In Vivo Attenuation of Acute CCl$_4$ Hepatotoxicity by Alcoholic Red Wine

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Abstract

The primary routes of potential human exposure to CCl$_4$ are inhalation, ingestion, and dermal contact. High exposure to CCl$_4$ can cause liver, kidney and central nervous system damage, and liver is especially sensitive to CCl$_4$ because of its role as the body's principal site of metabolism. This study was carried out to investigate the hepatoprotective potentials of red wine in CCl$_4$ intoxicated rat. Twenty-five animals were divided into 5 groups comprising of the negative and positive control groups, Reference group which was treated with standard drug-quercetin (100μg/ml; 1ml/kg BW), a 4th group which served as alcoholic red wine master control (500uL/ BW for 4 weeks), and another group of animals were pretreated with alcoholic red wine for 4 weeks before administering 1ml/kg body weight of CCl$_4$ after which the blood and liver were excised out for biochemical estimation. Hepatic damage due to CCl$_4$ intoxication was assessed by employing biochemical parameters; markers of hepatic oxidative damage were measured in terms of GSH and enzyme antioxidants (GST, CAT, SOD, GPx) levels. Antioxidant test assays, enzyme level and activities were carried out on the homogenized liver. The results indicated that there was ameliorative effect of the red wine against the toxic effect caused by CCl$_4$. It was observed that red wine exacted a potent amelioration when compared to the standard drug quercetin and this can be due to the presence of polyphenols working against the effect of the CCl$_4$ intoxication.

Keywords: Alcoholic red wine, Hepatotoxicity, CCl$_3$–CCl$_4$

INTRODUCTION

Free radical induced lipid peroxidation is believed to be one of the major causes of cell membrane damage leading to a number of pathological situations (Slater, 1984). CCl$_4$ was formerly used for metal degreasing and as dry cleaning, fabric-spotting, and fire extinguisher fluids, grain fumigant and reaction medium. Because of its harmful effects, these uses are now banned and it is only used in some industrial applications (DeShon, 1979). Carbon tetrachloride (CCl$_4$) is a potent hepatotoxin and is rapidly bio-transformed by cytochrome P-450 to trichloro methyl (CCl$_3$) radical and Cl (Adewole *et al*., 2007). In general, a number of chemicals including various environmental toxicants and even clinically useful drugs can cause severe cellular damages in different organs of the body through metabolic activation to highly reactive substances such as free radicals (Noguchi *et al*., 1982).

In the liver and kidneys after the initiation of lipid peroxidation, CCl$_3$ react with oxygen of cellular proteins and lipids producing a trichloro methyl peroxyl radical which attacks rapidly lipid membrane of endoplasmic reticulum. Living cells continuously react with oxygen leading to the production of free radicals (Adewole *et al*., 2007). The reactive oxygen species are predisposing factors for many diseases including cancer, arthritis, aging and immunodeficiency diseases (Khalid *et al*., 2012). The damage caused by free radicals is partly ameliorated by a large group of protective agents called antioxidants that react with free radicals stopping the damage they cause.

Red wine basically come from the fruit (skins and seeds) and vine stems. The more represented phenol groups in wine are flavonoids and non-flavonoids, which are again present in a larger amount in red than in white wines, and include free and conjugated myricetin, quercetin, kaempferol, and isorhamnetin; (+)-catechin, (-)-epicatechin, gallic acid, p-coumaric acid, caffeic acid, caftaric acid, trans-resveratrol, cis-resveratrol, and trans-resveratrol glucoside (Burns *et al.*, 2000). The delphinidin-like flavonols myricetin, laricitrin, and syringetin were missing in all white varieties, indicating that the enzyme flavonoid 3′, 5′-hydroxylase is not expressed in white grape varieties (Mattivi *et al.*, 2006).

This study aimed to evaluate the hepatoprotective tendencies of alcoholic red wine (11%) (Ibukun and Oladipo, 2016) against CCl$_4$ intoxication as well as to evaluate the effect of alcoholic red wine consumption on the liver.

MATERIALS AND METHODS
Chemicals, Reagents and Samples

Reagents and Chemicals used in this experiment were obtained from different sources such as British Drug House (BDH) and Sigma limited and were all of good analytical grades. All the solutions, buffers and reagents were prepared using glass distilled water.

Sample Collection

The red wine used was bought from supermarkets in Akure, Ondo State. And the wine was concentrated using rotary evaporator and rediluted with distilled water to concentration which served as the stock from which various concentrations (100-500μg/ml) used for the assays were calculated.

In vitro Antioxidant Assays

Since the determination of antioxidant activities is a quantitative analysis, all assays were performed in triplicate. For each test performed, various concentrations of the extract and standards were prepared using the serial dilution method.

Assay for total antioxidant activity

Total antioxidant activity of the sample extract was carried out using reagent solution of sulphuric acid, sodium phosphate and ammonium molybdate. To the reagent solution; sulphuric acid (0.6 M), sodium phosphate (28 mM)and ammonium molybdate (4 mM); 0.3 ml of sample was added and incubated at 95 ^{0}C in a water bath for 90 min. After cooling to room temperature absorbance was recorded at 765 nm against reagent blank. The absorbance of the sample was extrapolated on the ascorbic acid standard curve to obtain concentration of the sample in mg/ml then the total antioxidant activity (mg/g ascorbic acid equivalence) was calculated.

Reducing Power Assay

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Extract (0.5 ml) was mixed with 1.25 ml each of phosphate buffer and potassium ferricyanide ($C_6N_6FeK_3$). The mixture was incubated at 50 ^{0}C for 20 min. Trichloroacetic acid (1.25 ml) was then added and the mixture centrifuged at 3000 rpm for 10 min. Thereafter, 1.25 ml of the upper layer of the solution was mixed with 1.25 ml of distilled water and 0.25 ml of $FeCl_3$. The absorbance was read at 700 nm. Higher absorbance of the reaction mixture indicates greater reductive potential.

DPPH Radical Scavenging (1, 1-diphenyl-2-picrylhydrazyl) Activity Assay

The DPPH radical scavenging activity of the extracts was evaluated according to the method described by Leong and Shui (2002). Exactly 1 ml of 0.3mM DPPH prepared in methanol was added to 1 ml of extract of various concentrations and allowed to react at room temperature for 30 min. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophometrically at 517nm and the percentage scavenging activity was calculated using the formula below.

% scavenging activity = ((Ac – As)/Ac) x 100

Where Ac is the absorbance of control and As the absorbance of the extract.

Metal Chelating Activity Assay

The metal chelating activity was determined according to the method of Haro-Vicente *et al.* (2006). Extract (1 ml) was added to 100 μl of 1 mM $FeSO_4.7H_2O$. The reaction mixture was left at room temperature for 2 min. After which 0.5 ml of 0.5 mM 1, 10-phenanthroline was added and the mixture was incubated for 10 min at room temperature. The absorbance was read at 510 nm. The Fe^{2+} chelating capacity was calculated thus:

Fe $^{2+}$ chelating activity (%) = ((Ac – As)/ Ac) x 100

Hydroxyl Radical Scavenging Activity Assay

The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+} /ascorbate/EDTA/H_2O_2 system according to the method of Halliwell *et al.* (1987). The reaction mixture contained 1 ml (3.0 mM deoxyribose, 0.1 mM EDTA, 2 mM H_2O_2, 0.1 mM L-Ascorbic acid, 0.1 mM $FeCl_3.6H_2O$ in 10 mM phosphate buffer, pH 7.4) and various concentrations of the extracts (1-5 mg/ml). The reaction mixtures were incubated at 37 ºC for 1 h, followed by the addition of 1 ml of 1 % (w/v) TBA (in 0.25 N HCl) and 1.0 ml 10 % (w/v) TCA. The reaction mixtures were heated in boiling water bath at 100 C for 20 min and the pink chromogen (malondialdehyde-(TBA) adduct) was extracted into 1.0 ml of butan-1-ol and the absorbance was read at 532 nm against reagent blank. The hydroxyl radical scavenging activity calculated thus: Hydroxyl radical (OH$^-$) scavenging activity (%) = ((Ac – As)/Ac) X 100

In vivo Biochemical Evaluations

Experimental Design

The experiment was designed such that the alcoholic red wine was rationed and administered to the groups. 25 male albino rats weighing 180-200g were used according to the standard guidelines of the Care and Use of Experimental Animal Resources. The rats were housed in cages under standard laboratory conditions (12h light/ dark cycle, 23±2ºC).

