and intestines and beneath the diaphragm. The liver performs more than 500 vital metabolic functions (Naruse *et al.*, 2007). It is involved in the synthesis of products like glucose derived from glycogenesis, plasma proteins, clotting factors and urea that are released into the blood stream. It regulates blood levels of amino acids. Liver parenchyma serves as a storage organ for several products like glycogen, fat and fat soluble vitamins. It is also involved in the production of a substance called bile that is excreted to the intestinal tract (Saukkonen *et al.*, 2006).

Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics. The substance that cause liver injury are referred to as hepatotoxins or hepatotoxicants. The hepatotoxic response elicited by a chemical agent depends on the concentration of the toxicant which may be either parent compound or toxic metabolite, differential expression of enzymes and concentration gradient of cofactors in blood across the acinus (Navarro and Senior 2006). Hepatotoxic response is expressed in the form of characteristic patterns of cytolethality in specific zones of the acinus (Kedderis, 1996). Hepatotoxicity related symptoms may include a jaundice appearance causing yellowing of the skin, eyes and mucous membranes due to high level of bilirubin in the extracellular fluid, pruritus, severe abdominal pain,

nausea or vomiting, weakness, severe fatigue, continuous bleeding, skin rashes, generalized itching, swelling of the feet and/or legs, abnormal and rapid weight gain in a short period of time, dark urine and light colored stool (Bleibel *et al.*, 2007).

Cassia occidentalis, which is commonly called ‘Rai Dorai’ in Hausa, ‘Akidi ogbara’ in Igbo, ‘Abo rere’ in Yoruba and ‘Coffee senna’ in English. It is said to belong to the family *Leguminosae*, sub family *Caesalpinoidae*, and botanically classified as both *Cassia occidentalis* and *Senna occidentalis* (Vijayalakshmi, 2013). Extract of several parts of this plant has been widely reported for its pharmacological activities, which ranges from antibacterial, anti-histamine release, antiplatelet aggregation, hepato-curative activities, memory protection and neuroprotection (Jafri *et al.*, 1999 and Sadique *et al.*, 1987). The plant is widely used by the local people of Hausa-Fulani tribe in northern Nigeria for the prevention and treatment of various diseases. This research therefore assess the potency of leaves extract of *Cassia occidentalis* on carbon tetrachloride induced hepatotoxic rat model.

2. Materials and Methods

2.1. Study Animals

Albino rats (both male and female) weighing 100-150g were obtained from Department of Biological Sciences, Bayero University Kano. Animals were housed in colony cages at an ambient temperature and relative humidity. The animals had free access to standard palletized grower feed and drinking water. All authors hereby declare that Principle of laboratory animal care and ethical guidelines for investigation of experimental pain in conscious animals were observed during experimentation (NIH, 1996 and Zimmermann, 1983).

2.2. Plant Material

The leaves of *Cassia occidentalis* were collected at Sabon Gari Fagge LGA of Kano State and authenticated at the Herbarium unit of the Department of Biological Sciences, Faculty of Sciences, Bayero University Kano, with the accession number of BUKHAN 0306. The collected plant samples were rinsed in clean water and air dried at room temperature with all foreign matter removed. The dried samples were pulverized into powder using mortar and pestle, the powder obtained was used to prepare the extracts. Extraction was performed by soaking 500g of the powder in two Liters of distilled water for two Days. It was filtered using three layers of cheesecloth before using Whitman no1 filter paper to obtain a clear debris free extract. The filtrate was then evaporated to dryness in water bath at 40°C. The solvent free extract was dissolve in distilled water and administered to rats.

The volume to be administered to animals was calculated using the method Alhassan *et al.*, (2017)

$$\text{Volume to be administered (ml)} = \frac{\text{weight of rat (kg)} \times \text{Dose (mg/kg)}}{\text{Concentration of the extract (mg/ml)}}$$

2.3. Experimental Protocol

2.3.1. Induction of liver damage

The CCl₄ was mixed with olive oil, in 1:1, ratio the value of CCl₄ administered was determined by the weight of the rabbit according to the following relationship

$$\text{Volume to be administered (ml)} = \frac{\text{weight of rat (kg)} \times \text{Dose (mg/kg)}}{\text{Concentration of the extract (mg/ml)}}$$

2.3.2. Medicinal properties of the extract

Fifteen (25) rats were placed into five (5) groups of three (3) rats each, group II, III, IV and V were induced with liver damage using CCl₄ at a dose of 120mg/kg bodyweight (BW) intramuscularly according to Alhassan (2009)

Group I: Normal control

Group II: Test control. Liver damage induced without extract.

Group III: Liver damage induced, administered with 50mg/kg of the extract

Group IV: Liver damage induced, administered with 100mg/kg of the extract

Group V: Liver damage induced, administered with 10mg/kg of standard drug. (Livolin)

The rats in all groups were sacrificed 24hours after two weeks of administration and sera obtained for biochemical analysis. Aspartate aminotransferase (AST) and Alanine Aminotransferases Assay (ALT) were assayed using Reitman and Frankel (1957) method, Alkaline Phosphatase (ALP) activity assayed using the method developed by Roy (1970), Bilirubin by method of Malloy and Evolyn (1939), Total protein was determined by Biuret method of Tietz (1995).

