Evaluation of Ginger Oleoresin in Carbon Tetrachloride Induced Hepatotoxicity in Rats

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ARTICLE INFORMATION

ABSTRACT

The present study evaluated the hepatoprotective activity of ginger oleoresin against Carbontetrachloride induced liver toxic damage in rats. Rats were divided into six groups. Hepatotoxicity was induced by the administration of a single intraperitoneal dose (2ml/kg) of Carbontetrachloride in experimental rats. Post-treatment with Ginger oleoresin at 300 and 600mg/kg dose given by oral routewas carried out to find their protective effectsagainst carbontetrachloride induced hepatic injury. Biochemical parameterfor oxidative stress, inflammation and lipid profile along with genotoxicity and histological changes in rat serum and liver were studied. Silymarin was used as standard hepatoprotective agent. Extracted oleoresin dose dependently provided hepatoprotective effects.The hepatoprotective action of ginger oleoresin may be related to its free radical scavenging,anti-inflammatory and hypolipidemic activity and concluded to be partly mediated by its active constituent's 6-gingerol, shogaol and zingerone. -phosphate; CCl_3*, Trichloromethyl free radical; CCl_3OO*, Trichloromethyl peroxy radical; ROS, Reactive oxygen species; iNOS, inducible nitric oxide synthase; NO, Nitric oxide, VLDL, Very low density lipoprotein.

1. Introduction

Liver is the largest gland of body that plays a pivotal role in regulating various physiological processes in the body such as metabolism, secretion and storage. Hepatotoxicity is a term that indicates damage to the cells, tissues, and structure or liver functions. Currently, millions of people are suffering from hepatic damage known to be induced by alcohol, chemicals, and drugs, infections, and immune response (Al-Harbi *et al.,* 2014). Genetic condition modifies susceptibility to various types of causative factors (Bose *et al.,* 2011).Chemicals, in the form of some drugs such as paracetamol, and antitubercular drugs (isoniazid, rifampicin), toxic compounds like carbontetrachloride (CCl_4), thioacetamide, dimethylnitrosamine, D-galactosamine/ lipopolysaccharide, gamma radiations, cadmium and arsenic heavy metals, mycotoxin (aflatoxin) are unquestionably various risk factors for hepatic injury (Deepa *et al.,* 2013; Domenicali *et al.,* 2009; Larson *et al.,* 2005).

Carbontetrachloride (CCl_4) is a clear, colorless, volatile, heavy and noninflammable liquid. (Deepa *et al.,* 2013; Domenicali *et al.,* 2009; Larson *et al.,* 2005; Alkreathy *et al.,* 2014) $CCl_4$is a lipophillic molecule metabolized in liver and spread easily in the lipid compartments of the body. $CCl_4$is a well-known hepatotoxin or classic model used extensively to investigate the hepatotoxicity in animals by initiating lipid peroxidation (Harbi *et al.,* 2014; Weber *et al.,* 2013; Recknagel *et al.,* 1989). Lipid peroxidation induce membrane disintegration of liver hepatocytes, which in turn increases the release of cytosolic enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) (Suzek *et al.,* 2015; Mohamed *et al.,* 2014). CCl_4 also causes the activation of immune system through the infiltration of inflammatory cells to the site of injury, responsible for the release of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF-α), interleukin-6 (IL-6) and c-reactive protein (CRP), which further enhance hepatotoxicity through repeated cycle of inflammation (Mohamed *et al.,* 2001a). Administration of CCl_4 increased triglycerides (TG), total cholesterol (TC), low density lipoprotein (LDL), whereas lowered the high density lipoprotein (HDL) (Boll *et al.,* 2001a; Andritoiu *et al.,* 2014). CCl_4 induced hepatotoxicity lead toaccumulation of Ca^{2+} in mitochondria, activate many membrane damaging enzymes, causes disruption of mitochondrial metabolism, decreased ATP synthesis and damage micro-filaments that support cell structure(Deepa *et al.,* 2013; Nicotera *et al.,* 1990). Free radicals of CCl_4 induced an increase in the number of Agyrophillic nucleolar organizer region (AgNORs) and enhance activity of telomerase enzyme hepatic architecture (Khanna *et al.,* 2003). CCl_4 caused various histological

changes to the liver, including cell necrosis, change in hepatic architecture, Kupffer cell hyperplasia, central vein congestion, inflammation, fatty changes, infilteration of the liver by lymphocytes and hydropic degeneration of hepatic cells is common (Moreira *et al.,* 2014; Al-Sayed *et al.,* 2014).

