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Effect of Aqueous-Methanol Leave Extract of *Cassia Occidentalis* on CCl_4 Induced Hepatotoxic Rabbits

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Abstract
The use of the different part of *Cassia occidentalis* in treatment of several deseases has lead to the several researches about the plant capability in desease prevention and curation. This research investigates the effects of aqueous methanol leaves extract of *Cassia occidentalis* on most of the liver function indices (ALT, AST, ALP, T.BIL, D.BIL, T.P, Albumin, Globulin) in carbon tetrachloride induced hepatotoxicity. A total of fifteen rabbits were used for the research, which were divided into five groups (GroupI to V) three rabbits per each group. Group I served as normal control, Group II served as test control, Group III, IV and V were induced with hepatic toxicity and administered with the extract at a dose of 50mg/kg, 100mg/kg and 10mg/kg of livolin respectively, per day for two weeks. A significant decrease ($p<0.05$) in liver function indices was observed in all groups compared to test control. Administration of the extract lead to a significant decrease ($p<0.05$) in liver function indices in a dose dependent manner compared to test control. Similar pattern of activity was observed between 50mg/kg of the extract and 10mg/kg of livolin (standard drug) in hepatocurative activity. The observed hepatocurative ability of the plant may be due to the presence of phytochemicals.

Keywords: *Cassia occidentalis*; Carbon Tetrachloride; Aqueous-Methanol and Hepatoxocity.

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1.0 Introduction

Medicinal plant have continued to attract attention in the global search for effective methods of using plants' parts (e.g. seeds, stems, leaves, roots and bark etc) for the treatment of many diseases affecting humans (Sofowora, 1993). Many important drugs used in medicine today are directly or indirectly derived from plants due to its bioactive constituents. In recent years, secondary plant metabolites previously with unknown pharmacological activities have been extensively investigated as sources of medicinal agents (Krishnaraju *et al.,* 2005).

Cassia occidentalis, commonly called '*Rai Dorai*' in Hausa, '*Akidi ogbara*' in Igbo, '*Abo rere*' in Yoruba and '*Coffee senna*' in English belong to the family *Leguminosae*, sub family *Caesalpinoidae*, and botanically classified as both *Cassia occidentalis* and *Senna occidentalis* (Jafri *et al.,* 1999) Extract of several parts of this plant has been widely reported for its pharmacological activities, which ranges from antibacterial, anti-histamine release, antiplatelet aggregation, memory protection and neuro protection (Sadique *et al.,* 1987).

A model organism is a species that is extensively studied to understand particular biological phenomena, with the expectation that discoveries made on the organism model will provide insight into the workings of other organisms. In particular, model organisms are widely used to explore potential causes and treatments for human disease when human experimentation would be unfeasible or unethical (Fox, 1986).

Liver is the largest organ of the human body weighing approximately 1500g, 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). The central role played by liver in the clearance and transformation of chemicals exposes it to toxic injury (Saukkonen *et al.,* 2006). Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics (Navarro and senior, 2006). The chemicals that cause liver injury are called hepatotoxins or hepatotoxicants. Hepatotoxicants are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals, natural chemicals like microcystins, herbal remedies and dietary

supplements (Willett *et al.,* 2004). Certain drugs may cause liver injury when introduced even within the therapeutic ranges. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediated response affecting hepatocytes, biliary epithelial cells and/or liver vasculature (Deng *et al.,* 2009). The hepatotoxic response elicited by a chemical agent depends on the concentration of the toxicant which may be either parent compound or toxic metabolite (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.

The current study was carried out to evaluate hepatocurative activity of aqueous-methanol extract of *Cassia occidentalis* against CCl_4 induced Hepatotoxicity rabbits.

2.0 Materials and methods

2.1 Sample Collection

The leaves of *Cassia occidentalis* were collected at sabbon gari town, Fagge LGA of Kano State and authenticated at the Herbarium unit of the Department of Biological Sciences, Faculty of Sciences, Bayero Universty, 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 were used to prepare the extracts.

2.2 Extract preparation

The powdered plant material (250g) was percolated into 2.5 liter capacity bottle containing methanol and water in a ratio of 70:30 for 48 hours with intermittent shaking. The percolate were filtered with clean sieving materials. It was then subjected to another filtration via Whatman filter paper using vacuum pump to obtain clear debris free extract. The extract was placed into water bath at 40^0C degree centigrade for three days till the solvent was completely evaporated. The extract solution was prepared by dissolving 1g of dried extract in 100ml distilled water to make a concentration of 10mg/ml.

