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Protective effect of *Allium sativa* extract against carbon tetrachloride- induced hepatic oxidative stress and hyperlipidemia in rats

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Pathogenesis of several chronic liver diseases has been attributed to overwhelmed antioxidant protective system against reactive oxygen species (ROS). The present study ascertained the capacity of short-term administration of ethanolic extract of *Allium sativa* to neutralize ROS and ameliorate hyperlipidemia. Hyperlipidemia was induced in rats by single intra-peritoneal injection of CCl_4 (dosage = 2.0 mL/kg), followed by treatment with ethanolic extract of *A. sativa* (dosage: 200 and 400 mg/kg) at a regular interval of 16 for 64 h. Blood samples were drawn from the rats at *t* = 0 h and *t* = 76 h, that is, 12 h after the end of 64 h treatment with CCl_4/*A. sativa* extract, to ascertain for hepatic function and serum lipid profile (SLP). In addition, liver post mitochondrial supernatant (PMS) fraction was measured for oxidative stress indicators: lipid peroxidation (LPOx), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and reduced glutathione (GSH). On the average, short-term administration of ethanolic extract of *A. sativa* caused reduction of SLP in the following magnitude: total cholesterol (TC) = 19.48%, triacylglycerol (TAG) = 48.59%, VLDL-C = 48.57%, LDL-C = 19.49% and increase in HDL-C = 32.43%. Also, improvement in oxidative stress indicators gave SOD = 10.20%, GPx = 30.92%, CAT = 18.18%, LPOx = 35.92% and GSH = 51.09%. Although the administration of *A. sativa* extract to the rats did not restore full therapeutic benefits within the experimental time (*t* = 76 h), the capacity of the plant extract to ameliorate oxidative stress and hyperlipidemia in the animals was fairly at par with the standard hepatic drug-hepaticum.

Key words: *Allium sativa,* hepatocyte, hyperlipidemia, lipid profile, oxidative stress.

INTRODUCTION

The liver is often referred to as an organ of homeostasis by virtue of the fact that the metabolic concern of the hepatocyte is to ensure constancy in the internal environment of vertebrates. The capability of the liver to achieve this physiologic feat is hinged on high vascularization of the organ, capacity to serve as storage site for macromolecules and micronutrients as well as abode for enzymes involved in carbohydrate, protein and lipid metabolism. In addition, the central roles of the liver in xenobiotic and endogenous detoxification reactions have been well reported (Sugatani et al., 2006; Shaker et al., 2010; Singh et al., 2011). The biosynthesis of most

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plasma lipoproteins and apolipoproteins occur in the hepatocytes (Mensenkamp et al., 2000; Jiang et al., 2006). Therefore, agents/factors that compromise hepatocellular functionality and integrity alter plasma lipid profile patterns (Wolf, 1999; Ramcharran et al., 2011). Hyperlipidemia describes the elevation in plasma lipid components; triacylglycerol (TAG), low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC), but reduced levels of high-density lipoprotein cholesterol (HDL-C) (Ochani and D'Mello, 2009; Kaur and Meena, 2013). According to Shaker et al. (2010), hepatic dysfunction is associated with acute hepatitis, hepatocellular carcinoma, apoptosis, necrosis, inflammation, immune response, fibrosis, ischemia, altered gene expression and regeneration.

The hepatocyte is well furnished with antioxidant defense systems. Notwithstanding, pathogenesis of several chronic liver diseases has been attributed to overwhelmed antioxidant protective system by accumulation and elevated levels of ROS (Czuczejko et al., 2003; Novo et al., 2006; Chikezie, 2011). Notably, the antioxidant scavenging enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione *S*-transferase (GST) and glutathione peroxidase (GPx), which offer primary protection to the hepatocyte and by extension, to other peripheral tissues, against oxidative injury (Halliwell, 1994; Bonnefont-Rousselot et al., 2000; Avti et al., 2006; Pasupathi et al., 2009). Some non-enzymatic antioxidants are reduced glutathione (GSH), α-tocopherol, β-carotene and ascorbate (Avti et al., 2006; Surapanen, 2007; Singh et al., 2011; Necib et al., 2013).

Despite disparities in the distribution and metabolism plasma lipoprotein between humans and rats (Uetrecht, 2006), the use of animal model as tool for lipid and biomedical research is reliable and still popular. Also, applications of plant extracts for the treatment/-management of lipidemia have been severally reported with promising prospects (Kaur and Meena, 2013; Reach and Ernst, 1995).

Accordingly, among several medicinal benefits, *A. sativa* (garlic) have been demonstrated to be an agent of glycemic control (Banerjee and Maulik, 2002; El-Demerdash et al., 2005; Ibegbulem and Chikezie, 2013). The phytochemical and nutritive contents, coupled with previously reported medicinal usefulness of *A. sativa* extract (Auer et al., 1990; Reach and Ernst, 1995; Qidwai et al., 2000; Ibegbulem and Chikezie, 2013) informed the trial of *A. sativa* extract in the present investigation. The present study ascertained the capacity of short-term administration of ethanolic extract of *Allium sativa* to neutralize ROS and ameliorate hyperlipidemia in CCl_4 induced hyperlipidemic rats.

