

Full Length Research Paper

Hepato-protective potentials of *Sterculia setigera* stem-bark extract on acetaminophen induced hepato toxicity in Wistar albino rats

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The study was set to investigate the potency of stem bark extract of *Sterculia setigera* as a hepato-protective agent against acute administration (overdose) of acetaminophen in experimental animals. Experimental animals were grouped into six treatments with each group containing five rats. Group 1 was the placebo, Group II was the standard treatment orally administered acetaminophen at a dosage of 250 mg/kgbw and thereafter treated with the standard drug silymarin at 100 g/kgbw after 6 h, to Group III (negative treatment) was orally administered acetaminophen only, at a dosage of 250 mg/kgbw without follow up treatment with standard drug (silymarin). Groups IV, V and VI were orally administered 70% methanol stem bark extract at a dosage of 200, 400 and 600 mg/Kgbw six hours after being orally administered with the hepatotoxic acetaminophen. The trial treatment was carried out for a period of three weeks. The inadequacy of herbs used in curing of liver diseases and other dysfunctions caused by allopathic drugs is enough reason to focus on systematic scientific research to evaluate some species of plants that are traditionally claimed to possess hepato-protective activities.

Key words: *Sterculia setigera*, stem-bark, extract, Wistar albino rats.

INTRODUCTION

Liver is the largest organ in the body, contributing about 2% of the body weight in the average human. It is connected with most of the physiological processes which include growth, immunity, nutrition, energy

metabolism, reproduction, synthesis and secretion of bile, albumin, prothrombin and the reduction of the compliments which are the major effectors of the hormonal branch of the immune system (Dey and Saha,

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2013). Hepatotoxicity is the inflammation of the liver general associated with various drugs used in the modern medicine, different chemicals, toxins and viruses (Ravikumar et al., 2005; Stierum et al., 2005).

Hepatic problems along with heart problems are the major causes of death across the world. Roger and Pamplona (2001) and EllahiBukish et al. (2014) reported that, two million people die annually from liver related disorders with 60,000 occurring from hepatitis B alone. The WHO fact sheets (2005) also reveals that, more than 170 million people have long term liver infections with hepatitis C virus (Bartholomew et al., 2014).

Numerous medicinal plants and their formulations are being used for disorders in ethnomedical practices and in traditional system of medicine in different parts of the world. This situation arose from the fact that, conventional drugs used in the treatment of liver diseases are often unavailable, inaccessible and unaffordable particularly to the rural poor that suffer most, the burden of the disease (Anurag et al., 2013).

For instance, cirrhosis was the twelfth leading cause of death in the United States in 2007 and represented a large economic burden with the national cost of treatment ranging from \$14 million and above, in addition to the \$2 billion as an indirect cost due to loss of work productivity and reduction in the health-related quality of life (HRQOL). Depending on the disease aetiology, this has been on the progressive rise and is expected to significantly increase to \$2.5million and \$10.6 million respectively by the year 2028 (Teschke et al., 2012; Christopher and Taosheng 2017). Unwanted side effects experienced with most of the orthodox drugs are another major reason for a search of an alternative source of drugs against liver diseases.

The plant *Sterculia setigera* Del, of the family: Sterculiaceae is a savannah, widespread in the savannah areas of the tropical Africa. It is mostly found in the open savannah woodlands often characterised by stony hills. It is widespread in Nigeria and called by different tribes with different names. For instance, it is called Kukuuki in Hausa, Kokongiga in Nupe, Bo' boli in Fulfulde, Sugubo in Kanuri, Ose-awere/Esofunfun/Etula in Yoruba, Ompla/Upula in Idoma, Upuru in Igede, Ufia in Igala and Kaume-ndul/Kumenduur in Tiv languages (Igoli, et al., 2005).

This plant has wide application as a traditional remedy to various ailments in Nigeria. For instance, the Yorubas use its burnt stem bark in the preparation of black soap against dermatosis (Adjanahun et al., 1991), the Igedes also employ the stem bark decoction to treat diarrhoea (Almagoul et al., 1985). Several tribes in the North central Nigeria use the mercerated bark from the plant for the treatment of dysentery (Igoli et al., 2003). This work therefore, set to evaluate the potency of this plant (*Sterculia setigera*) as a hepato-protective agent that will be more affordable, accessible and readily available to the rural poor in Nigeria and the rest of sub-Saharan Africa.