Group 1: Untreated, unintoxicated group-Negative control group (C-),

Group 2: Rats intoxicated with CCl_4 (1 ml/kg body weight, s.c.) in a 1:1 solution with olive oil intraperitoneally (i.p) (Adewole *et al.,* 2007) after 4 weeks without red wine pretreatment (test control (C+))

Group 3: Rats were pre-treated with alcoholic red wine (500uL/BW) for 4 weeks orally and CCl_4 intoxication at the end of the 4th week ($RCCl_4$ group)

Group 4: Rats which served as alcoholic red wine master control (500uL/ BW for 4 weeks) (RC group)

Group 5: Reference group which was treated with standard drug-quercetin (100µg/ml; 1ml/kg BW) (Q)

Preparation of serum and tissue homogenates

Blood samples was collected by ocular punctures into plain bottles. Serum was prepared by aspiration of the clear liquid after clotting and centrifuged for 10 minutes at 3000g in a bench centrifuge. Rats were anaesthetized by cervical dislocation and sacrificed loss of consciousness. The liver was excised, washed in ice cold 1.15% potassium chloride solution, blotted with filter paper and weighed, and homogenized in ice cold 5 % w/v sodium phosphate buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenates were stored at 4 ^{0}C and then used for biochemical analysis.

Hepatotoxicity Study Assays

Evaluation of Aspartate Amino Transferase (AST) Activity

Activity of AST was evaluated using manufacturer protocol of Randox AST Kit based on the principle of Reitman and Frankel (1957). Diluted sample (0.1ml) was mixed with 0.5 ml of R1 (phosphate buffer (100 mmol/L, pH 7.4), L-aspartate (100 mmol/L), and α-oxoglutarate (2 mmol/L)) and the mixture incubated for 30 min at 37 ^{o}C after which 0.5 ml of R2 (2, 4-dinitrophenylhydrazine (2 mmol/L)) was added to the reaction mixture and allowed to stand for another 20 min at 25 ^{0}C. Then, 5.0 ml of NaOH (0.4 mol/L) was added and the absorbance was read against the reagent blank after 5 min at 546 nm.

The activity of AST in homogenate was obtained following the extrapolation of absorbance value on AST standard curve.

Evaluation of Alanine Amino Transferase (ALT) Activity

Assay of alanine amino transferase ALT activity was carried out using the manufacturer protocol of Randox ALT Kit based on the principle described by Reitman and Frankel (1957). Reagent1 (0.5 ml) containing Phosphate buffer (100 mmol/l, pH 7.4), L-alanine (200 mmol/l) and α-oxoglutarate (2.0 mol/l) was added to a test tube already containing 0.1 ml of serum sample and the mixture was incubated at 37ºC for 30 min. Then, 0.5ml of R2 containing 2, 4-dinitrophenylhydrazine (2.0mmol/l) was added and the mixture incubated again at 20 ^{o}C for 20 min. Finally, 5 ml of NaOH was added. The mixture was allowed to stand for 5 min at room temperature and the absorbance was read at 546nm. The activity of ALT in the homogenate was obtained from a standard curve

Evaluation of Alkaline Phosphatase (ALP) Activity

Assay of alkaline phosphatase (ALP) activity was carried out according to the procedure provided by Randox Kit Manufacturer which is based on the method of Englehardt *et al*. (1970). ALP activity was measured by monitoring the concentration of p-nitrophenol formed when ALP reacted with p-nitrophenyl phosphate. Exactly 1.0 ml of the reagent (1 mol/l, pH 9.8 Diethanolamine buffer, 0.5 mmol/l MgCl$_2$; substrate: 10 mmol/l p-nitrophenolphosphate) was added to 0.02 ml of sample and then mixed. The absorbance was read for 3 min at intervals of 1 min at a wavelength of 405nm.

ALP activity was determined using the formula:

U/l = 2760 x A405 nm/min

Evaluation of Gamma-Glutamyl Transferase (GGT) Activity

The assay was conducted using a Randox kit manual. Using a water-bathe set at 37C for the incubation of the reaction, the reaction is constituted of 100µl of the sample extract 1000µl of the reagent at a pH of 8.25 using tris-buffer of 100mmol/l concentration. The reagent contains glycylglycine (100mmol/l) and L-γ-glutamyl-3-carboxy-4-nitroanilide (2.9 mmol/l). The absorbance of the mixture was read at 405nm.

To calculate the GGT activity use the following formula.

U/L = 1158 x A 405 nm/min

Total protein in liver homogenate

This was carried out using the manufacturer protocol of Randox Total Protein Kit based on Weichselbaumin, 1995. 1ml of reagent R1; Sodium hydroxide (100 mmol/l), sodium-potassium tartrate (16 mmol/l), Potassium iodide (15 mmol/l) and copper II sulphate (6 mmol/l)) was added to 0.02 ml of the test sample, the mixture was incubated at 25 ^{o}C and the absorbance was then measured against the reagent blank at a wavelength of 546 nm.

Total Protein Concentration =   ((Abs Sample/ Abs Standard) x standard concentration

Antioxidants Assays Evaluation

Superoxide Dismutase Activity (SOD)

Liver SOD activity was assayed by the method of Kakkar *et al.,* (1984). Reaction mixture contained 1.2 ml of sodium pyrophosphate buffer (0.052 mM, pH 7.0), 0.1 ml of phenazine methosulphate (PMS) (186 µM), 0.3 ml of nitro blue tetrazolium (NBT) (300 µM). 0.2 ml of the supernatant obtained after centrifugation (1500 x g, 10 min followed by 10,000 x g, 15 min) of 10% liver homogenate was added to reaction mixture. Enzyme reaction was initiated by adding 0.2 ml of NADH (780 µM) and stopped precisely after 1 min by adding 1 ml of glacial acetic acid. Amount of chromogen formed was measured by recording color intensity at 560 nm. Results are expressed as units/mg protein.

Glutathione Peroxidase Activity (GSH-Px)

Glutathione peroxidase (GSH-Px) activity was measured by NADPH oxidation, using a coupled reaction system consisting of glutathione, glutathione reductase, and cumene hydroperoxide (Tappel, 1978). 100 μL of enzyme sample was incubated for five minutes with 1.55 ml stock solution (prepared in 50 mM Tris buffer, pH 7.6 with 0.1 mM EDTA) containing 0.25 mM GSH, 0.12 mM NADPH and 1 unit glutathione reductase. The reaction was initiated by adding 50 μL of cumene hydroperoxide (1 mg/ml), and the rate of disappearance of NADPH with time was determined by monitoring absorbance at 340 nm. One unit of enzyme activity is defined as the amount of enzyme that transforms 1 μmol of NADPH to NADP per minute. Results are expressed as units/mg protein.

