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Hepatoprotective effects of *Allium cepa* (onion) extracts against paracetamol-induced liver damage in rats

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Liver damage due to paracetamol hepatotoxicity is a major health challenge worldwide. It is against this background that this study was designed to determine the hepatoprotective effects of the increasing dosage of *Allium cepa* methanolic extracts on paracetamol induced hepatotoxic rats. Fifty-four (54) adult male albino rats comprising of nine normal and 45 paracetamol hepatotoxic rats were used for this study. The experimental design was the three by three Latin square design. Paracetamol hepatotoxicity was induced by single administration of paracetamol at 750 mg/kg ip on the first day of the experiment. The different biochemical parameters assessed were determined before the start of the study and subsequently monthly for the duration of the study. Blood samples were collected from the rat through the eye monthly for analysis and serum was obtained by centrifugation (5000 rpm for 10 min) and stored at -20°C prior to analysis. The effects of duration and increasing dosages (200, 300 and 450 mg/kg) of *A. cepa* methanolic extracts produced a duration dependent significant ($p < 0.05$) reductions in the alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and total serum bilirubin (TSB) of paracetamol hepatotoxic rats after the duration of study when compared with those of the paracetamol, normal and silymarin control rats. *A. cepa* reduced alanine aminotransferase and total serum bilirubin in a dose dependent fashion whereas it reduced aspartate aminotransferase, alkaline phosphatase and lactate dehydrogenase level in a dose independent manner. *A. cepa* extracts studied showed potent hepatoprotective properties. It was evident that *A. cepa* extracts was able to reduce significantly all the elevated biochemical parameters due to paracetamol hepatotoxicity and this was collaborated by results of histopathological studies.

Key words: *Allium cepa,* paracetamol, hepatoprotective effects, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, total serum bilirubin.

INTRODUCTION

The liver is one of the most vital organs that functions as a centre for metabolism of nutrients and excretion of waste metabolites. The liver handles the metabolism and excretion of drugs from the body thereby providing protection against foreign substances by detoxifying and eliminating them (Mohamed-Saleem et al., 2010). Given

the liver's strategic function in the body, it is continuously and variedly exposed to xenobiotics, environmental pollutants and chemotherapeutic agents (Gupta and Misra, 2006). Hepatic dysfunction due to paracetamol overdose is increasing worldwide and medicinal plants are a major constituent of various alternative systems of medicines used worldwide since ancient times. To rationalize the use of herbal preparations in the management of hepatotoxicity, a scientific research on them is needed. This is moreso as therapies developed along the principles of western medicine often carry the risk of adverse effects and are often too costly especially for the developing countries of the world. *Allium cepa* (onion) is a bulbous plant widely cultivated with leading production in China, India and United states. It is rich in proteins, carbohydrates, sodium, potassium and phosphorus. Traditionally, onion has been used to treat intestinal infections (Shaik et al., 2012). It has been reported to be an antibacterial, antiviral, antiparasitic, antifungal and has antihypertensive, hypoglycemic, antithrombotic, anti-hyperlipidemic, anti-inflammatory and antioxidant activity (Abdul et al., 2010; Ozougwu et al., 2008; Eyo et al*.,* 2011; Ozougwu, 2011). This present study was designed to determine the hepatoprotective effects of increasing dosage of *A. cepa* (onions) methanolic extracts against paracetamol induced liver damage in rats viz a viz biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and total serum bilirubin (TSB) level of paracetamol induced hepatotoxic rats.

MATERIALS AND METHODS

Plant materials

The *A. cepa* used for the study was bought from the Ogige market, Nsukka, Enugu state, Nigeria. The plants were identified (Gbile, 1980) to species level at the Herbarium Unit, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Animal model

Fifty- four (54) adult white wistar strain male albino rats (*Rattus norvegicus*) weighing 180 to 200 g were used for the study. They were fed *ad labitum* with 18% crude protein (Guinea feed) commercial feed and allowed to acclimatize for two weeks under standard photoperiodic condition in a clean rat cage with three rats per cage in the research laboratory. All animals were maintained under the standard laboratory condition for temperature (26 ± 2°C), humidity (50 ± 5%) and light (12 h day length) and were allowed free access to food and water.

Preparation of plant extracts

Fresh healthy *A. cepa* bulbs were washed, cut into small pieces and homogenized in a warring blender. The resulting mixture was soaked in 2 L of 80% methanol. The mixture was allowed to stand for 24 h with intermittent shaking. Following filtration, the filtrate obtained was concentrated to dryness at 40°C using a rotary evaporator under reduced pressure. The dried extracts were weighed and then stored in a refrigerator.

Induction of paracetamol hepatotoxicity in rats

The minimum dose of paracetamol that causes death in rats is 1060 mg/kg and the median lethal dose (LD_{50}) is 765 mg/kg (Boyd and Hogan, 1968). Paracetamol hepatotoxicity was induced by single administration of solution of paracetamol at 750 mg/kg intraperitoneally. After 4 days only rats with ALT levels above 65 U/l were considered as hepatotoxic and used for the study. Several researchers have induced hepatotoxicity in rats by single administration of solution of paracetamol on rats at 750 mg/kg ip (Hamid et al., 2011; Reddy et al., 2012; Rafi et al., 2013).