MATERIALS AND METHODS

Collection of plant samples and preparation of extract

Fresh samples of *A. sativa* were obtained in July, 2012 from a local market at Umoziri-Inyishi, Imo State, Nigeria. The plant specimen was identified and authenticated by Dr. F.N. Mbagwu at the Herbarium of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. A voucher specimen was deposited at the Herbarium for reference purposes. Ethanol/water extract (1:2 *v/v*) of *A. sativa* was prepared by methods of Ibegbulem and Chikezie (2013) with modifications according to Lam et al. (2003). Fresh bulbs of *A. sativa* were washed under a continuous stream of distilled water for 15 min and air-dried at room temperature (25 ± 5°C) for 5 h. The bulbs were chopped and further dried for 5 h in an oven at 60°C and subsequently ground with ceramic mortar and pestle. Twenty-five grams (25 g) of the pulverized specimen was suspended in 250 mL of ethanol/water mixture (1:2 *v/v*) in stoppered flask and allowed to stand in a thermostatically controlled water bath at 40°C for 24 h. The suspension was filtered with Whatman No. 24 filter paper, concentrated in a rotary evaporator at 50°C and dried in vacuum desiccator. The yield was calculated to be 3.6% (*w/w*). The extract was re-dissolved in 20 mL of PBS (pH = 7.4) and incubated at 37°C for 30 min with thorough shaking. The dissolved content was quickly frozen at -80°C before lyophilization. The required amount of lyophilized extract was reconstituted in 400 µL distilled water (DW) and administered by intra peritoneal injection to the rats at doses of 200 and 400 mg/kg (Giri et al., 2012**)** at regular time intervals of 16 to 64 h.

Experimental animals

Male rats *Rattus norvegicus* (8-10 weeks old) weighing 150 to 200 g were generous gift from Professor A.A. Uwakwe (Department of Biochemistry, University of Port Harcourt, Nigeria). The rats were maintained at 25 ± 5°C, 30-55% of relative humidity on a 12-h light/12-h dark cycle, with access to water and food *ad libitum* for two weeks acclimatization period. The handling of the animals was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978).

Study design

The animals were deprived of food and water for 16 h before commencement of treatments (control and test experiments) as previously described (Ibegbulem and Chikezie, 2013). Also, hyperlipidemia was induced in the rats by single intra-peritoneal injection of CCl_4 (dosage = 2.0 mL/kg) 16 h before commencement of study. A total of 20 rats were categorized into five groups of four (n = 4) each as follows:

(i) Group C1: Control/Normal rats received only DW (vehicle; 2.0 mL/kg/16 h, i. p.) for 64 h.
(ii) Group C2: Control/Hyperlipidemic rats received 2.0 mL/kg CCl_4 + DW (vehicle; 2.0 mL/kg/16 h, i. p.) for 64 h.
(iii) Group T1: Hyperlipidemic rats received 2.0 mL/kg CCl_4 + *A. sativa* (200 mg/kg/16 h, i. p.) for 64 h.
(iv) Group T2: Hyperlipidemic rats received 2.0 mL/kg CCl_4 + *A. sativa* (400 mg/kg/16 h, i. p.) for 64 h.
(v) Group T3: Hyperlipidemic rats received 2.0 mL/kg CCl_4 + Hepaticum (100 mg/kg/16 h, i. p.) for 64 h.

Collection of blood

Blood samples were drawn from the tail vein of each rat prior to anaesthetization under light ether that is, at experimental t = 0 h for measurement of serum lipid profile (SLP) and levels of γ-glutamyl transferase (γ-GT), alanine transaminase (ALT) and aspartate transaminase (AST) activities. Finally, blood samples were obtained

by carotid artery puncture for measurement of SLP and enzyme activities 12 h after the end of 64 h treatment with *A. sativa* extract that is (*t* = 76 h).

Serum lipid profile

Total cholesterol (TC), triacylglycerol (TAG) and high-density lipoprotein cholesterol (HDL-C) were determined using commercial kits (Randox Laboratory Ltd., UK). Low-density lipoprotein cholesterol (LDL-C) concentration was determined by difference according to the formula described by Friedewald et al. (1972): LDL-C = TC - (HDL-C) - (TAG/5), as reported by Shaker et al. (2010). Very low-density lipoprotein cholesterol (VLDL-C) concentration was estimated using the methods of Burnstein and Sammaille (1960), where the value in mg/dL is based on the assumption that in fasting animals, the VLDL-C to TAG ratio is relatively fixed at 1:5 (Ibegbulem and Chikezie, 2013). Atherogenic index (AI), which was a measure of atherogenesis in normal and treated rats was calculated thus: [TC-(HDL-C)]/(HDL-C) (Suanarunsawat et al., 2011).

