British Journal of Pharmaceutical Research
6(3): 141-154, 2015, Article no.BJPR.2015.057
ISSN: 2231-2919

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation of Antioxidant Activity of Giant African Snail (*Achachatina maginata*) Haemolymph in CCl4- Induced Hepatotoxixity in Albino Rats

Bashir Lawal[1*], Oluwatosin K. Shittu[1], Prince C. Ossai[1], Asmau N. Abubakar[1], and Aisha M. Ibrahim[1]

[1]Department of Biochemistry, Federal University of Technology, Tropical Disease Research Unit, PMB65, Minna, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author BL designed the study and carried out the laboratory work. He equally wrote the first draft of the manuscript and undertook the final editing of the paper. Authors OKS and PCO took part in the laboratory work, part of the draft and undertook in the initial and final editing of the paper. Authors ANA and AMI took care of the literature searches, data analysis and participated in designing the experiment. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2015/15887
Editor(s):
(1) Hua Naranmandura, Department of Pharmacology, Toxicology and Biochemical Pharmaceutics, College of Pharmaceutical Sciences, Zhejiang University, China.
(2) Ali Nokhodchi, School of Life Sciences, University of Sussex, UK.
Reviewers:
(1) Ndatsu Yakubu, Department of Biochemistry, IBB University Lapai, Niger State, Nigeria.
(2) Anonymous, Nigeria.
(3) Anonymous, Egypt.
(4) Atef Mahmoud Mahmoud Attia, Biochemistry department, Biophysical laboratory, National Research Centre, Egypt.
(5) Anonymous, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=984&id=14&aid=8274

Original Research Article

Received 23rd December 2014
Accepted 9th February 2015
Published 26th February 2015

ABSTRACT

Objective: To evaluate the acute toxicity, *in vitro* antioxidant and hepatoprotective activities of Giant African Snail (*Achachatina maginata)* haemolymph against carbon tetrachloride (CCl_4) induced liver damage in albino rats.
Place and Duration of Study: Department of Biochemistry, Federal University of Technology, Minna, Nigeria. It was carried out between September and December, 2014.
Methods: Antioxidant activity of the haemolymph was assayed by DPPH radical scavenging,

**Corresponding author: Email: bashirlawal12@gmail.com;*

reducing properties, and inhibition of lipid peroxidation by the haemolymph. In addition, the phenol and flavonoid contents were also evaluated. The safety of the extract was also investigated by the acute oral toxicity limit test. The hepatoprotective activities of the haemolymph were compared with a known hepatoprotective drug, silymarin. For this purpose, twenty adult albino rats were assigned into 5 groups. Group 1 and 2 serves as normal control and CCL_4 control respectively. Group 3-5 were treated with 200 mg/kg, 400 mg/kg haemolymph and 100 mg/kg Silymarin respectively.

Results: The lethal dose (LD_{50}) of haemolymph determined was greater than 5000 mg /kg in rats. The amount of total phenolics and flavonoids were estimated to be 9.30±0.11 mg/g GAE and 15.20±0.59 mg/g catechin equivalent respectively. The haemolymph exhibited concentration dependent *in vitro* antioxidant activity with an IC_{50} value of 579.66±2.69 µg/mL in the DPPH radical scavenging activity and 310.75±3.12 µg/mL in lipid peroxidation inhibitory activity but shows low reducing properties compare to ascorbic acid. The liver damage was evidenced by the elevated levels of serum and liver ALT, ALP, serum AST, hepatic thio-barbituric acid reacting substances (TBARS) and reduced serum catalase in CCl_4-hepatotoxic rats. Administration of haemolymph (200/400 mg/kg) and standard control drug silymarin (100 mg/kg) significantly ($P<0.05$) reduced CCL_4- induced elevations of the levels of AST, ALT, ALP, and TBARS while the reduced concentration of catalase due to CCL_4 was reversed. However, the SOD activities was significantly ($P<0.05$) higher in CCL_4 treated group compare to the control group.

Conclusion: The present study proved the antioxidant activity of Giant African Snail (*Achachatina maginata)* haemolymph can be used as accessible source of natural antioxidants with potential application to reduce oxidative stress induced liver damage.

Keywords: Achachatina maginata; haemolymph; hepatoprotective; antioxidant biochemical parameters; sylimarin.

1. INTRODUCTION

The recent growth in knowledge of free radicals and reactive oxygen species (ROS) in biological systems is producing a medical revolution that promises a new age of health [1]. Under a situation of oxidative stress, reactive oxygen species such as superoxide ($O2^{\cdot-}$), hydroxyl (OH.), and peroxyl (·OOH, ROO·) radicals are generated. These reactive oxygen species play an important role in degenerative or pathological processes, such as aging [2], cancer, coronary heart disease, Alzheimer's disease, atherosclerosis, cataracts, and inflammation [3].

Liver is a vital organ which regulates many important metabolic functions and is responsible for maintaining homeostasis of the body [4]. It plays a vital role in xenobiotics metabolism and is responsible for protection of human body against adverse effect of drugs, chemicals toxin, bacteria, virus and parasite. Hence, in the process of these activities liver itself is subjected to variety of diseases and disorders and obviously needs protection [5]. So far no effective treatments in conventional or synthetic medicine that gives protection to the liver against damage or helps to regenerate hepatic cells [6]. Because of this fact efforts are being made to find suitable curative agents in natural products for the treatment of liver diseases [7].

A number of chemical agents and drugs which are used on a routine basis produce cellular as well as metabolic liver damage [8]. Most of hepatotoxic chemicals affected liver cells mainly by inducing lipid peroxidation and oxidative stress in liver. Chemicals such as CCl_4 get converted into reactive toxic metabolites by hepatic microsomal cytochrome P450 [9]. CCl_4catabolised radicals induced lipid peroxidation, damage the membranes of liver cells and organelles, causing the swelling and necrosis of hepatocytes and resulted in release of cytosolic enzymes into the blood [10].

The principle function of antioxidants is in delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and they may reduce oxidative damage to the human body [11]. Many antioxidants compounds are present in natural product. Depending on the type and source of the natural product, the mode of action of natural antioxidants involves multiple mechanisms of action [12].