A flavonoligan mixture of milk thistle, Silymarin (Silybum marianum), is an vital herbal hepatoprotective drug (Abenavoli *et al.,* 2010). Altered cytoplasmic membrane architecture of Silymarin prevented the penetration of hepatotoxic substances, such as carbon tetrachloride, thioacetamide and D-galactosamine, into cells (Abenavoli *et al.,* 2010; Basiglio *et al.,* 2009). Hepatoprotective effects of silymarin are due to several mechanisms including antioxidation, membrane stabilization, immunomodulation, inhibition of lipid peroxidation, anticarcinogen, stimulated protein synthesis and enhanced liver detoxification (Kim *et al.,* 2009; Pradhan *et al.,* 2006; Lieber *et al.,* 2003; Skottova *et al.,* 1999; Luper 1998). Ginger oleoresin also known as gingerin, is widely used herbal remedies obtained by percolating the powdered rhizomes of *Zingiber Officinale,*which is belonging to the family of *Zingiberaceae* (Harimurtia *et al.,* 2011). Oleoresin ginger is dark golden brown viscous oil. It consists of essential oils, organic soluble resins and other non-volatile pungent components, which comprised homologous series of phenolic ketones such as gingerols, shogaols and zingerone (Singh *et al.,* 2005; Deline, 1985). These components have reported antioxidant, anti-inflammatory effects, antitumorogenic, antipyretic, antimicrobial card-iotonic, analgesic and antitussive effects (Kumar *et al.,* 2014; Huetal, 2012; Dugasani *et al.,* 2010; Singh *et al.,* 2009; Young *et al.,* 2005; Shin *et al.,* 2005). 6-gingerol was effective in preventing hepatic and renal damage in type-II diabetic db/db mice by reducing oxidative stress (Singh *et al.,* 2009). 6-Gingerol-rich fraction lowers total bilirubin and further restores ALT, AST, ALP, and gamma glutaryl transpeptidase (GGT) enzymes the sensitive indicators of hepatocellular damage and dysfunction (Salihu *et al.,* 2016; Sabina *et al.,* 2011). 6-gingerol decreased significantly liver weight index and restore the levels of triglycerides, total cholesterol and free fatty acid in the plasma and liver, which may be the primary protective mechanism exerted by the compound in high fat diet fed hamsters (Tzeng *et al.,* 2015). 6-Shogoal was comparatively more significant in decreasing the hepatic malondialdehyde (MDA) than 6 gingerol possibly due to its dominant antioxidant property. 6-Shogoal is a, 5- lipoxygenase inhibitor (Flynn *et al.,* 1986), contributed to the increased intrahepatic vascular resistance of the cirrhotic rat liver which resulted in prevention from hepatotoxin induced necroinflammatory injury (Titos *et al.,* 2005; Graupeara *et al.,* 2002). 6-Shogoal exhibited a significant hepatoprotective activity by reducing serum activities of AST, ALT and ALP, in to diclofenac sodium intoxicated rats (Alqasouami *et al.,* 2011). Zingerone was found as a hepatoprotective agent due to its free radicals scavenging and anti-inflammatory ability (Kumar *et al.,* 2014; Shin *et al.,* 2005). Zingerone prevents lipid peroxidation in rat liver microsomes (Reddy and Lokesh, 1992). Therefore ginger oleoresin can be used for developing new drugs to treat drug/chemical-induced liver toxicity.

2. Materials and Methods

2.1 *Animals*

Wistar albino rats of either sex (weighing 130-300g) procured from the 'Institutional animal house' was employed in the studies. The animals were kept in polyacrylic cages with wire mesh top and soft bedding. Animals were housed under standard environmental conditions 12h light and dark cycle and maintained with free access to water and a standard laboratory diet. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) IAEC/SIBP/14/CR-010 as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Government of India. Animals were acclimatized to laboratory conditions prior to experimental work.

2.2 *Drugs and Chemicals*

Ginger oleoresin from Dayal food products (Indore, India) and Silymarin from Tejkamal Pharmaceuticals Pvt. Ltd. (Bangalore) were used in the study. ATP (Adenosine-tri-phosphate) was purchased from Sigma-Aldrich, St (Louis, MO, USA). Carbon tetrachloride, Thiobarbituric acid, 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB) and Silver Nitrite ($AgNO_3$) were purchased from Otto Chemika-Biochmica (Mumbai, India). All other solvents and chemicals used for experimental work were of analytical grade.

2.3 *Experimental Design*

In the present study, rats were randomly divided into group of six having six animals in each group. Intra-peritoneal (*i.p.*) administration of a single dose of 2ml/kg $CCl_4$was used to induce hepatic injury in rats . Silymarin (100 mg/kg, *p.o.*) was used as a reference standard. 300mg/kg and 600mg/kg doses of ginger oleoresin were dissolved in 1% gum acacia and administered orally in wistar rats. The experimental design for study was as follows: Group1 served as normal healthy control rats. Group 2 injected with CCl_4 (2ml/kg *i.p.*). Group 3 CCl_4 co-administered with silymarin drug (100mg/kg *p.o.*).Group 4 was normal healthy rat administered ginger oleoresin (600mg/kg *p.o.*). Groups 5 and 6 CCl_4, co-administered with ginger oleoresin (300mg/kg and 600mg/kg *p.o.*) respectively.

