



CURATIVE POTENTIAL OF AQUEOUS STEM BARK EXTRACT OF *Cassia sieberiana* IN RATS

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ABSTRACT

Liver disease is still a global health concern and the drugs used to treat the condition are also implicated in exacerbating the problem. Many are sources are constantly screened for bioactivity in order to compensate shortcomings from current treatments. Thus, the current study evaluates the hepatocurative potential of aqueous stem bark extract of *Cassia sieberiana* in liver-damaged albino rats. Phytochemical analysis, acute toxicity study and effect of aqueous stem bark extract of *Cassia sieberiana* in rats induced with CCl_4 liver damaged were conducted. The rats were randomly divided into five groups of six rats each. With one group being normal control while the others were induced with 150 mg/kg CCl_4 to induce liver damage followed by daily administration of graded doses (50, 100 and 150 mg/kg) of aqueous stem bark extract of *Cassia sieberiana* (ASBEC) for four weeks. Three rats from each group were randomly selected and sacrificed 48 hours after CCl_4 induction in order to confirm liver damage, while the remaining three rats in all the groups were sacrificed after four weeks. The blood samples were collected to determine serum levels of liver enzymes, total proteins, bilirubin(s) and albumin. Histopathological examination on the liver section was carried out. The phytochemical analysis revealed the presence of tannins, saponins, cardiac glycosides, alkaloid and flavonoids. The acute toxicity of ASBEC shows no sign of toxicity at a maximum dose of 5000mg/kg. The extract produced dose-dependent reduction in the biochemical markers of liver injury ($P \leq 0.05$) compared to the negative (CCl_4 -only treated group) control. There is no statistically significant change ($P > 0.05$) in the serum biomarkers of liver injury in the extract treated groups compared to the positive (normal saline) control. The results of the biochemical parameters indicate some hepatocurative effects of the extract against CCl_4 induced liver toxicity and this was supported by the histopathological examination of rat's liver. Hence, the curative latent of the *Cassia sieberiana* may be due to the presence of high concentration of tannins and saponins.

Keywords: Albino rats, Carbon tetrachloride (CCl_4), *Cassia sieberiana*, Hepatoprotection, Liver

INTRODUCTION

Medicinal plants have been identified and used for treatment purposes in different parts of the world. The use of traditional medicine has increased due to lower cost and safety as compared to synthetic drugs (Abdolrasool *et al.*, 2013). Treatment of liver damage by traditional medicine is an essential constituent of alternative medicine (Obidah *et al.*, 2009). *Cassia sieberiana* is an important plant used in Nigeria for alleviation and treatment of diseases. It belongs to Acacia family and is distributed across the Semi-Arid North-eastern Nigeria and ranges from giant trees to small annual herbs (Biu *et al.*, 2013). The plant is used in treatment of malaria, jaundice, diarrhoea and rheumatism (Abdulrazak *et al.*, 2015). It was also reported that the fruits are used for the treatment of ulcer, piles and stomachache (Toma *et al.*, 2009). The aqueous decoctions of the roots, stem bark and the fruit pulp have been used traditionally in North-

eastern Nigeria for the treatment of inflammatory conditions (Madusolomuo *et al.*, 1999) and tiredness (Abdolrasool *et al.*, 2013).

Liver is an organ responsible for metabolism of xenobiotics that may be harmful to the body and detoxify them to harmless substances. It also has a wide range of biochemical functions, including protein synthesis and other biomolecules (Donkor *et al.*, 2014). Liver damage can result from xenobiotics (Friedman, 2014) or viral hepatitis and inherited metabolic diseases (Bolanle *et al.*, 2014). It has been reported that carbon tetrachloride (CCl_4) induces free radical mediated lipid peroxidation and liver damage (Murtala *et al.*, 2016). Many reports showed similarities between human liver cirrhosis and CCl_4 induced liver damage (Ellahi *et al.*, 2014); this justifies the use of CCl_4 to induced liver damage in experimental animals.

Therefore, there is the urgent need to investigate a medicinal plant that has hepatocurative effect to determine its potential in treating liver diseases.