MATERIALS AND METHODS

Plant material

The ethno-botanical survey was carried out in the surrounding villages namely, Old/New Awuru, Koro, Popo, Kere, Lubaruru and Dogongari villages around New-Bussa in Borgu local government area of Niger State. The main aim was to ascertain from the local people (particularly the elderly ones), the plant species commonly utilised in the traditional management of liver diseases. Part(s) utilised, method of preparation and period of harvest were also enquired from the interviewees. The identity of the plant was confirmed by Mr Musa Idris in the Department of Forestry, Federal College of Wildlife Management, New Bussa, Nigeria. The plant was deposited at the Forestry Research Institute Herbarium with an assigned voucher number FIH/Garba/NBS/1467.

Preparation of the extract

The crude extract was prepared based on the method described by Garba et al. (2015). Briefly, fifty gram of the dried sample was pulverised to powdered form and cold extracted in 400 ml of 70% v/v (methanol/water mixture). Extraction lasted for 48 h. The extract was filtered using muslin cloth and the solvent was removed and recovered using rotary evaporator. The extract was then transferred into a sterile universal bottle and stored at 4°C until required for use. The yield of the extract was 5.46 g/50 g or 10.92% of the whole sample extracted.

Phytochemical analysis

The phytochemical analysis of the extract from stem bark of *S. setigera* was carried out based on coloration and precipitation test as described by Trease and Evans (2002) and Sofowara (1982).

Test for alkaloids

0.5 g of extract was diluted into 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggen dorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggen dorff's reagent) was regarded as positive for the presence of alkaloids.

Test for phenols

1 ml of crude extract and Iron (III) chloride were mixed for 2 min. Formation of a deep bluish green colouration of the mixture indicate the presence of phenols.

Test for tannins

0.5 g of the extract was boiled with 10 ml of distilled water in a test tube and then filtered. A few drops of 10% of ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for terpenoids (Salkowski test)

To 0.5 g of the extract was added 2 ml of chloroform. Concentrated

H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for cardiac glycosides

1 g of the extracts was treated with 2 ml of glacial acetic acid, a drop of 10% FeCl₃ and 1 ml of concentrated H₂SO₄. The appearance of brown coloration indicates the glycosides.

Test for flavonoids

5 ml of dilute ammonia was added to the aqueous portion of the extract followed by concentrated sulphuric acid (1 ml). A yellow coloration that disappears on standing indicates the presence of flavonoids.

Test for saponins

To 0.5 g of extract was dissolved in 5 ml of distilled water in test tube. The solution was shaken and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken after which it was observed for the formation of an emulsion.

Test for anthraquinones

1 ml of the plant crude extract was mixed with 1 ml of chloroform, then 10% NH₃ solution was added to the mixture. A brick red precipitate indicate the presence of anthraquinones.

Test for phlobatannins

0.2 g of the crude extract was mixed with 5 ml of 1% HCl in a test-tube and heated for 2 min. A red precipitate indicates the presence of phlobatannins.

Test for steroids

Five drops of concentrated H₂SO₄ was added to 0.2 g of the extract. A reddish brown colour indicates the presence of steroids.

Experimental animals

Thirty experimental animals (Wister rats) were purchased from animal house of the Federal University of Technology, Minna, Niger State. The rats were housed in a rat Pen(s) measuring 3 m × 2 m × 2.5 m. The floor surface was overlaid with sawdust which was changed at three days intervals to prevent mould growth. They were properly fed with rat's pellets and water *ad libitum*. They were allowed twelve days to get properly acclimatised with our laboratory conditions. The handling of the animals in the course of experimental work was done strictly based on the Canadian Council on Animal Care guidelines (CCAC, 1999).

Experimental design

Thirty experimental animals (Wister rats) were grouped into six of five rats each (n=5).
Group I was the placebo
Group II was the standard treatment orally administered acetaminophen at a dosage of 250 mg/kgbw and thereafter treated

with the standard drug silymarin at 100 g/kgbw after six hours. Group III (negative treatment) was orally administered acetaminophen only, at a dosage of 250 mg/kgbw without follow up treatment with standard drug (silymarin). Groups IV, V and VI were orally administered 70% methanol stem bark extract at a dosage of 200, 400 and 600 mg/Kgbw six hours post oral administration with the hepatotoxic acetaminophen. The trial of induced toxicity and follow-up treatments with both standard drug and the extract was carried out for a period of three weeks.