2.4 *Assessment of Weight*

2.4.1 *Estimation of body weight*

The body weight of rats belonging to different groups were accessed on first day and final day of experimental protocol and compared for the changes.

2.4.2 *Estimation of liver weight*

The liver tissue in all groups was taken out after scarifying animals on 15th day. The tissue was cleaned with saline, blotted dry, placed on aluminum foil and weighed on electronic balance.

2.4.3 *Estimation of relative liver weight*

The remnant, regenerated liver was eviscerated and weighed (A) and total body weight (B) was measured at the time of sacrifice. The acquired data was expressed as percentage of the ratio between remnant liver weight, divided by the total body weight times 100.

$$\text{Liver/ Body Weight Ratio (\%)} = A/B \times 100$$

2.5 *Biochemical Parameters*

2.5.1 *Preparation of serum and liver homogenate*

On 15th day of protocol schedule, animal were weighed and sacrificed after 24h of the last treatment. 3ml of blood sample from each animal was taken. The collected blood was allowed to clot for 15minutes. At 3000 rpm samples were centrifuged for 15 min and serum was used for biochemical analysis. Liver was removed and weighed andthen perfused in ice-cold saline solution. A 10% (w/v) tissue homogenates was prepared in 0.1M phosphate buffer (pH 7.4). The homogenate was centrifuged at $10,000 \times g$ for 15 min. Collectedsupernatant was and used for biochemical estimation.

2.5.2 *Assessment of total protein in tissue homogenate*

The protein content was assessed by Biuret method (Nicotera *et al.,* 1990) using diagnostic protein estimation kit. The working reagent containing potassium iodide, potassium sodium tartrate, copper sulphate and sodium hydroxide (1000 μl) was added to 20 μl standard protein and 20 μl sample to prepare standard and test. Blank contains 1000 μl of reagent only. All the tubes were incubated at 37º C for 10 min, the absorbance for sandard and test were measured against blank at 546 nm spectrophotometrically. when treated with cupric ions in alkaline solution protein presents in sample formed a blue color complex and intensity of color is proportional to proteins present in sample.

$$\text{Total protein concentration (g/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} 1 \times \text{Concentration of standard (g/dL)}$$

2.5.3 *Assessment of Oxidative Stress*

2.5.3.1 Estimation of malondialdehyde (MDA) in tissue homogenate

Lipid peroxidation assay was carried out by the method of (Habig *et al.,* 1979).[41] Biomarker Malondialdehyde (MDA) is of lipid peroxidation and reacts with thiobarbituric acid (TBA) to form a pink chromogen.Liver homogenate (1ml) was mixed with trichloroacetic acid (TCA) 1ml (10% w/v) was centrifuged at 1850 g for 15 min followed by addition of 1ml of TBA solution (0.67% w/v) to 1ml of supernatant and was boiled for 45 min. Absorbance was read after cooling at 530 nm against a blank containing all the reagent except liver homogenate. Standard curve was used to measure the concentration of MDA in the supernatant and results were expressed as MDA equivalents in nmol/g liver.

2.5.3.2 Estimation of reduced glutathione (GSH) in tissue homogenate

(Jollow *et al.,* 1974) method was used to estimate reducedglutathione content. 1.0 ml of sample homogenate was precipitated with 1ml of (4%) sulfosalicylic acid. For 1h samples were kept at 4°C for and then centrifuged at

1200g for 20min . 3.0ml assay mixture contained 0.1ml filtered aliquot, 2.7ml phosphate buffer (0.1 mol; pH 7.4) and 0.2ml 5,5-dithio-bis (2-nitro benzoic acid) (DTNB) (100mmol). The yellow color developed was estimated at 412 nm on spectrophotometer and was expressed as μmol GSH/g tissue.

2.5.3.3 Estimation of superoxide dismutase (SOD) in tissue homogenate

SOD activity was measured by well established method described by (Misra and Frodvich, 1972). Supernatant 0.2 ml of the liver homogenate was mixed with 0.8 ml 50 mmol of pH 10.4 glycine buffersfollowed by reaction with addition of 0.02 ml of epinephrine. After 5 min the absorbance was measured at 480 nmspectrophotometrically. SOD activity was expressed as percent activity.

2.5.3.4 Estimation of catalase (CAT) in tissue homogenate

(Aebi *et al.,* 1974) method was used by measuring the changes in absorbance of a solution of 10mM H_2O_2 in phosphate buffer, pH 7.0. The decreased absorbance per unit time is a measure of catalase activity. 20 μl liver homogenate was added to the cuvette containing 980 μl of the substrate

mixture (0.30 ml of H_2O_2 in 50 ml of 0.05 M phosphate buffer, pH 7.0). Initial absorbance was recorded after 1min followed by final absorbance after 6 min. The reaction was estimated at 230 nm, CAT activity was expressed as percent activity.