Catalase Activity (CAT)

The activity of CAT was measured using its perioxidatic function according to the method of Johansson and Borg (1988). 50 μL potassium phosphate buffer (250 mM, pH 7.0) was incubated with 50 μl methanol and 10 μL hydrogen peroxide (0.27%). The reaction was initiated by addition of 100 μL of enzyme sample with continuous shaking at room temperature (20°C). After 20 minutes, reaction was terminated by addition of 50 μL of 7.8 M potassium hydroxide. 100 μL of purpald (4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole, 34.2 mM in 480 mM HCl) was immediately added, and the mixture was again incubated for 10 minutes at 20oC with continuous shaking. Potassium peroxidate (50 μL 65.2 mM) was added to obtain a colored compound. The absorbance was read at 550 nm in a spectrophotometer. Results are expressed as micromoles of formaldehyde produced/mg protein.

Reduced glutathione (GSH)

Reduced glutathione (GSH) level in the hepatocytes was assayed following the method of Ellman (1959), modified by Hissin and Hilf (1973). The serum (720 μl) was double diluted and 5% TCA was added to it to precipitate the protein content of the homogenate. After centrifugation (10, 000 x g for 5 minutes) at 4oC the supernatant was taken, 5,5´-dithiolbis-2-nitrobenzoic acid (DTNB) solution (Ellmam's reagent) was added to it and the absorbance was measured at 412 nm on a spectrophotometer. A standard graph was drawn using different concentrations of standard GSH solution (1 mg/ml). With the help of the standard graph, GSH contents in the homogenates of the experimental animals were calculated.

Glutathione-S-transferase (GST)

GST catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. GST activity was measured by the method of Habig and Jakoby (1974). The reaction mixture contained suitable amount of the enzyme (25 μg of protein in homogenates), 1 ml of KH_2PO_4 buffer, 0.2 ml of EDTA, 0.1 ml of 1-chloro-2,4-dinitrobenzene (CDNB), and GSH. The reaction was carried out at 37°C and monitored spectrophotometrically by the increase in absorbance of the conjugate of GSH and CDNB at 340 nm. A blank was run in absence of the enzyme. One unit of GST activity is 1 μmol product formation per minute.

The results of replicate readings were pooled and expressed as mean ± standard deviation. One way analysis of variance was used to analyze the results and Duncan multiple test was used for the post hoc (DMRT). Statistical package for Social Science (SPSS) 17.0 for Windows was used for the analysis. The significance level was set at $p < 0.05$.

RESULTS

Antioxidant Activity

The in vitro antioxidant abilities of the extracts were typified by the Total Antioxidant Activity (Table 1). DPPH Radical Scavenging Activities (Figure 1), Hydroxyl Radical Scavenging Activities (Figure 2), Metal Chelating Activities (Figure 3) and Reducing Power Activities (Figure 4). The in vitro antioxidant evaluations, typified by these assays showed a concentration dependent red wine which exhibited competitive activity against the different standards.

The red wine extract was derived from the alcoholic red wine by concentrating, this is to evaluate the antioxidant capacities of the polyphenlic content of the red wine with respect to ability to scavenge free radicals.

Table 1: total antioxidant (mg/g AAE) and total phenolic content (mg/g TAE).

	TOTAL ANTIOXIDANT (mg/g AAE)
RED WINE	96.57±6.441

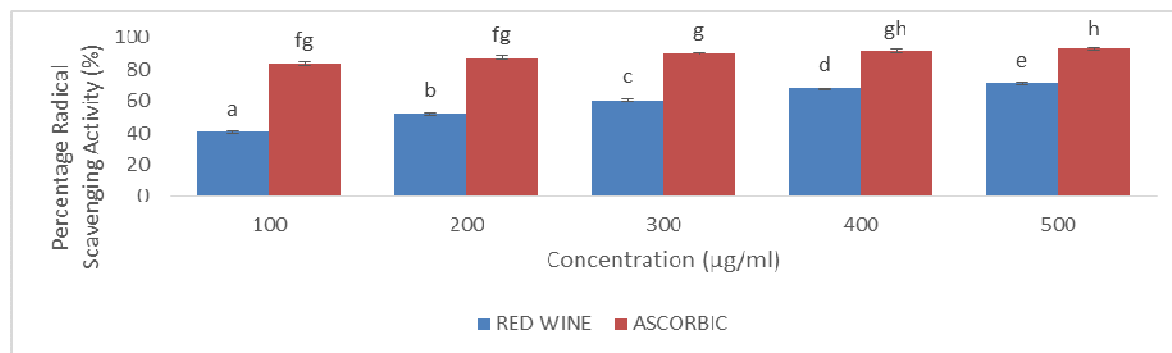Values are expressed as mean ± standard deviation (n=3) (P<0.05).

Figure 1: DPPH radical scavenging activities of red wine. Each value represents mean (n=3). Values with different superscript are significantly different (P<0.05).
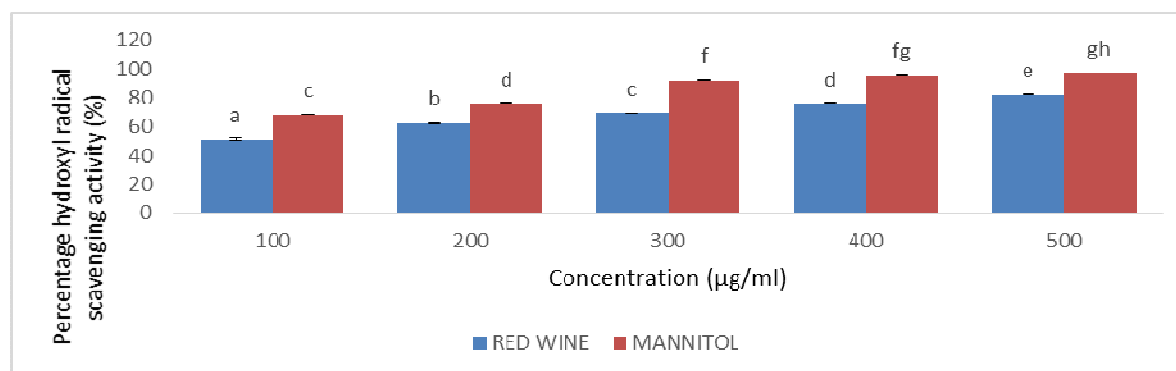

