Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Biochemistry 9 (2): 86-97, 2014
ISSN 1815-9923 / DOI: 10.3923/ajb.2014.86.97

Hepatocyte Oxidative Stress Indicators of Carbon Tetrachloride Induced Hyperlipidemic Rats (*Rattus norvegicus*) Treated with *Allium sativa* Extract

[1]Chikezie Paul Chidoka and [2]Uwakwe Augustine Amadikwa
[1]Department of Biochemistry, Imo State University, Owerri, Nigeria
[2]Department of Biochemistry, University of Port-Harcourt, Port-Harcourt, Nigeria

Corresponding Author: Chikezie Paul Chidoka, Department of Biochemistry, Imo State University, Owerri, Nigeria Tel: +2348038935327

ABSTRACT

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^{-1}), followed by treatment with ethanolic extract of *A. sativa* (dosage: 200 and 400 mg kg^{-1}) at a regular interval of 16 h for 64 h. Blood samples were drawn from the rats at t = 0 and t = 76 h, i.e., 12 h after the end of 64 h treatment with CCl_4 per *A. sativa* extract treatment, to ascertain 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 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 against 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 antioxidant defense structures are reduced glutathione (GSH), a-tocopherol, β-carotene and ascorbate (Avti *et al.*, 2006; Surapaneni, 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; Resch 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; Resch 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 *A. 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 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). Freshbulbs 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^{-1} (Giri *et al*., 2012) at regular time intervals of 16 for 64 h.

Experimental animals: Male rats *Rattus norvegicus* (8-10 weeks old) weighing 150-200 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 light 12 h dark cycle, with access to water and food *ad libitum* for 2 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.

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). Hyperlipidemia was induced in the rats by single intra-peritoneal injection of CCl_4 (dosage = 2.0 mL kg^{-1}) 16 h before commencement of study. A total of 20 rats were categorized into 5 groups of 4 (n = 4) each as follows:

- **Group C1:** Control/Normal rats received only DW (vehicle; 2.0 mL kg^{-1} 16 h^{-1}, i.p.) for 64 h
- **Group C2:** Control/Hyperlipidemic rats received 2.0 mL kg^{-1} CCl_4+DW (vehicle; 2.0 mL kg^{-1} 16 h^{-1}, i.p.) for 64 h
- **Group T1:** Hyperlipidemic rats received 2.0 mL kg^{-1} CCl_4+*A. sativa* (200 mg kg^{-1} 6 h^{-1}, i.p.) for 64 h
- **Group T2:** Hyperlipidemic rats received 2.0 mL kg^{-1} CCl_4+*A. sativa* (400 mg kg^{-1} 16 h^{-1}, i.p.) for 64 h
- **Group T3:** Hyperlipidemic rats received 2.0 mL kg^{-1} CCl_4+Hepaticum (100 mg kg^{-1} 16 h^{-1}, i.p.) for 64 h

Collection of blood: Blood samples were drawn from the tail vein of each rat prior to anesthetization under light ether i.e., 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 treatment i.e., (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 Friendwald *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^{-1} 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: Organhomogenate 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 γ_{max} = 595 nm by methods of Bradford (1976) using bovine serum albumin as standard.

Lipid peroxidation: Measurement of LPOx was according the methods of Ohkawa *et al.* (1979) with minor modifications by according to Chikezie (2011). Briefly, the reaction mixture consist of PMS fraction in 50 mM Tris-HCl buffer (pH = 7.4), 500 μM 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) to the reaction mixture. 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 at 95°C. After cooling to 24°C, the mixture was centrifuged at 3,000×g for 10 min. The TBA reactive substances (TBARS) in the supernatant were measured in supernatant at γ_{max} = 532 nm; molar extinction coefficient (S) = 1.53×10^5 M^{-1} cm^{-1}. The level of LPOx was expressed in terms of nM of TBARS per 90 min mg^{-1} protein.

