Scientia Africana, Vol. 16 (No. 1), June 2017. Pp 82-88
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HEPATOPROTECTIVE EFFECT OF AQUEOUS LEAF EXTRACT OF SOURSOP ON SOME BIOCHEMICAL PARAMETERS OF PARACETAMOL-INDUCED LIVER DAMAGE OF WISTAR RATS

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Received: 11-10-16
Accepted:14-11-16

ABSTRACT

The hepatocurative effect of aqueous leaf extract of Annona muricata on paracetamol induced hepatotoxicity in rats was studied. Three different dosages were orally administered (0.7g/kg, 1.2g/kg and 1.5g/kg) once daily for 3 weeks. The levels of liver enzymes; aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), total cholesterol (CHOL), and triglycerides (TGs) were studied using standard protocols. Significant ($p<0.05$) increase of serum levels of ALT, AST, GGT, CHOL and TGs in paracetamol poisoned rats, with significantly ($p<0.05$), decreased serum levels of TP, and HDL were recorded. These findings confirmed induction of hepatotoxicity by the Annona muricata leaf extract was found to significant ($p<0.05$) reduce the serum levels of AST, ALT, CHOL, and TGs and significantly ($p<0.05$) increased the serum concentration of high density lipoprotein (HDL). These indicate the possible hepatocurative effects of aqueous leaf extract of Annona muricata on paracetamol induced liver toxicity in rats.

Key words: aqueous leaf extract, hepatotoxicity, liver enzymes.

INTRODUCTION

The use of herbal products for medicinal benefits has played an important role in nearly every culture on earth. Herbal medicine was practiced by ancient people of Africa, Asia, Europe and the Americans (Wargovich *et al.*, 2001).

Liver is a metabolically active organ responsible for many vital life functions (Keith, and Robert, 2001). Nearly all enzymes are protein found in our body that speed up certain chemical reactions. Liver enzymes perform this function within the liver. The two common are known as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Price and Stevens, 2003).

The objectives of the study were to know effect of soursop leaves extract on biochemical parameter of paracetamol-induced liver damaged Wistar rats.

MATERIALS AND METHODS

Preparation of Aqueous *Annona muricata* extract

Fresh leaves of *Annona muricata* was obtained from soursop tree at Port Harcourt, Rivers State, where it was found growing, and the species was properly identified, confirmed and kept at the herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt, Choba, Rivers State (Herbarium Number-UPH/P/088) for reference purposes. The

leaves were carefully plucked, thoroughly washed and dried properly for 7 days, pulverized and then sieved. Seven (7) grams of the powdered leaf was weighed out and dissolved in 70 ml (1:10 dilution) of distilled water. The mixture was vigorously shaken for about 5 minutes and kept in the laboratory bench for 24 hours before filtration. The extract (filtrate) served as stock solution of soursop extract, from the stock, 8, 14 and 18 ml/kg solution were prepared.

Preparation of the Animals

A total of twenty five (25) Wistar albino rats (3 months old), weighing 100 -225 g were obtained from the Department of Animal and Environmental Biology, University of Port Harcourt Animal House. They were housed in stainless steel cages (5 rats per cage) and kept in a well-ventilated room. The rats were fed with standard diet (Livestock Feeds Nig. Ltd. Ikeja, Nigeria) and water *ad libitum*. The standard guidelines for the use of experimental animals (including applying humane actions during sacrifice) were adhered to.

Effect of Extracts

The rats were divided into five (5) experimental groups. At the end of two weeks (14 days) following grouping and acclimatization; 0.7 mg/kg, 1.2 mg/kg, and 1.5 mg/kg of soursop extract was orally administered to the rats in groups 3, 4, 5 (group 1 was the general control i.e no liver damage; and group 2 was the paracetamol control i.e the rats were induced or administered orally, with Paracetamol and was kept without administering soursop extract).

For analysis, one rat from each group was sacrificed at determined time intervals, 24-hour for control and paracetamol-treated rats and 1,- 2 and 3-week for all groups. After anesthetics with chloroform, fresh blood was drawn by cardiac puncture and serum obtained. For histopathology, fresh livers obtained from the sacrificed rats were immediately placed in 10% formaldehyde.

