Antioxidant and Hepatoprotective Effect of Hibiscus sabdariffa Methanolic Extract (HME) against Carbon Tetrachloride (CCL_4) Induced Damage in Rats

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Abstract: The study was aimed to investigate the antioxidant activity of Hibiscus sabdariffa methanolic extract (HME). The preliminary study showed that HME was able to scavenge the ABTS and DPPH radicals and these radicals scavenging abilities were found to be dose-dependent. The antioxidant bioactivity was evaluated on CCl_4 (carbon tetrachloride) treated Wistar albino rats. CCl_4 injection induced oxidative stress by a significant rise in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and thiobarbituric acid reactive substances (TBARS) along with reduction of superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). Pretreatment of rats with different doses of HME (50 and 100 mg/kg) significantly lowered serum ALT, AST, ALP, LDH and TBARS levels against CCl_4 treated rats. GSH and hepatic enzymes like SOD and CAT were significantly increased by treatment with the HME, against CCl_4 treated rats. According to this study, the total phenolic content of HME was found to be 54.19 mg/g in GAE/g dried weight. The presence of these phenolics compounds may be responsible for the antioxidant and hepatoprotective activities observed in this study which could be due to the ability of phenolics compounds to absorb, neutralize and quench free radicals.

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1. Introduction

Diets appear to play an important role in human health and in the development of certain diseases such as cancer and cardiovascular disease (Liu, 2004). Consumption of plant based foods, which contain significant amounts of bioactive phytochemicals (e.g Polyphenols) may provide desirable health benefits beyond basic nutrition to reduce the risk of chronic diseases (Kerio, et. al., 2013; Anokwuru, et. al., 2011; Liu, 2004; Zeinab, et. al., 2012).

Hibiscus sabdariffa is a medicinal plant that is consumed for its health benefits in Nigeria, juice/concoction prepared from the plant is taken as a preventive/curative measures against diabetes and hypertension. The antihypertensive and other pharmacological properties of Hibiscus sabdariffa have been demonstrated in previous studies (Wahabi, et. al., 2010; Lin, et. al., 2012). *Hibiscus sabdariffa* is a complex mixture of phytochemicals, especially polyphenols, which include flavonoids, phenolic acids and anthocyanins (Salah et al., 2002; Ali et al., 2005; Lin, 2005). Hibiscus polyphenolic rich extract which is a group of phenolic compounds isolated from the dried flowers of Hibiscus sabdariffa has been showed to induce cell death in human gastric carcinoma (AGS) (Lin et al., 2005) while the constituent of the extract *Hibiscus* anthocyanins and protocatechuic acid have been demonstrated to have strong antioxidant (Tseng, et. al., 1997) and antitumor effects (Tseng, et. al., 2000). It has been postulated in several studies that effectiveness of plant extract against many diseases are due to their antioxidant action (Nithiyanantham, 2013; Jenner, 2003; Dalle-Donne et al., 2006).

Oxidative stress and liver damage have been induced in rats in many studies with carbon tetrachloride (CCl_4) and antioxidants have been demonstrated of capable of preventing this toxicity (Tsai, et. al., 2013; Olurunnisola, et. al., 2011). However, it is unclear if HME also has protective effects against CCl_4 toxicity. Therefore the purpose of this study was to investigate the antioxidant effect of HME in-vitro as well as examine its protective effect on the CCl_4 -induced oxidative stress and liver damage in rats.

2. Materials and Methods

2.1. Reagents

6-Hydroxy- 2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzthiazoline-6-sufonic acid) ABTS, Gallic acid, thiobarbituric acid (TBA), nicotinamide adenine dinucleotide reduced (NADH) were obtained from Sigma–Aldrich Chemical Co. Ltd. (England). Nitrobluetetrazolium (NBT), 5,5_-dithiobisnitro benzoic acid (DTNB) was obtained from Fluka

(Buchs, Switzerland). All other chemicals used were analytical grade.

2.2. Plant Material (Hibiscus sabdariffa)

The dried flowers of Hibiscus sabdariffa were bought from Sabo market, in Ogbomoso and authenticated at Department of Pure and Applied Biology of Ladoke Akintola University of Technology, Ogbomoso, by Dr A.J. Ogunkunle and Dr. Akintola. The dried calyces were further dried at 40°C until a constant weight was obtained and reduced to coarse powder.