Superoxide dismutase: SOD was estimated according to the methods of Kono (1978). Briefly, the reaction mixture contained 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^- anion generated due to photo-activation of hydroxylamine hydrochloride was measured at γ_{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^{-1} 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 γ_{max} = 340 nm was recorded for 5 min. The change in absorbance was defined as nmole of NADPH oxidized to

NADP; S = 6.2×10^3 M^{-1} cm^{-1} at γ_{max} = 340 nm. The levels of GPx were expressed in terms of nmole NADPH consumed min^{-1} mg^{-1} protein (U mg^{-1} 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 min^{-1} at γ_{max} = 240 nm was recorded for 5 min. CAT activity was calculated using S = 43.6 M^{-1} cm^{-1} and expressed in terms of mole H_2O_2 consumed min^{-1} mg^{-1} protein (U mg^{-1} 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 γ_{max} = 405 nm after 10 min. A standard curve was obtained with standard µg GSH. The level of GSH was expressed as g GSH mg^{-1} protein.

Statistical analysis: The data collected were analyzed by the analysis of variance procedure while treatment means were separated by the Least Significance Difference (LSD) incorporated in the statistical analysis system (SAS) package of 9.1 version.

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 showed 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, i.e., 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^{-1}; ALT = 71.89±1.57, 75.68±0.95 U L^{-1} 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 ($p>0.05$).

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

	Enzyme activity (U L^{-1})					
	t = 0 h			t = 76 h		
Groups	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^{bc}	71.89±1.57^{bc}	130.80±0.94^{bc}	27.06±1.96^{c}	55.67±1.25^{c}	115.89±1.95^{bc}
T2	40.49±0.68^{bcd}	73.91±1.05^{bcd}	125.23±1.22^{bcd}	25.41±0.77^{cd}	50.03±1.75^{cd}	109.96±1.62^{bcd}
T3	41.43±0.99^{bcde}	75.11±0.98^{bcde}	123.70±1.09^{bcde}	20.98±0.92^{ae}	47.09±0.99^{ade}	102.08±1.91^{de}

Mean (X)±SD of three (n = 3) determinations, Mean in the columns with the same letter are not significantly different at p>0.05 according to LSD

Although at t = 76 h, $T1_{\gamma\text{-GT}}$ activity was significantly ($p<0.05$) 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 the corresponding enzyme activity at t = 0 h.

Although, $T1_{ALT}$ activity and was not significantly different ($p>0.05$) activity 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^{-1}<$C1_{ALT}$ activity = 46.14±1.64 U L^{-1}; $p>0.05$ (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^{-1} (Table 1). Serum AST activity was in the order: $T1_{AST}$ activity = 115.89±1.95 U L^{-1}>$T2_{AST}$ activity = 109.96±1.62 U L^{-1}>$T3_{AST}$ activity = 102.08±1.91 U L^{-1} (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 ($p<0.05$) than $C2_{r\text{-GT}}$ 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^{-1} >$C1_{\gamma\text{-GT}}$ activity =17.78±0.75 U L^{-1}; $p<0.05$ (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^{-1} >$C1_{\gamma\text{-GT}}$ activity =17.78±0.75 U L^{-1}; t = 76 h (Table 1). $C2_{ALT}$ activity was highest, representing 1.48 folds increase in enzyme activity compared to $C1_{ALT}$ activity ($p<0.05$).

SLP indicated $C1_{TC}$ = 33.75±1.02 mg dL^{-1} (Fig. 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

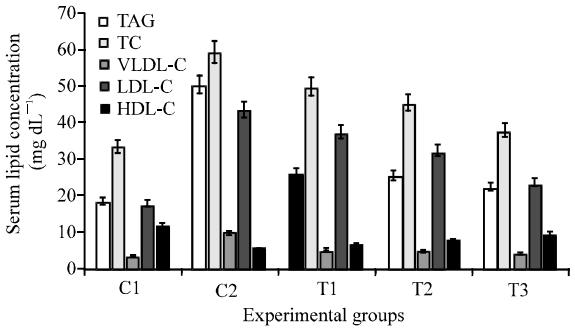

Fig. 1: SLP at t = 76 h of normal and hyperlipidemic rats treated with *A. sativa* extract

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

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

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

	Enzyme activity (U mg^{-1} protein)		
Groups	SOD	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^{bc}	6.39±0.14^{bc}	5.17±0.34^{bc}
T2	0.89±0.05^{bcd}	6.44±0.09^{bcd}	5.49±0.39^{bcd}
T3	0.77±0.07^{cde}	7.09±0.08^{bcde}	5.97±0.31^{bcde}