Determination of Serum Protein-The Biuret Method

The cupric ions in the Biuret reagent in an alkaline solution gives a purple colour with peptide bonds found only in proteins. The colour is proportional to the concentration of the protein in the sample and is measured spectrophotometrically at 540nm.

Enzyme Assays

The determination of aspartate aminotransferase in the serum samples were performed at 37^0C using the Randox kit by measuring the amount of oxaloacetate hydrazone formed in the presence of L-aspartate, α-oxoglutarate and 2,4-dinitrophenyl hydrazine reported by Ibekwe *et al.*, (2007). For alanine aminotransferase, L-alanine replaced L-aspartate (Ibekwe *et al., (2007)*). CHOL was assayed according to the Method of Enzymatic Endpoint. GGT and TG was assayed according to the method of Colorimetric (Third Report of the National Cholesterol Education Programme (NCEP) Expert Panel, 2001). HDL was assayed according to the method of CHOD-PAP (Third Report of the National Cholesterol Education Programme (NCEP) Expert Panel, 2001).

Histopathology

This sections of the preserved liver slices obtained with the use of a tissue slicer were fixed on microscopic slides and stained before observing under the microscope following the method described by Baker and Silverton (2005).

Scientia Africana, Vol. 16 (No. 1), June 2017. Pp 82-88
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Statistical Analysis

All data were expressed as mean ± SEM (Standard error of mean) and statistically analyzed with ANOVA (Analysis of variance) at 95 % confidence level. A p value of < 0.05 was considered statistically significant.

RESULTS

The results obtained for the serum enzyme level of aspartate aminotransferase (AST) are shown in Table 1. Table 2-6 shows that for GGT, CHOL, TGs and HDL respectively.

Table 1: Comparison of the parameters (AST, ALT, GGT CHOL, TGs, HDL) mean concentration values and SEM for each group (grp) of rats.

	AST	ALT	GGT	CHOL	TGs	HDL
Grp1	8.500±0.707	10.500±0.707	26.500±0.707	4.150±0.071	0.650±0.071	1.150±0.071
Grp2	22.500±0.707	28.500±0.707	35.500±0.707	4.850±0.071	1.350±0.071	0.650±0.071
Grp3	22.500±0.707	19.700±0.424	25.700±0.424	4.600±0.141	0.950±0.071	1.150±0.071
Grp4	20.500±0.707	18.500±0.707	25.700±0.424	4.350±0.071	0.790±0.014	1.790±0.071
Grp.5	16.500±0.707	15.500±0.707	24.500±0.707	4.350±0.071	0.650±0.071	0.150±0.071

Groups of rats treated with paracetamol exhibited significant ($p<0.05$) increase in the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), when compared to normal control rats. However, serum total protein (TP) were significantly ($p<0.05$) reduced in paracetamol-treated rats. Groups of rats treated with paracetamol demonstrated significant ($p<0.05$) increase in the serum levels of total cholesterol (CHOL), when compared to normal control rats. However, serum levels of high density lipoprotein (HDL) and Gamma-glutamyltransferase (GGT) were significantly reduced in paracetamol-treated rats. Soursop leaf extract produced no mortality after 48 hours of observation. The leaf extract in the dosage range administered to liver damaged rats apparently accelerated the reversion of the liver damage and lowered the high levels of serum ALT and AST activities when compared to rats treated with paracetamol alone. The effect was time and dose dependent. The results indicate that treatment with soursop leaf extracts after establishment of paracetamol-induced liver damage significantly reduced and even reversed the liver damage in rats.

Table 2: Summary of the Histological Examination of the Liver Architecture of Experimental Animals

Tissue (Liver) Pathology-Result of Liver Fibrosis

Group	Results
1	Normal Architecture
2	Abnormal Architecture
3	Fatty change and necrosis
4	Enlarged hepatocytes with granules cytoplasm with macrovesicular steatosis
5	Fatty change not involving all logule of liver