The use of wild animals and their products constitutes essential ingredients in the preparation of drugs in traditional medicine [13,14]. The African giant snail (*Archachatina marginata*) is one of the most important minor forest products in West Africa and Nigeria in

particular. Soewu [14] reported fifty-five fauna species used for various traditional preparations of which *Achachatina marginata* is included. In the traditional ethnomedicine of South West Nigeria, various preparations containing snails are useful in restoring fertility, virility, cure smallpox, relieve in labour pains, blood loss in pregnancy and during delivery [15]. Suppositories containing 5% and 7% of the mucin isolated from *A. marginata* showed marked and consistent blood glucose lowering effect [16]. A special form of calcium phosphate extracted from snails has been implicated in the cure of some kidney diseases, tuberculosis, diabetes, asthma, heart diseases and circulatory disorders [17]. The low sodium, fat, cholesterol and reasonable amounts of iron in *A. marginata* make it useful in the treatment of anaemia, hypertension, obesity, arteriosclerosis and other heart-related diseases [17].

However, there are no scientific reports in the literature regarding its usefulness as hepatoprotective agent. This study, therefore, was designed to investigate the acute toxicity, *in vitro* antoxidant and hepatoprotective activities of *Achachatina maginata* hemolymph against CCl_4 induced hepatotoxicity in rats.

2. MATERIALS AND METHODS

2.1 Snail Collection

African Giant Snails (*Achachatina maginata)* weighing 110-200 g were bought from Sabon kasuwa Kagara, Niger state in September, 2014. They were housed in a ventilated container and fed with cucumbers and melon.

2.2 Drugs and Chemicals

Sylimarin (silybon-140) was purchased from micro labs limited. Chemicals like 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), Folin-Ciocalteu reagent, Gallic acid were purchased from Sigma (St. Louis, Missouri, USA). All enzyme assay kits were products of Randox Laboratories Ltd, United Kingdom. All other reagents used were of analytical grade and were prepared in distilled water.

2.3 Experimental Animals

Healthy albino rats (1:1 male to female ratio) of average weight 120-150 g were purchased from Animal House, University of Ibadan, Oyo State Nigeria. The rats were kept in clean plastic cages and maintained under standard laboratory conditions in the biochemistry laboratory, Federal University of Technology Minna till they reached the desired weight. They were allowed unrestricted access to rat pellets and water *ad-libitum*. The study was carried out according to the Guide for the Care and the Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council, USA [18].

2.4 Haemolymph Collection

The apex of the snails was opened by method adopted from Akinloye and Olorode [19]. The haemolymph was drained into a clean conical flask and concentrated using water bath at low temperature.

2.5 Acute Toxicity Studies (Determination of LD_{50})

Acute toxicity of the haemolymph was evaluated as described by Lorke [20]. In 2 phase at dose of 10, 100 and 1000 mg kg^{-1} weight, respectively (PHASE 1) and dose of 1600, 2900 and 5000 mg kg^{-1} (PHASE 2). The haemolymph was administered once and the rats were observed for death and sign of toxicity within 24 hrs.

2.6 Determination of Total Phenolic and Flavonoid Content

The total phenolics concentration in the hemolymph was determined according to the Folin-Ciocalteu method as described by [21] using gallic acid as the standard. Total flavonoid was measured by the aluminium chloride colorimetric method as described by [22], using catechin as the standard to construct a calibration curve. All determinations were carried out in triplicate, and the results are expressed as mg gallic acid equivalent (GAE) /g and catechin equivalents (CE) in mg/g for phenol and flavonoid respectively.

2.7 *In vitro* Antioxidant Assay

The free radical scavenging ability of the haemolymph against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals were evaluated according to method described by Kaur et al. [23], using ascorbic acid as the reference. Lipid oxidation was measured as increases in thiobarbituric acid-reactive substances (TBARS) according to the method described by Ohkawa et al. [24]. The extract concentration providing 50% inhibition (IC50) was calculated from the plot of

inhibition (%) against extract concentration. The reducing property was determined by assessing the ability of extracts to reduce ferric chloride ($Fecl_3$) solution as described by Pulido et al. [25]. Ascorbic acid at the same concentrations was used as a positive control for haemolymph.

2.8 Hepatoprotective Study

2.8.1 Experimental design

The hepatoprotective activity of the haemolymph was evaluated according to the method of Guntupalli et al. [26] as modified by Hassan et al. [27]. The animals were randomly assigned to five experimental groups of 4 animals each. The experimental groups are illustrated as follows:

Group1:- serve as Normal control and received no treatment.
Group2:- serve as negative control and were administered 30% carbon tetrachloride in liquid paraffin (1 ml/kg body weight, intra peritoneal) daily for 5 days.
Group3:- serve as standard group received CCl_4 daily and Silymarin, a known anti-hepatotoxic drug at a dose of 100 mg/kg intra peritoneal daily for 5 days.
Group 4:- received CCl_4 daily for 5 days and *Achachatina maginata* hemoymph at a daily dose of 200 mg/kg intra peritoneal,
Group5:- received CCl_4 (daily for 5 days) and *Achachatina maginata* hemoymph at a daily dose of 400 mg/kg intra peritoneal for five days. All the animals were sacrificed on the 6th day. The blood and liver was collected for estimation of biochemical parameters.

2.8.2 Collection of blood and liver

Collection of sample for biochemical analyses was as described previously [28;29]. The animals were anasthesized with chloroform and blood was collected through cardiac puncture into a clean, dry centrifuge tubes. The blood sample was allowed to stand for 10 minutes at room temperature and then centrifuged at 1000 rpm (503xg) for 15 minutes to get the serum. The rat was dissected to reveal the internal organ and the liver was removed and placed in sample bottles containing 0.25 M sucrose solution to maintain a normal physiological environment. The liver was homogenized and the supernatant were stored in sample bottles for subsequent used.

2.8.3 Determination of biochemical parameters

The biochemical analyses were determined for Alkaline phosphatase (ALP) based on methods of Tietz, [30], Aspartate transaminase (AST) and alanine transaminase (ALT) as described by Reitman and Frankel, [31]. Catalase activities was determined as described by Bock et al. [32], superoxide dismutase (SOD) activities was estimated using epinephrine by the method of Misra and Fridovich, [33]. While the lipid peroxidation was estimated by measuring the concentration of Thiobarbituric acid reactive species (TBARS) in liver homogenate as described by [1,34]. The results were expressed as n.mol of MDA/mg of protein.

