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## **Modulatory Effect of Aqueous Stem Bark Extract of *Psidium guajava* Linn. against CCl<sub>4</sub> Induced Liver Damage in Rats**

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### **ABSTRACT**

The present study was aimed to evaluate the stem bark aqueous extract of *Psidium guajava* for modulatory effect against CCl<sub>4</sub> induced liver damage in rats. A total of thirty six male rats, were randomly divided into six groups of six rats each. The extract was administered orally for 15 days at 125, 250 and 500 mg kg<sup>-1</sup> b.wt. The results obtained showed that treatment with the extract significantly (p<0.05) restored liver weight. There was significant (p<0.05) increase in the level of Packed Cell Volume (PCV), haemoglobin (Hb) and Red Blood Cell (RBC) counts and significant (p<0.05) decrease in White Blood Cell (WBC) counts compared to toxin control group. Also administration of the extract caused significant (p<0.05) decrease in the activities of Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP) and the level of total bilirubin and significant (p<0.05) increase in total protein level compared to toxin control group. Similarly the extract caused a significant (p<0.05) increase in the activities of Catalase (CAT) and Superoxide Dismutase (SOD) and significant (p<0.05) decrease in reduced Glutathione (GSH) and Thiobarbituric Reactive Substances (TBARS) level compared to group 2 (toxin control group). The histopathological study indicated that treatment with the extract restored and regenerated hepatic cells compared to toxin control group. This study found that administration of aqueous stem bark extracts ameliorated hepatotoxicity induced by CCl<sub>4</sub> in rats.

**Key words:** Liver damage, oxidative stress, ameliorative effect, *Psidium guajava*, CCl<sub>4</sub>

### **INTRODUCTION**

Liver is a vital internal organ and part of the digestive system; it plays an important role in maintaining health and regulates many important metabolic functions such as detoxification and secretory functions in the body. Liver diseases are a major cause of illness and death worldwide; Hepatitis and cirrhosis are particularly common liver disorders (Cubero and Nieto, 2006; Ajith *et al.*, 2007). These diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Carbon tetrachloride (CCl<sub>4</sub>) is a xenobiotic that produces hepatotoxicity in human as well as in various experimental animals (Lee *et al.*, 2007; Rudnicki *et al.*, 2007). Covalent binding of the metabolites of CCl<sub>4</sub>, trichloromethyl (CCl<sub>3</sub>·) free radicals and subsequent derivative to cell proteins is considered to be the initial step in a chain of events that eventually lead to membrane lipid peroxidation and finally to cell death (Weber *et al.*,

2003). Silymarin has been used for over 20 years in clinical practice for the treatment of toxic liver diseases (Messner and Brissot, 1990). In this study, silymarin was used as a standard drug against CCl<sub>4</sub> induced hepatic damage in rats. Herbal antioxidants have become a vital area of research since past few years (Premeanth *et al.*, 2011; Yahaya *et al.*, 2012), due to their potentials of scavenging reactive oxygen species and reduce free radical induced tissue injury (Gupta and Flora, 2005). It is being acknowledged that plants contain non-nutritional constituents with beneficial health effects, such as anti-inflammatory and anti-carcinogenic properties (Bissell, 1998), hepatoprotective and cardio protective properties (Croft *et al.*, 1999). *Psidium guajava* Linn. belongs to the family of Myrtaceae. It is cultivated throughout Asia and Africa including Nigeria. Besides being consumed as fresh fruit, it can be processed into juices and jams and preserved products. Apart from these uses *Psidium guajava* contains numerous phytochemical compounds which can effectively scavenge free radicals. The plant was reported to have antidiarrheal, antimicrobial, antigenotoxic, hepatoprotective, lipid-lowering, hypoglycemic and antioxidant activities (Kamath *et al.*, 2008; Gutierrez *et al.*, 2008). Also various parts, like roots stem bark, leaves and fruits were reported to possess many pharmacological properties and it is used in the treatment of various disorders such as respiratory and gastrointestinal disorders (Begum *et al.*, 2002; Kaneria and Chanda, 2011). Therefore, the present study was undertaken to evaluate the ameliorative effects of aqueous stem bark extract of *P. guajava* Linn against CCl<sub>4</sub> induced liver damaged in rats.

## MATERIALS AND METHODS

**Chemicals, reagents and drugs:** Diagnostic kits for the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum bilirubin were purchased from Randox Laboratories Ltd, (United Kingdom). Bovine Serum Albumin (BSA), Trichloro Acetic Acid (TCA), thiobarbituric acid (TBA), reduced glutathione (GSH), Sodium pyrophosphate, Ethylene Diamine Tetra Acetic acid disodium salt (EDTA) 5,5-dithiobis(2-nitrobenzoic acid) (DTNB),  $\beta$ -nicotinamide Adenine Dinucleotide Hydrogen (NADH) were obtained from Sigma Chemical (St. Louis, MO, USA). Silymarin was purchased from Vellore, India. Pyridine (C<sub>5</sub>H<sub>5</sub>N), disodium hydrogen phosphate (Na<sub>2</sub>HPO<sub>4</sub>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), dihydrogen potassium phosphate anhydrous (KH<sub>2</sub>PO<sub>4</sub>), Potassium heptochromate (VI), Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), thiobarbituric acid (TBA), dimethylsulfoxide (DMSO), carbontetrachloride (CCl<sub>4</sub>) were purchased from Merck India Ltd (Mumbai, India). All other chemicals and reagents were of analytical grade.