Blood collection and measurement of parameters

On the 22nd day, the experimental animals were sacrificed and the blood sample was collected in a heparinised and non-heparinised sample bottles for haematological and serum biochemical analysis respectively. The haematological parameters were determined using the automated haemato-analyser Sysmex kx21, (product of Sysmex corporation, Japan).

Calculation of absolute values

The different absolute values such as, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from values of RBC, PCV and Hb as follows: MCV (millimicron) = PCV% × 10 / RBC count (x million per mm³); MCH (picogram) = Hb g/dl × 10 / RBC count (x million per mm³) and MCHC (picogram) = Hb g/dl × 100 / PCV %

Determination of biochemical parameters

The biochemical analyses were determined for Alkaline phosphatase (ALP) based on methods of Tietz (1995) (Gornall et al., 1949), Aspartate transaminase (AST), Alanine transaminase (ALT), Gamma glutamyl transferase (γGT), and Isocitrate dehydrogenases (ICDH) as described by Reitman and Frankel (1957). While the serum total protein concentration was estimated by Biuret method as described by Gornall et al. (1949).

Statistical analysis

The data are presented as mean ± S.E.M. All the data were analysed by one-way ANOVA and differences between the means were assessed with Duncan Multiple comparison test. Differences were considered significant at p ≤ 0.05. All analyses were carried out using Statistical Package for Social Science (SPSS) version 2.0 (USA).

RESULTS AND DISCUSSION

From the result obtained in Figure 1, it is clear that the extract substantially protects the liver from the oxidative damage that usually characterise the continuous administration of acetaminophen. This becomes vivid from the ALT concentrations in all the other treatments (Placebo inclusive) that appear not to be significantly different (p ≤ 0.05) from one another, but with quite significant difference (p ≤ 0.05) from the values obtained from the negative control. Of interest is the fact that, the extracts from this plant appeared to be nearly as effective