Serum enzyme assay

AST and ALT activities were measured using the automated enzymatic methods (EliTech Diagnostic, Sees, France); whereas, γ-GT activity was according to the methods as described by Fiala et al. (1972).

Preparation of liver homogenates

Organ homogenate was prepared according the procedures of Adekunle et al. (2013). Quickly, the liver was excised and placed between blotting papers to remove accompanying blood. Next, the organ was rinsed in 1.15% KCl solution to obliterate residual hemoglobin molecules. The sample was homogenized using a Teflon homogenizer in aqueous $K_2PO_4/KHPO_4$ buffer (0.1 M; pH = 7.4); in 4:1 volume of buffer to organ weight. Subsequently, the homogenate was centrifuged at 10,000 × *g* for 20 min at 4°C to obtain the post mitochondrial supernatant (PMS) fraction and collected into sample bottles. The PMS fraction was finally stored at -80°C before used for analyses. The homogenate was used to assay the following oxidative stress indicators: Lipid peroxidation (LPOx), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and GSH. Protein concentration was measured at λ_{max} = 595 nm by methods of Bradford (1976) using bovine serum albumin as standard.

Lipid peroxidation

Measurement of LPOx was according to the methods of Ohkawa et al. (1979) with minor modifications according to Chikezie (2011). Briefly, the reaction mixture consist of PMS fraction in Tris-HCl buffer (50 mM, pH = 7.4), ter-butyl hydroperoxide (BHP) (500 µM in ethanol) and 1.0 mM $FeSO_4$. The reaction mixture was incubated for 90 min at 37°C, after which the reaction was stopped by introducing 0.2 mL of 8% sodium dodecyl sulfate (SDS) followed by 1.5 mL of 20% acetic acid (pH = 3.5). The quantity of malondialdehyde (MDA) produced during the incubation period was determined by adding 1.5 mL of 0.8% thiobarbituric acid (TBA) and further heating the mixture at 95°C for 45 min. After cooling to 24°C, the mixture was centrifuged at 3,000 × *g* for 10 min. The TBA reactive substances (TBARS) were measured in supernatant at λ_{max} = 532 nm; molar extinction coefficient (Σ) = 1.53×10^5 M^{-1} cm^{-1}. The level of LPOx was expressed in terms of nM of TBARS per 90 min/mg protein.

Superoxide dismutase

SOD was estimated according to the methods of Kono (1978). Briefly, the reaction mixture containing solution A (50 mM Na_2CO_3, 0.1 mM EDTA, pH = 10.0), solution B (96 µM nitrobluetetrazolium [NBT] in solution A), and solution C (0.6% Triton X-100 in solution A) were incubated at 37°C for 10 min. Reaction was started by introducing 100 µL of solution D (20 mM hydroxylamine hydrochloride, pH = 6.0). The rate of NBT dye reduction by $O_2^{\cdot-}$ anion generated due to photo-activation of hydroxylamine hydrochloride was measured at λ_{max} = 560 nm in the absence of PMS fraction. Next, 10 µL aliquot of PMS were added to the reaction mixture and 50% inhibition in the rate of NBT reduction by SOD present in the enzyme source was measured. A unit (U) of SOD activity was defined by the 50% inhibition of NBT. SOD activity was expressed in U/mg protein.

Glutathione peroxidase

GPx activity was measured by the method of Paglia and Valentine (1967). Briefly, the reaction mixture contained aliquot of PMS in 50 mM $K_2PO_4/KHPO_4$ buffer (pH = 7.0), 1.0 mM EDTA, 1.0 mM NaN_3, 0.2 mM NADPH, 1.0 U glutathione reductase, and 1.0 mM GSH. The reaction mixture was allowed to equilibrate at 25°C for 5 min. The reaction was started by introducing 0.1 mL of 2.5 mM H_2O_2. Increase in absorbance at λ_{max} = 340 nm was recorded for 5 min. The change in absorbance was defined as nanomoles of NADPH oxidized to NADP; Σ = 6.2×10^3 M^{-1} cm^{-1} at λ_{max} = 340 nm. The levels of GPx were expressed in terms of nmole NADPH consumed/min/mg protein (U/mg protein).

Catalase

Measurement of PMS fraction CAT activity was according to the method of Luck (1963). The final reaction volume of 3.0 mL contained 0.05 M Tris-buffer, 5 mM EDTA (pH = 7.0), and 10 mM H_2O_2 (in 0.1 M $K_2PO_4/KHPO_4$ buffer; pH = 7.0). A hundred microliters (100 µL) aliquot of the PMS fraction was added to the above mixture. The rate of change of absorbance per min at λ_{max} = 240 nm was recorded for 5 min. CAT activity was calculated using Σ = 43.6 $M^{-1}cm^{-1}$ and expressed in terms of mole H_2O_2 consumed/min/mg protein (U/mg protein).

