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Original Research Article

Effect of Aqueous Root Extract of *Cassia occidentalis* on Acetaminophen Induced Hepatorenal Toxicity Rat Model

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Abstract: Indiscriminate usage of *Cassia occidentalis* in treating many diseases has been locally practiced, the need for ascertaining the epicacy of the extract in management of liver and kidney damage become imperative. This research investigate the effects of aqueous root extracts of *Cassia occidentalis* on some liver and kidney parameters in acetaminophen induced liver and kidney toxicity. A total of thirty five rats were used for the research, in phase I, ten rats were used to confirm the inducement of hepato-renal toxicity by acetaminophen. They were grouped into two of five rats each, group I serve as normal control, group two serves as test control and administered with 750mg/kg body weight of acetaminophen. The animals were euthanized after 24 hours of acetaminophen administration and liver function indices (ALT, AST, total protein, Albumin total and direct Bilirubin) and kidney function indices (urea and creatinine) were assayed. In phase II, twenty five rats were grouped into five groups (GI – GV) of five rats each. G I served as normal control, GII served as test control, GIII, GIV and GV were induced with hepato-renal toxicity and administered with the extract at a dose of 50mg/kg, 100mg/kg and 150mg/kg body weight per day for two weeks. A significant increase ($p<0.05$) in both liver and kidney function indices was observed in test control group compared to normal control in both phases. Administration of the extract lead a significant decrease ($p<0.05$) in liver parameters in a dose dependent manner compared to test control. Contrary to this however, a slight decrease in serum urea and creatinine was observed in the extract administered groups. The observed hepatocurative ability of the plant may not be unconnected with the presence of various phytochemicals in the plant.

Keywords: *Cassia occidentalis;* acetaminophen, hepatocurative; nephrocurative and histology.

INTRODUCTION

Traditional medicine (indigenous medicine or folk medicine) describes medical knowledge systems which developed over centuries with various societies before the era of modern medicine. The World Health Organization (WHO) defines traditional medicine as the health practices, approaches, knowledge and belief incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illness or maintain well-being [1]. Medicinal plants are plants that possess therapeutic properties or exert beneficial pharmacological effect on animals. It is now been established that the plants, which naturally synthesizes and accumulate some secondary metabolites e.g alkaloids, glycosides, tannins, volatile oils and certain minerals and vitamins possess some medicinal properties. According to WHO traditional medicines, need to be evaluated, given due recognition and developed so as to improve their efficacy, safety, availability and wider application at low cost [2].

Cassia occidentalis is widely distributed and very commonly used plant. Cassia occidentalis, which is commonly called 'Dora rai' in Hausa, 'Akidi ogbara' in Igbo, 'Abo rere' in Yoruba and 'Coffee senna' in English has been reported to contain many phytochemicals including alkaloids, anthocyanosides, phenolics, proteins, phlobatannins, steroids, tannins, flavonoids, anthroquinone, saponins, terpenes, resins, balsams, amino acids, carbohydrates, sugars and cardiac glycosides [3]. *Cassia occidentalis* have been reported to have many pharmacological effects including antimicrobial, anthelmintic, insecticidal, antioxidant, antianxiety, antidepressant, antimutogenic antidiabetic, and wound healing, hepatoprotective, anti-inflammatory, analgesic, antipyretic and other effects

[4]. The plant is widely used by the local people of Hausa-Fulani tribe in northern Nigeria for the prevention and treatment of various diseases (liver and kidney diseases inclusive).

Liver is the largest and heaviest internal organ of the body weighting about 1.4 – 1.6 kg, it is a soft, reddish-brown triangular organ with two lobes, averaging about the size of an American football in adults [5]. Liver performs more than 500 different functions which includes fats, proteins and carbohydrates metabolism. The liver also metabolizes all drugs and other foreign bodies hence, plays a major role in homeostasis [6].

Kidneys are the major organs in eliminating toxic compound metabolized by the liver. It receives about 1200ml of blood per minute [7], 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 [8]. 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 [9].

MATERIALS AND METHODS

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 [10] and ethical guidelines for investigation of experimental pain in conscious animals [11] were observed during experimentation.