Experimental design

The study was carried out on paracetamol- induced hepatotoxic rats for 12 weeks. The experimental design was the three by three Latin square design. Fifty-four rats used were divided into two major groups: Group I, Nine non-hepatotoxic rats (Normal control); Group II, Forty-five paracetamol induced hepatotoxic rats.

The group I rats were three rats each in three different cages and each received 1 ml/kg of 5% methanol solution daily throughtout the duration of the study. The Group II rats (paracetamol induced hepatotoxic rats) were divided into three subgroups (IIa, IIb, IIc). The subgroup IIa was the paracetamol control, three rats in a cage, and was replicated thrice and had 3 rats each which received 750 mg/kg of paracetamol only (Sumy et al., 2011; Iqbal et al., 2007). Subgroup IIb was divided into 3 replicates (IIb1, IIb2, and IIb3) respectively each replicate had 3 rats and received 200, 300 or 450 mg/kg of *A. cepa* methanolic extracts orally daily. The subgroup IIc, three rats each in a cage, and replicated thrice received the standard drug silymarin at 100 mg/kg (Yuvaraj and Subramoniam, 2009). The different biochemical parameters (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase and total serum bilirubin) assessed were determined first before the start of the experiment and subsequently monthly for the duration of the study. Blood samples were collected from the rat through the eye monthly for analysis. Serum was obtained by centrifugation (5000 rpm for 10 min) and stored at -20°C prior to analysis.

Evaluation of biochemical parameters

Serum alanine aminotransferase and aspartate aminotransferase levels were determined by colorimetric method of Reitman and Frankel (1957) and absorbance was read at 505 nm using spectrophotometer. Alkaline phosphatase level in serum was

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Abbreviations: ALT, Alanine aminotransferase; **AST,** aspartate aminotransferase; **ALP,** alkaline phosphatise; **LDH,** lactate dehydrogenase; **TSB,** total serum bilirubin.

Table 1. Effects of the increasing dosage of *A. cepa* extracts on alanine aminotransferase level of paracetamol induced hepatotoxic rats.

Treatments	Dosage	Alanine aminotransferase level (U/L)				% change after the study
		Week 0	Week 4	Week 8	Week 12	
ME	1.0 ml/kg	$49.70 \pm 3.27^{1,a}$	$48.77 \pm 3.10^{1,a}$	$51.00 \pm 2.52^{1,a}$	$50.63 \pm 3.30^{1,a}$	-
PARA	750 mg/kg	$49.27 \pm 3.15^{1,a}$	$72.41 \pm 4.00^{2,b}$	$75.36 \pm 4.52^{3,2,b}$	$78.34 \pm 5.40^{4,b}$	8.19
AC	200 mg/kg	$49.31 \pm 2.66^{1,a}$	$63.33 \pm 2.67^{2,c}$	$60.67 \pm 3.41^{3,2,c}$	$60.98 \pm 2.31^{4,2,c}$	-15.79
AC	300 mg/kg	$49.31 \pm 2.76^{1,a}$	$64.82 \pm 2.16^{2,d,c}$	$58.33 \pm 2.33^{3,d,c}$	$57.44 \pm 2.40^{4,3,d}$	-20.67
AC	450 mg/kg	$48.73 \pm 3.12^{1,a}$	$68.48 \pm 4.67^{2,e}$	$56.74 \pm 2.57^{3,e,d}$	$56.49 \pm 2.56^{4,3,e,d}$	-21.99
SL	100 mg/kg	$50.57 \pm 3.83^{1,a}$	$52.41 \pm 3.621^{l,g,j}$	$51.94 \pm 2.18^{1,a}$	$50.58 \pm 2.56^{1,a}$	-30.15

Values given represents the Mean ± SD of 9 observations, mean values labeled with the same number superscripts along the same row are not significantly different at 5% significance level ($p < 0.05$). Mean values labeled with the same alphabets superscripts on the same column are not significantly different at 5% significance level ($p < 0.05$). ME = 5% Methanol solution representing the normal control; PARA = Paracetamol control; AC = *Allium cepa* ; SL= Silymarin representing standard drug. % change = % change from para treated at weeks 4 compared to all treatments groups at week 12. Negative % change denotes decrease, Positive % change denotes an increase.

determined by the method of King and King (1954). Serum was incubated with disodium phenylphosphate as substrate buffered at pH 10 for 15 min at 37°C. The hydrolytic products, phenol was condensed with 4-amino antipyrine and then oxidized with alkaline ferrcyanide and the red complex developed was read at 510 nm using spectrophotometer. Lactate dehydrogenase level was estimated by the method of Babson and Babson (1973), where the reduction of nucleoside derived amino acids (NAD) was coupled with the reduction of tetrazolium salt and the produced formazan was measured using spectrophotometer at 503 nm. Total serum bilirubin was determined following the method of Mallory and Evelyn (1937). Diazotised sulphonilic acid reacts with bilirubin in diluted serum and forms purple colored azobilirubin which was read at 540 nm using spectrophotometer.