Reduced glutathione

Level of GSH in organ homogenate was determined by the procedure according to Moron et al. (1979) with minor modification. The 100 µL aliquot of the PMS fraction was mixed with 25% of $CHCl_3$ and centrifuged at 2000 ×*g* for 15 min to precipitate proteins. The supernatant was aspirated and diluted to 1.0 mL with 0.2 M $Na_2PO_4/NaHPO_4$ buffer (pH = 8.0). Later, 2.0 mL of 0.6 mM 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) was added. The absorbance of the developed yellow-colour complex maintained at 25 ± 5°C was measured at λ_{max} = 405 nm after 10 min. A standard curve was obtained with standard GSH. The level of GSH was expressed as µgGSH/mg protein.

Statistical analysis

The data collected were analyzed by the analysis of variance procedure while treatment means were separated by the least

Table 1. Serum γ-GT, ALT and AST activities of normal and hyperlipidemic rats treated with *A. sativa* extract.

Group	Enzyme activity (U/L)					
	t = 0 h			*t* = 76 h		
	γ-GT	ALT	AST	γ-GT	ALT	AST
C1	17.89±0.95[a]	44.09±1.04[a]	94.98±1.35[a]	17.78±0.75[a]	46.14±1.64[a]	98.18±1.81[a]
C2	38.08±1.05[b]	75.68±0.95[b]	123.68±1.99[b]	41.09±1.01[b]	68.07±1.04[b]	124.94±2.64[b]
T1	39.99±1.00[b,,c]	71.89±1.57[b,c]	130.80±0.94[b,c]	27.06±1.96[c]	55.67±1.25[c]	115.89±1.95[b,c]
T2	40.49±0.68[b,c,d]	73.91±1.05[b,c,d]	125.23±1.22[b,c,d]	25.41±0.77[c,d]	50.03±1.75[c,d]	109.96±1.62[b,c,d]
T3	41.43±0.99[b,c,d,e]	75.11±0.98[b,c,d,e]	123.70±1.09[b,c,d,e]	20.98±0.92[a,e]	47.09±0.99[a,d,e]	102.08±1.91[d,e]

The mean ± S.D of three (*n* = 3) determinations. Means in the columns with the same letter are not significantly different at $p > 0.05$ according to LSD. γ-GT, Levels of γ-glutamyl transferase; ALT, alanine transaminase; AST, aspartate transaminase.

significance difference (LSD) incorporated in the statistical analysis system (SAS) package of 9.1 version (2006).

RESULTS

At the end of the experimental time, *t* = 76 h, serum γ-GT, ALT and AST activities of group C1 ($C1_{\gamma\text{-GT}}$, $C1_{ALT}$ and $C1_{AST}$) did not show significant difference ($p > 0.05$) compared to corresponding enzyme activities at *t* = 0 h. Table 1 shows that the ratio of $C1_{ALT}$ activity to $C1_{AST}$ activity at *t* = 0 h and *t* = 76 h was 1:2 approx. In addition, relative marginal variation in $C1_{\gamma\text{-GT}}$ activity within the experimental time was 0.73%; $p > 0.05$. Although γ-GT, ALT and AST activities of group C2 were significantly ($p < 0.05$) elevated compared to group C1, group C2 exhibited marginal variations in the three serum enzyme activities at *t* = 76 h compared to the values at *t* = 0 h; increase in $C2_{\gamma\text{-GT}}$ activity = 7.90%, decrease in $C2_{ALT}$ activity = 10.06% and increase in $C2_{ALT}$ activity = 1.02%. However, group C2 serum γ-GT, ALT and AST activities were relatively elevated at *t* = 76 h compared to groups C1, T1, T2 and T3. Specifically, at *t* = 76 h, $C2_{\gamma\text{-GT}}$, $C2_{ALT}$ and $C2_{AST}$ activities represented 2.31, 1.48 and 1.27 folds increase in corresponding enzyme activity compared to group C1; $p < 0.05$.

At the beginning of the experiment; that is, at *t* = 0 h, serum γ-GT, ALT and AST activities of rats in groups C2, T1, T2 and T3 were comparatively not significantly ($p > 0.05$) different. The three serum enzyme activities were within the range: γ-GT = 38.08 ± 1.05 - 41.43 ± 0.99 U/L; ALT = 71.89 ± 1.57 - 75.68 ± 0.95 U/L and AST = 123.68 ± 1.99 - 130.80 ± 0.94 U/L (Table 1). Furthermore, within the experimental time, serum γ-GT, ALT and AST activities of groups C2, T1, T2, and T3 were significantly different ($p < 0.05$) compared to group C1. Specifically, $C2_{\gamma\text{-GT}}$ activity represented 2.4 folds increase compared to $C1_{\gamma\text{-GT}}$ activity at *t* = 76 h; $p < 0.05$. Again, at *t* = 0 h, serum γ-GT, ALT and AST activities of groups T1, T2, and T3 were significantly different ($p < 0.05$) compared to group C2; whereas, at *t* = 76 h, the three serum enzymes activities were not significantly different ($p > 0.05$).