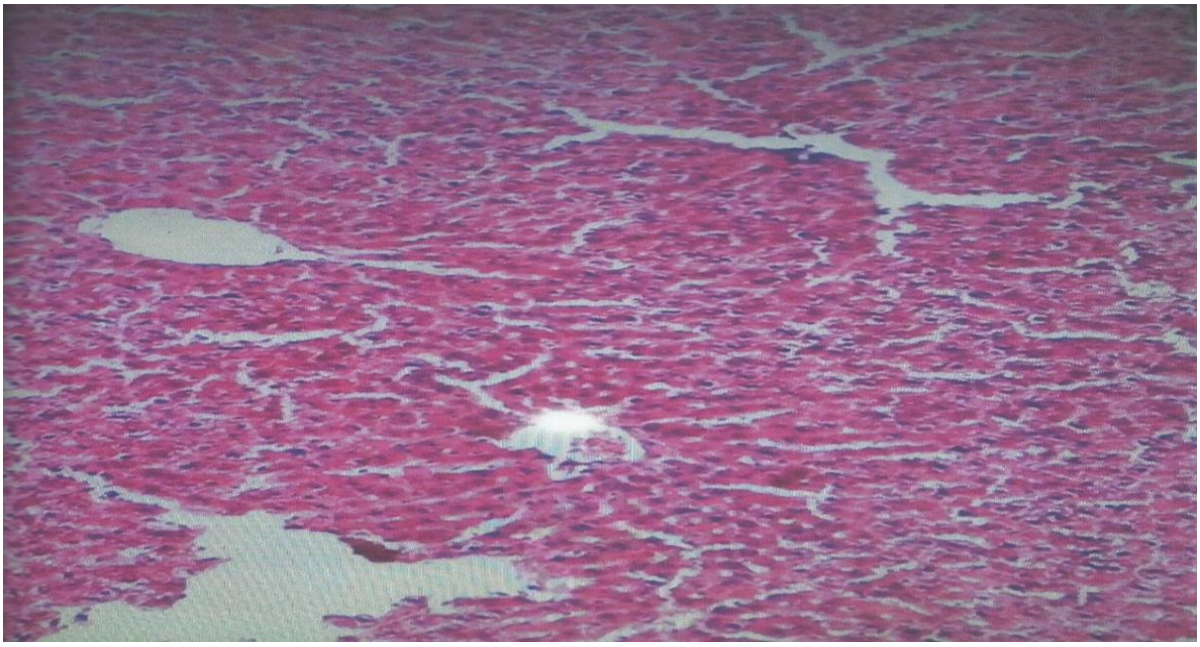

Figure 1: Photomicrograph of rat feed with only water and feed *ad libitum* (Group 1) showing Normal liver architecture. Fluorescein isothiocyanate Isomer I (FITC) (19365A) label used, eosin used and ~1nm (0.001µm) in this tissue section.