2.3.3. Statistical Analysis

Results were expressed as mean \pm standard error of mean and analyzed using ANOVA, with p value <0.05 considered significant followed by Tukey's post hoc test. A component of GraphPad InStat3 Software version 3.05 by GraphPadInc was used to analyze the data (GraphPad, 2000).

3. Result

Table 1 present liver function indices (AST, ALT, ALP, TP, ALB, D.BIL, T.BIL, D. BIL and Globulin) of hepatotoxic rats administered with varying dosage of the extract. A significant (p<0.05) increase in all parameters with the exception of Albumin which is lower in CCl₄ administered group compared to normal control. Administration of the extract lead to a significant fall in all parameters in a dose dependent pattern, similar to what was observed in standard drug administered group.

Table 1: Liver function indices of rats administered with aqueous methanol leaves extract of *Cassia occidentalis* for two weeks

Groups	AST (U/I)	ALT (U/I)	ALP (IU/L)	T.Protein (g/dl)	T.BIL (mg/dl)	D.BIL (mg/dl)	Albumin (g/dl)	Globulin (g/dl)
Group I	15.40 \pm 1.12 ^a	24.20 \pm 1.49 ^a	3.78 \pm 0.20 ^a	5.19 \pm 0.04	1.79 \pm 0.07 ^a	0.55 \pm 0.02 ^a	2.48 \pm 0.01 ^a	2.72 \pm 0.04 ^a
Group II	39.00 \pm 1.23 ^{a,b,c,d}	45.80 \pm 1.83 ^{a,b,c,d}	8.93 \pm 0.28 ^{a,b,c,d}	6.57 \pm 0.16	3.66 \pm 0.07 ^{a,b,c,d}	2.07 \pm 0.08 ^{a,b,c,d}	0.93 \pm 0.02 ^{a,b,c,d}	5.64 \pm 0.16 ^{a,b,c,d}
Group III	26.20 \pm 1.49 ^b	34.00 \pm 1.58 ^b	5.48 \pm 0.22 ^b	5.68 \pm 0.15	2.92 \pm 0.02 ^b	1.15 \pm 0.02 ^b	1.19 \pm 0.08 ^b	4.79 \pm 0.19 ^b
Group IV	18.60 \pm 1.29 ^c	27.60 \pm 2.18 ^c	4.00 \pm 0.13 ^c	5.22 \pm 0.09	2.04 \pm 0.04 ^c	0.69 \pm 0.04 ^c	2.03 \pm 0.04 ^c	3.18 \pm 0.11 ^c
Group V	30.00 \pm 2.45 ^d	38.80 \pm 1.43 ^d	4.91 \pm 0.10 ^d	5.68 \pm 0.16	2.52 \pm 0.17 ^d	1.47 \pm 0.08 ^d	1.45 \pm 0.05 ^d	4.24 \pm 0.23 ^d

Values are presented as mean \pm S.E, n=5. Values with the same supercripts along a column are significantly different compared to each other (p<0.05)

4. Discussion

The observed increase in serum levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Total bilirubin and direct bilirubin with concomitant decrease in total protein, albumin and globulin between normal and hepatotoxic rats 24 hours after intramuscular injection of 120mg/kg of CCl₄ confirm successful inducement of hepatotoxicity by the CCl₄. AST and ALT are non-plasma specific enzymes involved in transamination of aspartic acid and alanine respectively, the enzymes were reported to reach higher than normal levels in the blood when there is necrosis of the parenchymal cells of the liver as in viral or toxic hepatitis (Price and Stevens, 2003). ALP is also a non-plasma specific enzyme involved in the hydrolysis of a variety of phosphate esters at alkaline PH. It is also reported to reach higher than normal level in the blood in events of impaired liver function. Thus, they are used as serum markers of hepatic damage. These observations tally with the findings of Obi and Uneh, (2003) and Alhassan *et al.* (2009). Although certain factors such as haemolysis of red blood cells, presence of activators and inhibitors and presence of pyridoxine (vitamin B6), may influence the levels of AST in the serum since the concentration of AST in erythrocyte is roughly tenfold than normal serum level according to Price and Stevens (2003).

Administration of aqueous-methanol leave extract of *Cassia occidentalis* to CCl₄ induced hepatotoxicity rats resulted in significant fall in levels of hepatic marker enzymes (AST, ALT and ALP), Total bilirubin and direct bilirubin in a dose dependent pattern, with a significant rise in albumin. This finding is in support of the research of Jafri *et al* (1999) who reported that Aqueous-ethanolic extract (50%, v/v) of leaves of *Cassia occidentalis* possess a hepatoprotective effect on liver of rat induced by acetaminophen and ethyl alcohol. Alhassan *et al* (2017) also reported that aqueous root extract possess both hepatocurative and nephrocurative ability in acetaminophen induced hepato-renal toxicity.

The observed hepatocurative potential of the plant may not be unconnected with its phytochemical content. phytochemicals possess antioxidant properties which could counteract the toxic effect of CCl₄ by binding to the trichloro methyl-free radical, preventing its covalent binding to microsomal lipid and protein and thereby preventing lipid peroxidation which is thought to be the cause of liver damage by CCl₄ (Muhammad *et al.*, 2015).

5. Conclusion

The study demonstrates that aqueous-methanol leave extract of *Cassia occidentalis* possess hepatocurative ability against CCl₄ induced hepatotoxicity.

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