2.5.3.5 Estimation of reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) in tissue homogenate

Liver homogenate was added to 1.5 ml medium of (125mM sucrose, 65mM KCl and 10mM KOH, pH 7.4) and centrifuged at 8000g for 3 min.Fluorescence of collected supernatant was measured at 450nm spectrophotometericlaly. The activity of NADPH was expressed as percent activity (Moreira *et al.,* 2014).

2.5.4 *Estimation of adenosine-tri-phosphate (ATP)*

Aliqutos of homogenate (1ml) were sonicated immediately in 2ml of ice cold perchloric acid (0.1N) which was used to inactivate ATPases and freezed for 2 hour. Supernatants containing ATP was neutralized with 1N NaOH after centrifugation (3000 g, 4ºC, 5 min) and adjusted to pH 6-6.5. The sample was stirred again for 5 min and placed at ice-bath for 1hr. The absorbance was measured

spectrophotometrically at 365nm (Ramanathan *et al.,* 2012).

2.5.5 *Assessment of inflammation*

2.5.5.1 Nitrite/ nitrate level estimation in tissue homogenate

Nitrite which was accumulated in the supernatant is an indicator of the production of nitric oxide, was determined by colorimetric assay using Greiss reagent (0.1% N-(1-naphthyl) ethylene diamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by (Green *et al.,* 1982). Equal volumes of supernatant and Greiss reagent were mixed and mixture was incubated for 10 min at room temperature in the dark followed by estimation at 540nm spectrophotometrically (Green *et al.,* 1982).

2.5.6.4 Estimation of serum C-reactive protein (CRP)

C-reactive protein (CRP)increases significantly in most of tissue injuries, bacterial and viral infections, inflammation and malignant neoplasia is an acute phase protein present in normal serum. CRP is carried by CRP turbilatex method using CRP kit. The reagents and the photometer (cuvette holder) were maintained by 37°C. The wavelength was adjusted to 540 nm, temperature 37°C and with path length of 1 cm. Instrument was adjusted to zero with

$$CRP \ (mg/L) = \frac{(A_2 - A_1) \ sample}{(A_2 - A_1) \ calibrator} \times Calibrator \ concentration$$

distilled water. 5.0µl of serum/Calibrator was mixed with 900ul diluent (Tris buffer 20mmol/1 pH 8.2) and 100µl of latex. The absorbance was measured initialy (A_1) and after 2 minutes (A_2) of the sample addition.

2.5.6 *Assessment of lipid profiles*

2.5.6.1 Estimation of serum high density lipoprotein (HDL)

High density lipoproteins includes free and esterified cholesterol, triglycerides, phospholipids and apoproteins A, C and E. Chylomicrons, LDL and VLDL were precipitated

from serum by phosphotungstate in the presence of divalent cations such as magnesium. The HDL cholesterol remains unaffected in the supernatant and is estimated using cholesterol reagent of ERBA diagnostic kit.

Instrument was adjusted to zero with blank (1000 µl cholesterol working reagent mixed with 50 µl of distilled water). 50 µl of serum/standard was mixed with 1000 µl of cholesterol working reagent. Well mixed samples were incubated for 10 min. at 37°C. The wavelength was adjusted to 505 nm. The absorbance of standard and test were measured as

$$HDL \ Cholesterol \ (mg/dL) = \frac{Absorbance \ of \ test}{Absorbance \ of \ Standard} \times Concentration \ of \ standard \ (mg/dL) \times Dilution \ factor$$

2.5.6.2 Estimation of serum triglyceride (TG)

Elevation of triglycerides has been identified as a risk factor for atherosclerotic disease and liver dysfunctioning. Triglycerides were estimated using reagent of ERBA diagnostic kit. Instrument was adjusted to zero with blank

(1000 µl of working reagent mixed with 10 µl of distilled water). 10 µl of serum/standard was mixed with 1000 µl of working reagent. Well mixed samples were incubated for 10min at 37°C. The absorbance of standard and each test at 505 nm was measured spectrophotometrically against blank.

$$Triglycerides \ (mg/dL) = \frac{Absorbance \ of \ test}{Absorbance \ of \ standard} \times Concentration \ of \ standard \ (mg/dL)$$

2.5.6.3 Estimation of serum total cholesterol (TC)

To evaluate risk of the coronary arterial occlusion, atherosclerosis, myocardial infarction, liver function, biliary

function, intestinal absorption, thyroid function and adrenal disease serum cholesterol levels measurement are useful. Cholesterol levels were estimated using reagent of ERBA

diagnostic kit. Instrument was adjusted to zero with blank (20µl distilled water mixed with 1000µl of working reagent). 20µl of serum/standard was mixed with 1000µl of working

$$\text{Cholesterol (mg/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard (mg/dL)}$$

2.5.7 *Assessment of liver marker enzymes in serum*

2.5.7.1 Estimation of serum enzyme aspartate aminotransferase (AST)

AST is present in large amounts in. Increased levels of AST are associated with liver disease or damage, myocardial infarction, muscular dystrophy and cholecystitis as they are abundant in liver, renal, cardiac and skeletal muscle tissue. AST was estimated using reagent of ERBA diagnostic kit. Instrument was set as zero with distilled water. 100µl of serum was mixed with 1000µl of working reagent. Absorbance of each test was measured after every minute for four minute after proper mixing.