Mean (X)±SD of three (n = 3) determinations, Mean in the columns with the same letter are not significantly different at p>0.05 according to LSD

indicated decreased $T1_{TAG}$ and $T1_{VLDL-C}$ ($p<0.05$), whereas $T1_{TC}$ and $T1_{LDL-C}$ ($p>0.05$) were not significantly different. Furthermore, $T1_{HDL-C}$ was not significantly ($p>0.05$) elevated. Accordingly, $T2_{SLP}$ was significantly different ($p<0.05$) from $C2_{SLP}$. Conversely, $T3_{SLP}$ showed no significant difference ($p>0.05$) from $T2_{SLP}$, except in LDL-C concentration. An overview of Table 2 showed 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 0.770±0.7 U mg^{-1} 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^{-1} protein> $T2_{GPx}$ = 6.44±0.09U mg^{-1} protein> $T1_{GPx}$ = 6.39±0.14U mg^{-1} protein>$C2_{GPx}$= 4.90±0.10 U mg^{-1} protein (Table 3). A cursory look at Table 3 showed 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 showed 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

Table 4: Effects of *A. sativa* extract on hepatocyte LPOx and GSH levels

	Groups				
Parameters	C1	C2	T1	T2	T3
[LPOx] nM of TBARS 90 min^{-1} mg^{-1} protein	9.31±0.84^{a}	18.82±0.65^{b}	12.16±0.57^{bc}	11.96±0.63^{bcd}	10.52±0.77^{bcde}
[GSH] µg mg^{-1} protein	19.56±0.95^{a}	6.41±0.11^{b}	9.05±0.35^{bc}	10.32±0.85^{cd}	12.78±0.55^{cde}

Mean (X)±SD of three (n = 3) determinations, Mean in the rows with the same letter are not significantly different at p>0.05 according to LSD

those of $C2_{LPOx}$; p<0.05 and $C1_{LPOx}$; p>0.05. Level of $C2_{GSH}$ was relatively lowest, whereas $C1_{GSH}$ registered the highest concentration. Table 4 showed progressive increase in levels of hepatocyte GSH in the order: $T3_{GSH}$ = 12.78±0.55 µg GSH mg^{-1} protein> $T2_{GSH}$ = 10.32±0.85 µg GSH mg^{-1} protein>$T1_{GSH}$ = 9.05±0.35 µg GSH mg^{-1} 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 result of the present study confirmed the implication of ROS as promoters of hepatic damage which was indicated by disturbances in antioxidant defense systems and alterations of biopsy oxidative stress indicators. This 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), engendering 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 mechanism 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 showed 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) posited that the low serum level of HDL-C was a reflection of pathologic conditions and could indicate the 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 (Fig. 1) in the experimental rat groups (T1 and T2). In similar manner, Lau *et al*. (1983) had demonstrated by the use of both animal and human studies, that components of garlic lowered plasma TC and TAG levels with changes in blood lipoproteins and coagulation parameters. They further posited that their 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 these compounds are converted by allinase into thiosulfinates and polysulfides compounds which possess hypocholesterolaemic as well as antidiabetic, antibiotic and fibrinolytic properties.

The pattern of AI of the various experimental groups (Table 2) showed the propensity of hyperlipidemia, occasioned by hepatic 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 plays a major role in the etiology of atherosclerosis, hypertension, diabetes and several degenerative diseases (Vijayakumar *et al*., 2004; Du *et al*., 2010). In addition, mechanism generated by 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 has shown 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 standardhypolipidemic drug-hepaticum (T3). However, the short-term treatments did not provide for the animals, the requisit and anticipated full ther apeutic benefits. Likewise, previous authors have reported the therapeutic usefullness of *A. sativa* for 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, Durendic-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 productions of associated by-products such as malondialdehyde (MDA) and 4-hydroxyalkenals (4HNE) (Shaker *et al*., 2010; Al-Dosari, 2011; Durendic-Brenesel *et al*., 2013). Depleting $C2_{GSH}$ concentration confirmed increased oxidative stress (Surapaneni, 2007; Abdel-Moneim and Ghafeer, 2007) through ROS oxidation of sulfhydryl groups involved in cellular enzymatic cofactor and non-enzymatic reduction pathways. The present investigations showed that administration of *A. sativa* extract caused relief in oxidative stress to the experimental rats as indicated by decreased SOD but increasedGPx and CAT activities; 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^{-1}) of *A. sativa* extractto alleviate oxidative stress were comparable to the standard hepatic drug-hepaticum. Previous studies have shown that Buckwheat (*Fagopyrum esculentum*) (Durendic-Brenesel *et al*., 2013), *Ocimum sanctum* L. (Suanarunsawat *et al*., 2011) and Roselle (*Hibiscus sabdariffa* Linn.) (Ochani and D'Mello, 2009) share similar antioxidant phytochemicals 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 (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.