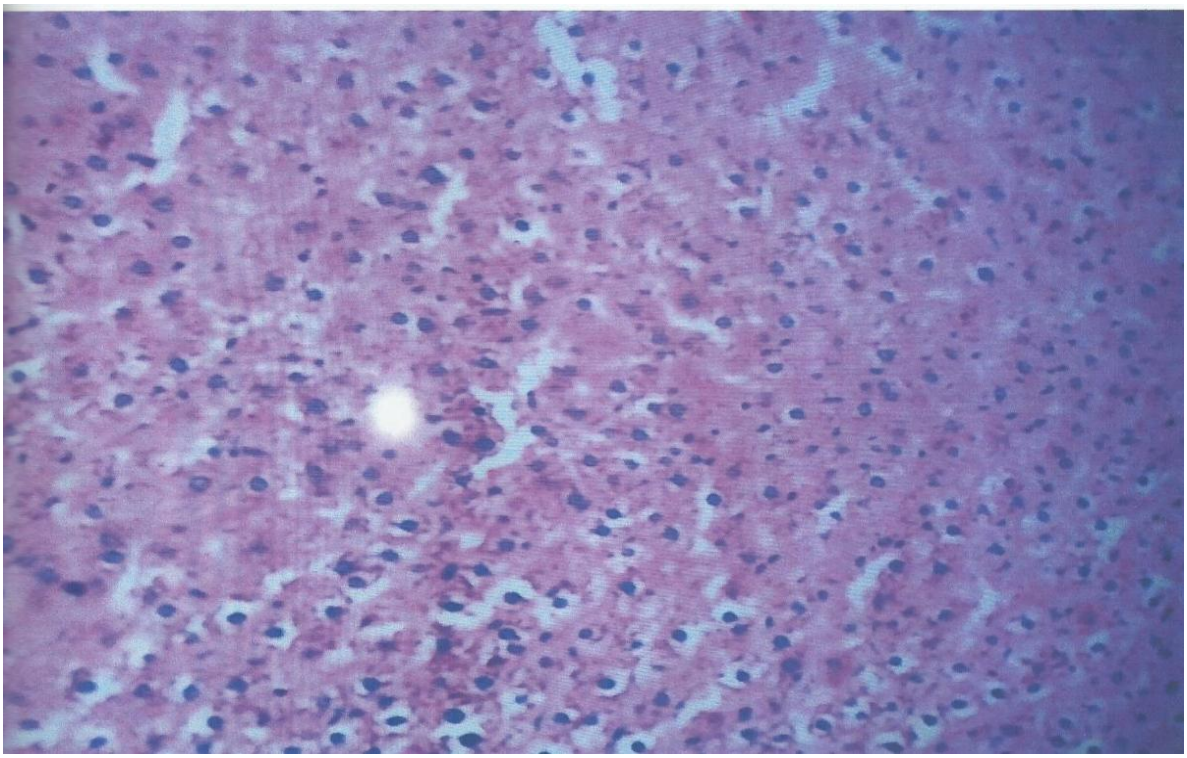

Figure 2: Photomicrograph of paracetamol-induced rat, (Group 2) –Abnormal liver architecture due to induced hepatotoxicity. Fluorescein isothiocyanate Isomer I (FITC) (19365A) label used, eosin used and ~1nm (0.001µm) in this tissue section.

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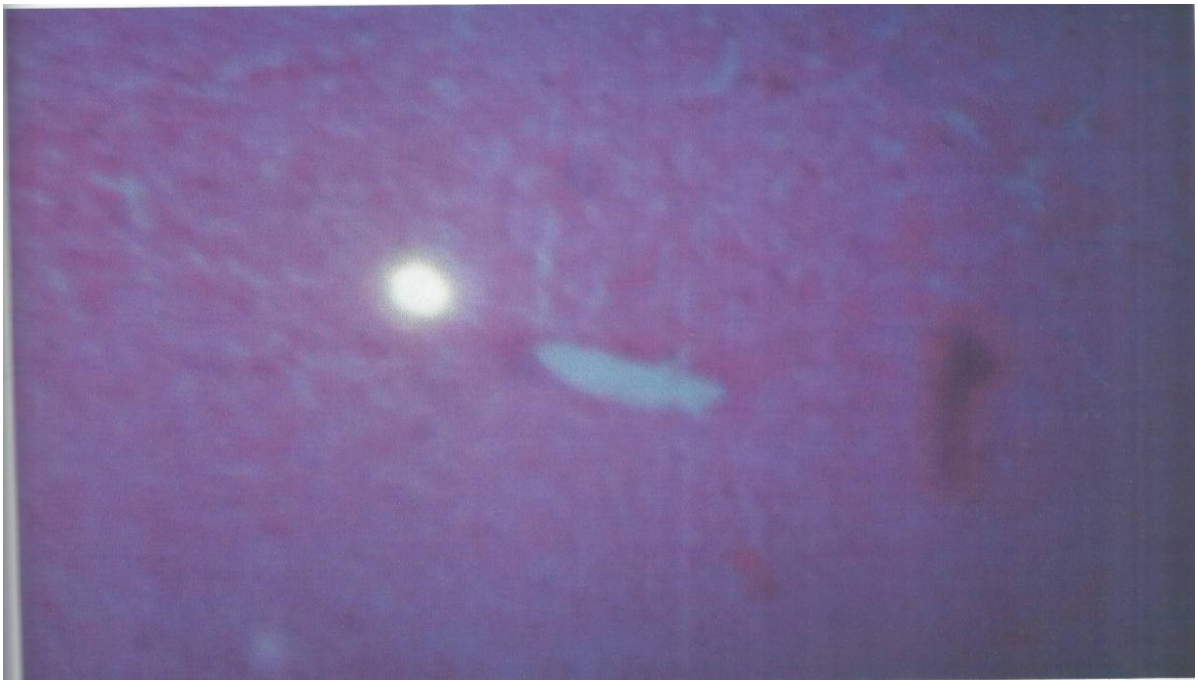

Figure 3: Photomicrograph of paracetamol-induced rat, administered with 8ml/kg of soursop leaf extract, (Group 3)-Fatty change and necrosis. Fluorescein isothiocyanate Isomer I (FITC) (19365A) and its derivatives label used, eosin used and ~1nm (0.001µm) in this tissue section.