2.3. Preparation of Hibiscus methanolic extract (HME)

Hibiscus sabdariffa was dried at room temperature and grounded with mortar and pestle. Weighed samples of this powdered material (200g) were loaded into extraction thimbles of Soxhlet extractor and were then extracted with methanol for 16 hours. The pooled methanolic solution of the extract was concentrated and evaporated to dryness at 50^{o}C with a rotary evaporator under reduced pressure. The extract was dissolved in water at a concentration of 4 g/100 ml, and aliquots of different concentrations were given orally to the animals with a gavage needle.

2.4. Determination of Total Phenolic Compounds in HME

The content of total phenolic compounds in HME was determined by Folin–Ciocalteu method as described by Miliauskas et al., (2004). Briefly, 1 ml aliquots of 0.024, 0.075, 0.0105 and 0.3 mg/ml ethanolicgallic acid solutions were mixed with 5ml Folin-ciocalteu reagent (diluted ten-fold) and 4ml (75g/L) sodium carbonate. The absorption was read after 30 min at 20^{0}C at 765 nm and the calibration curve was drawn. One ml of HME (1mg/ml) were mixed with the same reagents as described above, and after 1 hour the absorption was measured for the determination of plant phenolics. All determinations were performed in triplicate. Total content of phenolic compounds in plant methanol extracts in gallic acid equivalents (GAE) was calculated by the following formula:

$C = c \bullet V/m'$

Where: C-total content of phenolic compounds, mg/g plant extract, in GAE; c-the concentration of gallic acid established from the calibration curve, mg/ml; V- the volume of extract, ml; m´- the weight of pure plant methanolic extract, g.

2.5. Trolox Equivalent Antioxidant Capacity (TEAC) with Manganese dioxide

The assay was performed as previously described by Schelesier et al., (2002). The ABTS radical cation was prepared by filtering a solution of ABTS (in PBS) through manganese dioxide powder. Excess manganese dioxide was removed from the filtrate by passing it through a 0.2µm syringe filter. This solution was diluted in 5mM PBS pH 7.4, adjusted to an absorbance of 0.700 ± 0.020 at 734mm and preincubated at room temperature prior to use for 2 hours. 1 ml of $ABTS^{\bullet+}$ solution and various concentrations of the extracts (diluted with water) were vortexed for 45 seconds in reaction tubes, and the absorbance (734nm) was taken exactly 2 minutes after initiation of mixing. PBS blanks were run in each assay. The antioxidant activity of the extract was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

% antioxidant activity = $((A_{(ABTS\bullet+)} - A_{(Extracts)}) / (A_{(ABTS\bullet+)}) \times 100$.

2.6. Trolox Equivalent Antioxidant Capacity with Potassium Persulfate

The assay was performed essentially as described by Re et al., (1999). ABTS radical cation was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12–24 h before use. The $ABTS^{\bullet+}$ solution was diluted with water for the hydrophilic assay and with ethanol for the lipophilic assay and adjusted to an absorbance of 0.700 ± 0.020 at 734nm. For the photometric assay, 1ml of the $ABTS^{\bullet+}$ solution and various concentrations of the extracts were mixed for 45 seconds and measured immediately after 1 minute at 734nm. The antioxidant activity of the extract was calculated by determining the decrease in absorbance at different concentrations by using the following equation.

% antioxidant activity = $((A_{(ABTS^{\bullet+})} - A_{(Extracts)}) / (A_{(ABTS^{\bullet+})}) \times 100$.

2.7. DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging Activity

The assay was performed as previously described by Schelesier et al., (2002). The radical solution is prepared by dissolving 2.4 mg $DPPH^{\bullet}$ in 100 ml methanol. For the photometric assay 1.95 ml $DPPH^{\bullet}$ solution and 50 µl antioxidant solution were mixed. At first, the absorbance of the disposable cuvette with 1.95 ml $DPPH^{\bullet}$ was measured as blank, then the antioxidant solution was added and mixed. The reaction was measured at 5 min interval at 515 nm until $\Delta A=0.003$ min^{-1}. The anti-oxidative activity was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

%Inhibition activity = $((A_{(DPPH^{\bullet})} - A_{(Extracts)}) / (A_{(DPPH^{\bullet})}) \times 100$