**Experimental animals:** A total of thirty six apparently healthy male wister albino rats weighing between 160-180 g were purchased from the animal house, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria. The animals were kept in a clean plastic cage under 12 h light and dark cycles and were allowed free access of water and standard pellet diets *ad libitum*. The animals were allowed to acclimatize to the laboratory environment for one week before the commencement of the experiment.

**Plant sample collection and identification:** The stem barks of *P. guajava* Linn were collected from the garden of the Institute of Agricultural Research (IAR) Faculty of Agriculture Ahmadu Bello University Zaria, Nigeria in the month of April, 2012. The sample were identified and authenticated at the herbarium unit, Department of Biological Sciences, Ahmadu Bello University Zaria Nigeria, and a voucher number was given VN/2012/3253.

**Sample processing and preparation of extract:** The stem barks of *P. guajava* Linn were cleaned, washed with tap water and air-dried. The dried barks were broken into small pieces using pestle and mortar and then pulverized using electric blender into fine powder. One thousand grams (1000 g) of powder were weighed and soaked into 4 litres of distilled water. The mixture was shaken regularly at interval of 4 h and kept at room temperature for 48 h. After 48 h the homogenate was filtered using muslin cloth and the filtrate obtained were re-filtered using Whatman No. 1 filter paper, the filtrate obtained was concentrated using water bath set at 50°C for 10 h.

**Acute toxicity study:** The median Lethal Dose (LD<sub>50</sub>) of aqueous stem bark extract of *P. guajava* was carried out according to the method of described by Lorke (1983). The method involved two phases of which nine rats were grouped into three groups of three rats each. They received 10, 100 and 1000 mg kg<sup>-1</sup> b.wt. of the extracts, respectively. In the second phase also nine rats were grouped into three groups of three rats each and they received 1600, 2900 and 5000 mg kg<sup>-1</sup> b.wt. The rats were observed daily for any signs of toxicity including death throughout the period of study.

**Phytochemical analyses:** Quantitative phytochemicals analyses of stem bark extract of *P. guajava* were carried out according to the following methods: Tannins (Harborne, 1973), saponins and alkaloids (Obadoni and Ochuko, 2001), flavonoids (Bohm and Koupai-Abyazani, 1994).

**Experimental design and treatment:** A total of 36 male rats weighing 160-180 g were randomly divided into six groups of six rats each:

- Group 1:** It (served as normal control) was administered orally DMSO 1 mL kg<sup>-1</sup> b.wt. for the 15th days of the experimental period
- Group 2:** It (served as toxin control) was administered (i.p) 1 mL kg<sup>-1</sup> b.wt. CCl<sub>4</sub> in DMSO (1:1) on the 7th and 14th days only
- Group 3:** It was administered orally 50 mg kg<sup>-1</sup> b.wt. silymarin (standard drug) throughout the 15th days of the experimental period and then 1 mL kg<sup>-1</sup> b.wt. (i.p) of CCl<sub>4</sub> in DMSO (1:1) on the 7th and 14th days only
- Group 4:** It was administered orally 125 mg kg<sup>-1</sup> b.wt. of the extract throughout the 15th days and then 1 mL kg<sup>-1</sup> of CCl<sub>4</sub> in DMSO (i.p) on the 7th and 14th days only
- Group 5:** It was administered orally 250 mg kg<sup>-1</sup> b.wt. of the extract throughout the 15th days and then 1 mL kg<sup>-1</sup> b.wt. CCl<sub>4</sub> in DMSO (i.p) on the 7th and 14th days only
- Group 6:** It was administered orally 500 mg kg<sup>-1</sup> b.wt. of the extract throughout the 15th days and then treated with 1 mL kg<sup>-1</sup> b.wt. CCl<sub>4</sub> in DMSO (1:1) on the 7th and 14th days only

**Body and organ weights:** The initial and final body weights of all rats in each group were measured and recorded. Liver weights of all rats in each group were also measured after post treatment sacrifice.

**Evaluation of haematological parameters:** The blood sample was transferred into properly labelled sample bottle and centrifuged at 4000xg for 15 min. The plasma obtained was used for the

determination of Erythrocyte count (RBC), White Blood Cell (WBC), Packed Cell Volume (PCV) and haemoglobin (Hb) with the aid of an Auto Blood analyzer (Mindray Haematology analyzer, BC-2300).