2.9 Statistical Analysis

Values were analyzed using statistical package for social science (SPSS) version 16 and presented as means SE of the mean. Comparisons between different groups were carried out by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The level of significance was set at $P<0.05$ [35].

3. RESULTS

3.1 Acute Toxicity

In the acute toxicity studies no death was recorded during the treatment period at all doses of the haemolymph administered. The animals did not show any changes in general behavior and other physiological activities like giddiness, sniffing, aggressiveness, tachypnoea, or convulsion. From the above toxicity studies, the lethal dose 50 (LD_{50}) of haemolymph was greater than 5000 mg /kg in rats (Table 1).

Table 1. Acute toxicity profile of *Achachatina maginata* haemolymph

Dose (mg/kgbw)	Numbers of rat	Mortality
10	3	0/3
100	3	0/3
1000	3	0/3
1600	3	0/3
2900	3	0/3
5000	3	0/3

3.2 Total Phenolic and Flavonoid Content

Total phenolic content of haemolymph was determined to be 9.30±0.11 mg GAE per g of sample as measured by the Folin-Ciocalteu method. In addition, the total flavonoid content of haemolymph was 15.20±0.59 mg/g catechin equivalent (Table 2).

Table 2. Total phenolic and flavonoid content of *Achachatina maginata* hemoymph

Total phenolic	Total flavonoid content
9.30±0.11 mg GAE/g of sample	15.20±0.59 mg/g catechin equivalent

3.3 Antioxidant Assays

3.3.1. Scavenging activity on DPPH radical of haemolymph

The results of DPPH radical scavenging activity of haemolymph and the standard antioxidant (ascorbic acid) are presented in Fig. 1. The haemolymph and the standard antioxidant (ascorbic acid) promoted an inhibition of DPPH radical with increasing concentrations. However, the percentage inhibition of the DPPH radical by the haemolymph was lower than that of ascorbic acid. The IC_{50} (concentration that inhibits 50% of the DPPH radical) values of haemolymph and ascorbic acid were 579.66±2.69 and 31.33±0.47 µg/mL respectively.

3.3.2 Reducing power activity and lipid peroxidation

The antioxidant activity of haemolymph was determined by measuring its ability to transform Fe^{3+} to Fe^{2+} as illustrated in Fig. 2, haemolymph show a very low % ferric reducing antioxidant properties and a considerable % inhibition of Lipid peroxidation (*in vitro)* with an IC_{50} value of 310.75±3.12 µg/mL (Table 3). However, the reducing power and inhibition of Lipid peroxidation increased with an increase in concentration of the haemolymph.

3.4 Hepatoprotective Study

3.4.1 Alanine transaminase (ALT) activities

The ALT activities in serum and liver were presented in Fig. 3: The ALT activities in serum and liver were significantly ($p<0.05$) higher in CCl_4-hepatotoxic rats as compared with normal control rats. However, administration of haemolymph at 200 and 400 mg/kg to rats significantly ($P<0.05$) reduced the ALT activities as compared to the CCl_4-hepatotoxic rats, and similar to the positive control (sylimarin) as shown in Fig. 3.

Table 3. Inhibition of lipid peroxidation (*in vitro* assay) by *Achachatina maginata* hemolymph

Concentration (µg/ml)	% inhibition of Lipid peroxidation
25	25.31±0.33
50	32.41±0.24
100	36.01±0.13
150	39.21±0.46
200	43.96±0.13
400	55.78±1.67
IC_{50} value	310.75±3.12 µg/mL

Values are expressed as Mean±SEM of triplicate determination

Table 4. Effect of *A. maginata* haemolymph on lipid peroxidation (*in vivo)* in CCl_4-hepatotoxic rats

Groups	LPO (nmol MDA/mg liver protein) $_X10^{-6}$
CCl_4 alone	$20.87±1.23^{d}$
CCl_4 + 200 mg/kg Haemolymph	$4.15±0.76^{b}$
CCl_4 + 400 mg/kg Haemolymph	$2.52±0.22^{ab}$
CCl_4 + 100 mg/kg Silymarin	$2.22±0.91^{ab}$
Normal	$1.92±0.22^{a}$

Values are expressed as Mean ±SEM of four replicate (n=4).Values with different superscript are significantly different (p<0.05)

3.4.2 Aspartate transaminase (AST) activities

Fig. 4 present the results of serum and liver AST activities of different group. The serum AST activities was significantly ($p<0.05$) higher in CCl_4-hepatotoxic rats and significantly ($p<0.05$) lower in rats treated with 400 mg/kg of haemolymph when compared with normal control and other experimental group. The liver AST activity was significantly ($p<0.05$) higher in CCl_4-hepatotoxic rats treated with 200 and 400 mg/kg haemolymph when compared with normal control. However, no significant difference was recorded in the liver AST activities of CCl_4-hepatotoxic rats as well as those treated with standard control drug silymarin when compared with normal controls.

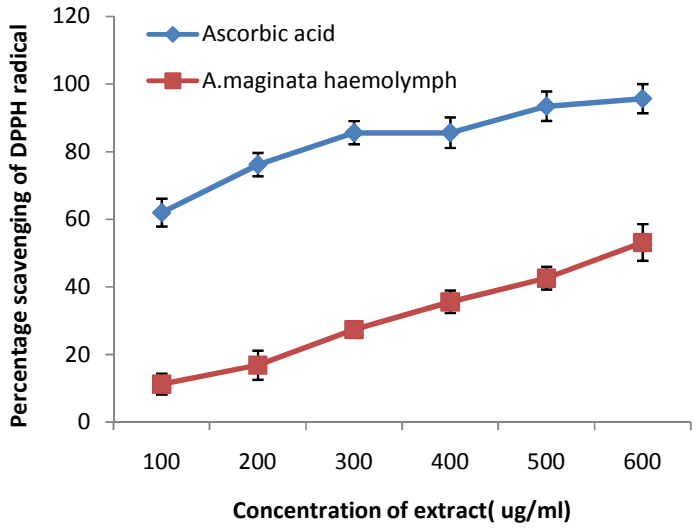

Fig. 1. DPPH radical scavenging activity of *Achachatina maginata* haemolymph. Each points represent Mean ±SEM of triplicate determination