2.8. Animal model and experimental design

Twenty four male wistar albino rats (180-220 g) were bought from the animal house at LAUTECH Agricultural department, Ogbomoso, Oyo state and they were maintained under standard environmental

conditions and had free access to feed and water. Seven days after acclimatization, the rats were divided into four groups with six rats each. Group 1 served as control, group 2 received single dose of equal mixture of carbon tetrachloride (CCl_4) and olive oil (50%, v/v, 1.25 ml/kg i.p.) on the 7th day. Group 3 and 4 animals were treated with Hibiscus methanolic extract (HME) at dose level of 50 and 100 mg/kg per day p.o., respectively for 7 days and on the 7th day; a single dose of equal mixture of carbon tetrachloride and olive oil (50% v/v 1.25 ml/kg i.p.) was administered.

2.9. Preparation of serum from whole blood

Twenty-four hours after the animals were administered with a single dose of carbon tetrachloride (CCl_4) they were sacrificed by chloroform anesthesia. Blood samples of each animal were collected by heart puncture and were allowed to clot for 45min at room temperature. Serum was separated by centrifugation at 4000×g for 5 min and analyzed for various biochemical parameters including serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatase (ALP), and serum lactate dehydrogenase (LDH).

2.10. Preparation of liver homogenates

Prior to biochemical analyses, the liver samples were cut into small pieces and homogenized in Phosphate buffer saline (PBS) with a homogenizer to give a 10% (w/v) liver homogenate. The homogenates were then centrifuged at 12,000 rpm for 15 min. The supernatant obtained was used for assay of superoxide dismutase, catalase, reduced glutathione, thiobarbituric acid reactive substances (TBARS) content, and protein estimation.

2.11. Biochemical analysis

Alkaline phosphatase (ALP), aspartate aminotransefrase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) in serum were determined using enzymatic kits (Labkit, Spain) according to the manufacturer's instructions

2.12. Measurement of hepatic lipid peroxidation

MDA levels were measured by the double heating method (Draper and Hadley, 1990). The method is based on spectrophotometric measurement of the purple color generated by the reaction of thiobarbituric acid (TBA) with MDA. Briefly, 0.5mL of liver homogenate was mixed with 2.5mL of tricholoroacetic acid (TCA, 10%, w/v) solution followed by boiling in a water bath for 15 min. After cooling to room temperature, the samples were centrifuged at 3000 rpm for 10 min and 2 mL of each sample supernatant was transferred to a test tube containing 1mL of TBA solution (0.67%, w/v). Each tube was then placed in a boiling water bath for 15 min. After cooling to room temperature, the absorbance was measured at 532 nm with respect to the blank solution. The concentration of MDA was calculated based on the absorbance coefficient of the TBA–MDA complex ($\varepsilon = 1.56\times10^5$ $cm^{-1}M^{-1}$) and it was expressed as nmol/mg protein.

2.13. Superoxide dismutase activity assay

SOD activity was measured according to method of Kakkar, et al. (1984). Assay mixture contained 0.1mL of supernatant,1.2 mL of sodium pyrophosphate buffer (pH8.3; 0.052 M), 0.1 mL of phenazine methosulphate (186 µM), 0.3 mL of nitrobluetetrazolium (300 µM) and 0.2mL of NADH (750 µM). Reaction was started by addition of NADH. After incubation at 30^0C for 90 s, the reaction was stopped by addition of 0.1 mLof glacial acetic acid. Reaction mixture was stirred vigorously with 4.0 mL of n-butanol. Colour intensity of the chromogen in the butanol was measured spectrophotometrically at 560 nm. One unit of enzyme activity was defined as that amount of enzyme which caused 50% inhibition of NBT reduction/mg protein.

2.14. Determination of Reduced Glutathione

GSH was assayed by the method of Jollow et al. (1974), with slight modification. An aliquot of 0.5mL of each tissue homogenate was precipitated with 0.5 mL of trichloroacetic acid (10% w/v). The precipitate was removed by centrifugation. 0.8 mL of the filtered sample was mixed with 0.3 DTNB (4 mg/mL) and 0.9mL phosphate buffer (0.1 M, pH 7.4). The yellow colour developed was read at 412 nm. Reduced glutathione was expressed as µg/mg of protein.