Data analysis

The data collected was pooled and analyzed for their central tendencies using descriptive statistic, values were given as mean ± standard deviation of the observations. Analysis of variance and LSD was employed to test the significant differences ($P < 0.05$) among treatment means. All analyses were performed using SPSS for windows statistical software package version 16. The resulting outputs were presented in tables.

RESULTS

Alanine aminotransferase level

The increasing dosages (200, 300 and 450 mg/kg) of *A. cepa* methanolic extracts produced a duration dependent significant ($p < 0.05$) reductions in the alanine amino-transferase levels of paracetamol hepatotoxic rats after the duration of treatment when compared with those of the paracetamol and silymarin control rats. Alanine aminotransferase levels were significantly higher in paracetamol control groups throughout the duration of the study compared to all other treatment groups whereas it was significantly higher in all groups at the same period compared to the normal group (1 ml/kg of 5% methanol solution). *A. cepa* reduced alanine aminotransferase level in a dose dependent fashion across the duration of the study with *A. cepa* at 200 mg/kg reducing alanine aminotransferase level by 15.79%, at 300 mg/kg it was reduced by 20.67% whereas at 450 mg/kg it was lowered by 21.99% after the duration of treatments when com-pared to paracetamol control at week 4. Silymarin reduced alanine aminotransferase level by 30.15% after the duration of treatment compared with paracetamol control at week 4 (Table 1). Normal control had no significant effect on alanine aminotransferase level whereas the paracetamol treated control raised alanine aminotransferase level by 8.19%.

Aspartate aminotransferase level

The increasing dosages (200, 300 and 450 mg/kg) of *A. cepa* methanolic extracts produced a duration dependent significant ($p < 0.05$) reductions in the aspartate aminotransferase level of paracetamol hepatotoxic rats after the duration of treatment when compared with those of the paracetamol and silymarin control rats. Aspartate aminotransferase level were significantly higher in parace-tamol control groups throughout the duration of the study compared to all other treatment groups whereas it was significantly higher in all groups at the same period compared to the normal group. *A. cepa* reduced aspartate aminotransferase level in a dose independent fashion across the duration of study with *A. cepa* at 200 mg/kg reducing aspartate aminotransferase level by 41.77% at 300 mg/kg it was reduced by 39.57% whereas at 450 mg/kg it was lowered by 38.59% after the duration of the study when compared to paracetamol control at week 4. Silymarin at 100 mg/kg reduced aspartate aminotransferase level by 62.26% after the duration of treatment compared with paracetamol control at week 4 (Table 2). Normal control had no significant effect on aspartate aminotransferase level whereas the parace-tamol treated control raised aspartate amino-transferase level by 8.97%.

Table 2. Effects of the increasing dosage of *A. cepa* extracts on aspartate aminotransferase level of paracetamol - induced hepatotoxic rats.

Treatments	Dosage	Aspartate aminotransferase level (U/L)				% change after the study
		Week 0	Week 4	Week 8	Week 12	
ME	1.0 ml/kg	86.76 ± 2.84^{1,a}	86.62 ± 2.49^{1,a}	86.32 ± 2.68^{1,a}	87.10 ± 2.58^{1,a}	-
PARA	750 mg/kg	87.17 ± 2.41^{1,a}	659.97 ± 12.00^{2,b}	688.42 ± 20.83^{3,b}	725.01 ± 12.09^{4,b}	8.97
AC	200 mg/kg	87.09 ± 2.44^{1,a}	451.21 ± 10.53^{2,c}	418.89 ±11.77^{3,c,d,e}	384.30 ± 18.95^{4,c}	- 41.77
AC	300 mg/kg	86.89 ± 2.81^{1,a}	430.18 ± 8.39^{2,d}	421.72 ± 9.28^{2,d,e}	398.80 ± 13.28^{3,d,e}	-39.57
AC	450 mg/kg	86.64 ± 2.76^{1,a}	460.36 ± 13.75^{2,e}	412.47 ± 12.46^{3,e}	405.31 ± 13.79^{3,e}	-38.59
SL	100 mg/kg	86.53 ± 2.63^{1,a}	278.68 ± 17.63^{2,l}	257.12 ± 10.03^{3,l}	249.09 ± 7.64^{3,l}	-62.26

Values given represents the Mean ± SD of 9 observations, mean values labeled with the same number superscripts along the same row are not significantly different at 5% significance level ($p < 0.05$). Mean values labeled with the same alphabets superscripts on the same column are not significantly different at 5% significance level ($p < 0.05$). ME = 5% Methanol solution representing the normal control; PARA = Paracetamol control; AC = *Allium cepa* ; SL= Silymarin representing standard drug. % change = % change from para treated at weeks 4 compared to all treatments groups at week 12. Negative % change denotes decrease, Positive % change denotes an increase.