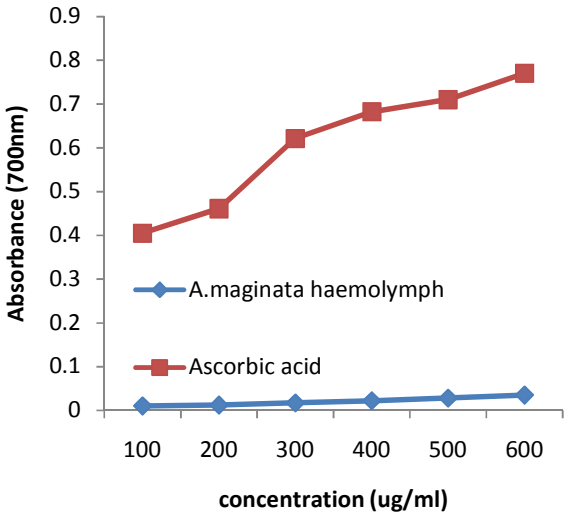

Fig. 2. Reducing power activity of *Achachatina maginata* haemolymph. Each points represent Mean ±SEM of triplicate determination

3.4.3 Alkaline phosphatase (ALP) activities

The serum and liver ALP activities were significantly ($p<0.05$) higher in CCl_4-hepatotoxic rats when compared with normal control and other experimental groups. The serum ALP activities were significantly ($p<0.05$) lower in sylimarin treated rat when compared with normal control. However, there was no significant difference in the serum ALP activities between 200 mg/kg hemolymph treated rat when compared with normal control (Fig. 5)

3.4.4 Super oxide dismutase (SOD) activities

The serum and liver SOD activities was significantly ($p<0.05$) higher in CCl_4-hepatotoxic rats and 200 mg/kg hemolymph treated rat when compared with normal control and other experimental groups. There was no significant

difference in the serum SOD activities of 400 mg/kg hemolymph treated rat, sylimarin treated and normal control. However, the liver SOD activity was significantly ($p<0.05$) higher in haemolymph treated group when compared with normal control (Fig. 6).

3.4.5 Catalase activities

The serum catalase activities were significantly ($p<0.05$) lower in CCl_4-hepatotoxic rats when compared with normal control and other experimental group. There was no significant difference in the serum catalase activities of 200 mg/kg, 400 mg/kg hemolymph treated rat and sylimarin treated group. The liver catalase activities was significantly ($p<0.05$) higher in CCl_4-hepatotoxic rats when compared with normal control and other experimental groups (Fig. 7).

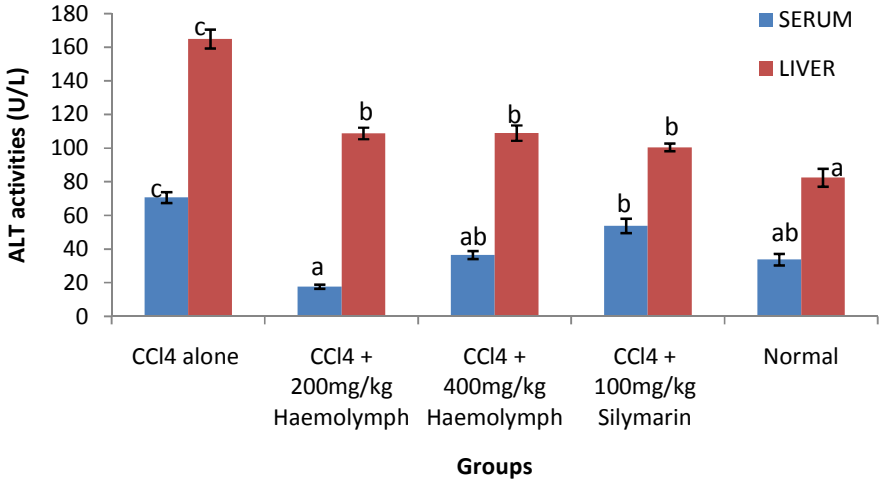

Fig. 3. Effect of *A. maginata* haemolymph on serum and liver alanine transaminase (ALT) activities in CCl4-hepatotoxic rats. Values are expressed as Mean ±SEM. Each mean is an average of four replicate (n=4). Column with different superscript are significantly different (p<0.05)

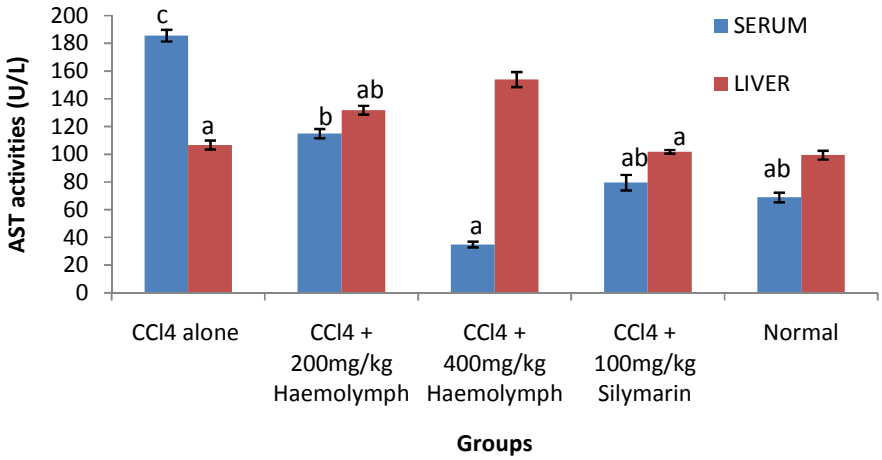

Fig. 4. Effect of *A. maginata* haemolymph on serum and liver aspartate transaminase (AST) activities in CCl4-hepatotoxic rats. Values are expressed as Mean ±SEM. Each mean is an average of four replicate (n=4). Column with different superscript are significantly different (p<0.05)