MATERIALS AND METHODS

Collection, Identification and Authentication

Cassia sieberiana stem bark was collected from Gwarzo town area, Kano state. It was identified and authenticated at Biological Science Department of Bayero University, Kano, by a botanist and a voucher number of 89 was assigned to it.

Preparation of the Plant Extract

The stem bark was washed with distilled water and dried at room temperature for seven days. The bark was then grounded into fine powder using pestle and mortar and 100g of the powder was soaked in 400cm³ of distilled water for 24hrs. The extract was filtered using Whatman filter paper No. 1. The filtrate was dried using rotatory evaporator, weighed and reconstituted

Phytochemical Analysis

The phytochemical analysis of *C. sieberiana* aqueous extract was carried out to determine that levels of tannins, saponins, alkaloids, flavonoids (as described by Gracelin *et al.*, (2013)) and cardiac glycosides (as described by Soladoye and Chukwuma (2012)).

Experimental Animals

Adult male Wistar rats of either of average body weight of 397.94±99.45 g were obtained from animal house facility of Department of Biological Sciences, Bayero University, Kano, Nigeria. The rats were kept in well ventilated animal house at room temperature

for 2 weeks to acclimatized. They were fed with standard animal diet (Top Feed®) and allow free access to water *ad libitum*.

Acute Toxicity

The LD₅₀ was determined using Lorke method (1983). Nine (9) albino rats were used in phase I of the method, the rats were divided into 3 groups of 3 rats each, The animals were treated with 10, 100 and 1000 mg/kg body weight of aqueous stem bark extract of *Cassia sieberiana* (ASBEC) and were monitored for 24hrs, for signs of toxicity and death. In phase II, four (4) rats were used and divided into four (4) groups of one rat each. They were treated with ASBEC at doses of 1600, 2900 and 5000 mg/kg body weight. They were monitored for 24 hours for signs of toxicity and death.

Experimental Design

A total of forty-three (43) rats were used in this study. Thirteen (13) rats were used for acute toxicity study, and thirty (30) rats were divided into five groups of 6 rats each for the assessment of ASBEC effect on CCl₄ induced liver damage. Three rats from each of the five groups were sacrificed after 48 hours of CCl₄ induction to confirm liver damage. The confirmation was followed by the daily administration of 50, 100 and 150mg/kg of the ASBEC to Groups III, IV and V animals respectively while Group I was neither induced with CCl₄ nor treated with the extract and therefore served as positive control. The rats in group II were induced with CCl₄ but not treated with the extract and therefore served as negative control.

Table 1: Experimental Design for Evaluation of the Effect of Administration of Aqueous Stem Bark Extract of *Cassia sieberiana* (ASBEC) on CCl₄-Induced Liver Toxicity

| Groups | No. of Animals | CCl ₄ Administered (mg/kg) | ASBEC (mg/kg) |
|-----------|----------------|---------------------------------------|---------------|
| Group I | 3 | Nil | Nil |
| Group II | 3 | 150 | Nil |
| Group III | 3 | 150 | 50 |
| Group IV | 3 | 150 | 100 |
| Group V | 3 | 150 | 150 |

After four weeks, the three remaining animals from each group (I to V) were sacrificed to assess liver status.

Induction of Liver Damage

Liver injury was induced by single administration of 150 mg/kg of CCl₄ intraperitoneally. A stock solution of CCl₄ was prepared 1:1 by dissolving 25cm³ of CCl₄ in 25cm³ of pure olive oil.

Biochemical Assays

The total protein (TP), alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), albumin (ALB), total bilirubin (TB), direct bilirubin (DB) levels in the serum of treated rats were evaluated using assay Randox Laboratories kits (Randox Laboratories LTD, United Kingdom, BT29 4QY).

Histopathology Analysis

The liver specimens of the sacrificed rats were transported in 10% buffered formalin to the Histopathology department of Aminu Kano Teaching Hospital, Kano, where histopathological analysis was conducted.