**Evaluation of hepatic biochemical parameters:** At the end of the experimental period, animals were fasted overnight for 12 h and sacrificed by cervical dislocation. Serum was harvested from the blood and was used for determination of biochemical parameters using commercial reagent kits (Randox Laboratories, United Kingdom) by the following methods: AST and ALT (Reitman and Frankel, 1957), ALP (Kind and King, 1954), total bilirubin (Mallay and Evelyn, 1937) and total protein (Lowry *et al.*, 1951).

**Preparation of liver for evaluation of antioxidant parameters:** The liver was immediately isolated and washed with normal saline, blotted with filter paper, weighed and homogenized with 10 times (w/v) using a homogenizer in ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 800 g for 5 min at 4°C to separate the nuclear debris. The supernatant so obtained was further centrifuged at 10,000g for 15 min at 4°C to get the post mitochondrial supernatant which was used to assays the activities of superoxide dismutase (SOD) according to the method described by Misra and Fridovich (1972) and Catalase according to the method of Sinha (1972). The levels of reduced glutathione (GSH) was determined by the method of Beutler *et al.* (1963) and Thiobarbituric Acid Reactive Substances (TBARS), assayed as malondialdehyde (MDA) was determined using the method described by Ohkawa *et al.* (1979).

**Histopathological study:** Small pieces of liver tissues in each group were collected in 10% neutral buffered formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections were cut and stained with haematoxylin and eosin (H and E). The tissue sections were examined microscopically at ×100 magnification.

**Statistical analysis:** The results were expressed as the Mean± standard deviation using one-way analysis of variance (ANOVA), followed by Duncan *Post hoc* test and p<0.05 was considered as statistically significant.

## RESULTS

The result obtained from the acute toxicity study indicated that, the LD<sub>50</sub> of aqueous stem bark extract of *P. guajava* Linn. was found to be greater than 5000 mg kg<sup>-1</sup> (Data not showed). The extract showed no sign of toxicity and no death was recorded throughout the time period of this study.

The result of phytochemical analyses (Table 1) indicated that, the amount of tannins, saponins, alkaloids, flavonoids and polyphenols were 0.05±0.02, 0.07±0.01, 0.09±0.02, 0.08±0.03 and 0.98±0.05, respectively. The amount of polyphenolics was the highest and the least amount recorded was tannins.

Table 1: Quantitative phytoconstituents of stem bark extract of *P. guajava*

Sample	Tannins (g g <sup>-1</sup> )	Saponins (g g <sup>-1</sup> )	Alkaloids (g g <sup>-1</sup> )	Flavonoids (g g <sup>-1</sup> )	Polyphenolics (g g <sup>-1</sup> )
Stem bark	0.05±0.02	0.07±0.01	0.09±0.02	0.087±0.003	0.98±0.05

Values are Mean±SD of triplicates determinations

Table 2: Effect of aqueous stem bark extract of *P. guajava* on liver weights in normal and CCl<sub>4</sub> intoxicated rats

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Liver weight (g/100g b.wt.)	3.79±0.01 <sup>a</sup>	6.22±0.02 <sup>b</sup> (I = 64.1)	4.27±0.06 <sup>a</sup> (I = 12.7)	5.86±0.05 <sup>b</sup> (I = 54.6)	5.47±0.06 <sup>b</sup> (I = 44.3)	4.88±0.03 <sup>a</sup> (I = 28.8)

Values are Mean±SD (n = 6), Values with different superscripts in a row are statistically different at p<0.05, D: Percentage decrease compared to control, I: Percentage increase compared to normal control

Table 3: Effect of aqueous stem bark extract of *P. guajava* on haematological parameters of CCl<sub>4</sub> intoxicated rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
PCV (%)	39.17±0.80 <sup>f</sup>	25.33±1.65 <sup>f</sup> (D = 35.3)	38.36±1.65 <sup>f</sup> (D = 2.1)	29.56±2.62 <sup>b</sup> (D = 24.5)	31.01±2.91 <sup>b</sup> (D = 20.8)	37.10±1.61 <sup>c</sup> (D = 5.3)
Hb (g dL <sup>-1</sup> )	14.07±0.21 <sup>d</sup>	9.06±0.76 <sup>a</sup> (D = 35.6)	13.15±0.26 <sup>d</sup> (D = 6.5)	11.11±1.01 <sup>b</sup> (D = 21.0)	12.00±0.45 <sup>bc</sup> (D = 14.7)	12.94±0.86 <sup>c</sup> (D = 8.0)
RBC (×10 <sup>6</sup> μL <sup>-1</sup> )	6.62±0.07 <sup>d</sup>	3.77±0.15 <sup>a</sup> (D = 43.0)	6.14±0.42 <sup>d</sup> (D = 7.3)	4.52±0.33 <sup>b</sup> (D = 31.7)	4.82±0.24 <sup>b</sup> (D = 27.2)	5.56±0.33 <sup>c</sup> (D = 16.0)
WBC (×10 <sup>3</sup> μL <sup>-1</sup> )	7.04±0.17 <sup>a</sup>	9.85±0.64 <sup>f</sup> (I = 39.9)	7.09±0.28 <sup>a</sup> (I = 0.7)	8.53±0.55 <sup>b</sup> (I = 21.2)	7.85±0.97 <sup>ab</sup> (I = 11.5)	7.40±0.52 <sup>a</sup> (I = 5.1)