Plant Material

The roots of *Cassia occidentalis* were collected fresh from a nearby forest at Daguru maje of Suleja, Niger state. Identified at Plant science department BUK with herbarium number BUK/HAN/073 They were then dried under the shade and grand to powder. The extraction was performed by soaking 400g of the crude powder of the roots of *Cassia occidentalis* in two Liters of distilled water for two Days. It was filtered using three layers of cheeses cloth 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^{o}C. The solvent free extract was dissolve in distilled water and administered to rats.

The volume to be administered to animals was calculated according to the method of [12].

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

Experimental protocol

Induction of liver and kidney damage

Ten albino rats were used in this phase. They were divided into two groups of five rats each.
Group I: Normal control
Group II: administered with 750mg/kg of acetaminophen.

The acetaminophen was dissolved in normal saline, the value of acetaminophen administered was determined by the weight of the rat according to the following relationship.

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

Rats were euthanized after 24 hours and the level of liver and kidney function indices were assayed in other to confirm the induction.

Medicinal properties of the extract

A total of 25 albino rats were divided into five groups of five rats each. Liver and kidney toxicity was induced by intramuscularly administration of 750mg/kg body weight.
Group I: normal control
Group II: Test control, administered intramuscularly with 750mg/kg body weight of Acetaminophen
Group III: administered with standard drug
Group IV: liver damage induced, administered with 50mg/kg of the extract
Group V: liver damage induced, administered with 100mg/kg of the extract
Group VI: liver damage induced, administered with 150mg/kg of the extract

The rats in all groups (I, II, III, IV and V) 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 [13] method, Alkaline Phosphatase (ALP) activity assayed using the method developed by Roy [14], Bilirubin by method of Malloy and Evolyn [15], Total protein was determined by Biuret method [16], Urea by the method of Weatherburn [17], Creatinine by the method of Bartels and Bohmer [18].

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 [11] version 3.05 by GraphPadInc was used to analyze the data.

RESULTS

Table 1 present liver function indices of (AST, ALT, ALP, TP, ALB, D.BIL, T.BILL and D. BIL) of rats administered intramuscularly with 750mg/kg acetaminophen rats after 24 hours. A significant ($p<0.05$) increase in all parameters was observed in acetaminophen administered group compared to control.

Table-1: Liver function indices of (AST, ALT, ALP, TP, ALB, D.BIL, T.BILL and D. BIL) of rats administered intramuscularly with 750mg/kg acetaminophen rats after 24 hours

Group	AST (IU/L)	ALT (IU/L)	TP (mg/dl)	ALB (g/dl)	T.BIL (mg/dl)	D.BIL (mg/dl)
G I	24.33±2.88[a]	27.67±2.31[b]	1.64±0.12[c]	2.42±0.37[d]	1.64±0.12[e]	0.30±0.05[f]
G II	50.76±3.00[a]	55.67±3.22[b]	4.50±0.67[c]	3.10±0.77[d]	4.50±0.67[e]	.36±0.20[f]

Values are presented as mean ± SD, n= 5. Values bearing superscripts in the same column are significantly different ($p<0.05$). Key: Key: AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALB: Albumin, DB: Direct bilirubin, TB: Total bilirubin, TP: Total Protein. Group I: normal control, Group II: Administered intramuscularly with 750mg/kg of acetaminophen

Table-2: serum level of urea and creatinine of rats administered intramuscularly with 750mg/kg acetaminophen rats after 24 hours

Group	Urea	Creatinine
G I	44.42±4.03[a]	46.00±0.01[a]
G II	146.74±6.16[a]	168.67±0.56[a]

Values are presented as mean ± SD, n= 5. Values bearing superscripts in the same column are significantly different ($p<0.05$). Key: Group I: normal control, Group II: Administered intramuscularly with 750mg/kg of acetaminophen

Table 3 present serum liver function indices (AST, ALT, ALP, TP, ALB, D.BIL, T.BILL and D. BIL) and kidney function indices (urea and creatinine) of rats administered intramuscularly with 750mg/kg acetaminophen and treated with varying doses of aqueous root extract of *Cassia occidentalis*. There was a significant increase in liver parameters except for albumin in group II compared to group I. a significant decrease in liver parameters was observed in test groups administered with the extract in a dose dependent pattern compared to test control.