REFERENCES

Abdel-Moneim, W.M. and H.H. Ghafeer, 2007. The potential protective effect of natural honey against cadmium-induced hepatotoxicity and nephrotoxicity. Mansoura J. Forensic Med. Clin. Toxicol., 15: 75-98.

Adekunle, A.S., A.L. Adedeji, E.O. Oyewo, O.T. Adedosu and A.T. Omotoso, 2013. Hyperlipidemia induced by atherogenic diet enhanced oxidative stress in the kidney and inflammatory responses: An *in-vivo* study. Asian J. Nat. Applied Sci., 2: 82-93.

Al-Dosari, M.S., 2011. Hypolipidemic and antioxidant activities of avocado fruit pulp on high cholesterol fed diet in rats. Afr. J. Pharm. Pharmacol., 5: 1475-1483.

Auer, W., A. Eiber, E. Hertkorn, E. Hoehfeld and U. Koehrle *et al.*, 1990. Hypertension and hyperlipidemia: Garlic helps in mild cases. Br. J. Clin. Pract., 69: 3-6.

Avti, P.K., S. Kumar, C.M. Pathak, K. Vaiphei and K.L. Khanduja, 2006. Smokeless tobacco impairs the antioxidant defense in liver, lung and kidney of rats. Toxicol. Sci., 89: 547-553.

Banerjee, S.K. and S.K. Maulik, 2002. Effect of garlic on cardiovascular disorders: A review. Nutr. J., Vol. 1. 10.1186/1475-2891-1-4

Benavides, G.A., G.L. Squadrito, R.W. Mills, H.D. Patel and T.S. Isbell *et al.*, 2007. Hydrogen sulfide mediates the vasoactivity of garlic. Proc. Nat. Acad. Sci. USA., 104: 17977-17982.

Bonnefont-Rousselot, D., J.P. Bastard, M.C. Jaudon and J. Delattre, 2000. Consequences of the diabetic status on the oxidant/antioxidant balance. Diabetes Metab., 26: 163-176.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Burnstein, M.A. and J. Sammaille, 1960. A rapid determination of cholesterol bound to A and B-lipoprotein. Clin. Chemical Acta, 5: 601-609.

Chikezie, P.C., 2011. Oxidative stress indicator of human sickel erythrocytes incubated in aqueous extracts of three medicinal plants. Asian J. Biochem., 6: 373-383.

Czuczejko, J., B.A. Zachara, E. Staubach-Topczewska, W. Halota and J. Kedziora, 2003. Selenium, glutathione and glutathione peroxidases in blood of patients with chronic liver diseases. Acta Biochim. Pol., 50: 1147-1154.

Du, H., X. Zhao, J.S. You, J.Y. Park, S.H. Kim and K.J. Chang, 2010. Antioxidant and hepatic protective effects of lotus root hot water extract with taurine supplementation in rats fed a high fat diet. J. Biomed. Sci., Vol. 17. 10.1186/1423-0127-17-S1-S39

Durendic-Brenesel, M., T. Popovic, V. Pilija, A. Arsic and M. Milic *et al.*, 2013. Hypolipidemic and antioxidant effects of buckwheat leaf and flower mixture in hyperlipidemic rats. Bosn J. Basic Med. Sci., 13: 100-108.

El-Demerdash, F.M., M.I. Yousef and N.I. El-Naga, 2005. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem. Toxicol., 43: 57-63.

Fiala, S., A.E. Fiala and B. Dixon, 1972. γ-Glutamyl transpeptidase in transplantable, chemically induced rat hepatomas and Spontaneous mouse hepatomas. J. Natl. Cancer Inst., 48: 1393-1401.

Friendwald, W.T., R.I. Levy and D.S. Frederickson, 1972. Estimation of concentration of LDL in plasma without use of preparative ultracentrifuge. Clin. Chem., 18: 499-502.