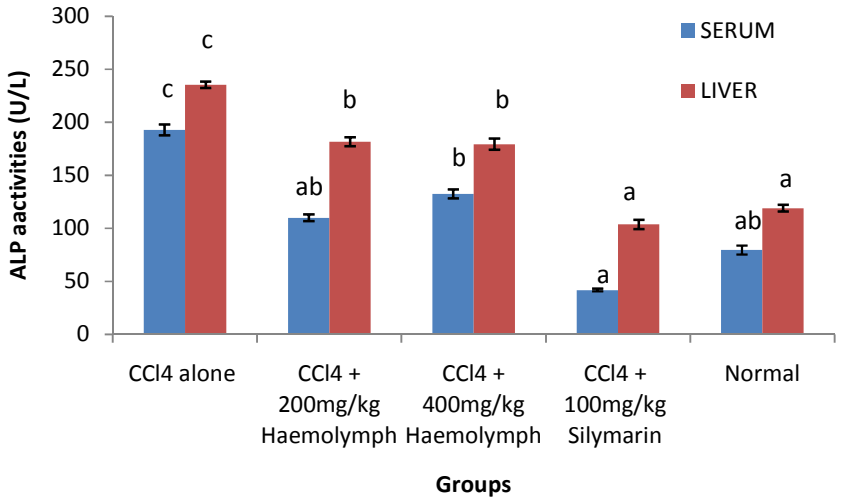

Fig. 5. Effect of *A. maginata* haemolymph on serum and liver alkaline phosphatase (ALP) activities in CCl4-hepatotoxic rats. Values are expressed as Mean ±SEM. Each mean is an average of four replicate (n=4). Column with different superscript are significantly different (p<0.05)

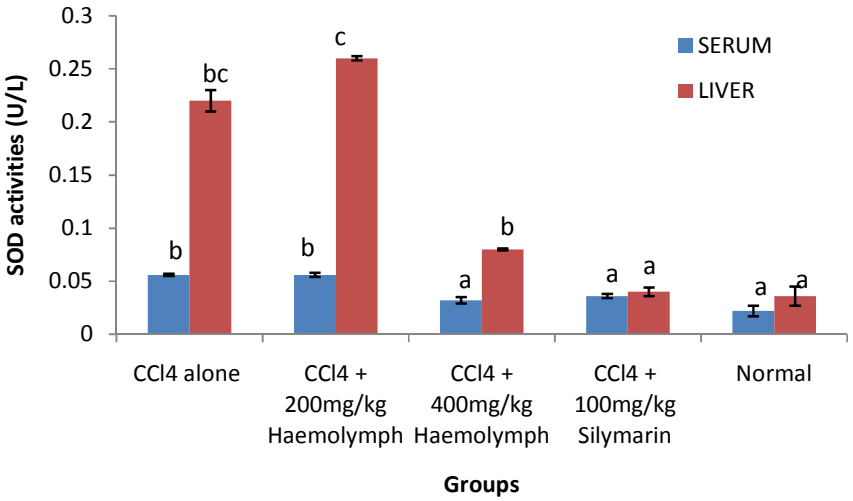

Fig. 6. Effect of *A. maginata* haemolymph on serum and liver super oxide dismutase activities (SOD) in CCl4-hepatotoxic rats. Values are expressed as Mean ±SEM. Each mean is an average of four replicate (n=4). Column with different superscript are significantly different (p<0.05)

3.4.6 Lipid peroxidation (*In vivo*)

The lipid peroxidation was significantly ($p<0.05$) higher in CCl_4-hepatotoxic rats than normal control and other experimental groups. Administration of 200 mg/kg and 400 mg/kg of haemolymph as well as the standard drug sylimarin significantly ($p<0.05$) reduced the level of LPO in liver homogenate when compared with CCl_4-hepatotoxic rats. The reduction in LPO was more significant at 400 mg/kg than 200 mg/kg haemolymph. However, there was no significant difference in LPO between 400 mg/kg haemolymph treated group and standard drug sylimarin treated group (Table 4).

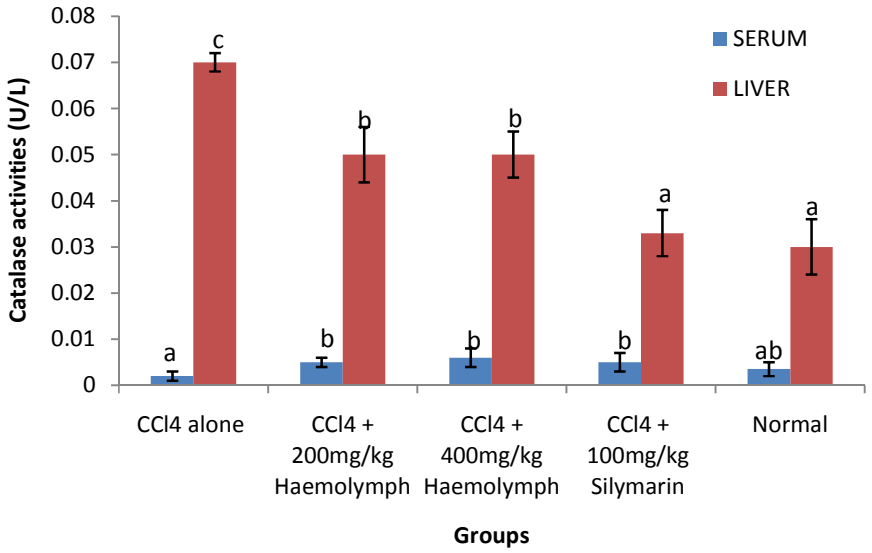

Fig. 7. Effect of *A. maginata* haemolymph on serum and liver catalase activities in CCl4-hepatotoxic rats. Values are expressed as Mean ±SEM. Each mean is an average of four replicate (n=4). Column with different superscript are significantly different (p<0.05)

4. DISCUSSION

About 80% of world's population is thought to depend chiefly on traditional medicine, for their primary health care needs. Emphases have however been laid that safety should be overriding criteria in the selection of natural medicine for use in health care [36]. In this study, the acute lethal effect of *A. maginata* haemolymph show that the haemolymph did not produce any grossly negative behavioral changes such as excitement, restlessness, respiratory distress, convulsions or coma. The LD_{50} is greater than 5000 mg/kg body weight, which is thought to be safe as suggested by [20]. Again, the absence of mortality among rats in all the dose groups after 48 hours of observation goes further to support this claim.

