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# EFFECT OF ETHANOL LEAF EXTRACT OF *CASSIA ANGUSTIFOLIA* EXTRACT ON LIVER OF WISTER RATS

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## Abstract

**Background/Aim:** The liver does many important things including makes bile, which helps carry away waste and break down fats in the small intestine during digestion makes certain proteins for blood plasma makes cholesterol and special proteins to help carry fats through the body. *Cassia angustifolia* is a plant regularly consumed in many part of Sub-Sahara Africa in management of various conditions such as infection, pain and diarrhea. The aim of this study is to determine the effect of *Cassia angustifolia* on the liver of Wister rats.

**Method:** Animals of either sex were selected. Group 1 received distilled water (10 ml/kg), while group 2, 3 and 4 received *Cassia angustifolia* 50, 100 and 200 mg/kg respectively. Animals were kept in standard cages and given access to the extract, water and food orally for 28 days, after which they were weighed and sacrificed. Blood was collected by cardiac puncture and taken immediately for hematological and chemo pathological analysis. The histological hepatotoxic potential of the plant was studied using haematotoxylin and eosin (H&E) staining technique.

**Result**: The result revealed Significant ($P<0.05$) decrease in RBC, HGB, MCV, while there was no change in the level of neutrophiles, basophiles, eosinophiles and platelets. The extract did not produce any significant change ($P<0.05$) in the level of ALB, AST and ALT when compared to the control. At 50 mg/kg dose level, *Cassia angustifolia* produced a decrease in BILD concentration in the treated rats while at 100 mg/kg dose level it caused slight increase in the levels of ALP, BILD AND BILT concentration. Histopathological evaluation also agrees with other parameters.

**Conclusion**: the result of the study suggests that the plant may be safe for consumption as it is been used by the locals for the purpose intended. The result also revealed that the drug may be of benefit in hepatic cases.

**Keyword:** *Cassia angustifolia*, rat, blood, liver

## Introduction

Weighing between 3.17 and 3.66 pounds (lb), or between 1.44 and 1.66 kilograms (kg), the liver is reddish-brown with a rubbery texture[1]. It is situated above and to the left of the stomach and below the lungs. The skin is the only organ heavier and larger than the liver. The liver is roughly triangular and consists of two lobes: a larger right lobe and a smaller left lobe. The lobes are separated by the falciform ligament, a band of tissue that keeps it anchored to the diaphragm. A layer of fibrous tissue called Glisson's capsule covers the outside of the liver[1]. This capsule is further covered by the peritoneum, a membrane that forms the lining of the abdominal cavity. This helps hold the liver in place and protects it from physical damage. An organ as complex as the liver can experience a range of problems. A healthy liver functions very efficiently. However, in a diseased or malfunctioning liver, the consequences can be dangerous or even fatal[2]. It is very vital to ensure that the liver function optimally. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, malaria, cardiovascular diseases and neurological disorders[2]. Plants have proven to be a novel source for bioacive natural products. They have evolved and adapted over millions of years to withstand bacteria, insects, fungi and weather to produce unique, structurally diverse secondary metabolites. Their ethnopharmacological properties have been used as a primary source of medicines for early drug discovery.

*Cassia angustifolia*, also known as Alexandrian Senna is a shrubby plant that reaches 0.5–1, rarely two, metres in height with a branched, pale-green erect stem and long spreading branches bearing four or five pairs of leaves. These leaves form complex, feathery, mutual pairs[4]. The leaflets vary from 4 to 6 pairs, fully edged, with a sharp top. The midribs are equally divided at the base of the leaflets. The flowers are in a raceme interior[5] blossoms, big in size, coloured yellow that tends to brown. Its legume fruit are horned, broadly oblong,

compressed and flat and contain about six seeds. When cultivated, the plants are cut down semi-annually, dried in the sun, stripped and packed in palm-leaf bags. It also serves as a fungicide[8].

Modern medicine has used extracts since at least the 1950s as a laxative[6]. If accidentally ingested by infants, it can cause side effects such as severe diaper rash[7]. The active ingredients have several senna glycosides which interact with immune cells in the colon[7]. This work was intended to investigate the effect of ethanol extract of Cassia angustifolia on the liver of rats.

## Materials and Method

### Animals

Male and female wister rats were obtained from Bingham University, Animal House. They were maintained on standard animal pellets and given water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee of Bingham University.