2.15. Catalase assay activity

Catalase activity was measured by the method of Aebi (1974). An aliquot (10µl) of each tissue supernatant was added to cuvette containing 1.99 µl of 50mM phosphate buffer (pH 7.0). Reaction was started by addition of 1000µl of freshly prepared 30mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometric ally at 240 nm. Activity of catalase was expressed as U/mg of protein.

3. Results

3.1. Trolox Equivalent Antioxidant Capacity (TEAC) assays [$mmoll^{-1}$ of three antioxidants; trolox, Gallic acid and Hibiscus methanolic extract (HME)

In the three versions of the TEAC assay; the TEAC value of Trolox is 1.00. Gallic acid responded in all the assays as the strongest antioxidant, but the TEAC value analyze in TEAC III (hydrophilic version) was lower when compared with the value obtained under TEAC II and TEAC III (lipophilic version), in addition, the two versions also showed comparable antioxidant activity both in Gallic and HME (Table 1).

Table 1. Trolox Equivalent Antioxidant Capacity (TEAC) assay of Trolox, Gallic acid and Hibiscus methanolic extract (HME)

Assay/Antioxidant	Trolox	Gallic	HSE
TEAC II	1.00	3.96±0.31	1.07±0.03
TEAC III(hydrophilic)	1.00	3.34±0.20	0.93±0.03
TEAC III (lipophilic)	1.00	3.99±0.16	1.28±0.02

3.2. Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of HME

The Hibiscus methanolic extract (HME) demonstrated a concentration dependent scavenging activity by quenching DPPH radicals (data not shown) and was compared with gallic acid, as a positive control. The IC50 values (defined as the concentration of test compound required to produce 50% inhibition) for DPPH scavenging by HME and gallic acid were 208.85 µg/dL and 16.33 µg/dL respectively (Table 2).

3.3. The Total Phenolic Content of Hibiscus methanolic extract (HME)

The phenolic content of Hibiscus methanolic extract was determined using Folin-Ciocalteu assay and by constructing a standard curve using gallic acid. The total amount of phenolic compounds present in HME was found to be 54.19 mg/g in Gallic acid equivalent (Table 2).

Table 2. Total phenolic content and DPPH radical scavenging value of HME

Sample	Total Phenol[a]	DPPH Scavenging activity (IC 50)[b]
HME	54.19 ± 1.8	208.85 ± 7.3
Gallic	–	16.30 ± 1.5

Each value represents the mean ± SEM. (n=3).

a Total phenolic content was expressed as mg gallic acid equivalents/g dried extract.

b Expressed as µg/mL

3.4. Effect of treatment with carbon tetrachloride on the levels of AST, ALT and ALP activities

CCl_4 treatment resulted in significant ($p < 0.05$) rise in the levels of AST, ALT and ALP when compared to the control group (Figs. 1). Oral administrations of HME at two different doses (50mg/kg and 100mg/kg) lower the levels of these marker enzymes, namely, AST, ALT and ALP significantly ($p < 0.05$).