Table 3. Effects of the increasing dosage of *A. cepa* extracts on alkaline phosphatase level of paracetamol induced hepatotoxic rats

Treatment	Dosage	Alkaline phosphatase level (U/L)				% change after the study
		Week 0	Week 4	Week 8	Week 12	
ME	1.0 ml/kg	112.44 ± 1.91^{1,a}	112.64 ± 1.86^{1,a}	112.07 ± 1.62^{1,a}	112.40 ± 1.72^{1,a}	-
PARA	750 mg/kg	112.16 ± 2.02^{1,a}	904.34 ± 13.31^{2,b}	941.61 ± 13.83^{3,b}	958.60 ± 19.67^{4,b}	6.00
AC	200 mg/kg	112.99 ± 1.93^{1,a}	449.06 ± 17.85^{2,c}	440.41 ± 26.60^{3,2,c}	425.62± 17.63^{4,c}	-52.94
AC	300 mg/kg	112.07 ± 2.15^{1,a}	418.96 ± 10.28^{2,d}	417.40 ± 10.57^{3,2,d}	418.12± 12.37^{4,2,d,c}	-53.77
AC	450 mg/kg	112.19 ± 2.28^{1,a}	460.24 ± 9.86^{2,e}	446.08 ± 8.72^{3,e,c}	446.08 ± 8.72^{4,3,e}	-50.68
SL	100 mg/kg	112.33 ± 1.80^{1,a}	249.69 ± 15.30^{2,l}	249.69 ± 15.29^{3,2,l}	219.78 ± 10.25^{4,l}	-75.70

Values given represents the Mean ± SD of 9 observations, mean values labeled with the same number superscripts along the same row are not significantly different at 5% significance level ($p < 0.05$). Mean values labeled with the same alphabets superscripts on the same column are not significantly different at 5% significance level ($p < 0.05$). ME = 5% Methanol solution representing the normal control; PARA = Paracetamol control; AC = *Allium cepa* ; SL= Silymarin representing standard drug. % change = % change from para treated at weeks 4 compared to all treatments groups at week 12. Negative % change denotes decrease, Positive % change denotes an increase.

Alkaline phosphatase level

The increasing dosages (200, 300 and 450 mg/kg) of *A. cepa* methanolic extracts produced a duration dependent significant ($p < 0.05$) reductions in the alkaline phosphatase level of paracetamol hepatotoxic rats after the duration of treatment when compared with those of the paracetamol and silymarin control rats. Alkaline phosphatase level were significantly higher in paracetamol control groups throughout the duration of the study compared to all other treatment groups whereas it was significantly higher in all groups at the same period compared to the normal group. *A. cepa* reduced alkaline phosphatase level in a dose independent manner with *A. cepa* at 200 mg/kg reducing alkaline phosphatase level by 52.94%, at 300 mg/kg it was reduced by 53.77% whereas at 450 mg/kg it was lowered by 50.68% after the duration of the study when compared to paracetamol control at week 4. Silymarin at 100 mg/kg reduced alkaline phosphatase level by 75.70% after the duration of treatment compared with paracetamol control at week 4 (Table 3). Normal control had no significant effect on alkaline phosphatase level whereas paracetamol treated control raised alkaline phosphatase level by 6.00%.

Lactate dehydrogenase level

The increasing dosages (200, 300 and 450 mg/kg) of *A. cepa* methanolic extracts produced a duration dependent significant ($p < 0.05$) reductions in the lactate dehydrogenase level of paracetamol hepatotoxic rats after the duration of the study when compared with those of the paracetamol and silymarin control rats. Lactate dehydrogenase levels were significantly higher in paracetamol control groups throughout the duration of the study compared to all other treatment groups whereas it was significantly higher in all groups at the same period compared to the normal group. *A. cepa* reduced lactate dehydrogenase level in a dose independent manner with *A. cepa* at 200 mg/kg reducing lactate dehydrogenase level by 42.16%, at 300 mg/kg it was reduced by 44.37% whereas at 450 mg/kg it was lowered by 43.61% after the duration of the study when compared to paracetamol control at week 4. Silymarin at 100 mg/kg reduced lactate dehydrogenase level by 63.08% after the duration of the

Table 4. Effects of the increasing dosage of *A. cepa* extracts on lactate dehydrogenase level of paracetamol induced hepatotoxic rats

Treatments	Dosage	Lactate dehydrogenase level (IU/L)				% change after the study
		Week 0	Week 4	Week 8	Week 12	
ME	1.0 ml/kg	$103.78 \pm 5.54^{1,a}$	$105.00 \pm 5.15^{1,a}$	$104.56 \pm 5.20^{1,a}$	$103.44 \pm 5.13^{1,a}$	-
PARA	750 mg/kg	$104.67 \pm 5.57^{1,a}$	$390.89 \pm 18.21^{2,b}$	$409.67 \pm 10.71^{3,b}$	$417.44 \pm 9.22^{4,b}$	6.79
AC	200 mg/kg	$103.11 \pm 4.96^{1,a}$	$250.22 \pm 9.50^{2,c}$	$234.33 \pm 10.26^{3,c}$	$226.11 \pm 10.26^{4,c}$	-42.16
AC	300 mg/kg	$104.22 \pm 6.04^{1,a}$	$267.33 \pm 13.64^{2,d}$	$248.00 \pm 14.44^{3,d}$	$217.44 \pm 11.81^{4,d,c}$	-44.37
AC	450 mg/kg	$105.67 \pm 5.43^{1,a}$	$105.67 \pm 5.42^{1,a}$	$261.44 \pm 9.32^{2,e}$	$220.44 \pm 9.36^{3,e,d,c,b}$	-43.61
SL	100 mg/kg	$103.22 \pm 5.26^{1,a}$	$165.11 \pm 18.15^{2,j}$	$165.11 \pm 18.15^{3,2,l}$	$144.33 \pm 13.10^{4,l}$	-63.08