Although at *t* = 76 h, $T1_{\gamma\text{-GT}}$ activity was significantly elevated compared to $C1_{\gamma\text{-GT}}$ activity, serum $T1_{\gamma\text{-GT}}$ represented 32.33% decrease in enzyme activity relative to $T1_{\gamma\text{-GT}}$ activity at *t* = 0 h. Likewise, decreases in serum enzyme activities at *t* = 76 h relative to *t* = 0 h were: $T1_{ALT}$ activity = 22.56% and $T1_{AST}$ activity =11.40%. The reduction in serum enzyme activities in group T2 was in the order: $T2_{\gamma\text{-GT}}$ activity = 37.24% > $T2_{ALT}$ activity = 32.31% > $T2_{AST}$ activity = 12.19%. $T3_{\gamma\text{-GT}}$ activity at *t* = 76 h represented 2 folds decrease compared to $T3_{\gamma\text{-GT}}$ activity at *t* = 76 h. $T3_{ALT}$ and $T3_{AST}$ activities at *t* = 76 h decreased by 1.60 and 1.20 folds respectively, compared to corresponding enzyme activity at *t* = 0 h.

Although, $T1_{ALT}$ activity and was not significantly different ($p > 0.05$) from $T2_{ALT}$ activity; these values represented corresponding 18.29 and 26.57% reduction in enzyme activities relative to $C1_{ALT}$ activity; $p < 0.05$. Conversely, $T3_{ALT}$ activity = 47.09 ± 0.99 U/L < $C1_{ALT}$ activity = 46.14 ± 1.64 U/L; $p > 0.05$ (Table 1). Likewise, $T3_{ALT}$ activity was not significantly different ($p > 0.05$) from $T2_{ALT}$ activity. Peak value of serum AST activity was registered in group C2; $C2_{AST}$ activity = 124.94 ± 2.64 U/L (Table 1). Serum AST activity was in the order: $T1_{AST}$ activity = 115.89 ± 1.95 U/L > $T2_{AST}$ activity = 109.96 ± 1.62 U/L > $T3_{AST}$ activity = 102.08 ± 1.91 U/L (Table 1). These values corresponded to 7.34, 11.99 and 18.30% reduction in $T1_{AST}$, $T2_{AST}$ and $T3_{AST}$ activities respectively, compared to $C2_{AST}$ activity.

Furthermore, compared to $C_{2\gamma\text{-GT}}$ activity, $T1\gamma_{\text{-GT}}$ activity was lower ($p < 0.05$), which was 59.20% reduction in enzyme activity. However, $T1_{\gamma\text{-GT}}$ activity was raised compared to $C1_{\gamma\text{-GT}}$ activity; *t* = 76 h, $T1_{\gamma\text{-GT}}$ activity = 27.06 ± 1.96 U/L > $C1_{\gamma\text{-GT}}$ activity =17.78 ± 0.75 U/L; $p < 0.05$ (Table 1). $T2\gamma_{\text{-GT}}$ activity was lower than $T1_{\gamma\text{-GT}}$ activity by 6.10%; $p > 0.05$. Nevertheless, $T2_{\gamma\text{-GT}}$ activity was significantly ($p < 0.05$) lower than $C2_{\gamma\text{-GT}}$ activity. $T3\gamma_{\text{-GT}}$ activity was not significantly ($p > 0.05$) different from $C1_{\gamma\text{-GT}}$ activity; specifically, $T3_{\gamma\text{-GT}}$ activity = 20.98 ± 0.92 U/L >$C1_{\gamma\text{-GT}}$ activity =17.78 ± 0.75 U/L; *t* = 76 h (Table 1). $C2_{ALT}$ activity was highest, representing 1.48 folds increase in enzyme activity compared to $C1_{ALT}$ activity ($p < 0.05$).