2.3 Experimental animals

Fifteen healthy rabbits of both sex were obtained 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. Principle of laboratory animal care and ethical guidelines for investigation of experimental pain in conscious animals were observed during experimentation (NIH, 1996; Zimmermann, 1983).

2.4 Induction of liver damage

5ml of CCl_4 was dissolved in 5ml of olive oil, making a ratio of 1:1. The concentration of CCl_4 was calculated as follows:

$$\frac{1.6v2+1.5vg/ml}{2} = 1.595g/ml$$

$$= \frac{1.595g \times 5}{10ml}$$

$$= \frac{7.975g}{10ml}$$

$$= 0.7975g/ml$$

$$= 797.5mg/ml$$

Therefore concentration of CCl_4 = 797.5mg/ml.

2.5 Experimental design

Fifteen (15) rabbits were placed into five (5) groups of three (3) rabbits each, group II, III, IV and V were induced with liver damage using CCl_4 at a dose of 120mg/kg bodyweight according to Alhassan (2009).

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

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

Group I: Normal control
Group II: Administered with 120mg/kg bodyweight CCl_4 without extract (positive control).
Group III: Administered with 120mg/kg bodyweight CCl_4 and 50mg/kg bodyweight of extract
Group IV: Administered with 120mg/kg bodyweight CCl_4 and 100mg/kg bodyweight of extract
Group V: Administered with 120mg/kg bodyweight CCl_4 and administering standard drug (livolin)

Rabbit in group I and II were sacrificed after 24hours of induction to confirm the induction of liver damage, while rabbits in groups (III, IV, and V) were sacrifice 24hours after two weeks of extract administration and sera obtained for biochemical analysis. Aspartate amino transferase (AST) and Alanine amino transferase (ALT) was determined by the method of Reitman *et al*. (1957) and the method is based on transamination reaction. Alkaline phosphatase (ALP) was assayed by Roy, (1970), and serum bilirubin by Molley and Evolyn. (1937)

2.6 Statistical Analysis
Results were expressed as mean ± standard deviation 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.

3.0 Results and discussion
3.1 Results
Table 1 present the liver function indices of rabbits induced with hepatotoxicity followed by administration of aqueous-methanol extract of *C.occidentalis* leaves. A significant (P<0.05) increase in all parameters was observed in CCl_4 administered groups compared to normal control except for albumin. Administration of the extract leads to a significant fall in the parameters in a dose dependent pattern compared to the positive control. However a similar hepatocurative ability was observed between group III administered with 50mg/kg of *C.occidentalis* leaves extract and group V administered with the standard drug (livolin).

Table 1: Liver function indices of rabbits administered with aquose methanol leave extract of *Cassia occidentalis* for two weeks (14 days)

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

Values are mean±S.E, n=3. Values with different supercripts along a column are significantly different (p<0.05)

3.2 Discussion

A successful induction of hepatoxocity was achieved via intramuscular administration of 120mg/kg of CCl_4. This was evidenced by remarkable increase in level of serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), Total bilirubin and direct bilirubin, T. protein, Albumin and Globulin between normal and hepatotoxic rabbits. AST, ALT and ALP are non-plasma specific enzymes and are mostly found in liver, kidney, heart, the enzymes were reported to be higher than normal in serum when there is liver necrosis. A findings supported by various researches (Alhassan *et al*., 2017 and Muhammad *et al*., 2015). 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 Lynch and Price (2007). The concurrent elevations of serum AST together with ALT and ALP indicates that factors may likely not be the cause of the elevated serum enzyme activities, but rather it is more likely to be due to the toxicity induced by CCl_4.

A fall in the levels of serum enzymes in extract administered groups may suggest the presence of a potential hepatocurative compound(s) in the extract, which is in accordance with the research of Alhassan *et al* (2017) who reported that *Cassia occidentalis* extract reduces or reversed the toxic effect of CCl_4 by restoring the hepatic membrane. Thus, preventing the leakage of enzymes into the blood circulation.

4.0 Conclusion

The research concludes that CCl_4 is an importand compound for the induction of hepatotoxicity in animal models, hence *Cassia occidentalis* is highly effective in revesing the effect of CCl_4 toxicity.

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