PCV: Packed cell volume, Hb: Haemoglobin, RBC: Red blood cell, WBC: White blood cell, Values are Mean±SD of triplicates determinations, Values with different superscripts on a row are statistically different at p<0.05, D: Percentage decrease compared to control, I: Percentage increase compared to normal control

Similarly there was significant (p<0.05) increase in liver weight of rats in the entire treated group compared to normal control group (Table 2). However, treatment with 125, 250 and 500 mg kg<sup>-1</sup> of stem bark aqueous extract significantly (p<0.05) prevented the increase in weight of liver in a doses related manner. For instance, the highest percentage increase of liver weight of 64.1% was recorded in group 2 (toxin control) compared to group 1 (normal control) and the lowest percentage increase of 12.7% was observed in group 3 (silymarin treated group).

The haematological parameters of group 2 (toxin control rats) were found to be significantly altered compared to those of normal control group (Table 3). The Packed Cell Volume (PCV) and haemoglobin (Hb) level of all, the treated group showed a decrease compared to group 1. However, group 2 had the highest percentage decrease of 35.3 and 35.6%, respectively for RBC count and Hb level. Similarly, Red Blood Cell (RBC) count significantly (p<0.05) decreased in all, the treated group compared to group 1, treatment with extract caused a dose related elevation of RBC counts, with group 4 having the lowest percentage decrease of 31.7% group 2 having the highest percentage decrease of 43% compared to group 1. Similarly, White Blood Cell (WBC) count was significantly (p<0.05) increased in all treated group compared to group 1. The lowest percentage increase of 0.7% was recorded in group 3 and the highest percentage increase of 39.9% was found in group 2.

The results of serum markers of liver damage (Table 4) indicated a significant (p<0.05) alterations compared to normal control. For instance in all, the treated group, a percentage increase in the activity of serum ALT, AST and ALP were recorded, with group 2 (toxin control group) having the highest increase of 131.5, 97.5 and 76.3% for ALT, AST and ALP, respectively. However the highest reduction in the activities of ALT, AST and ALP was found in group 3 which were treated with silymarin (50 mg kg<sup>-1</sup>) compared to normal control group. Also treated group showed a percentage increase in total bilirubin level compared to group 1, with group 2 having the highest percentage increase of 89.6%. While the total protein content of all treated group were significantly (p<0.05) decreased, with group 2 having the highest decrease of 53.5%.

Table 4: Effect of aqueous stem bark extract of *P. guajava* on the activity of serum ALT, AST, and ALP, total bilirubin and protein levels in CCl<sub>4</sub>intoxicated rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
ALT (IU L <sup>-1</sup> )	38.65±1.05 <sup>a</sup>	89.48±0.63 <sup>d</sup> (I = 131.5)	39.47±1.43 <sup>a</sup> (I = 2.1)	57.33±2.08 <sup>b</sup> (I = 48.3)	43.33±1.53 <sup>b</sup> (I = 12.1)	39.48±0.50 <sup>a</sup> (I = 2.1)
AST (IU L <sup>-1</sup> )	74.95±1.59 <sup>a</sup>	148.00±2.02 <sup>d</sup> (I = 97.5)	75.83±2.84 <sup>a</sup> (I = 1.2)	103.63±0.51 <sup>c</sup> (I = 38.3)	89.22±0.73 <sup>b</sup> (I = 19.0)	79.15±1.23 <sup>a</sup> (I = 5.6)
ALP (IU L <sup>-1</sup> )	68.25±0.68 <sup>a</sup>	120.30±1.14 <sup>e</sup> (I = 76.3)	70.60±0.63 <sup>b</sup> (I = 3.4)	97.67±0.58 <sup>d</sup> (I = 43.1)	78.33±0.58 <sup>c</sup> (I = 14.8)	70.93±0.60 <sup>b</sup> (I = 3.9)
TBIL (mg dL <sup>-1</sup> )	5.89±0.19 <sup>a</sup>	11.17±0.29 <sup>d</sup> (I = 89.6)	6.88±0.09 <sup>b</sup> (I = 16.8)	8.97±0.06 <sup>c</sup> (I = 52.3)	7.04±0.07 <sup>b</sup> (I = 19.5)	6.94±0.10 <sup>b</sup> (I = 17.8)
Protein (mg dL <sup>-1</sup> )	9.93±0.61 <sup>e</sup>	4.62±0.13 <sup>a</sup> (D = 53.5)	8.62±0.53 <sup>d</sup> (D = 13.2)	6.92±0.05 <sup>b</sup> (D = 30.3)	7.21±0.19 <sup>b</sup> (D = 27.4)	8.05±0.13 <sup>c</sup> (D = 18.9)

ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, TBIL: Total bilirubin, Values are Mean±SD of triplicates determinations. Values with different superscripts on a row are statistically different at p<0.05, D: Percentage decrease compared to normal control, I: Percentage increase compared to control

Table 5: Effect of aqueous stem bark extract of *P. guajava* on the activity of CAT and SOD and the levels of MDA and GSH in CCl<sub>4</sub>intoxicated rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
TBARS (MDA) (µmol m g mg <sup>-1</sup> tissue)	4.83±0.04 <sup>a</sup>	11.96±0.06 <sup>b</sup> (I = 147.6)	4.99±0.01 <sup>c</sup> (I = 3.3)	8.33±0.57 <sup>d</sup> (I = 72.5)	6.63±0.33 <sup>c</sup> (I = 37.3)	5.40±0.17 <sup>f</sup> (I = 11.8)
GSH (µmol mg <sup>-1</sup> tissue)	88.37±0.32 <sup>a</sup>	25.50±0.44 <sup>b</sup> (D = 71.1)	84.92±0.12 <sup>c</sup> (D = 3.9)	64.55±0.51 <sup>d</sup> (D = 26.9)	70.45±0.77 <sup>e</sup> (D = 20.3)	79.62±0.54 <sup>f</sup> (D = 9.9)
SOD (IU mg <sup>-1</sup> tissue)	78.35±0.31 <sup>a</sup>	34.88±0.20 <sup>b</sup> (D = 55.5)	75.48±0.05 <sup>b</sup> (D = 3.7)	60.67±0.73 <sup>c</sup> (D = 22.6)	67.13±0.22 <sup>d</sup> (D = 14.3)	70.56±0.39 <sup>f</sup> (D = 9.9)
CAT (IU mg <sup>-1</sup> tissue)	35.15±0.25 <sup>a</sup>	14.86±0.24 <sup>b</sup> (D = 57.7)	31.33±0.56 <sup>c</sup> (D = 10.9)	20.52±0.58 <sup>d</sup> (D = 41.6)	26.33±0.58 <sup>e</sup> (D = 25.1)	29.10±0.62 <sup>f</sup> (D = 17.2)

TBARS: Thiobabituric reactive substance, GSH: Reduced glutathione, SOD: superoxide dismutase, CAT: Catalase. Values are Mean±SD of triplicates determinations, Values with different superscripts on a row are statistically different at p<0.05, D: Percentage decrease compared to control, I: Percentage increase compared to normal control

The antioxidant parameters were significantly (p<0.05) altered (Table 5). There was elevation of MDA level in all, the treated groups compared to group 1. However, treatment with the extract caused a significant reduction of MDA level compared to normal control. The highest percentage increase of 147.6% was recorded in group 2 compared to normal control group. There was decrease in reduced glutathione (GSH) level in all, the treated group compared to group 1. The highest percentage decrease of 71.1% was recorded in group 2. However the percentage decrease of GHS at high dose was 9.9% compared to normal control. Also, the SOD and CAT activities were decrease in all, the treated group. Treatment with stem bark of *P. guajava* extracts significantly (p<0.05) increases the SOD and CAT activities compared to normal control group. For instance the highest percentage decrease of 55.5 and 57.7% for SOD and CAT respectively, were recorded in group 2 (toxin control group) and the lowest decrease of 3.7 and 10.9% were recorded in group 3. The histopathological examination of liver sections of control group (Fig. 1) showed normal cellular architecture with distinct hepatic cells with a well-preserve cytoplasm, sinusoidal spaces and central vein. However, Fig. 2 revealed disarrangement of normal hepatic cells with necrosis, vacuolization of cytoplasm, and feathery degeneration were observed in CCl<sub>4</sub> treated group. Liver sections of the rats treated with a standard drug for treatment of liver diseases (Fig. 3) showed marked

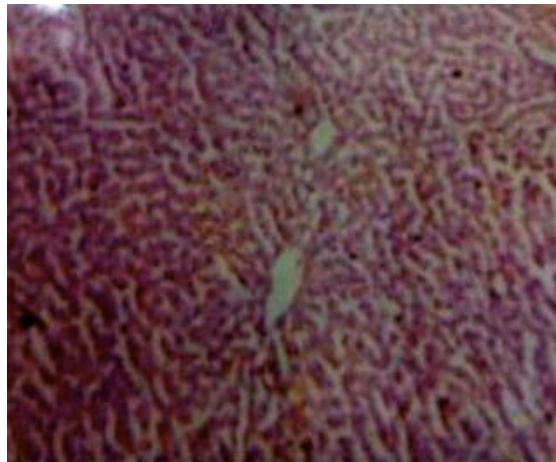


Fig. 1: Microphotograph of the normal control group rat liver section, H and E×100