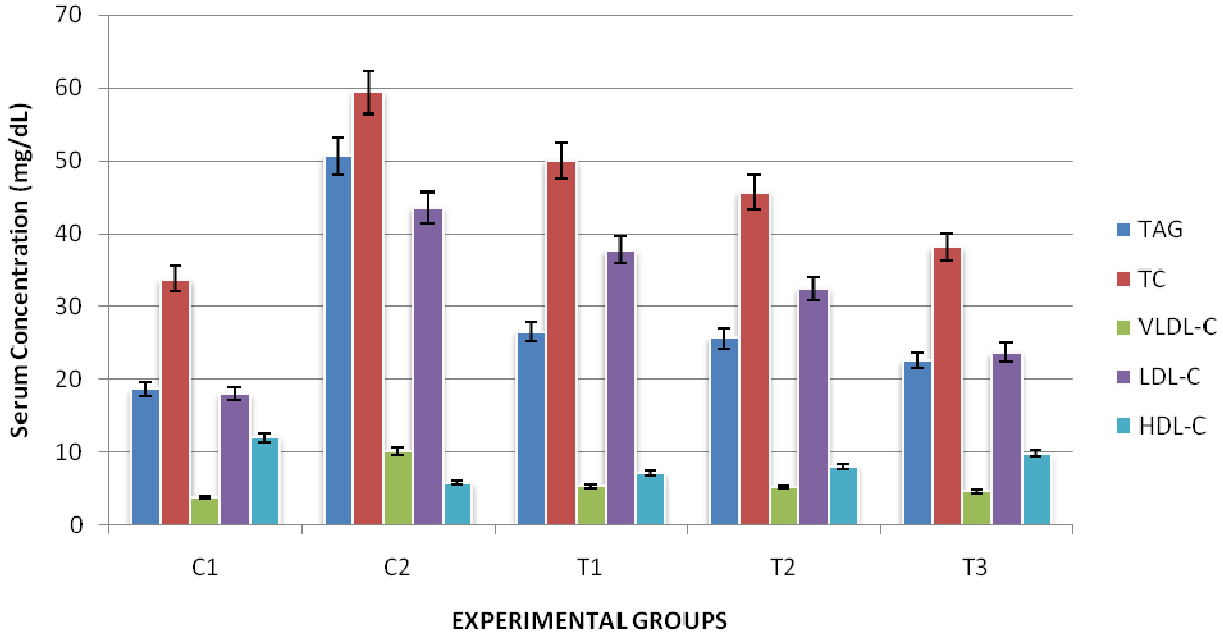

Figure 1. SLP at *t* = 76 h of normal and hyperlipidemic rats treated with *A. sativa* extract. TC, total cholesterol; TAG, triacylglycerol; VLDL-C, very low density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Table 2. Atherogenic index at *t* = 76 h of normal and hyperlipidemic rats treated with *A. sativa* extract.

Group	C1	C2	T1	T2	T3
AI	0.54	7.90	2.74	2.20	1.30

SLP indicated $C1_{TC}$ = 33.75±1.02 mg/dL (Figure 1), of which serum concentrations of VLDL-C, LDL-C and HDL-C accounted for 11.02, 53.21 and 35.76% of TC concentration respectively; AI = 0.54 (Table 2). $C2_{SLP}$ showed that serum lipids concentrations were profoundly altered. For instance, serum TAG, TC, VLDL-C and LDL-C concentrations were significantly ($p< 0.05$) elevated in group C2 by factors of 2.72, 1.76, 2.72 and 2.42 respectively, compared to group C1. The reduced levels of serum HDL-C in group C2 caused corresponding increase in AI (Table 2). Generally, $T1_{SLP}$ was not significantly different ($p> 0.05$) from $T2_{SLP}$. However, these values represented significant ($p< 0.05$) alteration in $T1_{SLP}$ compared to $C1_{SLP}$. The use of group C2 as reference point indicated decreased $T1_{TAG}$ and $T1_{VLDL-C}$ ($p< 0.05$); whereas, $T1_{TC}$ and $T1_{LDL-C}$ ($p> 0.05$). Furthermore, $T1_{HDL-C}$ was elevated ($p> 0.05$). Accordingly, $T2_{SLP}$ was significantly different ($p < 0.05$) from $C2_{SLP}$. Conversely, $T3_{SLP}$ showed no significant difference ($p> 0.05$) compared to $T2_{SLP}$, except in LDL-C concentration. An overview of Table 2 shows that the AI was in the order: C2 > T1 > T2 > T3 > C1.

Hepatocyte $C2_{SOD}$ gave the highest level of enzyme activity, representing 3.77 folds increase in activity compared to $C1_{SOD}$ activity ($p< 0.05$). Furthermore, hepatocyte $T1_{SOD}$, $T2_{SOD}$ and $T3_{SOD}$ exhibited elevated activities, which was significantly different ($p< 0.05$) from $C1_{SOD}$ activity. However, hepatocyte $T1_{SOD}$ and $T2_{SOD}$ activities were reduced compared to $C2_{SOD}$ activity ($p> 0.05$). Specifically, $T3_{SOD}$ activity gave 0.77±0.07 U/mg protein (Table 3), corresponding to 21.48% reduction in SOD activity compared to $C2_{SOD}$ activity. $C2_{GPx}$, $T1_{GPx}$, $T2_{GPx}$ and $T3_{GPx}$ activities were reduced relative to $C1_{GPx}$ activity. GPx showed progressive increase in enzyme activity in the order: $T3_{GPx}$ = 7.09±0.08 U/mg protein> $T2_{GPx}$ = 6.440.09U/mg protein> $T1_{GPx}$ = 6.39±0.14U/mg protein> $C2_{GPx}$= 4.90±0.10 U/mg protein (Table 3). A cursory look at Table 3 shows that hepatocyte CAT activity of the various experimental groups followed the same pattern as hepatocyte GPx activity. $T1_{CAT}$, $T2_{CAT}$ and $T3_{CAT}$ activities were reduced compared to $C1_{CAT}$ activity ($p> 0.05$). However, levels of activity of $T1_{CAT}$, $T2_{CAT}$ and $T3_{CAT}$ were not significantly different ($p> 0.05$).