Table-3: liver function indices (AST, ALT, ALP, TP, ALB, D.BIL, T.BILL and D. BIL) of rats administered with aqueous root extract of *Cassia occidentalis* for two weeks

	ALT (IU/L)	AST (IU/L)	TP (mg/dl)	ALB (g/dl)	T.BIL (mg/dl)	D.BIL (mg/dl)
GI	11.23±2.50[a]	12.95±2.49[a]	9.71±2.16[a]	2.10±1.13[a]	1.21±0.41[a]	0.80±0.10[a]
GII	31.23±3.01[a,b,c,d]	26.35±4.55 a,b,c,d	18.59±2.18 a,b,c,d	0.97±0.14 a,b,c,	4.93±0.71 a,b,c,	1.05±0.11[a]
GIII	20.50±2.10[b]	17.74±2.46[b]	13.39±3.19[b]	1.38±0.42	3.01±0.48	0.98±0.21
GIV	16.32±3.12[c]	15.09±3.15[c]	11.34±2.56[c]	1.57±0.53[b]	2.30±0.81[b]	0.94±0.39
GV	13.10±2.10[d]	13.56±1.76[d]	7.71±2.27[d]	1.62±0.35[c]	1.96±0.72[c]	0.90±0.45

Values are presented as mean ± SD, n= 5. Values bearing superscripts in the same column are significantly different ($p<0.05$). Key: AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALB: Albumin, DB: Direct bilirubin, TB: Total bilirubin, TP: Total Protein Group I: normal control, Group II: Administered intramuscularly with 750mg/kg of acetaminophen, Group III: administered with 50mg/kg of the extract, Group IV: administered with 100mg/kg of the extract, Group V: administered with 150mg/kg of the extract.

Table 4 shows the serum creatinine and urea level of rats administered with aqueous root extract of *Cassia occidentalis* after intramuscularly administration of 750mg/kg body weight of acetaminophen. A significant increase was observed in group II compared to group I. administration of the extract lowers the level of creatinine and urea in a dose dependent manner.

Table-4: Serum Activity of urea and creatinine of rats administered with root extract of *Cassia occidentalis* for two weeks

Group	Urea(µmol/L)	Creatinine (mg/dl)
GI	1.54±0.00[a]	0.85±0.72
GII	6.56±0.83[a,b,c]	5.38±1.40
GIII	5.72±2.08	4.47±2.35
GIV	4.84±6.55	3.37±0.72
GV	4.30±5.81	3.09±1.92

Values are presented as mean ± SD, n= 5. Values bearing superscripts in the same column are significantly different ($p<0.05$). Key: Group I: normal control, Group II: Administered intramuscularly with 750mg/kg of acetaminophen, Group III: administered with 50mg/kg of the extract, Group IV: administered with 100mg/kg of the extract, Group V: administered with 150mg/kg of the extract.

Histopathological analysis

The results of histopathological study in plate 1a and 1b, 2a and 2b, 3a and 3b, 4a and 4b, 5a and 5b shows a cross sections of liver and kidney of group I, group II, group III, group IV and group V respectively.

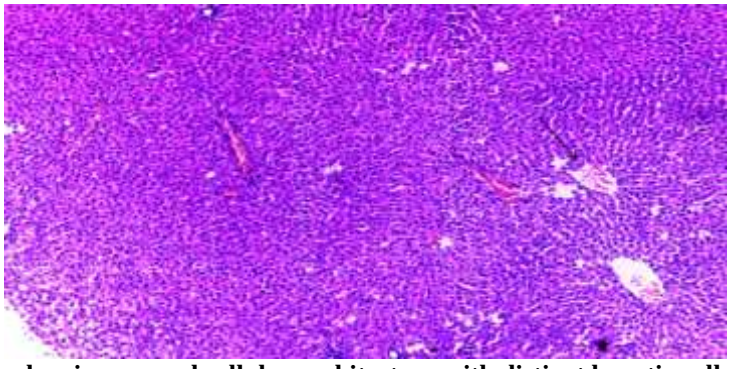

Plate-1a: section of liver showing normal cellular architecture with distinct hepatic cells, sinusoidal space and a central vein (H and E, mag.×100)