Activity of AST (IU/L) = Δ Absorbance/min $\times$ 1768 (factor)

Δ Absorbance = Change in Absorbance

Factor = 1768

2.5.7.2 Estimation of serum enzyme alanine aminotransferase (ALT)

ALT is present in high concentration in the liver and increased levels are generally a result of primary liver diseases such as cirrhosis, carcinoma, viral or toxic hepatitis and obstructive jaundice. ALT was estimated using reagent of ERBA diagnostic kit. The wavelength was adjusted to 340 nm. Instrument was set at zero with distilled water. 100µl of serum was mixed with 1000µl of working reagent and the absorbance of each test was measured after every minute.

Activity of ALT at 37°C (IU/L) = (ΔA/min) $\times$ Factor (1768)

2.5.7.3 Estimation of Serum enzyme alkaline phosphatase (ALP)

Alkaline phosphatase is present in high concentration in the liver, bone, placenta, intestine and certain tumors. Increase in Alkaline phosphatase activity in serum or plasma is related to disease of bone, biliary tract and liver. Alkaline Phosphatase hydrolyses para-nitrophenyl phosphate into paranitrophenol and phosphate, in the presence of magnesium ions in sample. The rate of increase in absorbance of the reaction mixture at 405 nm due to liberation of paranitrophenol is proportional to the alkaline phosphatase activity. ALP was estimated using reagents of Enzopak diagnostic kit. The wavelength was adjusted to 405 nm. Instrument was adjusted to zero with reagent blank. 20µl of serum was mixed with 1000µl of buffered substrate and the absorbance was measured at 30, 60, 90 & 120 seconds at 405 nm.

reagent and incubated at 37°C for 10 minutes. Aspiration of both blank, standard and test was performed followed by measurement of absorbancespectrophotometrically at 505 nm.

Alkaline Phosphatase activity (IU/L) = (ΔA/min)
$\times$ Factor (2713)

2.5.8 *Histopathological studies*

After removal of Liver tissue from the anterior portion of the left lateral lobe it was further subjected to histological analysis. After fixing Liver fragments in a 10% solution of formaldehyde, they were dehydrated in graduated ethanol (50-100%), cleared in xylene and finally embedded in paraffin. 4–5µm hepatic sections were analyzed by light microscopy with a magnification of 400× after staining with hematoxylin and eosin (H&E) using standard techniques.

2.5.9 *Genotoxicity study*
2.5.9.1 AgNORs count

Silver staining technique by well-established method by (Trere *et al.,* 1996) was performed on dried slides. Unstained fixed and dried slides were treated with one drop of colloidal solution (2% gelatin and 1% formic acid) and two drops of 50% $AgNO_3$ and were incubated at 35°C for about 8–12 min. The progressive staining was followed under microscope to get golden colored nuclei and brown/black NORs. After washing slides with distilled water it was further treated with 1% sodium thiosulphate for 1 min at room temperature to stop the reaction, and washed in tap water. Number of AgNORs was counted per cell. Counting was performed, using oil immersion at 100X. Areas with minimal cell overlap and no artifact were demarcated for counting. The appearance of black dots within the green colored nuclear background. Dots were defined as discrete homogenous silver precipitates with well-defined edges.

2.5.10 *Statistical analysis*

All the values are expressed as mean $\pm$ S.D. Data obtained from various groups was assessed at GraphPad Prism 5 and statistically analyzed using one way ANOVA, followed by Tukey test for comparison of all the groups. A 'P' value of less than 0.001 was considered statistically significant.