Fukao, H., H. Yoshida, Y. Tazawa and T. Hada, 2007. Antithrombotic effects of odorless garlic powder both *in vitro* and *in vivo*. Biosci. Biotechnol. Biochem., 71: 84-90.

Giri, R.K., S.K. Kanungo and N.K. Tripathi, 2012. Hypolipidemic activity of *Spinacia oleracea* L. in atherogenic diet induced hyperlipidemic rats. J. Biomed. Pharmaceut. Res., 1: 39-43.

Gull, I., M. Saeed, H. Shaukat, S.M. Aslam, Z.Q. Samra and A.M. Athar, 2012. Inhibitory effect of *Allium sativum* and *Zingiber officinale* extracts on clinically important drug resistant pathogenic bacteria. Ann. Clin. Microbiol. Antimicrob., Vol. 11. 10.1186/1476-0711-11-8

Halliwell, B., 1994. Free radicals and antioxidants: A personal view. Nutr. Res., 52: 253-265.

Hassan, H.M. and I. Fridovich, 1978. Superoxide radical and the oxygen enhancement of the toxicity of paraquat in *E. coli*. J. Biol. Chem., 253: 8143-8148.

Hsing, A.W., A.P. Chokkalingam, Y.T. Gao, M.P. Madigan, J. Deng, G. Gridley and J.F. Fraumeni Jr., 2002. Allium vegetables and risk of prostate cancer: A population-based study. J. Nat. Cancer Inst., 94: 1648-1651.

Ibegbulem, C.O. and P.C. Chikezie, 2013. Hypoglycemic properties of ethanolic extracts of *Gongronemalatifolium*, *Aloe perryi*, *Viscum album* and *Allium sativum* administered to alloxan-induced diabetic albino rats (*Rattusnorvegicus*). Pharmacog. Commun., 3: 12-16.

Jiang, J., P. Nilsson-Ehle and N. Xu, 2006. Influence of liver cancer on lipid and lipoprotein metabolism. Lipids Health Dis., Vol. 5. 10.1186/1476-511X-5-4

Kaur, G. and C. Meena, 2013. Evaluation of anti-hyperlipidemic potential of combinatorial extract of curcumin, piperine and quercetin in Triton-induced hyperlipidemia in rats. Sci. Int., 1: 57-63.

Kono, Y., 1978. Generation of Superoxide radical during autooxidation of hydroxylamine and an assay for superoxide dismutase. Arch. Biochem. Biophys., 186: 189-195.

Lam, E.W.N., E.E. Kelley, S.M. Martin and G.R. Buettner, 2003. Tobacco xenobiotics release nitric oxide. Tobacco Induced Dis., 1: 207-211.

Lau, B.H.S., M.A. Adetumbi and A. Sanchez, 1983. *Allium sativum* (garlic) and atherosclerosis: A review. Nutr. Res., 3: 119-128.

Luck, H., 1963. Catalase. In: Methods of Enzymatic Analysis, Bergmeyer, H.W. (Ed.). Vol. 3. Academic Press, New York, pp: 885-894.

Mahmoodi, M., M.R. Islami, G.R. Asadikaram, M. Khaksari, A. Sahebghadam Lotfi, M.R. Hajizadeh and M.R. Mizaee, 2006. Study of the effects of raw garlic consumption on the level of lipids and other blood biochemical factors in hyperlipidemic individuals. Pak. J. Pharm. Sci., 19: 295-298.

Mayes, P.A., 1983. Metabolism of Lipids: II. Role of the Tissues. In: Harper's Illustrated Biochemistry, Martin, D.W., P.A. Mayes and V.W. Rodwell (Eds.). 26th Edn., Medical Books/McGraw-Hill, California, pp: 224-247.

Mensenkamp, A.R., M.J. van Luyn, H. van Goor, V. Bloks and F. Apostel *et al*., 2000. Hepaticlipid accumulation altered very low density lipoprotein formation andapolipoprotein E deposition in apolipoprotein E3-Leiden transgenic mice. J. Hepatol., 33: 189-198.

Moron, M.S., J.W. Depierre and K.B. Manerwik, 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim. Biophys. Acta, 582: 67-68.

Necib, Y., A. Bahi and S. Zerizer, 2013. Amelioration of mercuric chloride toxicity on rat liver with Argan oil and sodium selenite supplements. Int. J. Pharm. Bio. Sci., 4: 839-849.