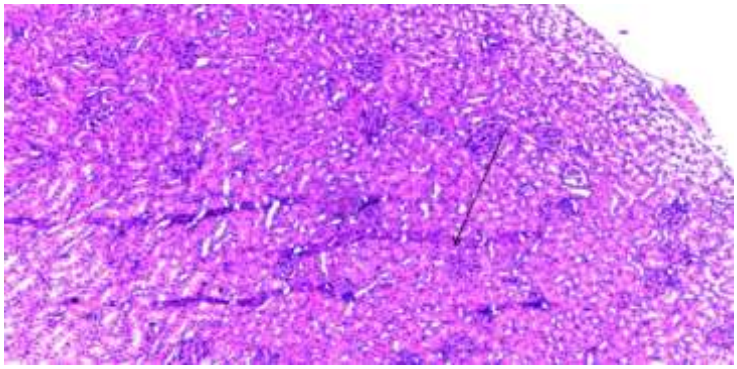

Plate-1b: section of kidney showing normal renal tissue with cortex containing glomeruli and medulla containing renal tubules (H and E, mag.×100)

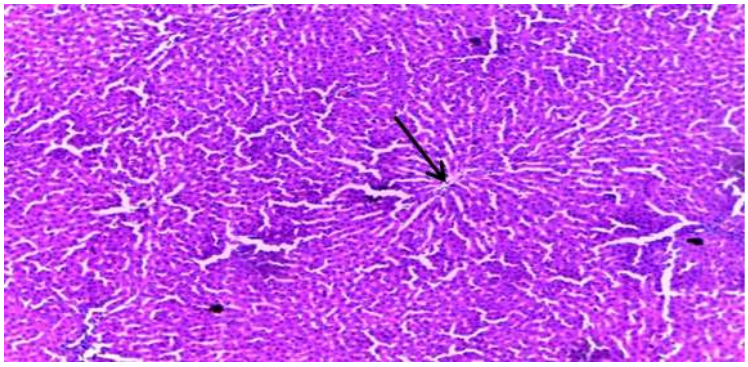

Plate-2a: section of liver of rat induced with 750mg/kg of acetaminophen showing hepatocyte degeneration, mild dilation of hepatic sinuses and necrosis. (H and E, mag.×100)

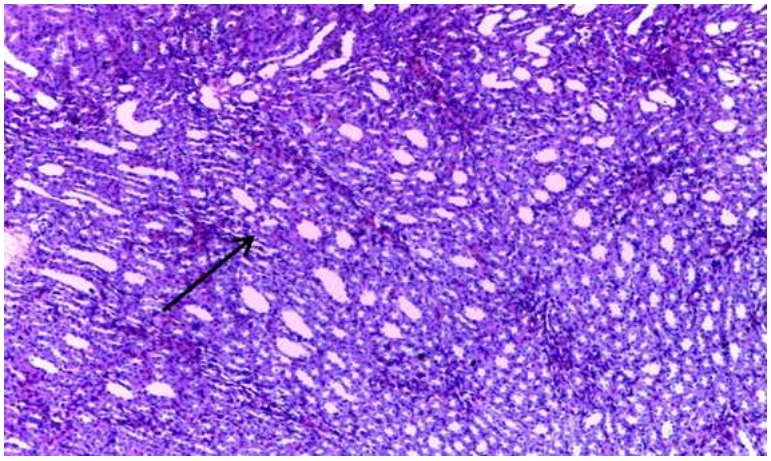

Plate-2b: section of Kidney of rat induced with 750mg/kg of acetaminophen showing mild dilation of renal tubules. (H and E, mag.×100)

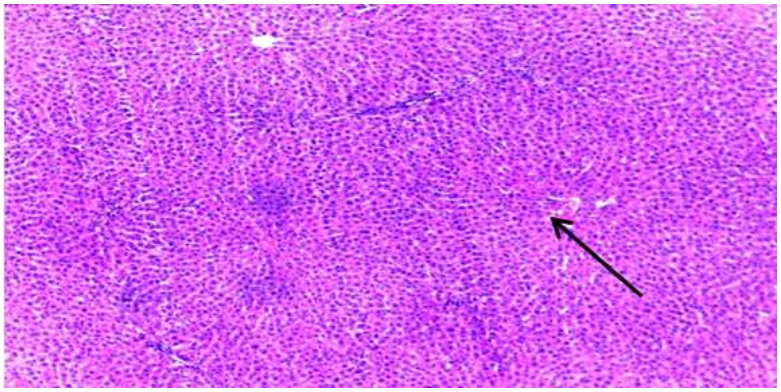

Plate-3a: section of Liver of rat induced with acetaminophen and administered with 50mg/kg of roots extract of *Cassia occidentalis* showing mild dilation of hepatic sinuses (H and E, mag.×100)