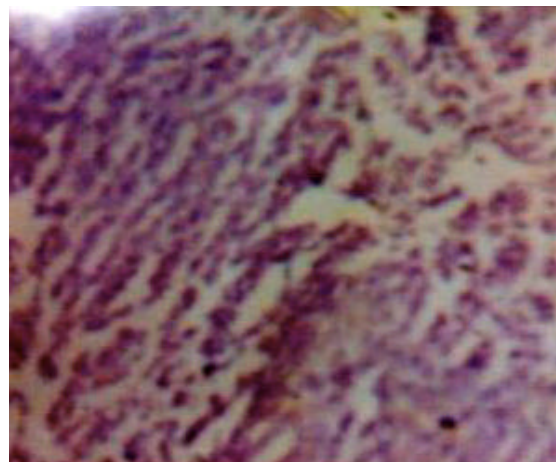


Fig. 2: Microphotograph of CCl<sub>4</sub> (1mL kg<sup>-1</sup> i.p) intoxicated group rat liver section, H and E×100

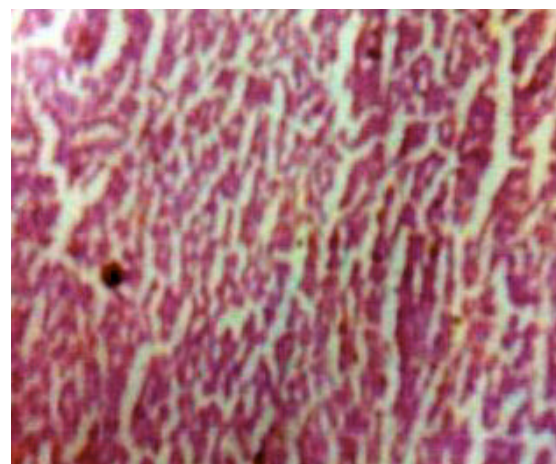


Fig. 3: Microphotograph of silymarin (50 mg kg<sup>-1</sup> p.o) treated group rat liver section, H and E×100



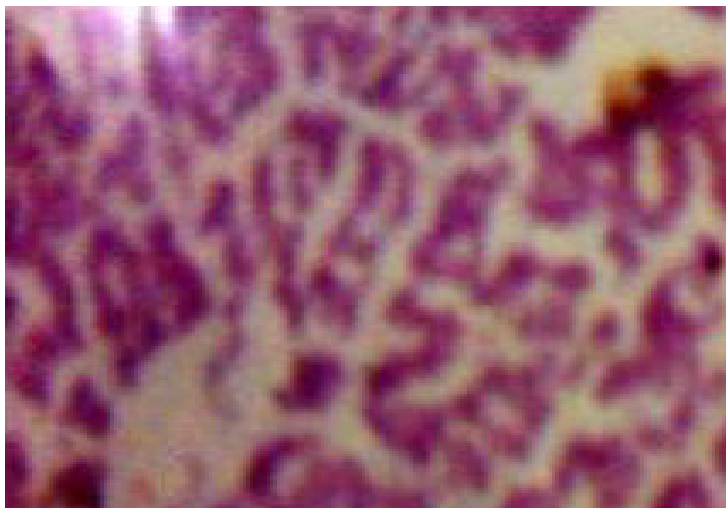


Fig. 4: Microphotograph of  $125 \text{ mg kg}^{-1}$  aqueous stem bark *P. guajava* treated group rat liver section, H and E $\times$ 100

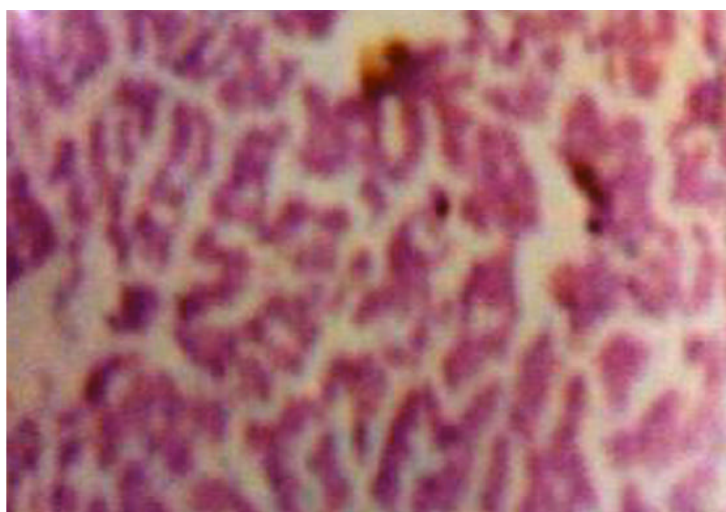


Fig. 5: Microphotograph of  $250 \text{ mg kg}^{-1}$  aqueous stem bark *P. guajava* treated group rat liver section, H and E $\times$ 100