### Plant collection

Leaves of *Cassia angustifolia* were collected from its natural habitat from nearby Karu village, Nasarawa State, Nigeria. The plant was authenticated from Department of Botany, Bingham University, Nasarawa State Nigeria.

### Plant extraction

The leaves were shadow dried for two weeks. The dried plant material was further reduced into small pieces and pulverized. The powdered material was macerated in 70% ethanol. The liquid filtrates were concentrated and evaporated to dryness at 40°C *in vacuum* using rotary evaporator. The ethanol extract was stored at -4°C until used.

### Animal study

Twenty four (24) rats of either sex (159-283g) were selected and randomized into four groups of six rats per group. Group 1 served as the control and received normal saline (10ml/kg) while the rats in groups 2, 3 and 4 were giving 50, 100, and 200 mg/kg of extract respectively. The weights

of the rats were recorded at the beginning of the experiment and at weekly intervals. The first day of dosing was taken as $D_0$ while the day of sacrifice was designated as $D_{29}$.

**Haematological analysis**

The rats were sacrificed on the 29$^{th}$ day of experiment. Blood samples were collected via cardiac puncture. One portion of the blood was collected into sample bottles containing EDTA for hematological analysis such as Hemoglobin concentration, white blood cell counts (WBC), differentials (neutrophils, eosinophils, basophils, lymphocyte and monocyte), red blood cell count (RBC), platelets and hemoglobin (Hb) concentration using automated Haematology machine (Cell-Dyn, Abbott, USA).

**Chempathology analysis**

Sera were separated from the blood samples and were stored at -20$^{o}$C until used for biochemical investigations such as measuring total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol, and total and direct bilirubin



**Histology study**

The livers of the animals were surgically removed and weighed and a part of each was fixed in 10% formaldehyde for histological processes.

**Statistical analysis**

Data were expressed as the Mean ±Standard Error of the Mean (SEM). Data were analyzed statistically using one-way Analysis of Variance (ANOVA) followed by Dunnett's post hoc test for multiple comparisons between the control and treated groups. Values of $P \leq 0.05$ were considered significant.

## Result

### Effect of 28 days oral administration of *Cassia angustifolia* on hematological parameters in rats.

*Cassia augustifolia* caused significant ($p<0.05$) decrease in the level of red blood cell, hemoglobin, platelet etc. and significantly ($p<0.05$) caused an increase in mean corpuscular hemoglobin concentration in the rats at the dose level of 50 mg/kg compared to the control. The level of basophiles, neutrophiles, eosinophils and lymphocytes were however not significantly ($p<0.05$) affected by mean corpuscular hemoglobin concentration (Table 1).

### Effect of 28 days oral administration of ocimum canum on hepatic indices in rats.

At 50 mg/kg dose level, *Ocimum canum* produced significant ($p<0.05$) decrease in BILD concentration in the treated rats while at 100 mg/kg dose significant ($p<0.05$) increase was obtained in ALP levels, BILD and BILT concentrations when compared to the control. All other parameters studied were however, not significantly affected (Table 2).

### Effect of 28 days oral administration of ethanol leaf extract of Ocimum canum on histology Liver of rats.

The liver showed slight vascular congestion, slight hepatic necrosis and lymphocyte hyperplasia at 50 mg/kg and 100 mg/kg, Sinusoidal congestion at 200 mg/kg. However, there was no sign of damage to the liver of the rats in control group (Plate 1).

**Table 1: Effect of 28 days oral administration of ethanol leaf extract of *Cassia angustifolia* on hematological parameters in wistar rats.**