There is growing evidence that oxidative stress may represent one of the agents involved in the initiation and/or progression of many human diseases [37]. Determination of the antioxidant compounds from natural product will help to develop new drug candidates for antioxidant therapy [38]. Soewu [14], reported fifty-five fauna species used for various traditional preparations of which *Achachatina marginata* is included.

In this study the haemolymph of *Achachatina marginata* exhibited considerable antioxidant properties. Phenolic and flavonoid compounds have been reported to be responsible for the antioxidant activity of natural products [39]. The antioxidant activities of flavonoids in biological systems are attributed to their ability to transfer electrons to free radicals, chelate metals, activate antioxidant enzymes, and reduce radicals of alpha-tocopherol or to inhibit oxidases while phenolic compounds exert it antioxidant effect by inactivating free radicals or preventing decomposition of hydroperoxide into free radicals [40]. The total phenolics and flavonoid content of *A. marginata* haemolymph was found to be 9.30 ±0.11 mg GAE per g and 15.20±0.59 mg/g catechin equivalent respectively. Therefore the flavonoids and phenol of haemolymph may be responsible for the observed antioxidant activity in this study.

The DPPH radicals were widely used to investigate the scavenging activity of some natural compounds. Fig. 1 shows the results of scavenging DPPH radical ability of *A. maginata* haemolymphat various concentrations in comparison with same doses of ascorbic acid. In DPPH scavenging assay the IC_{50} value of the haemolymph (579.66±2.69 µg/mL) was higher than IC_{50} value reported for some medicinal plant eg 55.62 µg/mL. for *S. surattense* [41]. The haemolymph showed dose-dependent DPPH radicals scavenging activity. The decrease in absorbance of DPPH caused by haemolymph is

due to the reaction between antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation. Similar findings have been documented by many authors [42,43]. The reducing power of a natural compound may serve as a significant indicator of its potential antioxidant activity. However in this study, *A. maginata* haemolymph was found to possess a very low reducing property compared to the ascorbic acid.

The results of in vitro antioxidant activity were further confirmed with in vivo hepatoprotective study. Evaluations of biochemical parameters are useful marker for assessment of tissue damage. Defeats in the activities of this marker are to a reasonable extent the toxicity of a test chemical compound [28]. Tissue or serum enzymes can also indicate tissue cellular damage caused by a test chemical compound long before conventional histological technique [44]. Previous study has reported that $CC1_4$ damages the liver cells causing leakages of the enzymes in the cells resulting to elevation of the levels of plasma transaminases [45]. This finding is in agreement with the results of the present study.

The liver damage was evidenced by the elevated levels of serum and liver ALT, ALP, serum AST, hepatic thio-barbituric acid reacting substances (TBARS) and reduced serum catalase in CCl_4-hepatotoxic rats.

ALT and AST are known as cytosolic marker enzymes and are used as indicator for hepatic damage. The significant increase in the activities of these enzymes may be attributed to increased membrane permeability or hepatocellular necrosis and cytosol leakage into the serum [46].

ALP is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum [47], and is often used to assess the integrity of the plasma membrane and endoplasmic reticulum [48]. The increase in the activity of ALP in CCl_4-hepatotoxic rats could be a consequence of leakage of the enzyme from other tissues or a reduced rate of clearance of the enzyme from the serum [49]. Such increase in ALP activities can constitute threat to the life of cells that are dependent on a variety of phosphate esters for their vital process since there may be indiscriminate hydrolysis of phosphate ester of the tissue [28]. However, improvement towards normalization of the biomarker enzymes following haemolymph (200 mg/kg and 400 mg/kg) and standard control drug silymarin (100 mg/kg) extract pretreatment suggested that the haemolymph have some functions in preserving structural integrity of hepatocellular membrane. However the effectiveness of the haemolymph in attenuating the elevated parameters was lower compared to that of standard hepatoprotective drug silymarin.

Antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4 -induced hepatotoxicity [50]. The body has an effective defense mechanism to prevent and neutralize free radicals-induced damage. This is accomplished by coordinate activities of set of endogenous antioxidant enzymes such as catalase and superoxide dismutase, these enzymes constitute a mutually supportive team of defense against reactive oxygen species (ROS) [51]. Super Oxide Dismutase (SOD) reduced the concentration of highly reactive superoxide radicals by converting it to H_2O_2 whereas catalase convert H_2O_2 into H_2O and O_2 and protect the mucosa tissue from a highly reactive hydroxyl radical [52]. The reduction in the activity of catalase may results in a number of deleterious effects due to accumulation of highly toxic metabolites and hydrogen peroxide on CCL_4 administration, which can induce oxidative stress in the cells. This finding agrees with the results of [27], who reported similar findings. However haemolymph treated groups showed significant ($P<0.05$) increase in the level of this enzyme, which indicates the antioxidant activity of the haemolymph.

Several studies have pointed out that CCL_4 induced oxidative stress results in gradually decrease SOD activity [4]. But in the present study administration of CCL_4 produced rise in SOD activity as compared to normal group. This finding is in agreement with Katarzyna et al. [53] who reported that the activity of SOD increased almost three times in the blood of CCL_4 intoxicated rabbits in comparison with the control group. Similarly, Sawomir et al. [54] reported an increase SOD activity in erythrocytes of human subject exposed to lead toxicity. The difference observed in our results and other published literatures could be attributed to the fact that SOD activity depend on the nature and intensity of oxidative stress. If the oxidative stress is at early stages or not very strong, the SOD activity increases. If the stress is persisting or its level very high, the proteins damage became profound and a decreased SOD activity may occur.

The metabolism of $CC1_4$ releases $CC1_3$ free radicals that cause the process of peroxidation by attacking the methylene bridge of unsaturated fatty acids side chain of microsomal lipids [45]. The level of lipids peroxide is a measure of membrane damage and alterations in structure and function of cellular membranes. In the present study, significant ($p<0.05$) elevation of lipid peroxidation were seen in the liver of CCl_4 treated group. The increase in MDA levels in the liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals [55]. This is also in agreement with the findings of [56], who established that high serum levels of MDA in patients with liver cirrhosis was correlated with lower serum levels of vitamin E. Treatment with standard silymarin and haemolymph treated groups showed significant ($p<0.05$) reduction in lipid peroxidation levels when compared with the CCl_4 treated control group. The observed protective effect of the haemolymph and silymarin against lipid peroxidation could be related to its antioxidant effects which assist in the preservation of membrane integrity. Similar results have been reported by other investigators [57].