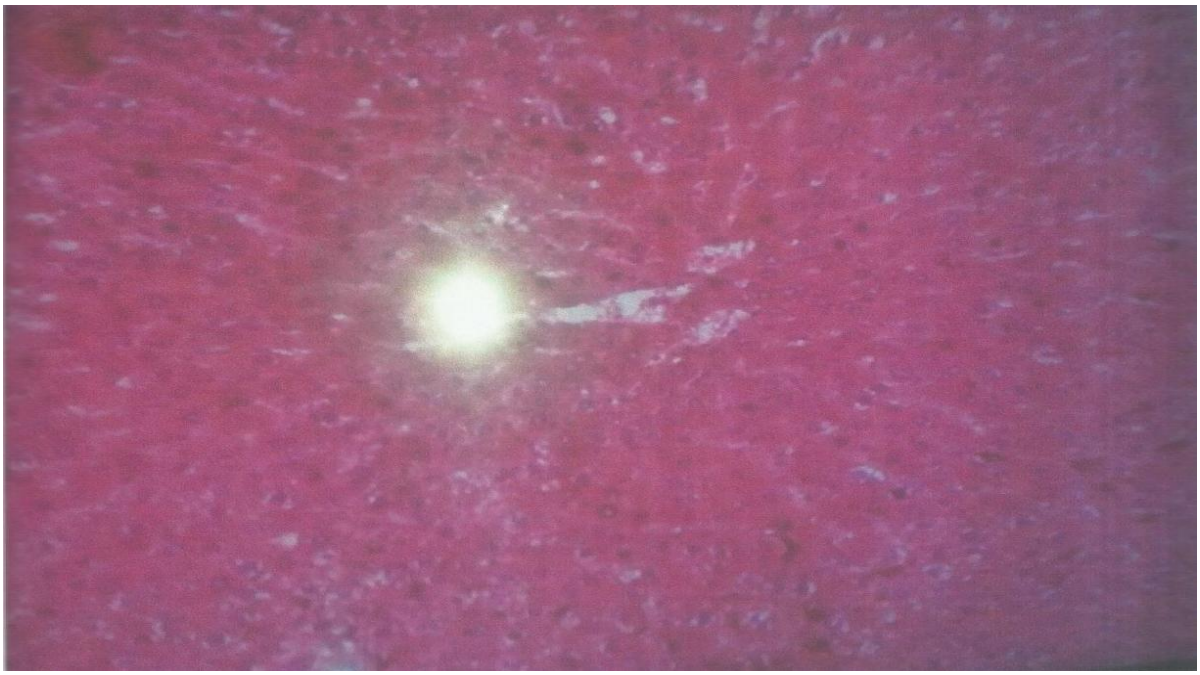

Figure 4: Photomicrograph of paracetamol-induced rat, administered with 14ml/kg of soursop leaf extract, (Group 4)-Enlarged hepatocytes with granules cytoplasm with macrovesicular steatosis. Fluorescein isothiocyanate Isomer I (FITC) (19365A) and its derivatives label used, eosin used and ~1nm (0.001µm) in this tissue section.