3. Results
3.1 *Effect of Ginger Oleoresin on Body Weight, Liver Weight and Relative Liver Weight*

Administration of CCl_4 significantly reduced ($p < 0.0001$) the body weight, while increased ($p < 0.0001$) the liver

weight of rats, when compared to normal group. Post treatment with ginger oleoresin at 300mg/kg and 600mg/kg *p.o.* dose for 14 days significantly ameliorates the changes

of body weight, liver weight and relative liver weight, when compared to CCl_4 treated group. (Table 1)

Table 1: Effect of Ginger oleoresin on body weight, liver weight and relative liver weight

Treatment	Body weight (g)	Liver weight (g)	Relative liver weight (%) to body weight	Total protein (g/dL)
Normal group	187.00 ± 8.59	5.67 ± 0.34	2.74 ± 0.19	7.45 ± 1.81
CCl_4 treated group	139.66 ± 5.16	6.67 ± 0.10	4.31 ± 0.14	3.00 ± 0.56
Silymarin pre-treated	175.33 ± 5.16	4.92 ± 0.42	2.75 ± 0.23	6.91 ± 0.74
ginger oleoresin, Postreated group -1	181.83 ± 2.04	6.25 ± 0.23	2.94 ± 0.16	5.50 ± 0.28
ginger oleoresin Postreated group -11	196.66 ± 5.16	6.46 ± 0.10	2.73 ± 0.07	8.21 ± 0.71

3.2 *Effect of Ginger Oleoresin on Total Protein in Tissue Homogenate*

Administration of CCl_4 significantly reduced (p<0.0001) total protein in liver, when compared to normal rats. Post treatment with ginger oleoresin at the dose 300mg/kg and 600mg/kg successfully revert (p<0.0001) the changes caused by CCl_4. Ginger oleoresin post treatment attenuate protein level in dose dependent manner with 600mg/kg (p<0.0001), when compared to 300mg/kg dose. (Table 1)

3.3 *Oxidative Stress Markers*

Oxidative stress causes the damage by production of free radicals and alteration of antioxidant defence system.
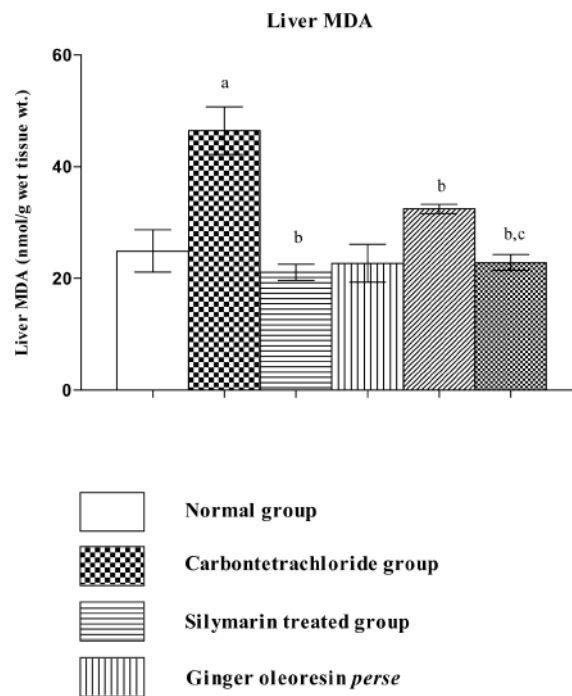
3.3.1 *Effect of ginger oleoresin on malondialdehyde (MDA) in tissue homogenate*

A significant increase (p<0.0001) in MDA level which is an end product of lipid peroxidation was observed in the liver of CCl_4 treated rats, when compared with the normal rats. Post treatment with ginger oleoresin at the dose of 300mg/kg and 600mg/kg significantly reduced (p<0.0001) the MDA level in liver, when compared with CCl_4 administered rats. The dose dependent effects were observed as ginger oleoresin 600mg/kg was found more significant in reducing (p<0.0001) MDA level in the liver as compared to ginger oleoresin 300mg/kg dose. (Fig.1)
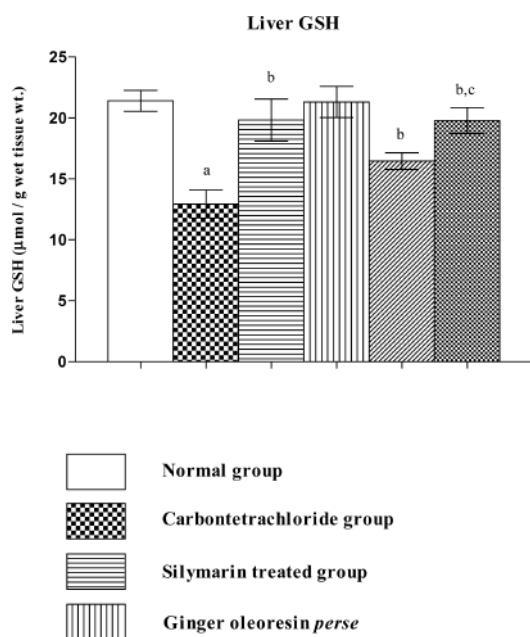
3.3.2 *Effect of ginger oleoresin on reduced glutathione (GSH) in tissue homogenate*