5. CONCLUSION

The results of the present study demonstrate that haemolymph possessed antioxidant activity and significantly inhibits the acute liver toxicity induced by CCL_4 in rat, as shown by a reduction in elevated serum and liver enzyme activities. The hepatoprotective activity of the haemolymph may be due to its free radical scavenging and antioxidant activity, resulting from the presence of Flavonoids and phenolic compounds in the haemolymph that enhanced the regeneration ability of liver.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical clearance was given by Federal University of Technology, Minna/Nigeria ethical review board (CUERB) in accordance with international standard on the care and use of experimental animals.

ACKNOWLEDGEMENTS

We would like to express our gratitude to Miss. Bolanle, Miss. Tawakaltu, Miss. Khadijat and all the technical staff of Wabilahi Teofiki Computer Center, Bosso, Minna. Niger State Nigeria, for typing this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gutteridge JMC, Wilkins S. Copper salt dependent hydroxyl radical formation. Damage to proteins acting as antioxidants. Biochem Biophys Acta. 1986;754:38-41.
2. Burns JPT, Gardner D, Matthews GG, Duthie M, Lean E, Crozier A. Extraction of phenolics and changes in antioxidant activity of red wines during vinification. J. Agric. Food Chem. 2001;49: 5797-5808.
3. Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. JAOCS. 1998;75:199-212.
4. Enas AKM. Hepatoprotective effect of aqueous leaves extract of Psidiumguajava and Zizyphusspina-christi against paracetamol induced hepatotoxicity in rats. Journal of Applied Sciences Research. 2012;8(5):2800-2806.
5. Rajkapoor BY, Venugopal J, Anbu N, Harikrishman MG, Ravichandran V. Protective effect of *Phyllanthus polyphllus* on acetaminphen induced hepatotoxicity in rats. Pak. J. Pharm. Sci. 2008;21(1):90-93.
6. Chattopadhyay RR. Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract. Part II. J. Ethnopharmacol. 2003;89(2-3):217- 219.
7. Bhandarkar M, Khan A. Anti-hepatotoxic effect of Numphaen stellata wild against carbon tetrachloride hepatic damage in albino rats. J. Ethnopharmacol. 2004;91: 61-64.
8. Thomas SH. Paracetamol poisoning. Pharmacology and Therapeutics. 1993;60: 91-120.
9. Buwa SS, Patil PH, Kulkarini, Hepatoprotective activity of Abhrak bhashma, an ayuvedic drug in albino rats against hepatitis induced by CCL_4. Ind J Exp Biol. 2001;39:1022-1027.

10. Singh B, Saxena AK, Chandan BK, Suri KK, Suri OP, Satti NK. Hepatoprotective activity of verbenal in on experimental liver damage in rodents. Fitoterapia. 1998;59(2):135-140.
11. Badarinath A, Rao KM, Chetty CM, Ramkanth S, Rajan T, Gnanaprakash K. A review on *in vitro* antioxidant methods: Comparisions, correlations and considerations. Int. J. Pharm. Tech Res. 2010;2(2):1276-1285.
12. Shahidi F. Antioxidants in food and food antioxidants. Nahrung. 2000;44:158 -163.
13. Adeola MO. Importance of wild animals and their parts in the culture, religious festivals and traditional medicine in Nigeria. Environ. Conserv. 1992;19:25-34.
14. Soewu DA. Wild animals in ethnozoological practices among the Yorubas of South western Nigeria and the implications for biodiversity conservation. Afr. J of Agric. Res. 2008;3(6):421-427.
15. Akinnusi O. Introduction to snail and snail farming. Triola Publishing Company, Abeokuta. 2002;70.
16. Adikwu MU. Evaluation of snail mucinmitifs as rectal absorption enhancer for insulin in non-diabetic rat models. Bio. Pharm. Bull. 2005;28(9):180 -4.
17. Abere SA, Lameed GA. The medicinal utilization of snails in some selected states in Nigeria. In: JC Onyekwelu, VAJ Adekunle and DO Oke (eds.) Proceeding of the first National conference of the Forests and Forest Products Society (FFPs) held in Akure, Ondo State. 2008;233-237.
18. Institute of laboratory animal resources, commission on life sciences, National Research Council. Occupational Health and Safety in the Care and Use of Research Animals, Washington, DC: National Academy Press; 1997.
19. Akinloye OA, Olorode BR. Effect of different feeding conditions on the performance, Haemolymph Biochemical and mineral value of African Giant land Snail. *Archachatina marginata*. J. of Agric and Environ. 2000;1:143-147.
20. Lorke D. A new approach to practical Acute Toxicity Testing. Arch. Toxic. 1983;54:275-287.
21. Sabir SM, Rocha JBT. Antioxidant and hepatoprotective activity of aqueous extract of *Solanum fastigiatum* (false "Jurubeba") against paracetamol-induced liver damage in mice. J. Ethnopharmacol. 2008;120:226-232.
22. Bothon FTD, Debiton E, Avlessi F, Forestier C, Teulade JC, Sohounhloue DKC. *In vitro* biological effects of two antidiabetic medicinal plants used in Benin as folk medicine. BMC Complem. Altern. Med. 2013;13:51.
23. Kaur P, Singh B, Kumar S, Kaur S. *In vitro* evaluation of free radical scavenging activity of *Rubia cordifolia* L. J. Chinese Clinic. Med. 2008;3(5):278-284.
24. Ohkawa H, Oishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analyti. Biochem. 1979;95:351-358.
25. Pulido R, Bravo L, Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J. Agric Food Chem. 2000;48:3396-3402.
26. Guntupalli MMR, Chandana VR, Palpu P, Annie S. Hepatoprotective effects of *rubiadin*, a major constituent of *Rubia cordifolia* Linn. J. Ethnopharmacol. 2006; 103:484-490.
27. Hassan SW, Salawu K, Ladan MJ, Hassan LG, Umar RA, Fatihu MY. Hepatoprotective, antioxidant and phytochemical properties of leaf extracts of newbouldia laevies. International Journal of Pharm Tech Research. 2010; (1):573-584.
28. Yakubu MT, Bilbis LS, Lawal M, Akanji MA. Effect of repeated administration of sildenafil citrate on selected enzyme activities of liver and kidney of male albino rats. Nigerian Journal of Pure and Applied Sciences. 2003;18:1395-1400.
29. Shittu OK, Habibat U, Usman YU. Effect of methanolic leaf extract of *Thymus vulgaris* on some biomarker enzymes in *Trypanosoma brucei* infected rats Int. J. Pharm. Biomed. Res. 2013;4(2):83-87.
30. Tietz NW. Clinical Guide to Laboratory Tests. 3rd edn. Philadelphia: W.B. Saunders. 1995;286-288.
31. Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology. 1957;28:56-63.
32. Bock PP, Kramer R, Pavelka M. Peroxisomes and related particles. In: Cell Biology Monographs, 1980;7:44-74.