Statistical Analysis

Results of biochemical parameters were expressed as mean plus or minus standard deviation (M ± SD) and analysed using One-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparison among groups. Probability value (*P*) of ≤ 0.05 was considered statistically significant.

RESULTS

Phytochemical Analysis

The quantitative phytochemical analysis showed that ASBECs is rich in tannins followed by saponins but contains low levels of cardiac glycosides, alkaloid and flavonoids (Table 2).

Table 2: Quantitative Phytochemical Analysis of Aqueous Stem Bark Extract of *Cassia sieberiana*

| Phytochemical constituents | Content (g %) |
|----------------------------|---------------|
| Tannins | 0.77± 0.04 |
| Saponins | 0.70± 0.09 |
| Cardiac glycosides | 0.08± 0.01 |
| Alkaloids | 0.04± 0.00 |
| Flavonoids | 0.01 ±0.00 |

Acute Toxicity Studies

The oral LD₅₀ of *Cassia sieberiana* in rats was estimated to be 5000 mg/kg. There was no mortality or obvious signs of toxicity in both phases. This shows that ASBECS is practically nontoxic to the rats.

CCl₄-induced Liver Damage

A significant increase ($P \leq 0.05$) in the mean serum level of liver enzymes (AST, ALT and ALP), total bilirubin (TB) and direct bilirubin (DB) were observed with a significant decrease ($P \leq 0.05$) in the mean level of total protein (TP) and albumin (AB) in CCl₄-induced rats compared to the standard (Group I) control (Table 3).

Hepatocurative Effect of Aqueous Stem Bark Extract of *Cassia sieberiana* in Rats

The aqueous stem bark extract of *Cassia sieberiana* had produced a dose-dependent and statistically significant ($P \leq 0.05$) reduction in all parameters of liver function test in the treated groups compared to the negative control (CCl₄-only treated). However, there is no statistically significant improvement in the deranged liver function parameters in the treated groups compared to the standard (normal saline only) control (Table 4).

Histological Analysis.

The results obtained from histopathological study is represented in Figure 1 through 5. Figure 1 shows a photomicrograph of cross section of a rat's liver in Group I. Figure 2 shows the liver of a rat induced with 150 mg/kg of CCl₄, Figure 3, 4 and 5 show the liver of rats treated with graded doses of the extract (50, 100 and 150 mg/kg) for four weeks after induction with CCl₄.

Table 3: Hepatotoxic Effect of CCl₄ on Liver Function Parameters in Rats

| Groups | AST (U/l) | ALT (U/l) | ALP (U/l) | TP (g/l) | TB (umol/l) | DB (umol/l) | AB (g/l) |
|-------------------------------------|-------------|-------------|--------------|-------------|-------------|-------------|------------|
| Group I (Control) | 24.17±2.84 | 21.00±3.00 | 111.00±2.00 | 78.33±8.50 | 14.67±0.58 | 10.33±1.53 | 10.10±2.2 |
| Group II (150mg CCl ₄) | 91.33±3.21* | 60.00±5.29* | 283.87±1.83* | 36.00±5.29* | 36.80±2.12* | 25.40±1.77* | 5.33±1.33* |
| Group III (150mg CCl ₄) | 92.33±3.11* | 60.33±2.09* | 293.00±1.99* | 32.00±4.39* | 36.80±3.02* | 24.34±1.66* | 5.33±1.00* |
| Group IV (150mg CCl ₄) | 91.00±1.00* | 59.00±4.79* | 280.99±4.23* | 36.33±1.26* | 37.70±1.10* | 23.70±0.99* | 4.67±2.03* |
| Group V (150mg CCl ₄) | 91.67±1.23* | 61.01±1.20* | 275.53±5.93* | 37.00±5.29* | 33.80±2.00* | 25.00±1.02* | 5.50±0.03* |

Values presented as mean ± standard deviation, n=3, *significantly different from normal control at $P \leq 0.05$ TP=Total Protein, ALP= Alkaline Phosphatase, ALT= Alanine Transaminase, AST= Aspartate Transaminase, ALB= Albumin, TB= Total Bilirubin, DB= Direct Bilirubin, CCl₄= Carbon tetrachloride