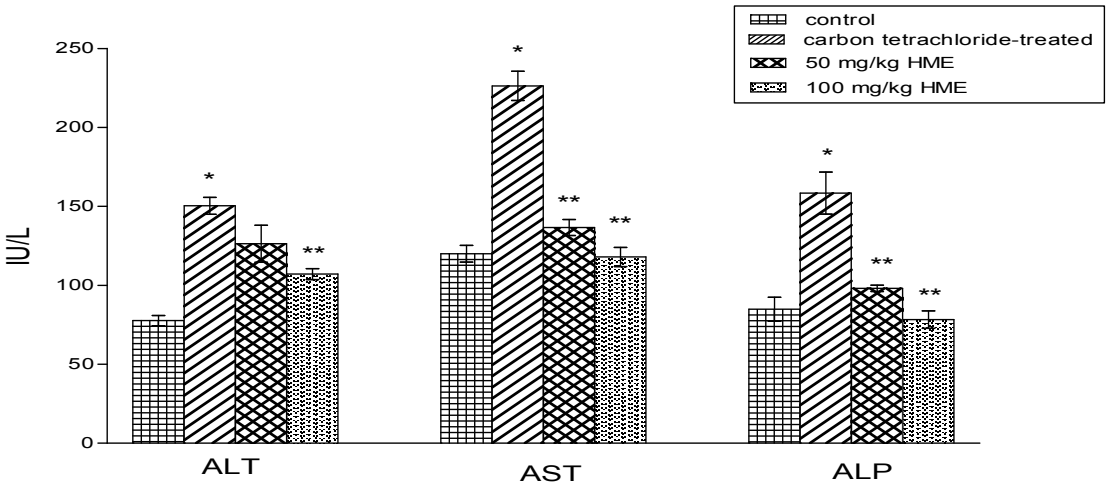

Fig.1 Effect of Hibiscus methanolic extract on serum levels of ALT (IU/L), AST (IU/L) and ALP (IU/L) during CCl4 treated oxidative stress in rats. Values are mean ± SEM. * Group 2 (CCl_4 treated rats) compared with Group 1 (control rats). ** Groups 3 and 4 (HME treated rats) compared with Group 2 (CCl_4 treated rats).

3.5. Effect of treatment with carbon tetrachloride on the level of Lactate dehyrogenase activity

CCl_4 treatment resulted in significant ($p < 0.05$) rise in the level LDH when compared to the control group (Figs. 2). Oral administrations of HME at two different doses (50mg/kg and 100mg/kg) lower the level of this marker enzyme, LDH significantly ($p < 0.05$).

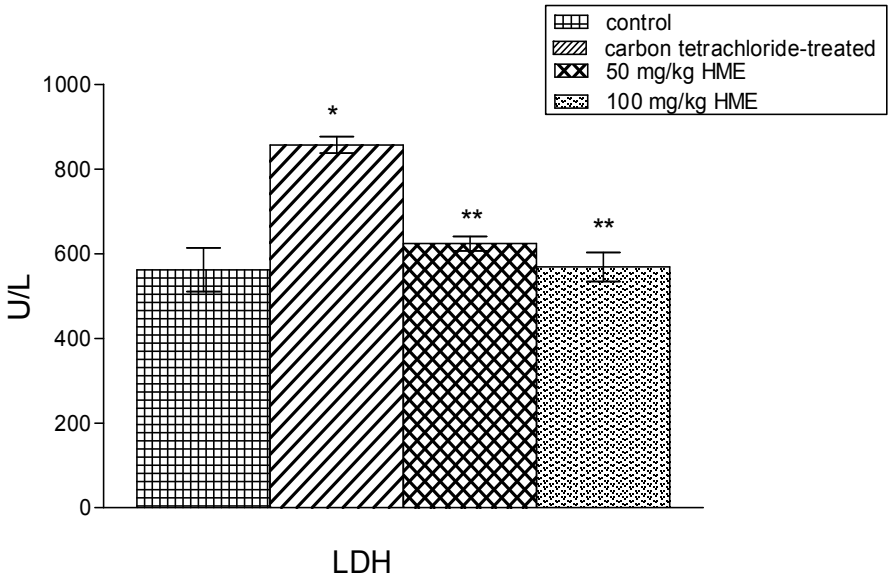

Fig.2 Effect of Hibiscus methanolic extract on serum levels of LDH (IU/L), during CCl_4 induced oxidative stress in rats. Values are mean ± SEM. * Group 2 (CCl_4 treated rats) compared with Group 1 (control rats). ** Groups 3 and 4 (HME treated rats) compared with Group 2 (CCl_4 treated rats).

3.6. Effect of treatment with carbon tetrachloride on the levels of superoxide dismutase and reduced glutathione

Administration of CCl_4 caused a significant ($p < 0.05$) decrease in SOD and GSH levels in rats when compared with normal animal. The HME at 50 and 100 mg/kg showed significant ($p < 0.05$) increase in SOD and GSH levels when compared to CCl_4 treated rats. (Fig. 3).