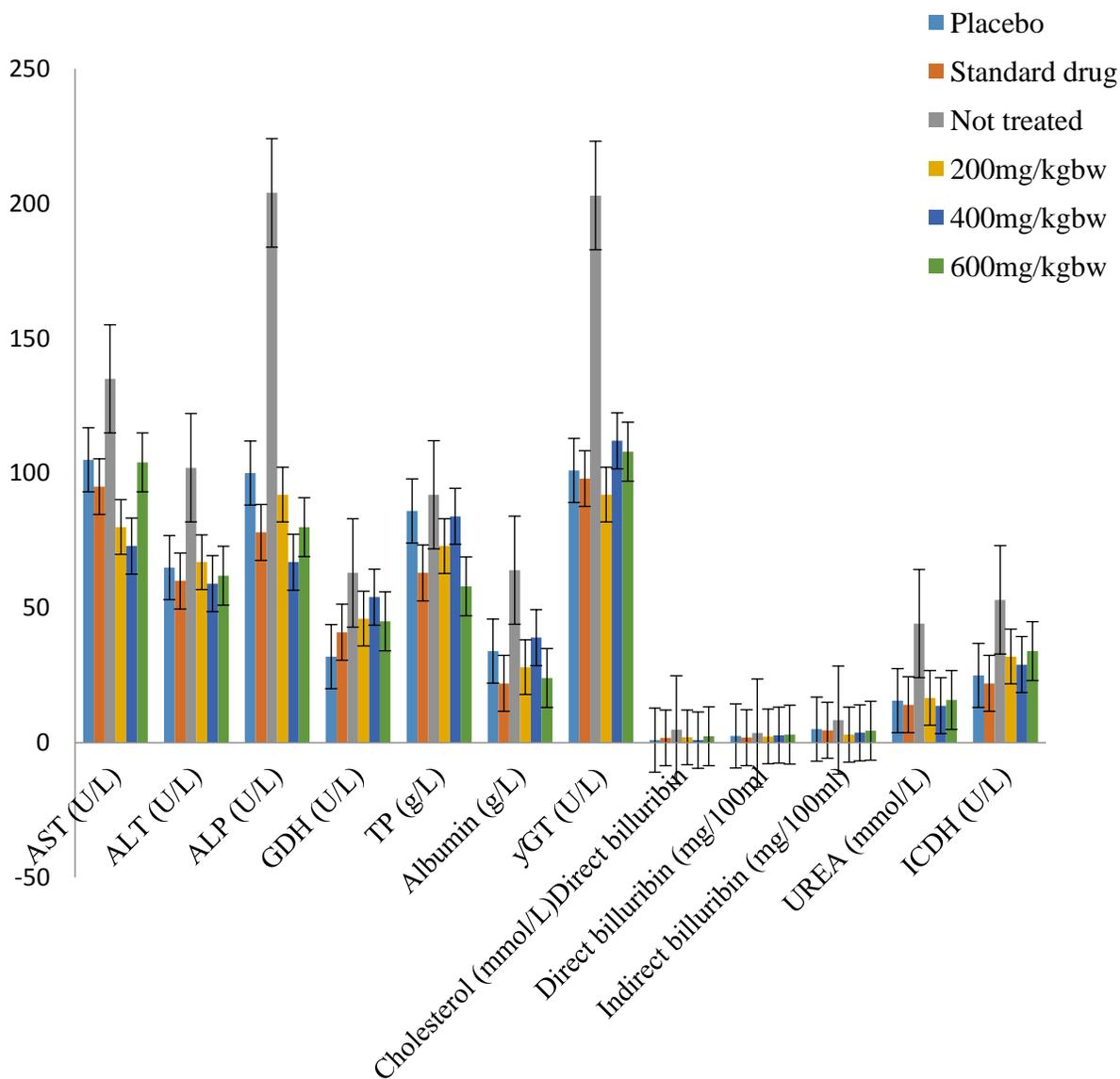


Figure 1. Biochemical parameters of rats administered different concentrations of *Sterculia setigera* stem bark extract. AST = Aspartate transaminase, ALT = Alanine transaminase, ALP = Alkaline phosphatase, GDH = Glucose dehydrogenase, TP = Total protein, γ GT = Gamma glutamyl transferase, ICDH = Isocitrate dehydrogenase.

as the standard drug (silymarinR) in maintaining the integrity of the liver cells, since high values of ALT is an indication of inflammation of the hepatocytes (Masto et al., 2018).

Conversely, while there is no significant difference ($p \leq 0.05$) between the AST concentration in the standard treatment, 200 and 400 mg/Kgbw, there appeared to be significant difference between these groups and the placebo, negative control and 600mg/kgbw and the former groups showed no significant difference ($p \leq 0.05$) in their AST concentrations. Myocardial injury/damage, might be the possible cause for the rise in the AST level in the former groups/treatments (Gao et al., 2015). The

ALP level is known to increase due to increased bone deposition, liver damage, hyperthyroidism, biliary tract disease, intestinal damage, hyperadrenocorticism, corticosteroid administration, barbiturate administration, and generalized tissue damage (including neoplasia) (Rangboo et al., 2016).

The levels falling within the normal range (in rats) in the standard group and the groups administered various concentrations (200, 400 and 600 mg/kgbw) is an indication of the extracts at these concentrations possessing similar protective and homeostatic role as the standard drug (silymarinR), while high value in the placebo group might not be unconnected to the raised

Table 1. Effect of 70% methanol stem bark extract of *S. setigera* on haematological parameters in acetaminophen induced hepatotoxic rats.