Table 4 shows that hepatocyte level of $C2_{LPOx}$ doubled that of $C1_{LPOx}$. However, levels of $T1_{LPOx}$, $T2_{LPOx}$ and $T3_{LPOx}$ were not significantly different ($p> 0.05$), but with values significantly lower than those of $C2_{LPOx}$; $p< 0.05$ and $C1_{LPOx}$; $p > 0.05$. Level of $C2_{GSH}$ was relatively

Table 3. Effects of *A. sativa* extract on hepatocyte SOD, GPx and CAT activities at *t* = 76 h.

Group	SOD	Enzyme activity (U/mg protein)	
		GPx	CAT
C1	0.26±0.05^{a}	14.89±0.04^{a}	8.17±0.51^{a}
C2	0.98±0.04^{b}	4.90±0.10^{b}	4.51±0.31^{b}
T1	0.87±0.02^{b,c}	6.39±0.14^{b,c}	5.17±0.34^{b,c}
T2	0.89±0.05^{b,c,d}	6.44±0.09^{b,c,d}	5.49±0.39^{b,c,d}
T3	0.77±0.07^{c,d,e}	7.09±0.08^{b,c,d,e}	5.97±0.31$^{b,c,d.e}$

The mean ± S.D of three (*n* = 3) determinations. Means in the columns with the same letter are not significantly different at *p* > 0.05 according to LSD.

Table 4. Effects of *A. sativa* extract on hepatocyte LPOx and GSH levels at *t* = 76 h.

Parameter	Group				
	C1	C2	T1	T2	T3
[LPOx]	9.31±0.84^{a}	18.82±0.65^{b}	12.16±0.57^{b,c}	11.96±0.63^{b,c,d}	10.52±0.77^{b,c,d,e}
[GSH]	19.56±0.95^{a}	6.41±0.11^{b}	9.05±0.35^{b,c}	10.32±0.85^{c,d}	12.78±0.55^{c,d,e}

The mean ± S.D of three (*n* = 3) determinations. Means in the rows with the same letter are not significantly different at *p* > 0.05 according to LSD. [GSH] = µgGSH/mg protein; [LPOx] = nM of TBARS per 90 min/mg protein.

lowest, whereas $C1_{GSH}$ registered the highest concetration. Table 4 shows progressive increase in levels of hepatocyte GSH in the order: $T3_{GSH}$ =12.78±0.55 µgGSH/mg protein> $T2_{GSH}$ = 10.32±0.85µgGSH/mg protein> $T1_{GSH}$ =9.05±0.35 µgGSH/mg protein, *p*> 0.05.

DISCUSSION

Short-term administration of CCl_4 to the experimental rats induced hepatocellular damage typified by raised levels of diagnostic liver functional enzymes in serum; γ-GT, ALT and AST (Table 1). The measurement of serum γ-GT, ALT and AST activities as a basis for ascertaining and confirmation of hepatocellular damage and dysfunction have been widely reported (Sugatani et al., 2006; Abdel-Moneim and Ghafeer, 2007; Shaker et al., 2010; Singh et al., 2011; Al-Dosari, 2011). The serum γ-GT, ALT and AST activities in groups T1, T2 and T3 relative to the group C2 was obvious indication of improvement of functional status of rats in groups T1, T2 and T3. The results of the present study confirm ROS as promoters of hepatic damage, which was indicated by disturbances in antioxidant defense systems and alterations of biopsy oxidative stress indicators. The mechanism by which CCl_4 compromised hepatic functionality and integrity was previously suggested by Shaker et al. (2010). They reported that the biotransformation of CCl_4 caused the production of highly unstable free radicals (CCl_3 or CCl_3O_2) that engendered endoplasmic reticulum lipid peroxidation and cellular damage. Mayes (1983) in another report stated that the short-term hepatotoxic effect of CCl_4 was because of the capability of CCl_4 to inhibit secretory mechanisms and conjugation of lipids with apolipoproteins within the hepatocytes and thereby causing fatty liver. In this regard, preceding studies have revealed distortions in plasma lipoproteins and lipid profile in animals with induced hepatocellular damage or impairments (Ooi et al., 2005; Jiang et al., 2006; Ramcharran et al., 2011). The reports presented here show perturbation in SLP patterns in the experimental rats, which was in concordance with previous observations. The alterations in SLP were reflections of compromised structural and functional integrity of the hepatocytes. Ooi et al. (2005) had previously suggested the clinical representation of serum low levels of HDL-C as a reflection of pathologic conditions and evaluation of severity of hepatic dysfunction.