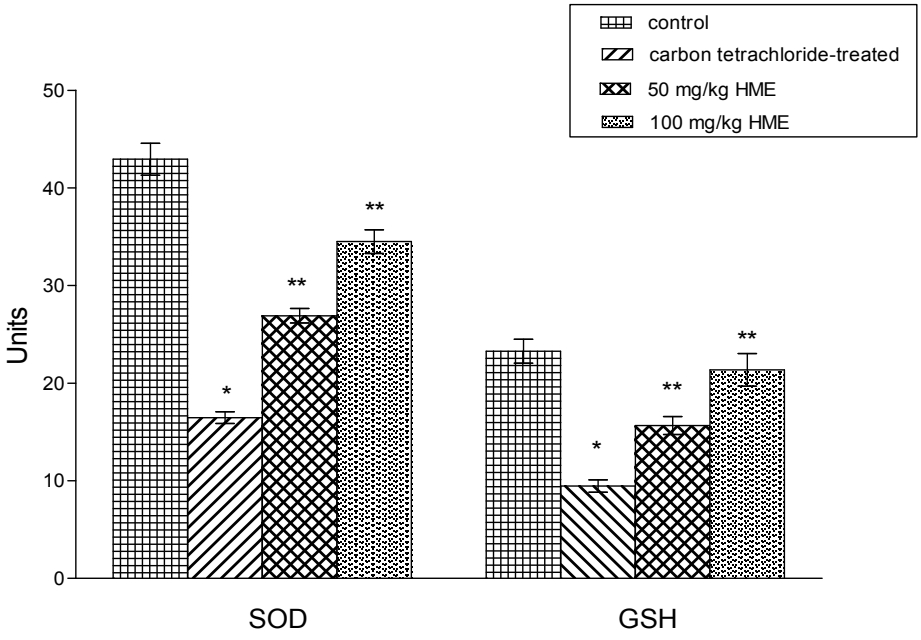

Fig.3 Effect of Hibiscus methanolic extract on hepatic level of SOD (U/mg protein) and GSH µg/mg protein) during CCl_4 induced oxidative stress in rats. Values are mean ± SEM.* Group 2 (CCl_4 treated rats) compared with Group 1 (control rats). ** Groups 3 and 4 (HME treated rats) compared with Group 2 (CCl_4 treated rats).

3.7. Effect of treatment with carbon tetrachloride on the level of catalase activity

The CCl_4-treatment caused significant ($p < 0.05$) decrease in the level of CAT in liver homogenate tissue, when compared with control group (Fig. 4). The pretreatment of plant extract at the dose of 50 and 100 mg/kg resulted in significant ($p < 0.05$) increase of CAT level when compared to CCl_4 treated rats.

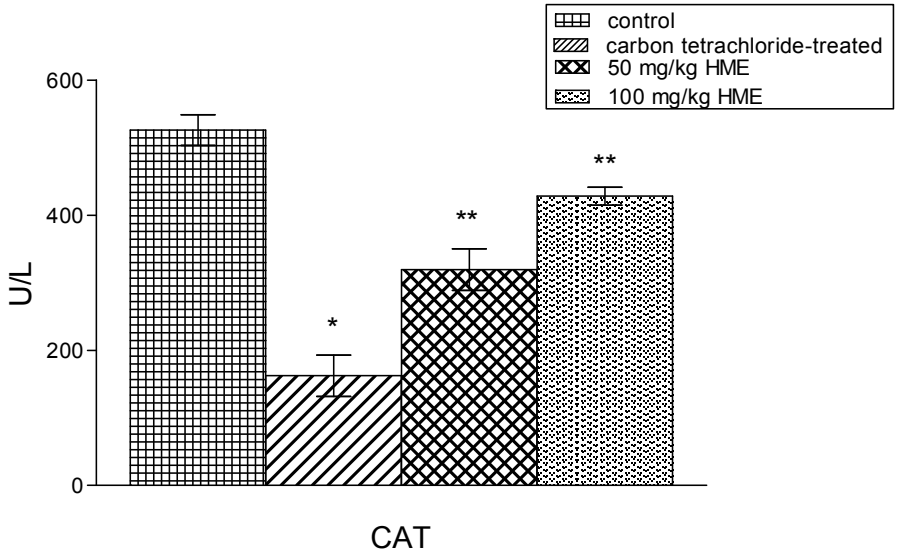

Fig.4 Effect of Hibiscus methanolic extract on hepatic level of catalase (U/mg protein) during CCl_4 induced oxidative stress in rats. Values are mean ± SEM.* Group 2 (CCl_4 treated rats) compared with Group 1 (control rats). ** Groups 3 and 4 (HME treated rats) compared with Group 2 (CCl_4 treated rats).