Novo, E., F. Marra, E. Zamara, L. Valfre di Bonzo and A. Caligiuri *et al.*, 2006. Dose dependent and divergent effects of superoxide anion on cell death, proliferation and migration of activated human hepatic stellate cells. Gut, 55: 90-97.

Ochani, P.C. and P. D'Mello, 2009. Antioxidant and antihyperlipidemic activity of *Hibiscus sabdriffa* Linn. Leaves and calyces extracts in rats. Ind. J. Exp. Biol., 47: 276-282.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

Ooi, K., K. Shiraki, Y. Sakurai, Y. Morishita and T. Nobori, 2005. Clinical significance of abnormal lipoprotein patterns in liver diseases. Int. J. Mol. Med., 15: 655-660.

Paglia, D.E. and W.N. Valentine, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70: 158-169.

Pasupathi, P., V. Chandrasekar and U.S. Kumar, 2009. Evaluation of oxidative stress, antioxidant and thyroid hormone status in patients with diabetes mellitus. J. Med., 10: 60-66.

Peng, Q., A.R. Buz'Zard and B.H. Lau, 2002. Neuro-protective effect of garlic compounds in amyloid-beta peptide-induced apoptosis *in vitro*. Med. Sci. Monit., 8: BR328-BR337.

Qidwai, W., R. Qureshi, S.N. Hasan and S.I. Azam, 2000. Effect of dietary garlic (*Allium sativum*) on the blood pressure in humans-a pilot study. J. Pak. Med. Assoc., 50: 204-207.

Ramcharran, D., A.S. Wahed, H.S. Conjeevaram, R.W. Evans, T. Wang, S.H. Belle and L.J. Yee, 2011. Serum lipids and their associations with viral levels and liver disease severity in a treatment-naive chronic hepatitis C type 1-infected cohort. J. Viral Hepatitis, 18: e144-e152.

Resch, K.L. and E. Ernst, 1995. [Garlic (*Allium sativum*): A potent medicinal plant]. Fortschr. Med., 113: 311-315.

Sahebkar, A., 2011. Potential efficacy of ginger as a natural supplement for nonalcoholic fatty liver disease. World J. Gastroenterol., 17: 271-272.

Shaker, E., H. Mahmoud and S. Mnaa, 2010. Silymarin, the antioxidant component and *Silybum marianum* extracts prevent liver damage. Food Chem. Toxicol., 48: 803-806.

Singh, A., T.K. Bhat and O.P. Sharma, 2011. Clinical biochemistry of hepatotoxicity. J. Clin. Toxicol., S4. 10.4172/2161-0495.S4-001

Suanarunsawat, T., W.D.N. Ayutthaya, T. Songsak, S. Thirawarapan and S. Poungshompoo, 2011. Lipid-lowering and anti-oxidative activities of aqueous extracts of *Ocimum sanctum* L. leaves in rats fed with a high-cholesterol diet. Oxidative Med. Cellular Longevity, Vol. 2011. 10.1155/2011/962025

Sugatani, J., T. Wada, M. Osabe, K. Yamakawa, K. Yoshinari and M. Miwa, 2006. Dietary inulin alleviates hepatic steatosis and xenobiotics-induced liver injury in rats fed a high-fat and high-sucrose diet: Association with the suppression of hepatic cytochrome P450 and hepatocyte nuclear factor 4 alpha expression. Drug Metabol. Disposition., 34: 1677-1687.

Surapaneni, K.M., 2007. Oxidant-Antioxidant status in gestational diabetes patients. J. Clin. Diagnos. Res., 1: 235-238.

Torres, M.D., J.R. Canal and C. Perez, 1999. Oxidative stress in normal and diabetic rats. Physiol. Res., 48: 203-208.

Uetrecht, J., 2006. Role of animal models in the study of drug-induced hypersensitivity reactions. AAPS J., 7: 914-921.

Vijayakumar, R.S., D. Surya and N. Nalini, 2004. Antioxidant efficacy of black pepper (*Piper nigrum* L.) and piperine in rats with high fat diet induced oxidative stress. Redox Rep., 9: 105-110.

Wolf, P.L., 1999. Biochemical diagnosis of liver diseases. Indian J. Clin. Biochem., 14: 59-90.