33. Misra HP, Fridovich I. The role of superoxide anion in the auto-oxidation of epinephrine and simple assay for superoxide dismutase. J. Biol. Chem. 1972;247:3170-3175.
34. Kimura Y, Okuda H, Okuda T. Studies on activities of tannins and related compounds. Planta Medica. 1984;50:473-76.
35. Adamu SO, Johnson TL. Statistics for Beginners, Book 1. SAAL. Publications, Ibadan, Nigeria. 1997;184-199.
36. World Health Organisation (WHO) Guidelines for the treatment of malaria, second edition. WHO Geneva; 2010. Available:www.who.int/malaria/publications
37. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999;64:555-559.
38. Erdemoglu N, Turan NN, Cakici I, Sener BB, Aydin A. Antioxidant activities of some Lamiaceae plant extracts. Phytotherapy Res. 2006;20:9-13.
39. Nehru SS, Zakaria Z, Sreenivasan S, Suryani SS. Free radical scavenging activity of Cassia spectabilis and Cassiafistula. Int. J. Nat. Eng. Sci. 2008; 2:111-112.
40. Ramde-Tiendrebeogo A, Tibiri A, Hilou A, Lompo M, Millogo-Kone H, Nacoulma OG. Guissou IP. Antioxidative and antibacterial activities of phenolic compounds from *Ficus sue Forssk*. International Journal of Biological and Chemical Sciences. 2012; 6(1):328-336.
41. Sridevi M, Senthil S, Pugalendi K V. *In vitro* antioxidant activities of *Solanum surattense* leaf extract. Asian Pac. J. Trop. Biomed. 2013;3(1):28-34.
42. Saha MR, Ashraful AR, Akter RJ. *In-vitro* free radical scavenging activity of *Ixora coccinea* L. Bangladesh J Pharmacol. 2008;3:90-96.
43. Naznin AH. *In vitro* antioxidant activity of methanolic leaves and flowers extracts of *Lippia alba*. Nur. Res. J. Med. Medical Sci. 2009;4(1):107-110.
44. Akanji MA, Adeyemi OS, Oguntoye SO, Sulyman F. *Psidium guajava* extract reduces trypanosomosis associated lipid peroxidation and raises glutathione concentrations in infected animals. EXCLI Journal. 2009;8:148-154.
45. Ashok K, Somayayi S, Bairy S, Hepatoprotective effect of *Ginkgo biloba,* Indian J. Pharmacol. 2002;33:260-266.
46. Teppema LJ, Nieuwenhuijs D, Sarton E, Romberg R, Olievier NC, Ward DS, Dahan A. Antioxidants prevent depression of the acute hypoxic ventilatory response by subanaesthetic halothane in men. J. Physiol. (London). 2002;544:931-938.
47. Wright PJ, Plummer DT. The use of urinary enzyme measurements to detect renal damage caused by nephrotoxic compounds. Biochemical Pharmacology, 1974;23:65-73.
48. Akanji MA, Olagoke OA, Oloyede OB. Effect of chronic consumption of metabisulphite on the integrity of the rat kidney cellular system. Toxicology. 1993; 81:173-179.
49. Mayne PD. Clinical chemistry in diagnosis and treatment. 6th Ed. Lloyd-Luke (Medical Books) Ltd. 2005;2:185.
50. Venukumar MR, Latha MS, Antioxidant activity of C*urculigo orchioides* in carbon tetrachloride- induced hepatopathy in rats. Indian. J. Clin. Biochem. 2002;17:80-87.
51. Hewawasam RP, Jayatilaka KAPW, pathirana C, Nudduwa LKB. Protective effect of *Asteracantha longifolia* extracts on mouse liver injury induced by carbon tetrachlride and paracetamol. J. Pharm. Pharmocol. 2003;55:1413-1418.
52. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiological Reviews. 1979; 59:527-605.
53. Katarzyna P, Maria W, Ewa A. The activity of antioxidative enzymes in the blood of rabbits exposed to the action of tetrachloromethane (CCl4). Poster presentation. 39th Meeting of the Polish Biochemical Society. 2003;1:107.
54. Sawomir K, Aleksandra K, Ewa B, Jolanta Z, Maria D. Activity of superoxide dismutase and catalase in erythrocytes of workers occupationally exposed to lead. Poster presentation. 39th Meeting of the Polish Biochemical Society. 2003;1:71.
55. Mahmoud AH, Motawa HM, Wahba HE, Ebrahim AY. Study of some Antioxidant parameters in mice livers affected with

Urtica pilulifera extracts. Asian J. Biochem. 2006;1(1):67-74.

56. Patrick-Iwuan YKC, Wegwu MO, Ayalogu EO. The protective nature of Garlic, Ginger and Vitamin E on CCL_4 - induced hepatotoxicity in rats. Asian J. Biochem. 2007;2(6):409-414.

57. Girish C, Koner BC, Jayanthi S, Ramachandra RK, Rajesh B, Pradhan SC. Hepatoprotective activity of picroliv, curcumin and ellagic acid compared to silymarin on paracetamol induced liver toxicity in mice. Fundam Clin Pharmacol. 2009;23:735-745.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=984&id=14&aid=8274