| | | Treatment (mg/kg) | | |
|---|---|---|---|---|
| Hematological parameters | DW(1ml/kg) | 50 mg/kg | 100 mg/kg | 200 mg/kg |
| WBC ($\times 10^9$/L) | 7.67±0.772 | 7.74±1.419 | 3.700±0.657* | 6.420±1.085 |
| RBC ($\times 10^{12}$/L) | 8.21±0.37 | 8.65±0.20 | 6.11±0.35* | 7.91±0.27 |
| HGB (g/dL) | 14.92±0.66 | 14.24±0.46 | 10.93±0.76* | 13.58±0.77 |
| HCT (g/dL) | 54.27±2.13 | 55.60±2.75 | 34.67±2.28* | 52.41±2.73 |
| MCV (fL) | 66.62±0.93 | 65.40±1.44 | 57.17±0.31* | 69.60±1.72 |
| MCH (pg) | 19.17±0.17 | 17.80±1.02 | 18.83±0.37 | 18.80±0.20 |
| MCHC (g/dL) | 34.17±0.16 | 32.30±0.72 | 35.50±0.53* | 30.62±0.74 |
| PLT ($\times 10^9$/L) | 626.86±46.71 | 577.20±93.32 | 242.13±45.28* | 688.40±45.26 |
| LYM (%) | 76.45±5.16 | 74.00±5.19 | 75.83±6.33 | 75.40±5.23 |
| NEUT ($\times 10^9$/L) | 20.86±4.57 | 21.83±2.69 | 25.33±5.66 | 21.36±4.17 |
| EOSI ($\times 10^9$/L) | 2.55±0.24 | 3.44±0.65 | 2.87±0.36 | 2.32±0.51 |
| BASO ($\times 10^9$/L) | 2.12±0.19 | 2.60±0.85 | 2.44±1.77 | 3.45±1.64 |

Data presented as Mean ± SEM: n = 6, One way ANOVA, followed by Dunnett's post hoc for multiple comparison *significantly different from the distilled water (DW) control at $p<0.05$. DW = distilled water (WBC = white blood cells, RBC = red blood cells, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT = platelet, LYM = lymphocyte, NEUT = neutrophils, EOSI = eosinophils, BASO = basophils).

**Table 2: Effect of 28 days oral administration of Ocimum canum on hepatic indices in wistar rats.**

| | | Treatment (mg/kg) | | |
|---|---|---|---|---|
| Hepatic indices | DW(10 ml/kg) | 50 | 100 | 200 |
| ALB (g/L) | 43.23±3.62 | 45.23±1.76 | 45.34±1.09 | 47.66±1.16 |
| ALP (IU/L) | 212.20±6.63 | 232.50±7.78 | 354.20±13.27* | 225.56±7.94 |
| ALT (IU/L) | 90.56±4.11 | 87.85±7.58 | 95.32±5.65 | 87.98±10.02 |
| AST (IU/L) | 194.80±79.60 | 195.25±5.76 | 171.43±20.78 | 193.30±6.73 |
| BILD (µmol/L) | 0.27±0.16 | 0.18±0.17* | 0.69±0.21* | 0.65±0.22 |
| BILT (µmol/L) | 2.22±0.41 | 2.45±0.25 | 3.46±0.80* | 2.73±0.56 |
| TP (g/L) | 77.20±3.08 | 65.07±2.77 | 68.13±3.27 | 81.11±3.22 |

Data presented as Mean ± SEM: n = 6, One Way ANOVA, followed by Dunnett's post hoc for multiple comparison *significantly different from the distilled water (DW) control at $p<0.05$. DW = distilled water (ALB = albumin, ALP = alanine phosphatase, ALT = alanine transaminase, BILD = unconjugated bilirubin, BILT = conjugated bilirubin, TP = total protein).