regeneration of cellular architecture and repair in response to the  $\text{CCl}_4$  induced hepatic damage more than the highest dose of the extract administered. However administration of stem bark aqueous extract of *P. guajava* orally at dose of  $125 \text{ mg kg}^{-1}$  (Fig. 4)  $250 \text{ mg kg}^{-1}$  (Fig. 5) and  $500 \text{ mg kg}^{-1}$  (Fig. 6), showed a dose related regeneration, from mild to marked regeneration of cellular architecture of hepatocytes, respectively. This was evident by the presence of megalocytes and absence of necrosis, showing the possibility of tissue repair taking place.

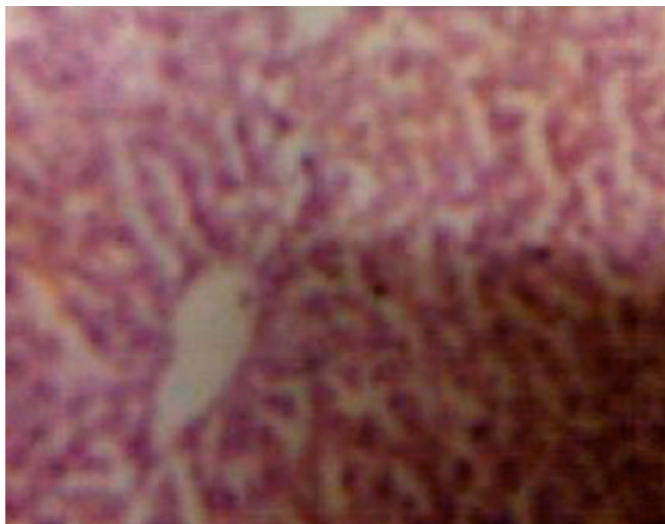


Fig. 6: Microphotograph of 500 mg kg<sup>-1</sup> aqueous stem bark *P. guajava* treated group rat liver section, H and E×100

## DISCUSSION

Liver maintains and regulates homeostasis in living systems. It is involved in some biochemical pathways which are necessary for growth and fight against diseases. It is also involved in the production and supply of nutrients and energy (Ward and Daly, 1999). Antioxidants appear to act against diseases by raising the levels of endogenous enzymatic and non-enzymatic antioxidants, for instance by up-regulating gene expressions of the antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) and Glutathione Reductase (GR) (Aruoma, 1994; Margail *et al.*, 2005). The results of the present study revealed that, acute toxicity study (LD<sub>50</sub>) of the stem bark aqueous extract of *P. guajava* was greater than 5000 mg kg<sup>-1</sup>, implying that the stem bark extract was relatively safe. Also the result of the quantitative phytochemical analyses revealed the presence of tannins, saponins, alkaloids, flavonoids and polyphenolics. These metabolites particularly flavonoids and polyphenolics have been attributed for the antioxidant potential and hepatoprotective activity observed. This observation was in agreement with the findings of Di Carlo *et al.* (1999). CCl<sub>4</sub> induced a significant (p<0.05) increase in liver weight, implying impaired animal growth and organ function, which is occur due to blocking of secretion of hepatic triglycerides into the plasma (Aniya *et al.*, 2005). However treatment with stem bark aqueous extract of *P. guajava* (500 mg kg<sup>-1</sup>, p.o) ameliorated the increase of liver weight in rats, restoring the altered liver weight to near normal. Evaluation of haematological parameters is relevant and vital indices to toxicity assessment in general. Thus decreased in the level PCV, Hb, and RBC counts along with the elevation in WBC counts in toxin control group could be attributed to destruction of erythrocytes, disturbed haematopoiesis, and reduction in the rate of their formation, and their enhanced removal from circulation. The result obtained was in agreement with that of Essawy *et al.* (2010). A reduction in the level of these haematological parameters may be attributed to the hyperactivity of bone marrow, which leads to the production of red blood cells with impaired integrity that are easily destroyed in the circulation, as well as marked leucopenia (Zaoui *et al.*, 2002; Adeneye *et al.*, 2006). However treatment with