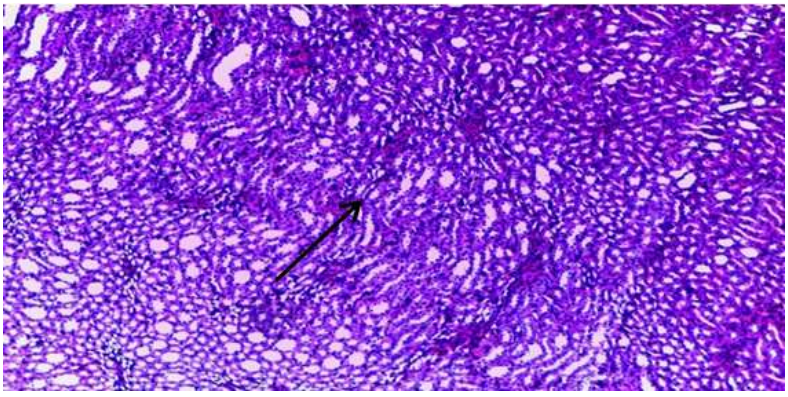

Plate-3b: kidney from rat induced with acetaminophen and administered with 50mg/kg of roots extract of *Cassia occidentalis* showing mild dilation mild dilation of renal tubules (H and E, mag.×100)

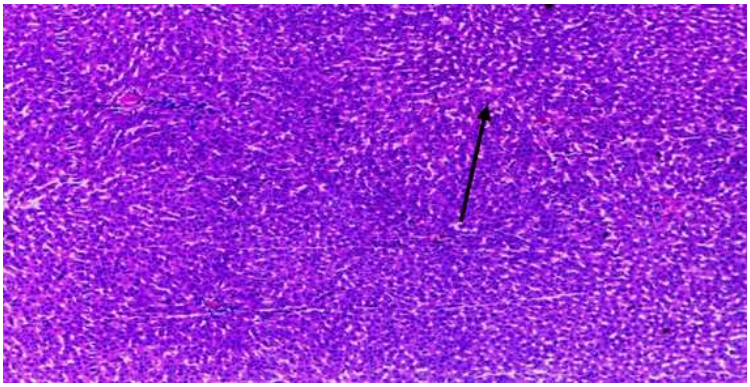

Plate-4a: section of Liver from rat induced with acetaminophen and administered with 100mg/kg of roots extract of *Cassia occidentalis* showing mild dilation of hepatic sinuses (H and E, mag.×100)

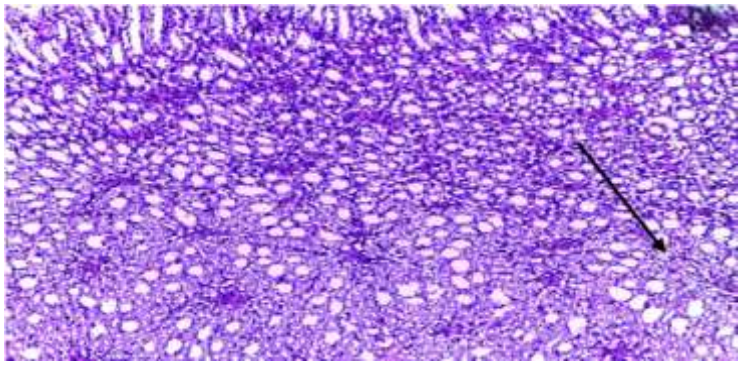

Plate-4b: section of kidney of rat induced with acetaminophen and administered with 100mg/kg of roots extract of *Cassia occidentalis* showing mild dilation of renal tubules (H and E, mag.×100)