Table 4: Effect of 4 Weeks Administration of ASBEC on Liver Function Parameters in Rats Induced with 150mg/kg CCl₄.

| Groups | AST (U/l) | ALT (U/l) | ALP (U/l) | TP (g/l) | TB (umol/l) | DB (umol/l) | AB (g/l) |
|---|----------------------------|----------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|---------------------------|
| Group I (Control) | 24.17±2.84 | 21.00±3.00 | 111.00±2.00 | 78.33±8.50 | 14.67±0.58 | 10.33±1.53 | 10.10±2.20 |
| Group II (CCl ₄) | 91.33±3.21 ^a | 60.00±5.29 ^a | 283.87±1.83 ^a | 36.00±5.29 ^a | 36.80±2.12 ^a | 25.40±1.77 ^a | 5.33±1.33 ^a |
| Group III (CCl ₄ + 50mg ASBEC) | 80.00±2.00 ^{a, b} | 50.33±3.51 ^{a, b} | 247.00±6.11 ^{a, b} | 42.00±2.65 ^{a, b} | 31.17±0.95 ^{a, b} | 23.57±0.57 ^{a, b} | 5.90±0.36 ^a |
| Group IV (CCl ₄ + 100mg ASBEC) | 75.00±1.00 ^{a, b} | 43.67±1.15 ^{a, b} | 204.67±6.11 ^{a, b} | 45.67±2.52 ^{a, b} | 31.33±6.66 ^{a, b} | 21.83±1.19 ^{a, b} | 6.10±0.17 ^{a, b} |
| Group V (CCl ₄ + 150mg ASBEC) | 72.33±2.52 ^{a, b} | 42.33±0.58 ^{a, b} | 196.67±1.53 ^{a, b} | 53.00±2.65 ^{a, b} | 28.67±0.58 ^{a, b} | 19.53±0.65 ^{a, b} | 6.80±0.10 ^{a, b} |

Values presented as mean ± standard deviation ($n=3$). Values in the same column bearing the superscripts 'a' and 'b' are significantly different at $P \leq 0.05$ when compared with Group I (Control) and Group II, TP=Total Protein, ALP= Alkaline Phosphatase, ALT= Alanine Transaminase, AST= Aspartate Transaminase, ALB= Albumin, TB= Total Bilirubin, DB= Direct Bilirubin, CCl₄= Carbon tetrachloride.

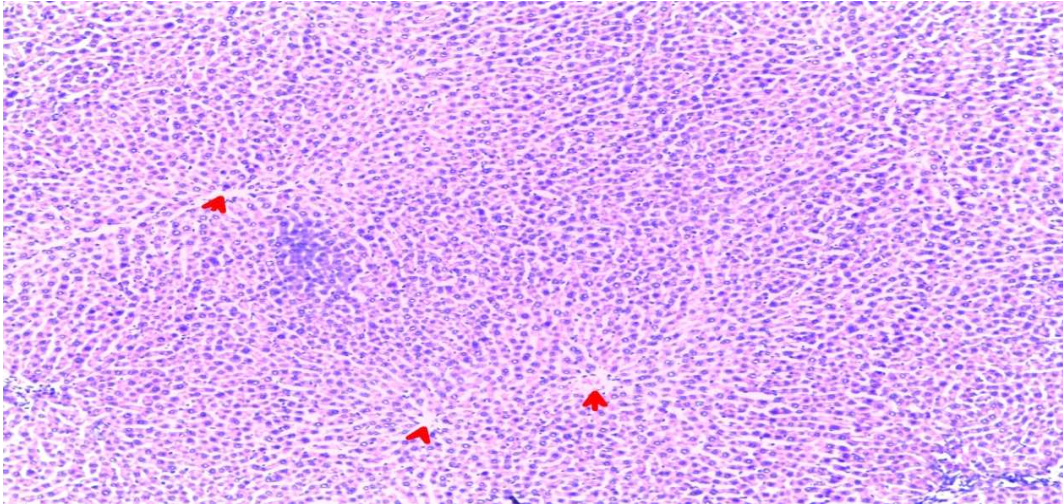


Figure 1: Stained cross section of a liver showing unremarkable liver architecture with hepatocytes arranged in cord radiating from central venule (H and E Stain ($\times 100$))