Treatment	HB(g/dl)	PCV (%)	MCV (mmicron)	MCH (pg)	MCHC (g/L)	RBC x10 ⁶ /mm ³	PLC (x10 ³ /mm ³)	TWBC (x10 ³ /mm ³)	NEU (%)	LEU (%)	MON (%)
Placebo	22.5±2.34 ^c	30.0±4.3 ^b	56.0±3.34 ^a	30±2.22 ^a	75±1.33 ^a	5.0±5.32 ^c	1050±32.45 ^a	32.0±3.23 ^a	24.0±1.34	50.0±3.23 ^a	26.0±2.32
Positive control(Std drug)	25.9±2.35 ^c	32.0±4.21 ^b	63.0±5.55 ^c	32.0±2.12 ^a	80.9±1.23 ^d	8.0±4.23	2298±21.14 ^d	53.7±2.32	0.6±1.23 ^a	81.0±5.11 ^d	13.0±2.11 ^a
Negative control	13.5±2.57 ^a	20.0±3.56 ^a	73.0±4.21 ^d	41.0±2.63 ^b	67.5±1.14 ^b	3.2±3.22 ^a	1287±22.08 ^c	74.0±1.11 ^d	0.9±1.22 ^b	74.0±7.23	17.0±2.23
200mg/kgbw	19.4±2.45 ^b	20.0±1.32 ^a	46.0±4.22 ^a	50.0±2.67 ^c	97.0±1.56 ^c	4.0±3.32 ^b	1132±22.89 ^b	62.0±3.32 ^c	10.0±1.23 ^b	72.0±4.33 ^c	18.0±2.32 ^b
400mg/kgbw	17.9±2.45 ^b	20.0±1.32 ^a	43.0±3.23 ^a	46.0±2.13 ^b	89.5±0.32 ^{bc}	4.0±4.11 ^b	1023±21.13 ^a	50.0±2.22 ^b	11.0±1.45	72.0±5.23 ^c	17.0±2.12 ^b
600mg/kgbw	19.8±2.34 ^b	20.0±1.34 ^a	66.0±5.76 ^c	67.0±2.65 ^d	99.0±1.23 ^c	4.5±4.33 ^b	1118±31.22 ^b	62.1±1.14 ^c	12.0±0.12 ^c	66.0±3.45 ^b	22.0±2.32 ^c

Values are mean ±SEM of 3 determinations. The values along the row with different superscripts are significantly different ($p \leq 0.05$).

Table 2. Qualitative phytochemical screening of *S. setigera*.

Plant sample	Tannins	Flavonoids	Alkaloids	Phenol	Saponin	glycoside
<i>Sterculia setigera</i>	+	+	+	+	+	+

levels of circulating steroids and biliary disease that may be inherent in some of the animals within the group (Tang et al., 2017) (Figure 1).

Despite its competing efficacy with the standard drug, it suffice to state that, the significant difference ($p \leq 0.05$) observed in the GDH concentration between the groups treated with the extract and the standard groups confirms that, liver necrosis which is one of the side effects of orthodox drugs against liver diseases (Lemasters, 1999) is more pronounced in the extract compared to the standard drug and this will require further fractional purification to eventually isolate the notorious compound responsible for the observed necrosis. Other parameters such as γ GT, Albumin, Cholesterol, Direct and indirect billuribin were all found not to be significantly different ($p \leq 0.05$) from the positive control but significantly different ($p \leq 0.05$) from the negative

control and this is a clear attestation to the fact that their levels increases due to fatty meals, hepatic or biliary diseases (Ashtari et al., 2015).

Moreover, one unique pharmacological properties that could be envisaged to be possessed by *S. setigera* extract is its non-polycytonaemic effect when compared with the standard drug as shown in Table 1. The RBC, Hb and PCV values were significantly higher ($p \leq 0.05$) in the group administered the standard drug when compared with the groups administered the extract. However, it remains to be established if the cause is relative or absolute. While the lower values obtained in the negative group might be attributed to the anaemia that usually characterised liver necrosis (Franchini et al., 2016) (Table 1). However, administration of both the standard drug and the *S. setigera* extract were observed to stimulate the immune mechanism of

the tested animals which might be extrapolated to mean the presence of some unusual compounds in both the standard drug and the extract since the placebo group were observed to have relatively lower values (Table 1). Additionally, higher Platelet counts observed in the group administered standard drug and the negative group further corroborates the earlier observation.

Qualitative analysis of the extract shows the presence of Flavonoids and phenols among other phytochemicals (Table 2). One of the flavones Naringin has been shown to play a significant role as a hepatoprotective agent (Lee et al., 2004). It was observed by Jayaraman and Namasivayam (2011) that, the Naringin is converted in monogastrics to Naringenin which was found to be a potent hepatoprotective agent. The possible presence of such compound in the flavonoids found to be contained in such plant might

attribute to the observed effect.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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