Reduced GSH is a cellular antioxidant which protect against protection against oxidative stress. Hepatic glutathione levels got significantly reduced (p<0.0001) in CCl_4 intoxicated

rats as compared to normal rats. Post treatment with ginger oleoresin at the dose of 300mg/kg and 600mg/kg restored the GSH levels in hepatic tissues (p<0.0001) as compared to CCl_4 treated group. The dose dependent effects were observed as ginger oleoresin 600mg/kg was found more significant in increasing (p<0.0001) GSH level in the liver as compared to ginger oleoresin 300mg/kg dose.(Fig.2)

Figure 1: Effect of ginger oleoresin on malondialdehyde (MDA) (nM/g wet tissue wt.) in tissue homogenate
Values were expressed as mean ± SD. a; P < 0.0001 versus Normal group; b P < 0.0001 versus Carbontetrachloride group; c p<0.0001 versus Ginger oleoresin post treated group-I

Figure 2: Effect of ginger oleoresin on reduced glutathione (GSH) (μM/g wet tissue wt.) in tissue homogenate

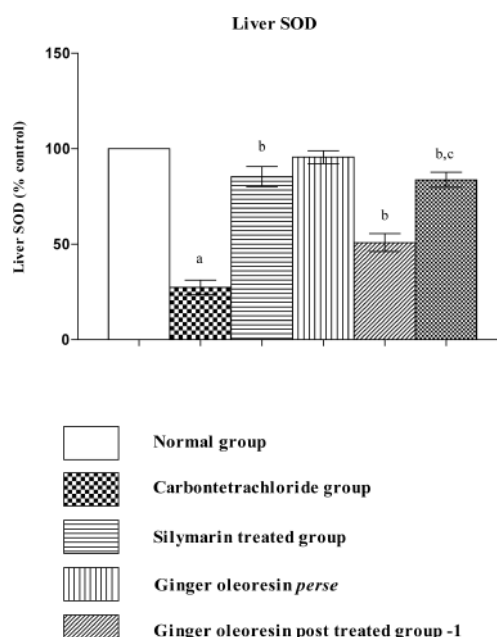Values were expressed as mean $\pm$ SD. a; P<0.0001 versus Normal group; b P < 0.0001 versus Carbontetrachloride group; c p<0.0001 versus Ginger oleoresin post treated group-I

3.3.3 *Effect of ginger oleoresin on superoxide dismutase (SOD) in tissue homogenate*

Antioxidant enzyme SOD plays a key role in detoxifying superoxide anions. CCl_4 administration significantly reduced (p<0.0001) the SOD levels, when compared to normal rats. In post treatment groups, 300mg/kg and 600 mg/kg administration showed significantly increased (p<0.0001) SOD level, when compared to CCl_4 treated group. Dose dependent effects were observed with ginger oleoresin 600mg/kg dose. Ginger oleoresin 600mg/kg significantly increased (p<0.0001) SOD levels, when compared to 300mg/kg dose. (Fig. 3)

3.3.4 *Effect of ginger oleoresin on catalase (CAT) in tissue homogenate*

Antioxidant enzyme catalasehas capability to detoxify oxidative free radicals. The liver catalase activity significantly decreased (p<0.0001) in CCl_4 intoxicated rats as compared to normal rats. In post treated, 300mg/kg and 600 mg/kg ginger oleoresin groups a significant increase (p<0.0001) in catalase activity was observed in hepatic tissues, when compared to CCl_4 treated group. Ginger oleoresin at the dose of 600mg/kg proved its dose dependent effect by significantly increasing (p<0.0001) catalase activity in rats when compared to 300mg/kg dose. (Fig.4)

Figure 3: Effect of ginger oleoresin on superoxide dismutase (SOD)(% control) in tissue homogenate
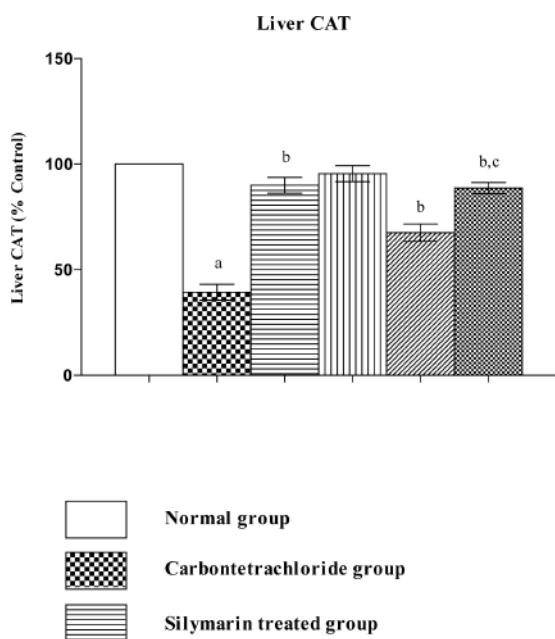
Values were expressed as mean $\pm$ SD. a; P < 0.0001 versus Normal group; b P < 0.0001 versus Carbontetrachloride group; c P< 0.0001Ginger oleoresin post treated group-I