different dose of stem bark aqueous extract of *P. guajava* and CCl<sub>4</sub> treated animals ameliorated CCl<sub>4</sub> induced haematotoxicity toward normal. These results indicated that stem bark of *P. guajava* has a potency to induce recovery in the haematological parameters towards normal values and the result obtained was comparable to that of silymarin. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effects. Hepatocellular necrosis or membrane damage leads to very high levels of serum ALT and AST released from liver to circulation. Among two, ALT is a better index of liver injury, since ALT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner, thus liver ALT represents 90% of total enzyme present in the body (Achliya *et al.*, 2003). The increased levels of serum marker enzymes are indicative of cellular leakage and loss of functional integrity of cellular membrane in liver (Drotman and Lawhorn, 1978). In the present study, treatment with stem bark extracts suppressed the elevated serum levels of AST, ALT towards the respective normal value this clearly indicates that, *P. guajava* extract has stabilizes the plasma membrane as well as helped in healing of the hepatic tissue damage. The serum ALP and total bilirubin levels on the other hand are also related to the status and function of hepatic cells. Increase in serum ALP is due to increased synthesis, in presence of increasing biliary pressure (Willianson *et al.*, 1996). Similarly the extract was able to improve the secretory mechanism of hepatic cells and reduces the elevated levels of ALP and total bilirubin. Similar result was obtained by Gopal and Rosen (2000), which reported that, most of the circulating proteins are synthesized in the liver and their concentrations indicate a synthetic ability of the liver since serum albumin accounts for 65% of serum proteins. The decreased level of total proteins observed in CCl<sub>4</sub> intoxicated rats indicated that liver damage and loss of functionality. The administration of stem bark aqueous extract significantly ( $p < 0.05$ ) prevented a decreased in serum total proteins level and restored the synthetic function of the liver to near normal. When ROS generation exceeds the antioxidant defense, the free radicals can interact with endogenous macromolecules and alter the cellular functions (Muthukumaran *et al.*, 2008). Malondialdehyde (MDA) is a breakdown product that is frequently quantified as a measure of lipid hydroperoxides, leading to term lipid peroxidation (LPO). MDA assay has been found to be one of the better predictor of oxidative damage and often shown excellent correlation with other markers, such as isoprostanes which are considered to be the most reliable markers of lipid peroxidation (Morrow, 2010). In the present study, it was observed that, there was significant ( $p < 0.05$ ) increase in the level of TBARS and significant ( $p < 0.05$ ) decrease in GSH level, SOD and CAT activities in toxin control group compared to normal control group, indicating the development of oxidative stress in the experimental animals. This observation was in agreement to that of Srilaxmi *et al.* (2010) and Kalu *et al.* (2011). The high significant elevation of MDA level in liver homogenate of toxin control group rats indicated excessive formation of free radicals and activation of lipid peroxidation of the hydrophobic core and cell damage (Fraga *et al.*, 1987). Similarly CCl<sub>4</sub> also induced highly significant reduction in the level of reduced glutathione (GSH) and activities of SOD and CAT in liver homogenate of toxin control group compared to normal control group. However, administration of aqueous stem bark extract of *P. guajava* and silymarin together with CCl<sub>4</sub> stimulated the antioxidant protective mechanisms against CCl<sub>4</sub> derived free radicals by reducing MDA level and elevating the level of reduced glutathione as well as the activities of both SOD and CAT enzymes in liver homogenate. This observation was in agreement with the findings of Roy and Das (2011). Reduced glutathione is the most abundant thiol in mammalian tissues involved in the protection of the cell against damage from electrophiles free radicals and ROS

formed during xenobiotics metabolism (Meister, 1991). To prevent lipid peroxidation by CCl<sub>4</sub>, reduced glutathione acts as a hydrogen donor instead of abstracting the hydrogen from methylene hydrogen of the membrane polyunsaturated fatty acids and the free radicals of CCl<sub>4</sub> abstract the hydrogen from SH group of reduced glutathione (Kosower and Kosower, 1978). SOD and CAT are the major enzymes, which catalyse and help in elimination of ROS derived from redox process of xenobiotics in liver tissues (Poli, 1993). These findings suggest that, *P. guajava* possesses potent hepatoprotective activity and could protect liver against CCl<sub>4</sub> induced oxidative stress probably via the alteration of cytochrome P450. Many compounds known to be beneficial against carbon tetrachloride-mediated liver injury and exert their protective action via a decreased production of carbon tetrachloride derived free radicals or through the antioxidant activity of the protective agents themselves (Javatilaka *et al.*, 1990; Thabrew *et al.*, 1987).

## CONCLUSION

The findings of the study is quite promising as the stem bark aqueous extract of *P. guajva* was found to possess hepatoprotective activity against CCl<sub>4</sub> induced liver damage in experimental animals. The protective effect observed could be attributed to the presence phytochemicals which might be responsible for restoration of liver damage. This is reflected in the reversal of the serum marker enzyme activity towards normal level and the correction of haematological parameters. The antioxidant activity stem bark extract of *P. guajva* at high dose was comparable to that of silymarin, which is a standard drug used in the treatment of many liver diseases and hepatic toxic injury. The actual mechanism is not clear and further biochemical and pharmacological investigations are needed to isolate and identify the active ingredient(s).

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