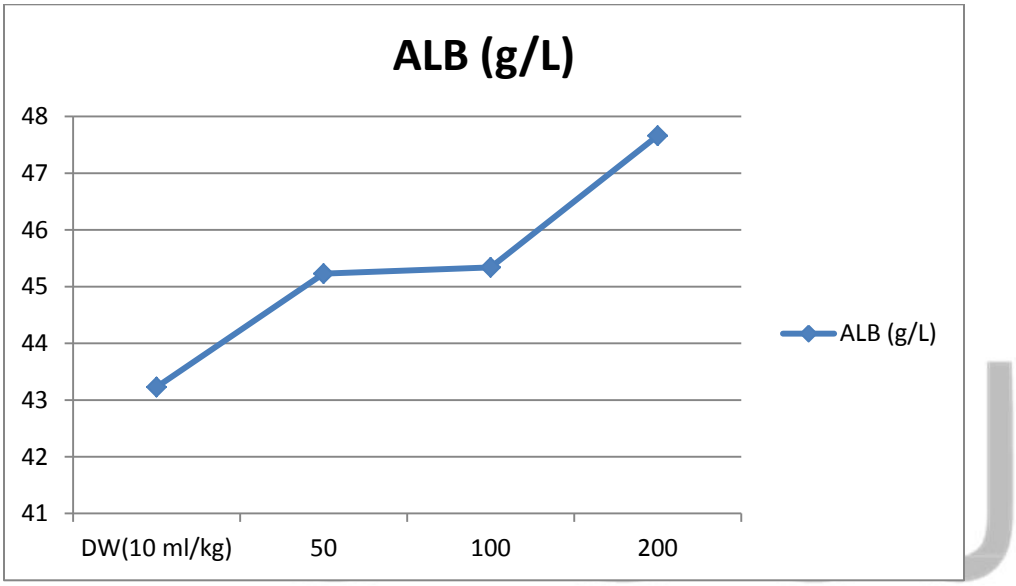


Fig 1: graph showing effect of the ethanol leaf extract of *Cassia angustifolia* on serum albumin level in rats

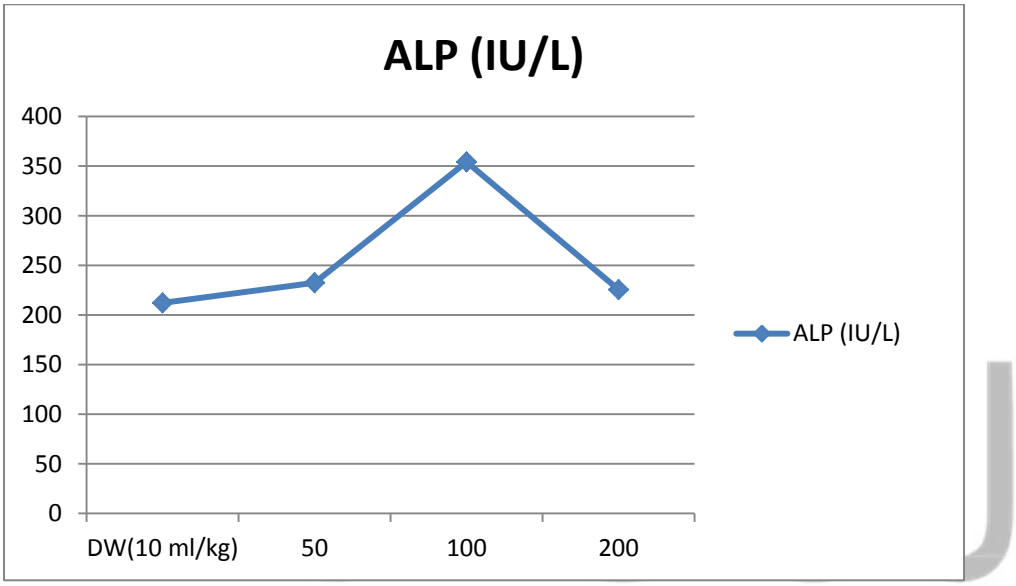


Fig 2: graph showing effect of the ethanol leaf extract of *Cassia angustifolia* on serum alkaline phosphatase level in rats.