Figure 2: Hydroxyl radical scavenging activities of red wine. Each value represents mean (n=3). Values with different superscript are significantly different (P<0.05).
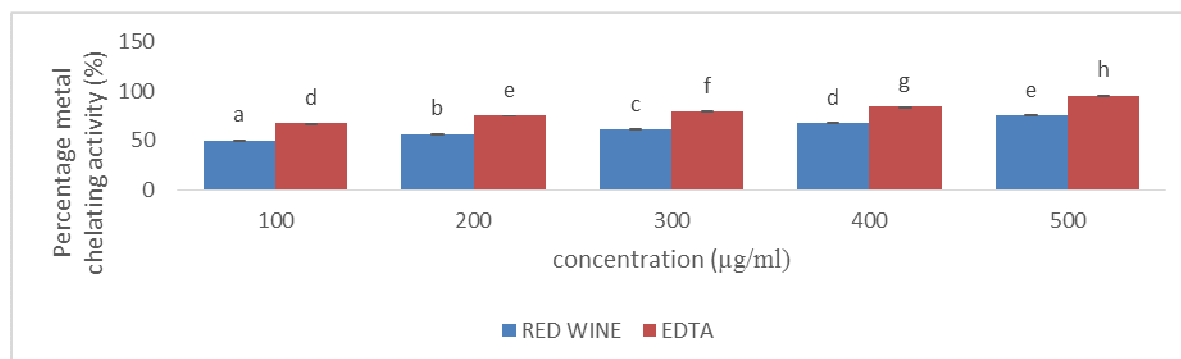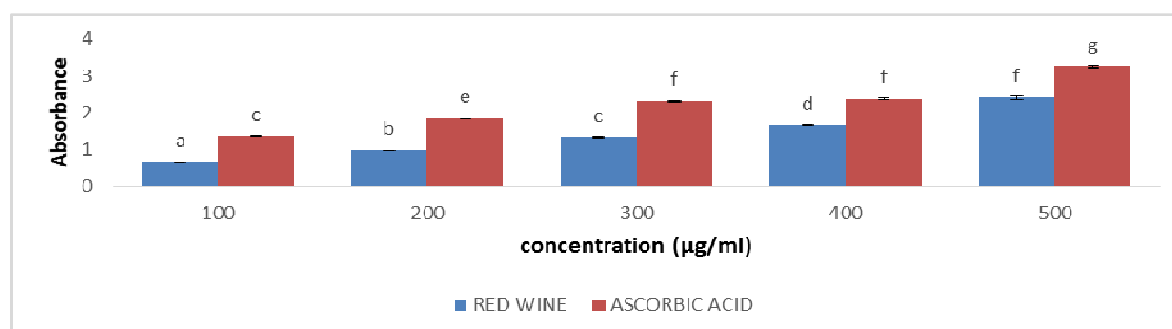

Figure 3: Metal chelating activities of red wine. Each value represents mean (n=3). Values with different superscript are significantly different (P<0.05).

Figure 4: Reducing power activities of red wine. Each value represents mean (n=3). Values with different superscript are significantly different (P<0.05).

Biochemical Estimations

Activities of antioxidant enzymes and liver function indices

Figures below revealed the effects of alcoholic red wine on biochemical variables in the serum and tissue homogenate of all experimental animal groups. CCl_4 intoxication markedly decreased antioxidants enzymes activities as well as reduced glutathione concentration, resulting in oxidative stress implication in CCl4-intoxicated rats. There was clear evidence that CCl_4-induced hepatic injury was associated with free radical injury and oxidative stress as revealed by the antioxidants activities. Effects of CCl_4, CCl_4 + red wine and alcoholic red wine treatments on hepatic tissue's GSH-Px, SOD, CAT, GSH and GST are presented in Figures. The liver antioxidant activity of GSH-Px, SOD, CAT, GSH and GST significantly decreased ($p<0.05$) in the CCl4-intoxicated group of rats. The positive control group maintained optimal activity of the antioxidants studied. Administration of alcoholic red wine significantly ($p<0.05$) decreased the activities of the endogenous antioxidant enzymes compared to the positive control. Alcoholic red wine demonstrated significant effect in restoring the altered activity of antioxidant enzymes like SOD, GSH-Px, CAT, GST and GSH towards their normal activities in the serum.
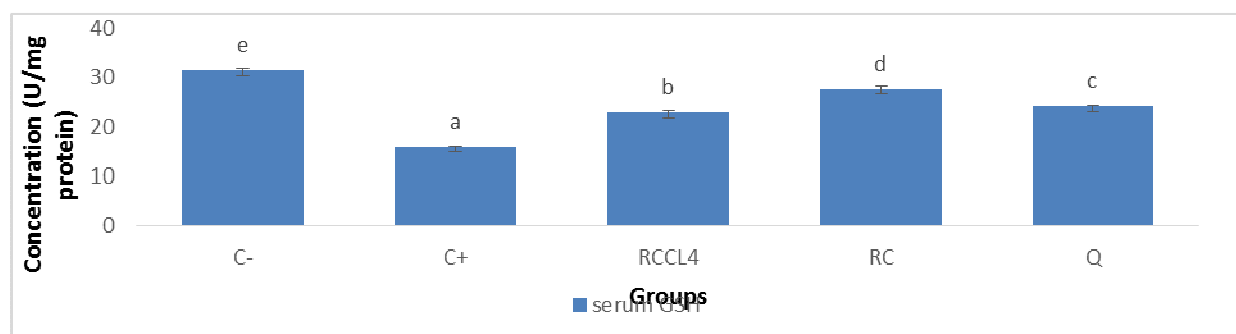

Figure 5: Effects of red wine on serum GSH activity (U/mg protein). Values are expressed as mean (n=5). Values with different superscript are significantly different (P<0.05).
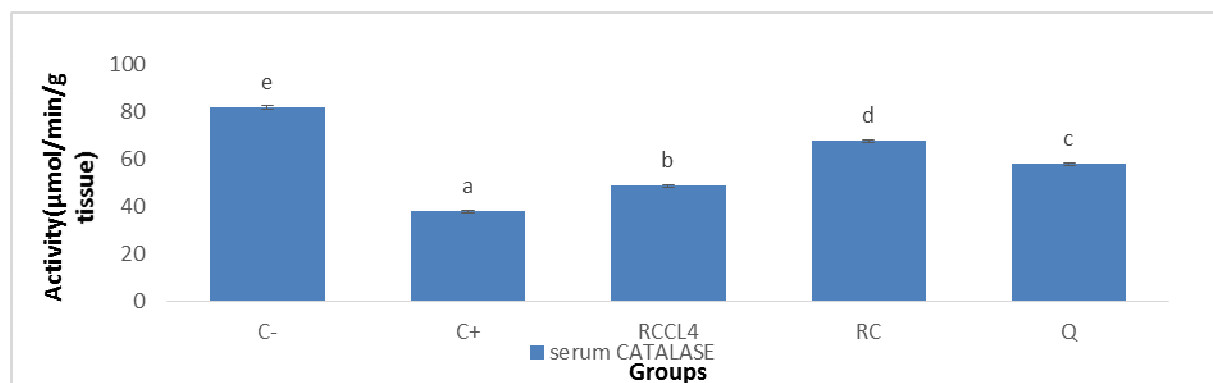

Figure 6: Effects of red wine on serum catalase activity (μmol/min/g tissue). Values are expressed as mean (n=5). Values with different superscript are significantly different (P<0.05).
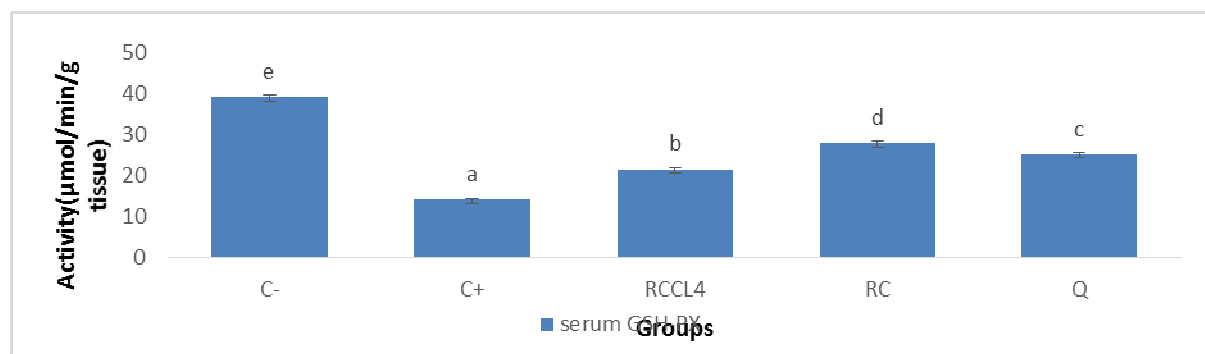