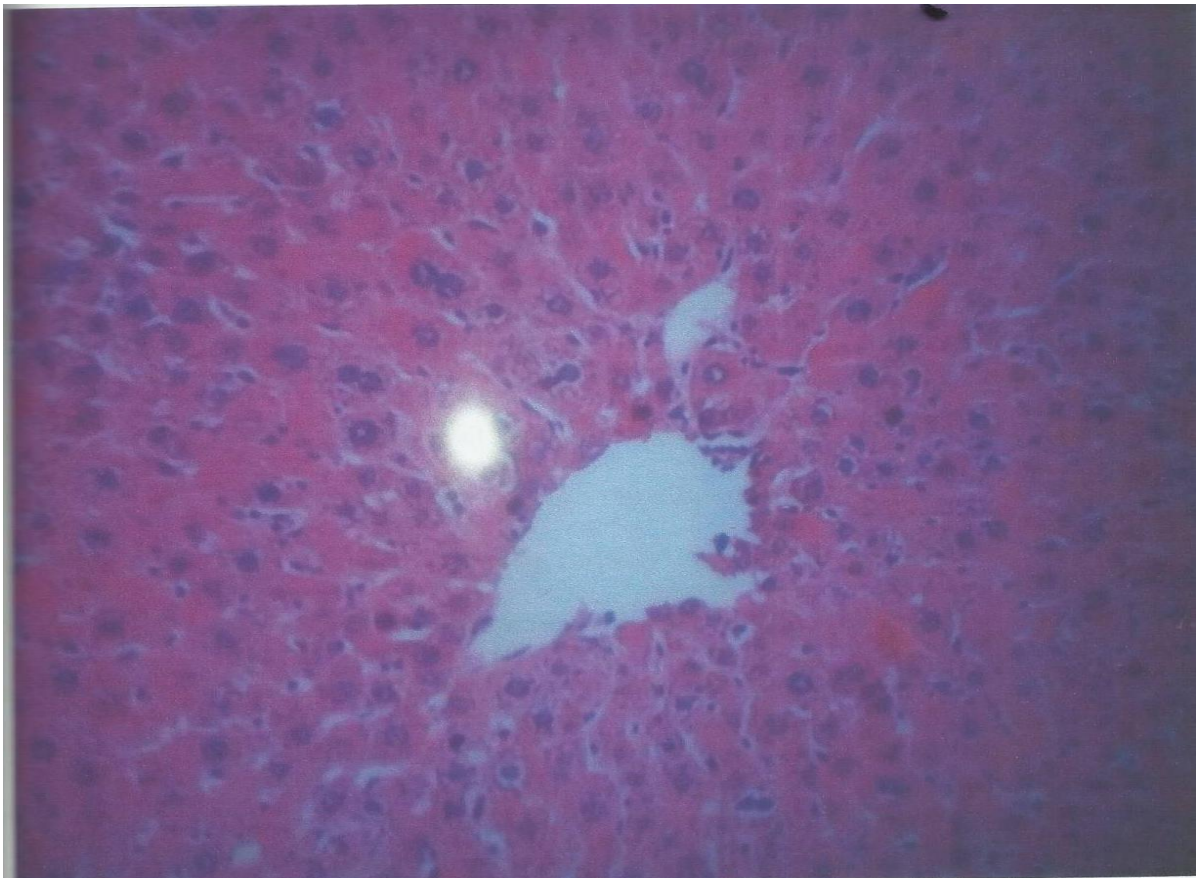

Figure 5: Photomicrograph of paracetamol-induced rat, administered with 18ml/kg of soursop leaf extract, (Group 5) – Fatty change not involving all logule of liver. Fluorescein isothiocyanate Isomer I (FITC) (19365A) and its derivatives label used, eosin used and ~1nm (0.001µm) in this tissue section.

DISCUSSION

Liver is the largest organ and it is target for toxicity because of its role in clearing and metabolizing chemicals through the process called detoxification (Larrey, 2003). Drug induced liver disorders occurred frequently can be life threatening and mimic all forms of liver diseases (Watkins and Seef, 2006). The covalent binding of N-acetyl-P-benzoquinoneimine, an oxidation product of paracetamol, to sulphydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutatjione in the liver as the cause of hepatotoxicity have been reported earlier (Jollow *et al.*, 2003).

Hepatotoxicity is the most remarkable feature of paracetamol overdose. Acute overdoses of paracetamol can cause potentially fatal liver damage and, in rare individuals, a normal dose can do the same. 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 value (Yadav and Dixit, 2003). Paracetamol biotransformation involves conjugation with glucoronide and sulphate. A small amount of paracetamol is metabolized by mixed function oxidase enzymes to form highly reactive compound NAPQI, which is immediately conjugated with glutathione

Scientia Africana, Vol. 16 (No. 1), June 2017. Pp 82-88
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and subsequently excreted as cysteine and mercapturic conjugates (Monago, 2012).

In overdose, large amounts of paracetamol are metabolized by oxidation because of saturation of the sulphate conjugation pathway, but once the protective intracellular glutathione stores are depleted, hepatic and renal damage may ensue (Monago, 2012).

Hence, the extracts of soursop leaf might be effective hepatoprotectors in administering it to patients with hepatopathies since the model of paracetamol-induced liver damage in rats simulates many of the features of human liver fibrosis (damage).

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