Values given represents the Mean ± SD of 9 observations, mean values labeled with the same number superscripts along the same row are not significantly different at 5% significance level ($p < 0.05$). Mean values labeled with the same alphabets superscripts on the same column are not significantly different at 5% significance level ($p < 0.05$). ME = 5% Methanol solution representing the normal control; PARA = Paracetamol control; AC = *Allium cepa* ; SL= Silymarin representing standard drug. % change = % change from para treated at weeks 4 compared to all treatments groups at week 12. Negative % change denotes decrease, Positive % change denotes an increase.

Table 5. Effects of the increasing dosage of *A. cepa* extracts on total serum bilirubin levels of paracetamol induced hepatotoxic rats.

Treatments	Dosage	Total serum bilirubin level (mg/dl)				% change after the study
		Week 0	Week 4	Week 8	Week 12	
ME	1.0 ml/kg	$0.52 \pm 0.16^{1,a}$	$0.53 \pm 0.15^{1,a}$	$0.54 \pm 0.16^{1,a}$	$0.51 \pm 0.13^{1,a}$	-
PARA	750 mg/kg	$0.55 \pm 0.15^{1,a}$	$3.57 \pm 0.37^{2,b}$	$4.19 \pm 0.36^{3,b}$	$4.78 \pm 0.49^{4,b}$	33.89
AC	200 mg/kg	$0.53 \pm 0.14^{1,a}$	$2.78 \pm 0.62^{2,c}$	$2.06 \pm 0.53^{3,2,c}$	$1.64 \pm 0.29^{4,c}$	-54.06
AC	300 mg/kg	$0.52 \pm 0.13^{1,a}$	$1.78 \pm 0.41^{2,d}$	$1.45 \pm 0.48^{3,d}$	$1.24 \pm 0.34^{4,3,d}$	-65.27
AC	450 mg/kg	$0.55 \pm 0.15^{1,a}$	$1.63 \pm 0.27^{2,e,d}$	$1.08 \pm 0.34^{3,e}$	$1.15 \pm 0.45^{4,3,e,d}$	-67.79
SL	100 mg/kg	$0.52 \pm 0.13^{1,a}$	$0.93 \pm 0.28^{2,l}$	$0.75 \pm 0.25^{1,2,a}$	$0.63 \pm 0.2^{4,1,a}$	-82.35

Values given represents the Mean ± SD of 9 observations, mean values labeled with the same number superscripts along the same row are not significantly different at 5% significance level ($p < 0.05$). Mean values labeled with the same alphabets superscripts on the same column are not significantly different at 5% significance level ($p < 0.05$). ME = 5% Methanol solution representing the normal control; PARA = Paracetamol control; AC = *Allium cepa* ; SL= Silymarin representing standard drug. % change = % change from para treated at weeks 4 compared to all treatments groups at week 12. Negative % change denotes decrease, Positive % change denotes an increase.

study compared with paracetamol control at week 4 (Table 4). Normal control had no significant effect on lactate dehydrogenase level whereas paracetamol treated control raised lactate dehydrogenase level by 6.79%.

Total serum bilirubin level

The increasing dosages (200, 300 and 450 mg/kg) of *A. cepa* methanolic extracts produced a duration dependent significant ($p < 0.05$) reductions in the total serum bilirubin level of paracetamol hepatotoxic rats after the duration of treatment when compared with those of paracetamol and silymarin control rats. Total serum bilirubin levels were significantly higher in paracetamol control groups throughout the duration of the study compared to all other treatment groups whereas it was significantly higher in all groups at the same period compared to the normal group. *A. cepa* reduced total serum bilirubin level in a dose dependent manner across the duration of the study with *A. cepa* at 200 mg/kg reducing total serum bilirubin level by 54.06%, at 300 mg/kg it was reduced by 65.27% whereas at 450 mg/kg it was lowered by 67.79% after the duration of treatments when compared to paracetamol control at week 4. Silymarin at 100 mg/kg reduced total serum bilirubin level by 82.35% after the duration of the study compared with paracetamol control at week 4 (Table 5). Normal control had no significant effect on total serum bilirubin level whereas paracetamol treated control raised total serum bilirubin level by 33.89%.