Figure 7: Effects of red wine on serum GSH-Px activity (μmol/min/g tissue). Values are expressed as mean (n=5). Values with different superscript are significantly different (P<0.05).
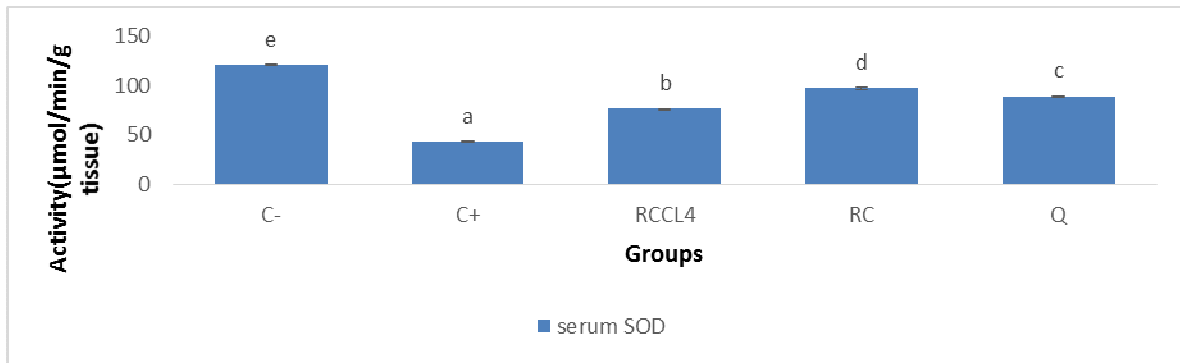
Figure 8: Effects of red wine on serum Superoxide Dismutase activity (μmol/min/g tissue). Values are expressed as mean (n=5). Values with different superscript are significantly different (P<0.05).

Figure 9: Effects of red wine on serum GST activity (μmol/min/g tissue). Values are expressed as mean (n=5). Values with different superscript are significantly different (P<0.05).

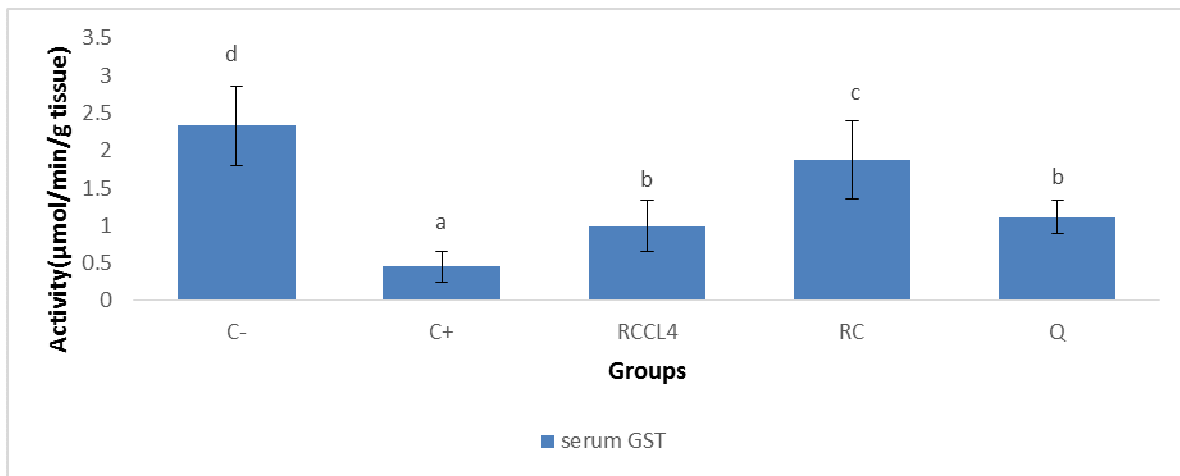Figure 10: Effects of red wine on serum ALP activity. Values are expressed as mean (n=5). Values with different superscript are significantly different (P<0.05).

Figure 11: Effects of red wine on serum AST activity. Values are expressed as mean (n=5). Values with different superscript are significantly different (P<0.05).
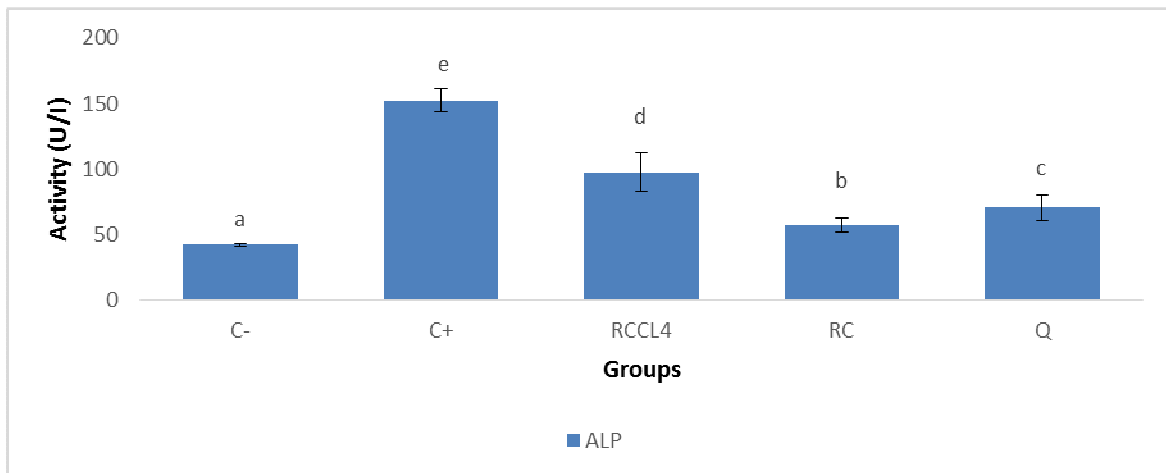

Figure 12: Effects of red wine on serum ALT activity. Values are expressed as mean (n=5). Values with different superscript are significantly different (P<0.05).
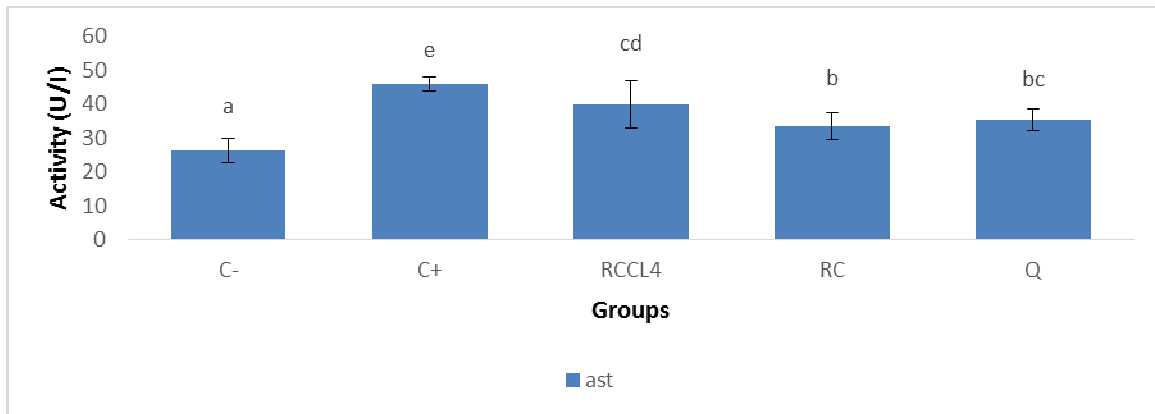