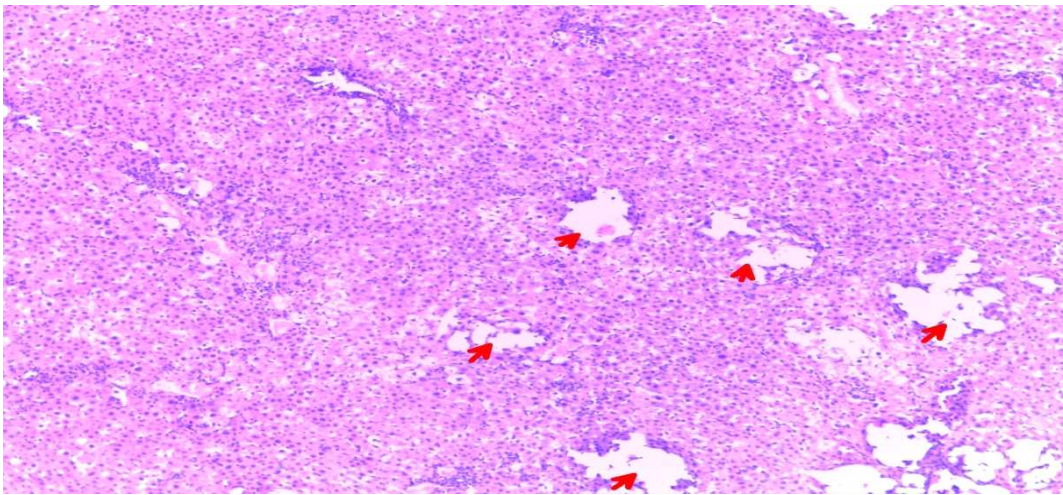


Figure 2: Stained cross section of a liver showing damages with the loss of normal architecture after CCl_4 administration (H and E Stain ($\times 100$))

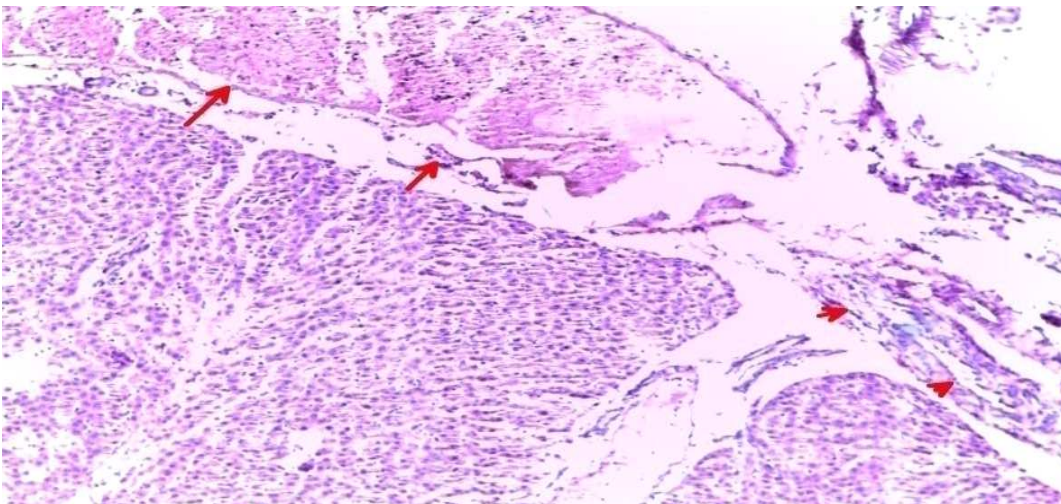


Figure 3: Stained cross section of a liver in CCl_4 induced hepatotoxic rat administered with daily dose of 50mg/kg of *Cassia sieberiana* for four weeks showing areas of fibrosis and vascular congestion. (H and E Stain ($\times 100$))