Histological examination of prepared tissue slides

The histological examination of liver sections under a light microscope basically supported the biochemical results. Liver sections from the normal control rats showed normal appearance of hepatic architecture, normal central vein, hepatocyte and normal hepatic sinusoid (Figure 1). Liver sections of paracetamol control rats showed cloudy swelling of fat droplets, very severely degenerated hepatocytes, very severely conjested sinusoids and damaged central vein (Figure 2). Liver section of rats treated with methanolic extracts of *A. cepa*

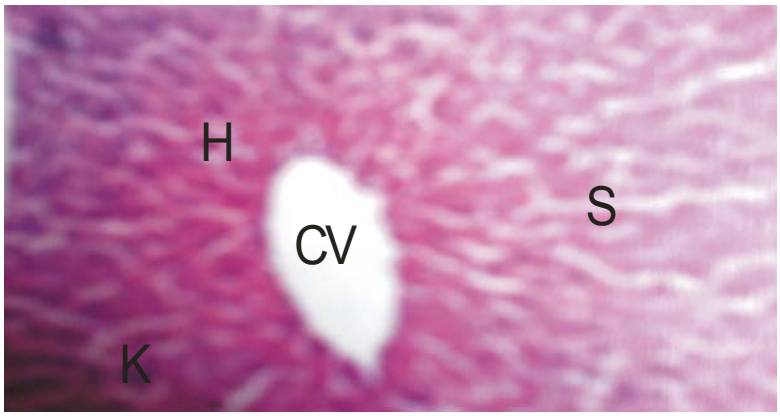

Figure 1. Photomicrograph of transverse section of normal liver treated with 1 ml/kg of 5% methanol solution showing normal appearance of hepatic architecture, normal central vein (CV), hepatocyte (H), Kupffer cells (K) and normal hepatic sinusoid. H & E X 400.

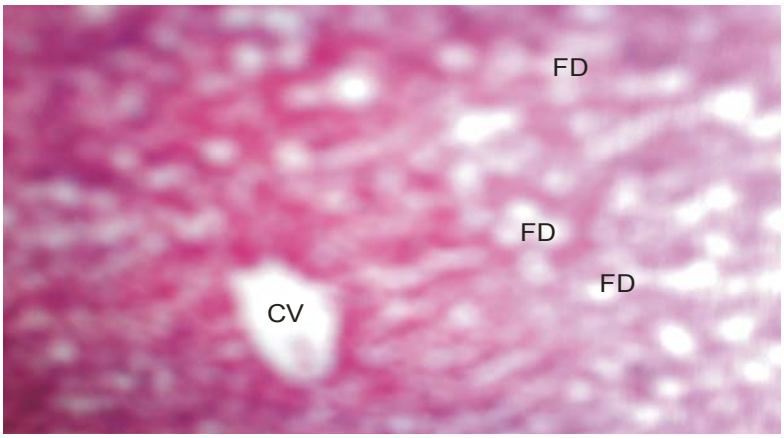

Figure 2. Photomicrograph of transverse section of liver treated with Paractamol at 750 mg/kg showing congested central vein (CV), very severe fat droplets (FD), very severely degenerated hepatocyte (H) and very severely conjested hepatic sinusoid. H & E X 400.

at 200 mg/kg showed distorted hepatic architecture, severely conjested central vein, moderately degenerated hepatocyte and severely congested sinusoid (Figure 3) whereas at 300 mg/kg it showed distorted hepatic architecture, severely congested central vein, moderately degenerated hepatocyte and severely congested sinusoid (Figure 4), at 450 mg/kg it showed severely congested central vein, moderately degenerated hepatocyte and severely congested sinusoid (Figure 5). The liver sections of rats treated with silymarin standard drug at 100 mg/kg showed mildly conjested central vein, moderately degenerated hepatocyte and moderately conjested sinusoid (Figure 6).

DISCUSSION

Paracetamol is a widely used analgesic and antipyretic drug, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man and rats at toxic doses (Mitchell et al*.*, 1973). Paracetamol-induced hepatic injury is commonly used as an experimental model for the study of hepatoprotective effects of medicinal plants and drugs (Plaa and Hewitt, 1982; Gite et al., 2010).

Liver aminotransferases (ALT and AST)

The aminotransferases (ALT and AST) are the most frequently utilized and specific indicators of hepatocellular necrosis (Dama et al*.*, 2011). The significant increase observed in the level of serum aminotransferase (AST and ALT) in paracetamol treated rats compared to the normal rats in this study could be due to hepatocellular damage because these enzymes are normally located in the cytoplasm and released into the circulation after

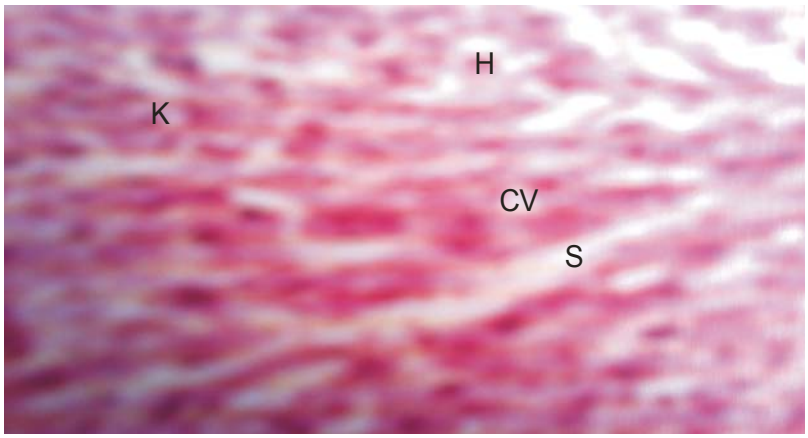

Figure 3. Photomicrograph of transverse section of liver treated with methanolic extracts of *Allium cepa* at 200 mg/kg showing severely congested central vein (CV), moderately degenerated hepatocyte (H), Kupffer cells (K) and severely conjested hepatic sinusoid. H & E X 400.