Figure 13: Effects of red wine on serum GGT activity. Values are expressed as mean (n=5). Values with different superscript are significantly different (P<0.05).
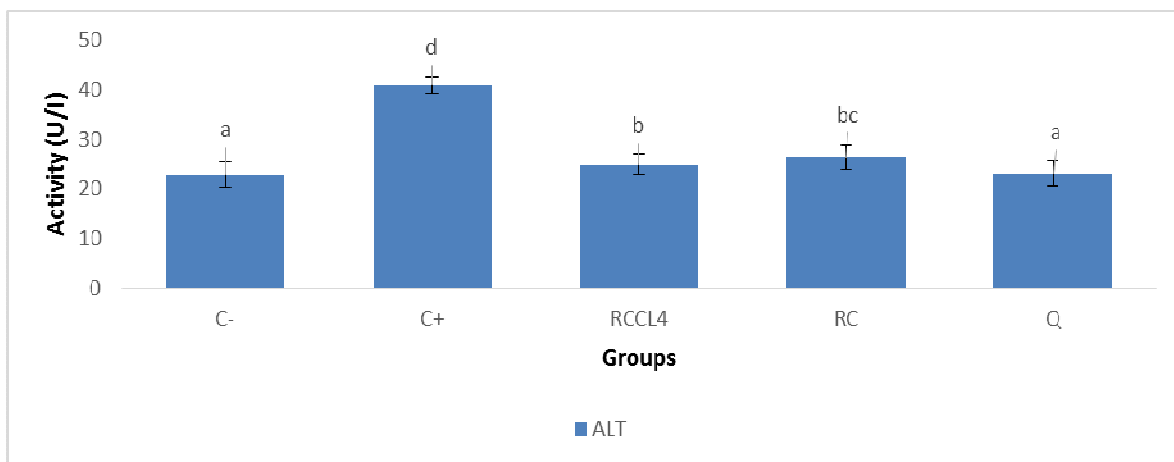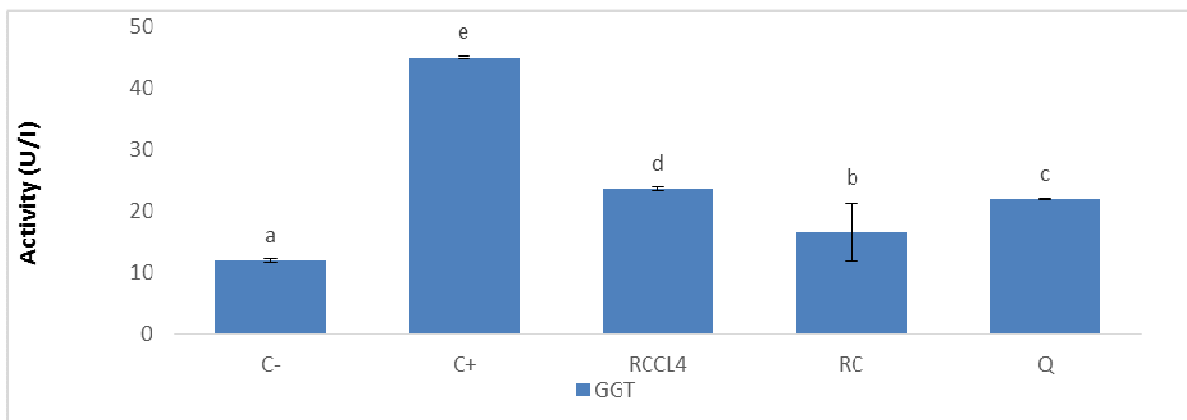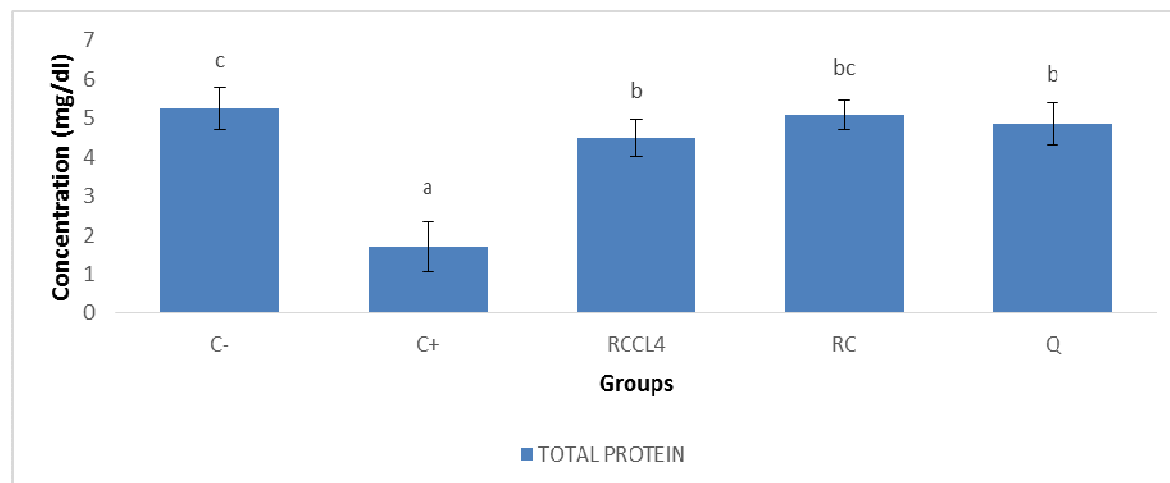
Figure 14: Effects of red wine on liver total protein. Values are expressed as mean (n=5). Values with different superscript are significantly different (P<0.05).

DISCUSSION

Polyphenolic compounds give wines color and account for differences in flavor between reds and whites (their concentration is in fact much lower in white wines, being 0.01% versus 0.2% in red wines) (Giuseppe *et al*., 2010). Antioxidant properties of non-alcoholic red wine can be traced to the natural substances present in grape which are: phenolic components of red wine are anthocyanins, catechins, resveratrol, phenolic acids, and the flavonols quercetin and myricetin, the concentrations of these components in the red wine can vary widely.

In vitro **antioxidant activity**

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. The extracts of the red wine scavenge DPPH radical in a concentration dependent manner. This study thus showed that the extract has the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

The hydroxyl radical is the most reactive of the reactive oxygen species, and it induces severe damage in adjacent biomolecules. The hydroxyl radical can cause oxidative damage to DNA, lipids and protein (Pham-Huy *et al*., 2008). In the present study, the extract of the red wine scavenged hydroxyl radical in a concentration dependent manner which compares favourably with mannitol. The hydroxyl radical in the cell can easily cross cell membranes at specific sites, react with most biomolecules and furthermore cause tissue damage and cell death. Thus, removing hydroxyl radical is very important for the protection of living systems (Ogunmoyole *et al.,* 2009). The high values obtained for the OH radical scavenging activity can be related to the major polyphenolic constituents of the extract.

Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydro peroxide decomposition reactions via Fenton chemistry ($H_2O_2 + Fe^{2+}$---> $Fe^{3+}OH^- +OH$) (Halliwell, 1997). The transition metal; iron, is capable of generating free radicals from peroxides and may be implicated in human cardiovascular disease (Halliwell and Gutteridge, 1990). Generally, the chelating of iron is regarded as an antioxidant mechanism to prevent oxidative assault on biological macromolecules such as lipids, proteins and nucleic acids. Free iron is a potential enhancer of reactive oxygen species formation as it leads to reduction of H_2O_2 and generation of the highly aggressive hydroxyl radical that can perpetuate the chain reaction (Singh *et al*., 2004). Metal ion chelating capacity is significant since it reduces the concentration of the transition metal that catalyzes lipid peroxidation. According to the results, the dealcoholized red wine chelated iron in a concentration–dependent manner which compares favourably with the standard.

Reducing potential of the extracts increased with increasing concentration. The increased reducing potential of the crude extract competitively with ascorbic acid could be as a result of phytochemicals and presence of other bioactive constituents with antioxidant properties. Reducing power of the red wine can be an indices for predicting its antioxidant ability. Reducing power is considered a defense mechanism which is related to the ability of the antioxidant agents to transfer electron or hydrogen atom to oxidants or free radicals (Ogunmoyole *et al.*, 2009). Antioxidant property of phenolics is due to their redox properties which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers.

In vivo **CCl₄-Hepatotoxicity ameliorating and antioxidant modulating activities**

These radicals are capable of initiating a chain of lipid peroxidation reactions by abstracting hydrogen from polyunsaturated fatty acids (PUFA). Peroxidation of lipids, particularly those containing PUFA, can dramatically

change the properties of biological membranes, resulting in severe cell damage and play a significant role in pathogenesis of diseases.

Liver is the major component in the body defenses against toxic agents due to its detoxifying ability, its excretory role and the fact that most injected or exogenous materials pass through the liver before entering the general circulation (Hayes, 1989). Liver damage could be confirmed by changes in the activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT). High levels of transaminases indicate hepatopathy (Zilva and Pannalla, 1975). High AST activities in the serum are always due to myocardial infarction, various types of liver diseases and sometimes with renal diseases, while ALT increases in the serum in infectious hepatitis. In fact, AST is employed in the diagnosis of myocardial infarction while ALT is useful in diagnosis of liver damage. The primary importance of measuring alkaline phosphatase is to check the possibility of bone disease or liver disease. Since the mucosal cells that line the bile system of the liver are the source of alkaline phosphatase, the free flow of bile through the liver and down into the biliary tract and gall bladder are responsible for maintaining the proper level of this enzyme in the blood. When the liver, bile ducts or gall bladder system are not functioning properly or are blocked, this enzyme is not excreted through the bile and alkaline phosphatase is released into the blood stream. Thus the serum alkaline phosphatase is a measure of the integrity of the hepatobiliary system and the flow of bile into the small intestine (Trevor, 2001). Gamma glutamyl transferase (GGT) is another enzyme that occurs primarily in hepatocytes. A high level of the enzyme is particularly associated with advent of stress.

Stress is a condition that lead to the proliferation of free radicals. Free radicals cause oxidation of lipids, proteins and bases of nucleic acids. Free radicals also cause damage to biomembranes, reflected by lipid peroxidation, thereby compromising cell integrity and function. Stress of toxicological and physiological forms had been reported to give progression in oxidative stress. Chida et al. (2006) reported that even though all of the interactions between stress and the liver are not completely understood, there appears to be a negative association between stress on liver disease progression (Chida et al., 2006). The result of the present study is in agreement with chida et al. (2006) and some previous report on the effect of stress on liver enzymes. Acute toxicological stress by CCl_4-intoxication in rats and mice causes increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities in the serum. The increases in the activities of these enzymes in the serum of experimental rats by CCl_4-intoxication could be predicted to result from damage of various tissues and protein denaturation, leading to over-flux of free amino acids exposing the R-groups of the amino acids. The exposure stimulates the liver to produce the enzymes which eventually served as liver markers. These enzymes which are majorly transaminases attempt to absorb the amino acids (majorly aspartic acid and alanine) into the tricarboxylic acid cycle (TCA). These attempts served as marker for oxidative stress, also when the hepatocytes are damaged by activities of free radicals, the release of these enzyme markers define the liver function indices. The primary role of cellular GGT is to metabolize extracellular reduced glutathione (GSH), allowing for precursor amino acids to be assimilated and reutilize for intracellular GSH synthesis (Lee et $al.,$ 2004). The evaluation of the gamma glutamyl transferase activity was evaluated in the serum and liver.

The ALP, AST, ALP and GGT activities revealed that the alcoholic red wine has ameliorating effect on the CCl_4-induced stress. The results (Figure 10-13) revealed that alcoholic red wine has ability to ameliorate toxicological stress induced by CCl_4 compared to the negative control group. CCl_3 react with oxygen of cellular proteins and lipids producing a trichloro methyl peroxyl radical which attacks rabidly lipid membrane of endoplasmic reticulum, damage caused by these radicals is partly ameliorated by a large group of polyphenols present in the wine. The result showed the dietary activity of the alcoholic red wine, it could be inferred that the alcoholic content of the red wine was responsible for the oxidative intolerance, leading to elevation of the liver function marker enzymes-ALP, AST, ALT and GGT. The total protein content of the liver thus confirmed the deteriorating effect of the alcoholic red wine, as a reduced concentration of the proteins were recorded. Previous study had been done and 500µL/BW had been shown not to have deleterious effect, thus prompting the use of 500µL/BW. However the result showed a contradicting effect and presented the alcoholic red wine as deleterious at the concentration. The hepatoprotective property of the alcoholic red wine could be attributed to the presence of polyphenols.

In $vivo$ studies indicated that CCl_4 enhances lipid peroxidation, reduces hepatic microsomal NADPH cytochrome P450, and reduced/oxidized glutathione ratio (GSH/GSSG) in the liver cells mitochondria (Walker et al., 1996). Evidence suggests that various enzymatic and non-enzymatic systems have been developed by mammalian cells to cope with ROS and other free radicals (Recknagel et al., 1989). However, when a condition of oxidative stress establishes, the defense capacities against ROS becomes insufficient (Halliwell and Gutteridge, 2000). ROS also affects the antioxidant defense mechanisms, by reducing the intracellular concentration of GSH and decreases the activity of SOD, CAT and GSH-Px. It had also been observed to decrease the detoxification system produced by GST (Yamamoto and Yamashita, 1999). It had been established that the liver plays very important role in maintaining the antioxidant status of the body.

In this study, it was observed that in CCl_4 intoxicated groups, there were significant decrease in SOD, CAT, GSH-Px and GST activities and depletion of GSH content in serum. It had been reported that SOD, CAT, GSH-Px and GST constituted mutually supportive team of defense against ROS (Bandhopadhy *et al*., 1999). The decreased activity of SOD in serum intoxicated with CCl_4 may be due to the inactivation of the antioxidant enzymes. This would cause an increased accumulation of superoxide radicals, which could cause deleterious effects by itself and also by generation of chlorinated radicals. Decreased GST activity by CCl_4-intoxication might be due to the decreased availability of GSH during the enhanced cellular oxidation.

Administration of alcoholic red wine prior to CCl_4 intoxication activated the expressions of the antioxidant machineries of the liver as revealed from enhanced levels of SOD, CAT, GSH-Px and GST activities, and increased GSH content. However the reduction in the activities of the endogenous antioxidant systems in alcoholic red wine master group (RC) compared with the normal control, could be as a result of radical generating effect of alcohol which was capable of causing depletion in the antioxidant status, thus resulting in oxidative stress.