3.8. Effect of treatment with carbon tetrachloride on the levels of thiobarbituric acid reactive substance

Rats treated with CCl_4 showed significant increase in Lipid peroxidation level (LPO) of liver homogenates when compared to rats in control group as shown in Fig. 5. Pretreatment with HME at two different doses (50mg/kg and 100mg/kg) significantly reduced the LPO level when compared with CCl_4 treated rats.

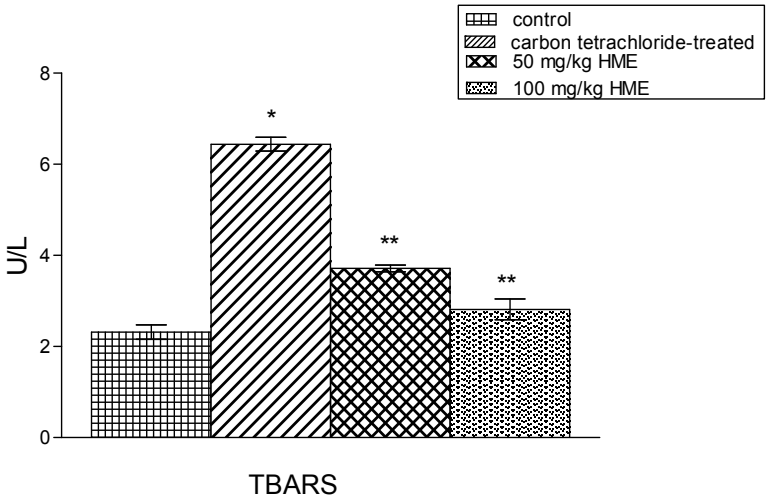

Fig.5 Effect of Hibiscus methanolic extract on hepatic level of thiobarbituric acid reactive substance (TBARS) (nM/mg protein) during CCl_4 induced oxidative stress in rats. Values are mean ± SEM. * Group 2 (CCl_4 treated rats) compared with Group 1 (control rats). ** Groups 3 and 4 (HME treated rats) compared with Group 2 (CCl_4 treated rats).

4. Discussions

Oxidative stress form when there is imbalance between free radical generating and scavenging systems has been implicated in the pathogenesis of wide range of disorders, including neurodegerative disorders, cardiovascular diseases, cancer, and ageing (Halliwell and Gutteridge, 1999). Several epidemiological studies suggest the importance of a high consumption of secondary plant products widely distributed in fruit and vegetables in ameliorating the effects of oxidative stress (Schlesier et al., 2002). Plant polyphenols are in particular presently gaining acceptance as responsible for the health benefits offered by fruit and vegetables (Nithiyanantham; 2013; Fraga, 2007). Studies suggest that plant polyphenols such as the flavonoids are potent antioxidant compounds both *in vitro* and *in vivo*. They have been shown to scavenge free radicals, chelate redox–active metal ions and inactivate other pro-oxidants (Ni´ciforovi´c et al., 2010; Fraga, 2007). Antioxidative effects of Hibiscus methanolic extract (HME) extracted from Hibiscus sabdariffa were determined in this study. Among the various methods used to evaluate the total antioxidant activity of vegetables or other plants, the $ABTS^{\bullet+}$ and DPPH radical scavenging assay are among the common applied methods. In the present study, HME showed DPPH radical scavenging activity which is attributed to its hydrogen donating ability. In still another approach, ABTS method was used to measure the radical scavenging activity of HME. Based on our results, HME showed significant radical scavenging activity in a concentration dependent manner.

To further evaluate the antioxidant properties of HME the ability of the extract to protect against CCl_4 induced liver damaged was evaluated. Carbon tetrachloride (CCl_4) is a toxic substance that is used to induce liver damage in rats (Tsai, et. al., 2013; Olurunnisola, et. al., 2011; Heba, et. al., 2011). Carbon tetrachloride (CCl_4) is biotransformed by the Cytochrome P_{450} in the liver endoplasmic reticulum to the highly reactive trichloromethyl free radical (Tsai, et. al., 2013; Clawson, 2009). CCl_4 can damage a number of tissues particularly the liver and kidney of many species (Drill, 2000). It has been hypothesized that one of the principle causes of CCl_4 induced liver injury is lipid peroxidation induced by free radical derivative of CCl_4. Thus, antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4 induced liver injury (Castro, 2004).