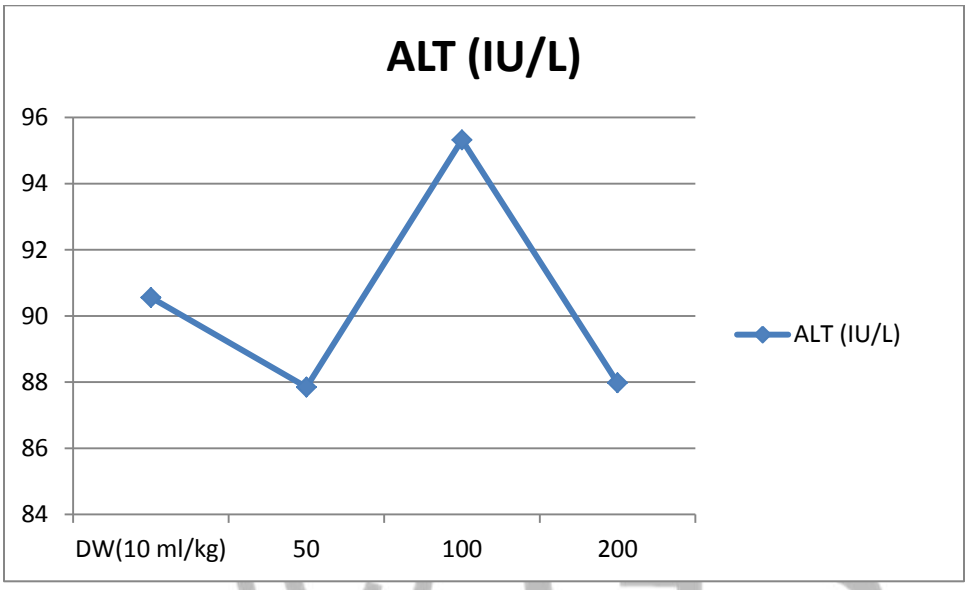


Fig 3: graph showing effect of the ethanol leaf extract of *Cassia angustifolia* on serum alkaline transferase level in rats.

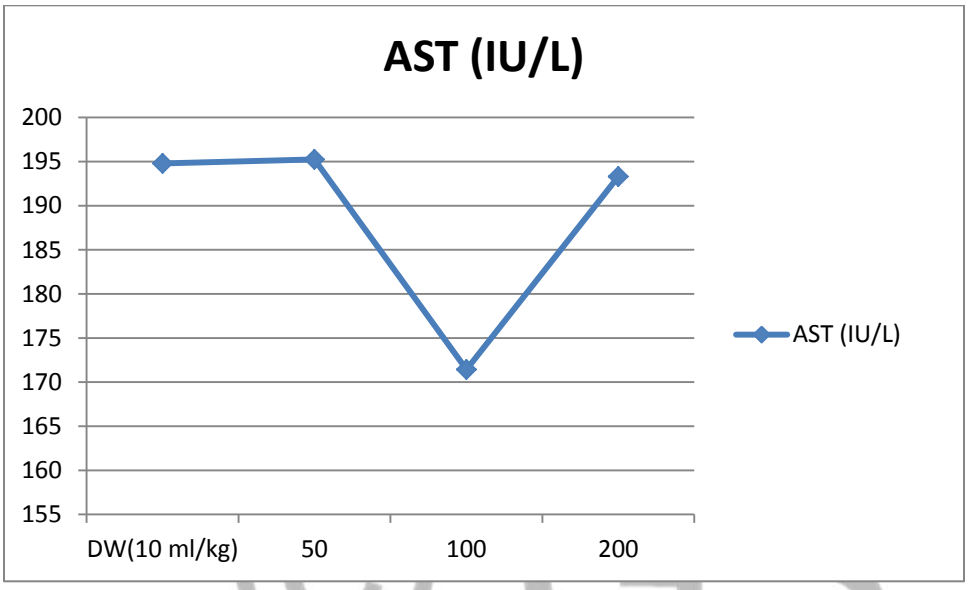


Fig 4: graph showing effect of the ethanol leaf extract of *Cassia angustifolia* on serum aspartate transferase level in rats.