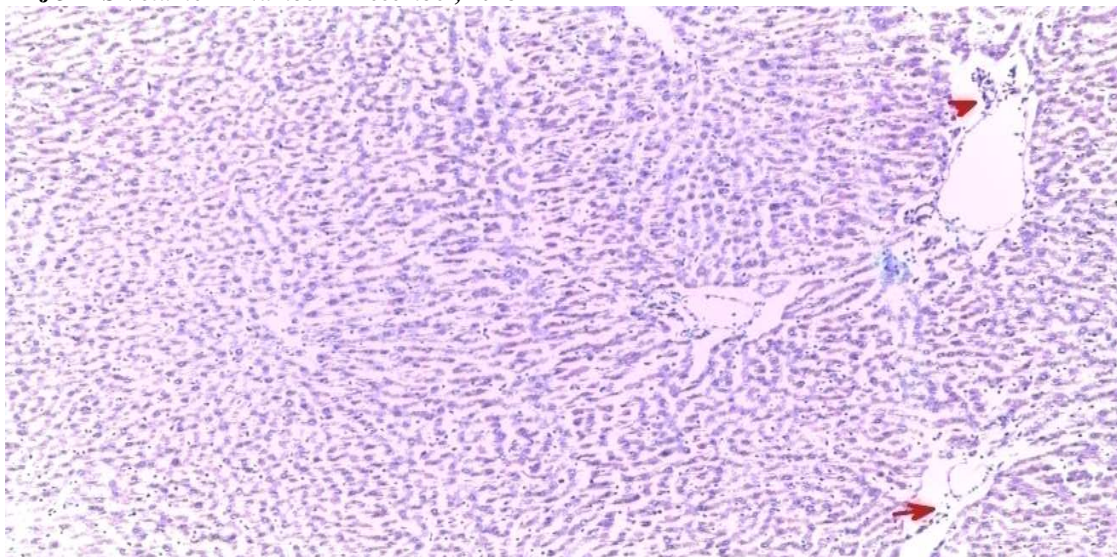


Figure 4: Stained cross section of a liver in CCl₄ induced hepatotoxic rat administered with daily dose of 100mg/kg of *Cassia sieberiana* for four weeks showing` areas of mild fibrosis. (H and E Stain (×100))

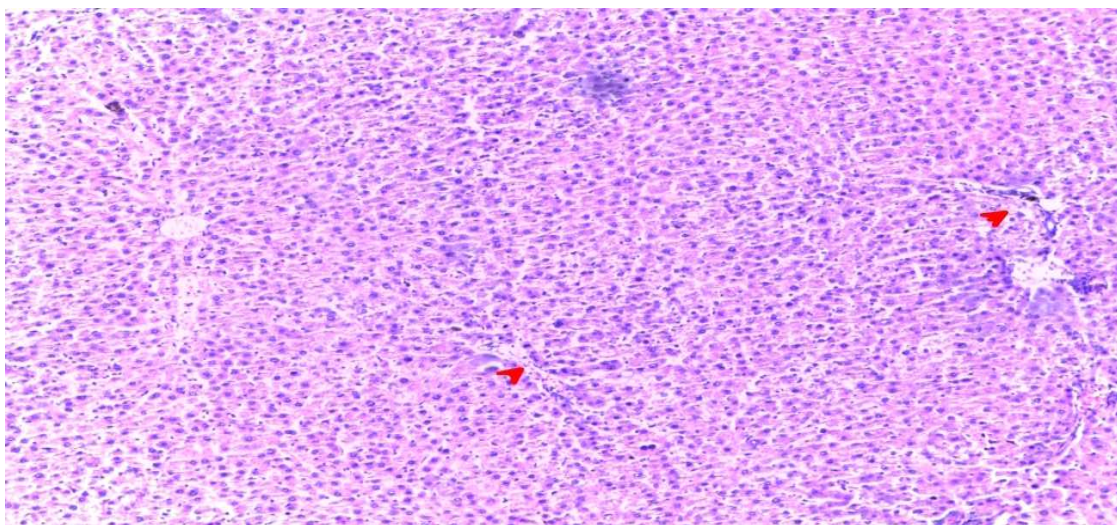


Figure 5: Stained cross section of liver in CCl₄ induced hepatotoxic rat administered with daily dose of 150mg/kg of *Cassia sieberiana* for four weeks showing areas of mild fibrosis. (H and E Stain (×100))