The hyperlipidemic ameliorative property of *A. sativa* extract is exemplified by its serum TC, TAG, VLDL-C and LDL-C lowering effect in a dose dependent manner (Figure 1) in the experimental rat groups (T1 and T2). In similar manner, Lau et al. (1983) had demonstrated by animal and human studies that component of garlic extract lowered plasma TC and TAG levels with changes in blood lipoproteins and coagulation parameters. They further posited that available data suggested that garlic may be of value in either the prevention or treatment of atherosclerotic diseases.

In another study, El-Demerdash et al. (2005) reported the presence of cysteine derivatives, notably, S-alkyl cysteine sulfoxides in *A. sativa*. They noted that during extraction process, these compounds are converted by allinase to thiosulfinates and polysulfides compounds,

which possess hypocholesterolaemic, antidiabetic, antibiotic and fibrinolytic properties.

The pattern of AI of the various experimental groups (Table 2) showed the propensity of hyperlipidemia, occasioned by hepatic injury and dysfunction, to promote atherogenic conditions. Studies have confirmed that hyperlipidemia elicits oxidative stress in organs such as the heart, kidney and liver (Suanarunsawat et al., 2011; Shaker et al., 2010), which is a major contributing factor in the etiology of atherosclerosis, hypertension, diabetes and several degenerative diseases (Vijayakumar et al., 2004; Du et al., 2010). In addition, ROS cause the oxidation of LDL-C, engendering cytotoxic events in endothelial cells and selective accumulation of modified LDL-C (Torres et al., 1999). This pathologic event is one of the various major contributing and causative factors of atherosclerosis. The present study shows the capacity of *A. sativa* extract to reverse oxidative stress and hyperlipidemia in the experimental rats (T1 and T2), which was comparable to those treated with the standard hypolipidemic drug-hepaticum (T3). However, the short-term treatments did not provide for the experimental animals the requisite and anticipated full therapeutic benefits. Nevertheless, previous authors have reported the therapeutic usefulness of *A. sativa* in the treatment and management of cardiovascular diseases (Mahmoodi et al., 2006), hypertension (Benavides et al., 2007), Alzheimer's disease (Peng et al., 2002), inflammation, thrombosis (Fukao et al., 2007) malignancy (Hsing et al., 2002) fatty liver (Sahebkar, 2011) and as antimicrobial (Gull et al., 2012).

Liver biopsy showed perturbations of enzymatic (SOD, GPx and CAT) and non-enzymatic (LPOx and GSH) oxidative stress indicators of experimental rats (Tables 3 and 4). In agreement with the present findings, Đurendić-Brenesel et al. (2013) reported increased SOD activity in the liver homogenates of the hyperlipidemic rats (Table 3). The reduced levels of $C2_{GPx}$ and $C2_{CAT}$ activities were the effect of raised and overwhelming levels of ROS (El-Demerdash et al., 2005; Avti et al., 2006); ROS has inhibitory effect on ROS scavenging enzymes such as CAT and GPx activities (Hassan and Fridovich, 1978; Avti et al., 2006). Consequently, raised levels of cytotoxic ROS engendered membrane lipid peroxidation with the production of associated by-products such as malondialdehyde (MDA) and 4-hydroxyalkenals (4HNE) (Shaker et al., 2010; Al-Dosari, 2011; Đurendić-Brenesel et al., 2013).

Depleting $C2_{GSH}$ concentration confirmed increased oxidative stress (Surapaneni, 2007; Abdel-Moneim and Ghafeer, 2007) mediated by ROS oxidation of sulfhydryl groups that are essential in cellular enzymatic cofactor and non-enzymatic reduction pathways. The present investigations show that administration of *A. sativa* extract caused relief in oxidative stress to the experimental rats, indicated by decreased SOD but increased GPx and CAT activities, coupled with decreased LPOx but increased GSH content in groups T1 and T2 compared to group C2 (Tables 3). Equally, oxidative stress indicators showed that short-term administration of *A. sativa* extract did not restore full therapeutic benefits to the experimental rats. However, the capacities of the two experimental doses (200 and 400 mg/kg) of *A. sativa* extractto ameliorate oxidative stress were comparable to the standard hepatic drug-hepaticum.

Previous studies have shown that Buckwheat (*Fagopyrum esculentum*) (Đurendić-Brenesel et al., 2013), *Ocimum sanctum* L. (Suanarunsawat et al., 2011) and Roselle (*Hibiscus sabdariffa* Linn) (Ochani and D'Mello, 2009) share similar antioxidant phytochemical profile with *A. sativa* extract (Ibegbulem and Chikezie, 2013). Accordingly, the presence of phytochemicals such phenolics, tannins and flavonoids in *A. sativa* extract, coupled with high content of antioxidant element-selenium (http.//www.complete-herbal.com/details/garlic.htm; Banerjee and Maulik, 2002) contributed to the antioxidant property of*A.sativa* extract.

Although the administration of *A. sativa* extract to the rats did not restore full therapeutic benefits within the experimental time (t = 76 h), the capacity of the plant extract to ameliorate oxidative stress and hyperlipidemia in the animals was fairly at par with the standard hepatic drug-hepaticum.

Conflict of Interests

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

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