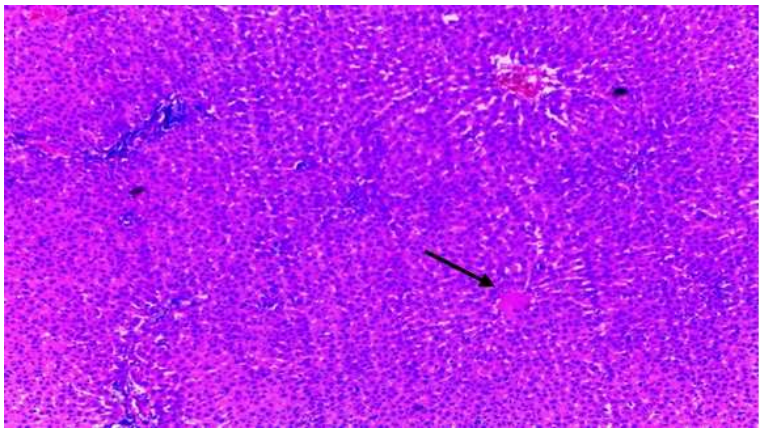

Plate-5a: section of Liver of rat induced with acetaminophen and administered with 150mg/kg of roots extract of *Cassia occidentalis* showing mild dilation of hepatic sinuses (H and E, mag.×100)

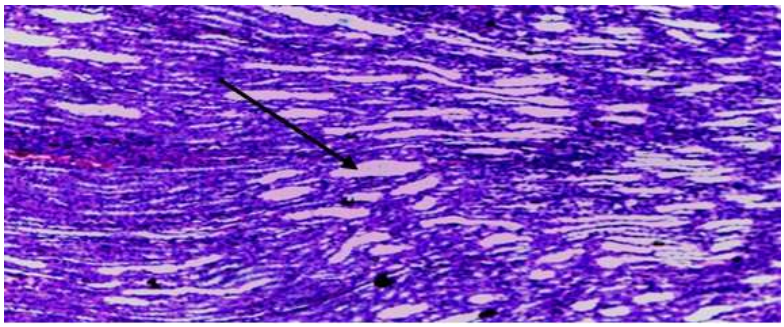

Plate-5b: section of kidney of rat induced with acetaminophen and administered with 150mg/kg of roots extract of *Cassia occidentalis* showing mild dilation induced of renal tubules (H and E, mag.×100)

DISCUSSION

A significant increase was seen in liver function indices, urea and creatinine in group II (Table 1 and 2) was as a result of administration of acetaminophen, a result that is consistent with several studies in rats. Uzzi *et al* [20] reported the inducement of Hepatotoxicity by administration of 800mg/kg body weight of acetaminophen using an orogastric tube. Sastry *et al* [21] also induced hepatotoxicity using 2g/kg body weight of acetaminophen. Ghosh *et al* [22] reported that acetaminophen induced renal insufficiency is consistent with hepatic necrosis and renal failure in humans and animals. The successful inducement of liver and kidney damage by acetaminophen was supported by result of histopathology (plates 2a and 2b). The mechanisism by which acetaminophen induces hepato-renal damage was taught to be through covalent binding of its metabolite "N-acetyl-p-benzoquinonemine", an oxidative product of acetaminophen to sulphydryl group of protein, which result in lipid peroxidative degradation of glutathione level and thereby cell necrosis [23].

Administration of the extract to rats induced with hepato-renal toxicity lead to a significant decrease in level of all liver parameters (AST, ALT, ALP, TP, ALB, D.BIL, T.BILL and D. BIL) compare to test control. The observed hepatocuraive ability of the extract was supported by histopathological analysis of the tissues which shows regeneration as well as healing of the hepatocytes as a result of extract administration. This finding is in support of the research of Jafri *et al* [24] 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.

The observed hepatocurative potential of the plant may be due to its phytochemical content. Phytochemical screening of the plant showed the presence of carbohydrates, saponins, sterols, flavonoids, resins, alkaloids, terpenes, anthraquinones, glycoside and balsam [25]. This phytochemicals possess antioxidant properties which could counteract the toxic effect of acetaminophen metabolite, 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 acetaminophen [26].

On the other hand, administration of the extract led to slight decrease in both serum urea and creatinine suggesting that the extract does possess a nephrocurative effect on the kidney. Tanimu and Wudil [27] reported that the leave extract has a curative effect on kidney using 300, 600, and 900mg/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; this was supported by slow regeneration of the mild dilation of renal tubules in the subsequent groups as seen in the result of histopathology (plate 3b, 4b, and 5b).

CONCLUSION

The study demonstrates that aqueous root extract possess both hepatocurative and nephrocurative ability in acetaminophen induced hepato-renal toxicity.

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