In this study, rat treated with single dose of CCl_4 developed a significant hepatic damage and oxidative stress, which was observed from a substantial increase in the activities of serum, AST, ALT, ALP and LDH. These are indicators of hepatocytes damage and loss of functional integrity. Any alteration in the activity of these enzymes causes tissue lesion and cellular impairment and dysfunction (Brito, et. al., 2012). Decrease in the serum levels of these enzymes with HME is an indication of the stabilization of plasma membrane as well as repair of liver damage caused by CCl_4, this observation is in agreement with the commonly accepted view that serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Brito, et. al., 2012).

During hepatic injury, there was decreased in the activity of superoxide dismutase (SOD) and catalase (CAT). This could be linked to exhaustion of the enzyme as a result of oxidative stress caused by CCl_4 (Raja, et. al., 2007). SOD and Catalase activities were however, brought to near normal in rats pretreated with the extract at 50mg/ml and 100mg/ml prior to CCl_4-treatment. In addition, there was decrease in liver reduced glutathione (GSH) level in rats treated with CCl_4 only, but a dramatic rise in the level of liver GSH was observed in rats pre-treated with HME at the dosage mentioned above. The stabilization of these enzymes by the extract is an indication of the improvement of the functional status of the liver. This can probably indicates that the Hibiscus methanolic extract (HME) either increase the biosynthesis of SOD, catalase and GSH or reduce the extent of oxidative stress leading to less degradation, or it may have both effects.

Enhanced lipid peroxidation expressed in terms of MDA (malonaldehyde) contents in CCl_4 treated rats as observed in our study indicates the damage to the hepatic cells which is confirmed by the earlier reports (Bhandarkar and Khan, 2004). Lipid peroxidation has been postulated to be the destructive process in liver injury due to toxicant (CCl_4) (Olorunnisola, et. al., 2011). In the present study, an elevation in the level of MDA in liver of animals treated with CCl_4 was observed. The increase in MDA level of the liver suggest enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense system to prevent formation of excessive free radicals. Pre-treatment with HME substantially reduce the level of lipid peroxidation. Hence, it may be possible that the mechanism of hepatoprotection by Hibiscus methanolic extract (HME) is due to its antioxidant potentials.

Plant phenolics are commonly found in both edible and non-edible plants and their antioxidant activities mainly due to their redox properties which allow them to act as reducing agents, hydrogen donators and singlet oxygen quencher (Huda-Faujan et al., 2009; Tawaha et al., 2007). Plants are thought to manufacture phenols for a variety of reasons but primarily as a part of their response to stress. Phenolic

compounds play an important role in plant resistance and defence against microbial infections which are intimately connected with reactive oxygen species (ROS) (Grassmann et al., 2002). Polyphenolic compounds in plants also have the potential to prevent oxidative-damage related diseases including cancer and atherosclerosis (Ni´ciforovi´c et al., 2010; Hogan, et al., 2007), In this study, phenolic content of HME were estimated using Folin-Ciocalteu assay which is a relatively simple method and widely used for estimation of polyphenolic content of plants. The total Polyphenolic content of the extract was found to be 54.19 mg in GAE/g dried weight. Polyphenolic are secondary plant metabolites and in addition to their various function in plants, some of these compounds act as anti-inflammatory, anti-microbial and anti-cancer agents in humans (Formica, et al., 1995). HME contain considerable amount of phenolic content, hence Hibiscus sabdariffa may be considered a medicinal plant for protection against free radical related diseases. It may however be possible that HME contain other natural antioxidants such as β-carotene, vitamin C and others which were not determined in this study that may be working synergistically with the phenolic compounds in the extract to produce the observed effects.

Conclution

HME possess strong antioxidant property which brought about its effect on the radicals and it may be mentioned that the altered biochemical profiles due to CCl_4 exposure is reversed towards normalization by Hibiscus methanolic extract. The contents of the extract not only protect the integrity of plasma membrane but, at the same time increase the regenerative and reparative capacity of the liver and can also terminate lipid peroxidation chain and restore antioxidant defence system. Beneficial effect of the extract may be due to the presence of some phenolic components that have membrane stabilizing effects. Also the results suggested that the phenolic compounds present in the extract possess strong antioxidant property against free radical and can efficiently work on the liver to keep it functioning normally, minimizing cell membrane disturbances and excessive formation of free radical.

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