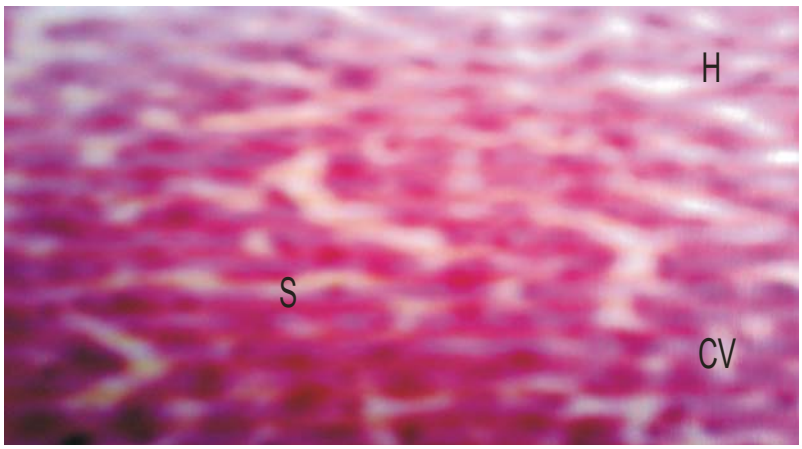

Figure 4. Photomicrograph of transverse section of liver treated with methanolic extracts of *Allium cepa* at 300 mg/kg showing distorted hepatic architecture, severely congested central vein (CV), moderately degenerated hepatocyte (H) and severely conjested sinusoid. H & E X 400.

cellular damage (Hassan and El-Gendy, 2003). The obtained results support the findings of Naziroglu et al. (1999) and Ahmed et al. (2000) who observed similar effects on hepatic enzymes after carbon tetrachloride intoxication in different animals. Administration of silymarin and *A. cepa* on paracetamol treated rats reduced the level of aminotransferase enzymes (AST and ALT) in the serum. The mechanism of action *A. cepa* could be by the prevention of the intracellular enzyme release and its membrane stabilizing and antioxidant effects (Sabina et al., 2011). This is because *A. cepa* are rich in strong antioxidant and are well documented against reactive oxygen species-mediated damage (Ippoushi et al., 2003; Lee et al*.,* 2009). The protective effects of silymarin observed in this study could be attributed to its antioxidant and free radicals-scavenging properties, which has been well established (Horvath et al., 2001).

Alkaline phosphatase (ALP)

In this study the reduction in ALP levels by extracts may suggest repairing of rats liver by *A. cepa* extracts. Possible mechanisms that may be responsible for the protection of paracetamol induced liver damage by *A. cepa* may be by the extract's ability to act as a free radical scavenger intercepting those radicals involved in paracetamol metabolism by microsomal enzymes and also its ability to inhibit rat hepatic microsomal membrane lipid peroxidation. Thus by trapping oxygen related free radicals, *A. cepa* could hinder their interaction with

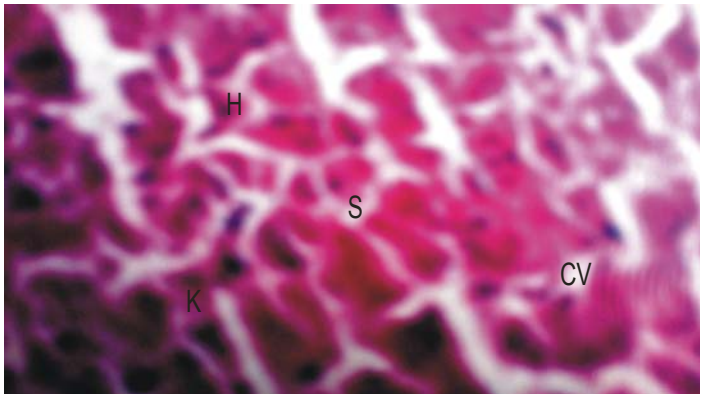

Figure 5. Photomicrograph of transverse section of liver treated with methanolic extracts of *Allium cepa* at 450 mg/kg showing distorted hepatic architecture, severely congested central vein (CV), moderately degenerated hepatocyte (H) and severely conjested sinusoid. H & E X 400.