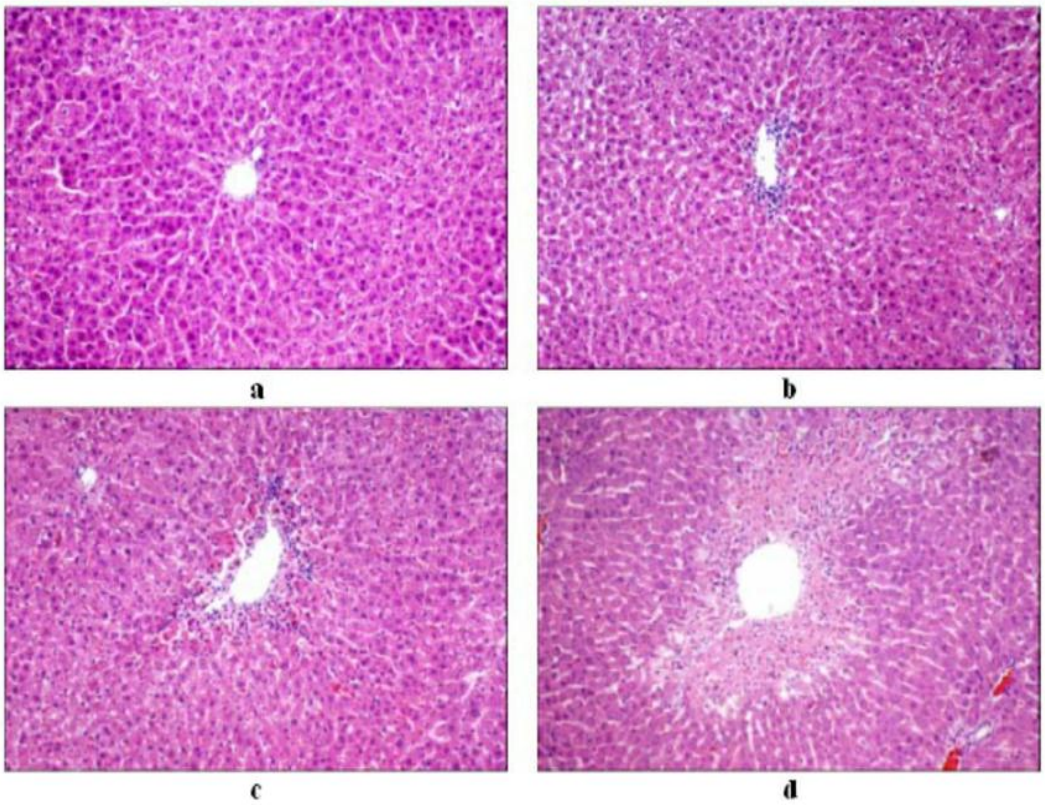


**Plate 1:**. figure of the liver (a) Control group, shows normal hepatocyte (H). (b) *Cassia angustifolia* 50 mg/kg (c) *Cassia angustifolia* 100 mg/kg,. d) 200 mg/kg *Cassia angustifolia*.

## Discussion

Environmental chemical-induced hepatotoxicity is a major public health concern[8]. Natural antioxidants ameliorate the effects of free radical induced oxidative stress. Plants have been of immense benefit in curing or managing numerous disease conditions[9]. From the results of this study, administration of ethanol leaf extract of *Cassia angustifolia* led to a decrease in platelet counts, red blood cell and haemoglobin in rats. Reduction in platelets count in experimental animals has been reported to indicate adverse effect on the oxygen carrying capacity of the blood as well as thrombopoietin[10,11]. Reduction in platelets counts obtained from the results of this study suggests that the administration of *Cassia angustifolia* may cause disruption in the oxygen carrying capacity of the blood. The study showed that *Cassia angustifolia* could disrupt hemoglobin production at high doses. Failure to produce hemoglobin occurs in many diseases, including iron deficiency anemia, thalassemia (an inherited disease in which globin chain production is deficient), and anemias associated with chronic infection or disease[12]. Also, the level of basophiles, neutrophiles, eosinophils and lymphocytes were not affected by the extract. This indicates that the plant may not interfere with the body immune.

Liver function was evaluated by assaying the activities and levels of serum ALT, AST, ALP, bilirubin (total and direct), total cholesterol, total protein and albumin which are naturally present in the cytoplasm[13]. When there is hepatopathy, these enzymes and molecules leak into the blood stream which serves as an indicator for the liver damage[14]. The most commonly used indicators of liver (hepatocellular) damage are alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The ALT is felt to be a more specific indicator of liver inflammation as AST is also found in other organs such as the heart and skeletal muscle[14,15,16]. The values obtained for liver function parameters showed that the conjugating ability of the liver was not compromised from the total and conjugating bilirubin levels; the synthetic ability of the liver was

also maintained judging from total protein and albumin values. There was also no hepatocellular damage as revealed by the ALT and AST values. Bilirubin is formed primarily from the breakdown of a substance called heme found in red blood cells[17,18,19]. It is taken up from the blood, processed, and then secreted into the bile by the liver. There is normally a small amount of bilirubin in the blood in healthy individuals (<17µmol/L). Conditions which cause increased formation of bilirubin, such as destruction of red blood cells, or decrease in its removal from the blood stream as in liver dysfunction, may result in an slight increase in the level of bilirubin in the blood[20,21,22].findings from the study revealed that there was significant increase in the level of ALP, BILD and BILT. These indicate that the plant extract may cause mild biliary obstruction, destruction of red blood cells and/or decrease in RBC removal from the blood stream. Slight hepatic necrosis with other normal hepatic features in histopathological study concurs with other parameters

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