CONCLUSION

The antioxidant activities of the extract of red wine showed scavenging ability against free radicals as well as ability to chelate metallic ions, thus could be a dietary source of bioactive compounds. This study described the hepatoprotective activities of the alcoholic red wine against CCl_4 intoxication. Despite the protective activity of the wine, the effect of the alcoholic content could not be neglected, limiting its dietary recommendation as a result of the alcoholic content in excessive consumption. However, further studies are recommended to demonstrate comparative study on dealcoholize and alcoholic red wine as well as histopathological studies on liver functions.

REFERENCE

1. Slater, T.F. and Sawyer, B.C. (1971). The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reaction in rat liver fractions in vitro. Inhibitory effects of free radical scavengers and other agents. *J Biochem*., 123: 823-828.
2. DeShon N. D. (1979). Carbon tetrachloride. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. Grayson M, and Eckroth D, eds. New York: John Wiley and Sons, Inc. 5:704-714
3. Adewole, O.S., Salako, A.A., Doherty, O.W., and Naicker, T. (2007). Effect of Melatonin on Carbon Tetrachloride-Induced Kidney Injury in Wistar Rats. *African Journal of Biomedical Research*, 10: 153 - 164
4. Noguchi, T., Fong, K. L., Lai, E. K., Alexander, S. S., King, M. M., Olson, L., Poyer, J. L. and Mccay P. B. (1982). Specificity of aphenobarbital-induced cytochrome P450 for metabolism of carbon tetrachloride to the trichromethyl radical. *Biochem Pharmacol*., 31:615-624.
5. Khalid, G.A., AlyaaMajid, M. A and Auda, M. H. (2012). The Role of Camel's Milk against Some Oxidant-Antioxidant Markers of Male Rats Treated With CCl4. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 3(1):385-389
6. Burns, J., Gardner, PT and O'Neil J, (2000). Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wines. *J Agric Food Chem*., 48:220–230.
7. Mattivi, F., Guzzon, R., Vrhovsek, U., Stefanini, M., Velasco, R. (2006). Metabolite profiling of grape: Flavonols and anthocyanins. *J Agric Food Chem*.;54:7692–7702.
8. Ibukun E. O. and Oladipo, G.O. (2016). Lipidomic modulation in stressed albino rats is altered by yolk and albumen of quail (*Coturnix japonica*) egg and poultry feed. *Biochemistry Research International*, Article ID 2565178.
9. Oyaizu, M. (1986). Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. *Japan Journal of Nutrition*, 44: 307-315
10. Leong, L.P. and Shui G. (2002). An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry*, 76:65-75.
11. Haro-Vicente, J.F., Martinez-Gracia, C. and Ros, G. (2006). Optimisation of invitro measurement of available iron from different fortification in citric fruit juices. *Journal of Food Chemistry,* 98:639-648.
12. Halliwell, B., Gutteridge, J.M.C., Aruoma, O.I. (1987). The deoxyribose method: A simple 'test tube' assay for determination of rate constants for reactions of hydroxyl radicals. *Anal Biochem*., 165: 215-219.
13. Reitman, S. and S. Frankel. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *American Journal of Clinical Pathology*. 28: 56-63.
14. Englehardt, A. *et al*. (1970). *Aerztl labor*.16:42.
15. Weichselbaumin, TE. (1995). *Amer. J.Clin. Path*., 16:40.
16. Kakkar, P., B. Das and P.N. Viswanathan, 1984. A modified spectrophotometeric assay of superoxide dismutase. *Ind. Biochem. Biophys*., 21: 130-132.
17. Tappel, A.L. (1978). Glutathione peroxidase and hydroperoxides. *Methods Enzymol*., 52: 506-513.

18. Johansson, L. H. and Borg, L. A. (1988). A spectrophotometric method for determination of catalase activity in small tissue samples. *Anal Biochem*., 174: 331–336.

19. Ellman, G. L. (1959). Tissue sulfhydryl group. Arch Biochem Biophys, 82: 70-77.

20. Habig, W. H. and Jakoby, W. B. (1974). Glutathione Stransferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem*., 249: 7130-7139.

21. Giuseppe Lippi, Massimo Franchini and Gian Cesare Guidi (2010). Red wine and cardiovascular health: the "French Paradox" revisited. International Journal of Wine Research:2 1–7

22. Pham-Huy, L.A., He, H. and Pham-Huyc, C. (2008). Free Radicals, Antioxidants in Disease and Health. *International Journal of Biomedical Science,* 4(2):89-96.

23. Ogunmoyole, T., Rocha, J.B.T., Okoronkwo, A.E. and Kade, I.J. (2009). Altered pH homeostasis modulates the glutathione peroxidase mimics and other antioxidant properties of diphenyldiselenide. *Chemico-Biological Interactions*. *Elsevier*. 182: 106–111.

24. Halliwell, B. and Gutteridge J.M.C. (1990). Role of free radicals and catalytic metal ion in human disease: an overview. *Methods in Enzymology,* 186:1-85.

25. Halliwell, B. (1997). Antioxidants: the basics - what they are and how to evaluate them. *Advance Pharmacology,* 38: 3- 20.

26. Singh, R.P., Sharad, S. and Kapur, S. (2004). Free Radicals and Oxidative Stress in Neurodegenerative Diseases: Relevance of Dietary Antioxidants. *Journal, Indian Academy of Clinical Medicine.* Vol.5, No. 3. 218-25.

27. Hayes, J.A. (1989). Systemic Responses to toxic Agents. In A guide to General Toxicology 2nd Revised Edition Krager AG. Basel Switzerland, pp. 24-61.

28. Zilva, J.F. and Pannalla, P.R. (1975). Clinical Chemistry in Diagnosis and Treatment. *Lloyd-luke London,* pp. 15-50.

29. Trevor, P. (2001). Application of Enzymatic Amalysis in Medicine and Industry. In Enzymes: 1ST ed. Horwood publishing limited, west Sussex, England. Pp. 268.

30. Chida, Y., Sudo, N. and Kubo, C. (2006). Does Stress Exacerbate Liver Disease? *Journal of Gastroenterology and Hepatology*, 20:202- 208.

31. Lee, K.W., Kim, Y.J., Lee, H.J., and Lee, C.Y. (2003). Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas andred wine. *Journal of Agricultural and Food Chemistry*, 51(25)*,* 7292-7295.

32. Walker, G., Kunz, D., Pignat, W., Wiesenberg, I., Van den Bosch, H. and Pfeilschifter, J. (1996). Tetranactin inhibits interleukin-1β and cAMP induction of group II phospholipase A2 in rat renal messangial cells. *Eur J Pharmacol*., 306(3): 265-270.

33. Recknagel, R. O., Glende, E. A., Dolak, J. A. and Waller, R. L. (1989). Mechanisms of carbon tetrachloride toxicity. *Pharmacol Ther.*, 43: 139-154.

34. Halliwell, B. and Gutteridge, J. M. C. (2000). Free radicals in Biology and medicine. Oxford University Press, pp. 148-149.

35. Yamamoto, Y. and Yamashita, S. (1999). Plasma ubiquinone to ubiquinol ratio in patients with hepatitis, cirrhosis, and hepatoma, and in patients treated with percutaneous transluminal coronary reperfusion. *BioFactors*, 9: 241- 145.

36. Bandhopadhy U., Das D., Banerjee K. R. (1999). Reactive oxygen species: Oxidative damage and pathogenesis. *Curr Sci.*, 77: 658-665.