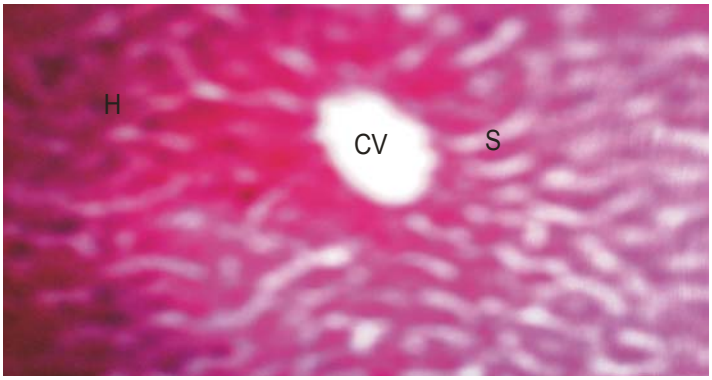

Figure 6. Photomicrograph of transverse section of liver treated with silymarin at 100 mg/kg showing mildly conjested central vein (CV), moderately degenerated hepatocyte (H) and moderately conjested sinusoid (S). H & E X 400.

polyester fatty acids and would abolish the enhancement of lipids peroxidative processes (Aniya et al., 2002; Achuthan et al., 2003; Chattopadhyay, 2003). Another possible mechanism is that the active ingredients in *A. cepa* allyl propyl disulfide could have increased the levels of glutathione which binds to the toxic metabolites of paracetamol such as N- acetyl- p- benzoquinone imine (NAPQI) and increased its rate of excretion from the body. Also, the active ingredients of *A. cepa* extracts might have inhibited the level of the cytochrome P- 450 enzyme system which decreased the formation of NAPQI from ingested paracetamol.

Lactate dehydrogenase level

In this present study, *A. cepa* reduced lactate dehydrogenase level in a dose independent fashion, after the duration of the study. Silymarin reduced lactate dehydrogenase level, paracetamol control raised lactate dehydrogenase level whereas normal control had no significant effect on lactate dehydrogenase level. Rusu et al. (2005) and De-Andrade et al. (2010) reported decrease in hepatic LDH after carbon tetrachloride intoxication but this present study showed increased levels of serum LDH after paracetamol intoxication. The difference could be due to the different hepatotoxin used, dosage, mode of administration and the physiological status of the experimental animals used. The observed increase in LDH in this study could be as a result of enzyme inhibition mainly due to increased membrane fluidity as a result of ROS involvement which led to enzyme leakage into circulation (Hamed, 2011).

Total serum bilirubin

Elevated total serum bilirubin observed in paracetamol

hepatotoxic rats suggested abnormal conjugation of bilirubin by the liver due to generalized hepatocellular damage (El-sherbiny et al., 2003). Total serum bilirubin was decreased in paracetamol hepatotoxic rats after treatment with silymarin and *A. cepa*, this supported the work of Fener et al. (1987) who reported that silymarin is known to have reductive effects on total serum bilirubin in different liver diseases. The possible mechanism of action of *A. cepa* extracts may be through their antioxidative effects. This is because *A. cepa* has active ingredients that are capable of free radical scavenging in living system (Mitra et al., 1998).

Histopathology

The efficacy of any hepatoprotective substance is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic histology and physiology that has been damaged by a hepatotoxin. Liver sections from the normal control rats showed normal appearance of hepatic architecture, normal central vein, hepatocyte, kupffer cells and normal hepatic sinusoid (Figure 1). This showed that there was no damage to hepatic architecture. Liver sections of rats treated with paracetamol control showed cloudy swelling of fat droplets, very severely degenerated hepatocytes, very severely conjested sinusoids and damaged central vein (Figure 2). This could be due to the formation of highly reactive radicals because of oxidative threat caused by paracetamol. Liver section of rats treated with methanolic extracts of *A. cepa* at 200 mg/kg showed distorted hepatic architecture, severely congested central vein, moderately degenerated hepatocyte and severely congested sinusoid (Figure 3) whereas at 300 mg/kg it showed distorted hepatic architecture, severely congested central vein, moderately degenerated hepatocyte and severely congested sinusoid (Figure 4), furthermore, at 450 mg/kg it showed distorted hepatic architecture, severely congested central vein, moderately degenerated hepatocyte and severely conjested sinusoid (Figure 5). The restorative ability of *A. cepa* on histological architecture could be due to its rich antioxidant effects and its documented effects against reactive oxygen species mediated hepatic damages (Ippoushi et al., 2003; Lee et al., 2009). The liver sections of rats treated with silymarin showed mildly conjested central vein, moderately degenerated hepatocyte and moderately conjested sinusoid (Figure 6). These improved histopathological changes could have been achieved because of the antioxidant effects of silymarin which may have reduced hepatic damage or improved hepatic architecture.

Conclusions

From the results of this experimental study, *A. cepa* showed potent hepatoprotective properties. It was evident that the *A. cepa* was able to reduce significantly all the elevated biochemical parameters due to parace-tamol hepatotoxicity. These encouraging results may have future clinical importance because of the increased use of natural herbs worldwide and Nigeria in particular. Pharmacological evidence at the molecular level is required to establish the actual mechanism of action of the active compounds and its nutraceutical role in human diet.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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