DISCUSSION

Phytochemicals exhibit a diverse physiological and pharmacological activities that includes disease preventive properties. The result obtained from phytochemical analysis of the *Cassia sieberiana* stem bark aqueous extract revealed high concentration of tannins, followed by saponins, alkaloids, cardiac glycosides and then flavonoids. These results agree with the findings of Aliyu, *et al.* (2013), Donkor, *et al.* (2014) and Awomukwu, *et al.* (2015) who reported that the aqueous leaf extract of *Cassia sieberiana* revealed high concentration of tannins in *Cassia sieberiana*. However, the types and amount of phytochemicals obtained during an extraction process depends significantly on solvent used (Bello *et al.*, 2016). Hence the lower concentrations of cardiac glycosides, alkaloids and flavonoids does not means that they are low in the plant but probably because

they are poorly soluble in aqueous solvent. Nevertheless, the presence of the phytochemical compound in the plant provide empirical basis for its traditional medicinal uses (Tella *et al.*, 2005). As such higher concentrations of tannins and saponins may be responsible for hepato-curative effect of the plant. Determination of LD₅₀ is usually the initial step in the evaluation of the toxic characteristics of a substance. It is aimed at estimating the toxicity profile of a compound following single exposure (Lorke, 1983). The result of the LD₅₀ of *Cassia sieberiana* aqueous bark extract does not show any sign of toxicity or mortality up to the maximum dose of a higher dose of 5000mg/kg. This shows that ASBEC is practically non-toxic to the rats. This agrees with the findings of Donkor, *et al.* (2014) who reported that the root of *Cassia sieberiana* shows no sign of toxicity.

Liver is an important organ in the body. It plays a key role in metabolic processes. Carbon tetrachloride (CCl₄) is a well-established hepatotoxin that acts via a highly reactive trichloromethyl radical generation that causes free radical-mediated lipid peroxidation (Alamgeer *et al.*, 2017) of the cytoplasmic membrane phospholipids causing functional and morphological changes in the cell membrane which leads to accumulation of lipid derived oxidants causing liver injury (Obidah *et al.*, 2009). These damages lead to the leakage of liver components (AST, ALP, ALT, total bilirubin, direct bilirubin) into the serum that are supposed to be contained within the hepatocytes, while at same time compromising the liver's synthesizing capacity (Donkor *et al.*, 2014) as observed in decrease in serum levels of total protein (TB) and albumin (AB). Hence, this provides a good model for inducing liver damage.

There was statistically significant reversal of the parameters of liver damage in dose dependent manner after ASBEC administration compared to the CCl₄ only treated group. The extract did not produce statistically significant reversal in the parameters of

liver damage compared to the positive (normal saline treated) control. This shows that the plant has active metabolites with hepato-regenerative and/or hepatocurative activity. Condensed tannins have been suggested to have free radical scavenging and antioxidant activities (Pithayanukul *et al.*, 2009) with anti-inflammatory property via cyclo-oxygenase 2 modulation (Smith *et al.*, 2000). Condensed tannins in ASBEC may be responsible for the hepatoprotective/hepatocurative activities observed. Similarly, saponins have been reported also to exhibit hepatoprotective activity via modulation of its antioxidant (Elekofehinti *et al.*, 2012) and anti-inflammatory activities (Akkol *et al.*, 2007).

CONCLUSION

The oral administration of aqueous stem bark extract of *Cassia sieberiana* shows a capacity for hepatocurative properties in rats. The presence of high concentrations of tannins and saponins in the ASBEC may be responsible for the hepato-curative effect observed.

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