3.3.5 *Effect of ginger oleoresin on reduced nicotinamide adenine dinucleotide phosphate (NADPH) in tissue homogenate*

For biosynthetic reactions NADPH provides the reducing equivalents and oxidation-reduction involved in protecting against the toxicity of ROS. After treatment of rats with CCl_4 significantly reduction (p<0.0001) in the level of NADPH was observed in the liver, as compared with the normal group. Post treatment groups, 300mg/kg and 600 mg/kg ginger oleoresin significantly prevented (p<0.0001) the changes of NADPH maintaining their levels near to normal range. The dose dependent effect was observed as ginger oleoresin 600mg/kg dose was found to be more effective (p<0.0001) as compared to 300mg/kg dose. (Fig. 5)

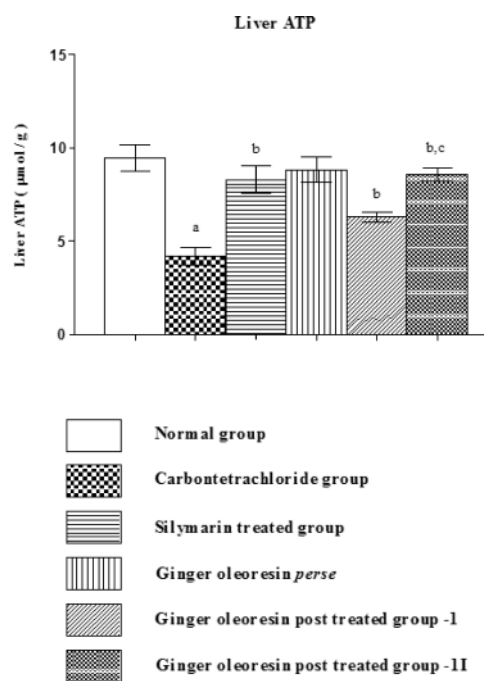## 3.4 *Effect of Ginger Oleoresin on Adenosine Triphosphate (ATP) in Tissue Homogenate*

Adenosine triphosphate (ATP) is a coenzyme used as an energy carrier in the cells. Hepatic ATP levels were significantly reduced (p<0.0001) in CCl_4 intoxicated rats as compared to normal rats. Post treatment with ginger oleoresin at the dose of 300mg/kg and 600mg/kg significantly attenuated (p<0.0001) the ATP levels in hepatic tissues as compared to CCl_4 treated group. In treatment doses of ginger oleoresin, 600mg/kg found to be effective (p<0.0001), when compared with ginger oleoresin 300mg/kg dose. (Fig. 6)

Figure 4: Effect of ginger oleoresin on catalase (CAT) (% control) in tissue homogenate

Values were expressed as mean ± SD. a; P < 0.0001 versus Normal group; b P < 0.0001 versus Carbontetrachloride group; c P < 0.0001Ginger oleoresin post treated group-I
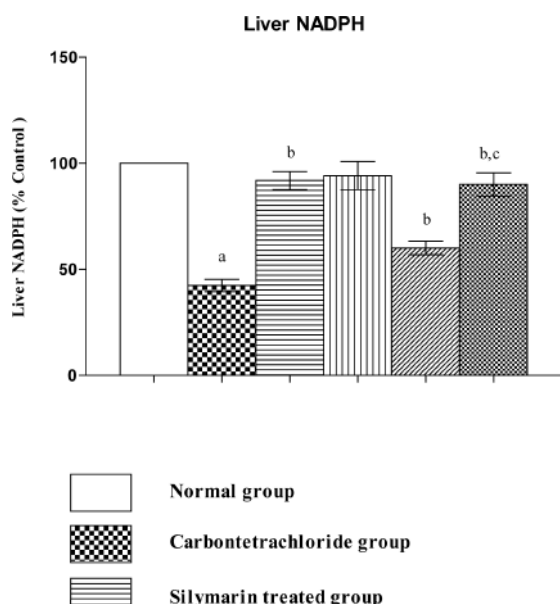
Figure 5: Effect of ginger oleoresin on reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) (% control) in tissue homogenate

Values were expressed as mean ± SD. a; P < 0.0001 versus Normal group; b P < 0.0001 versus Carbontetrachloride group; c p<0.0001 versus Ginger oleoresin post treated group-I

Figure 6: Effect of ginger oleoresin on adenosine-tri-phosphate (ATP) (µM/g tissue) in tissue homogenate

Values were expressed as mean ± SD. a; P<0.0001 versus Normal group; b P<0.0001 versus Carbontetrachloride group; c p<0.0001 versus Ginger oleoresin post treated group-I

3.5 *Inflammation*

Immune system activation through the infiltration of inflammatory cells at the site of injury resulted in the release of proinflammatory cytokines.

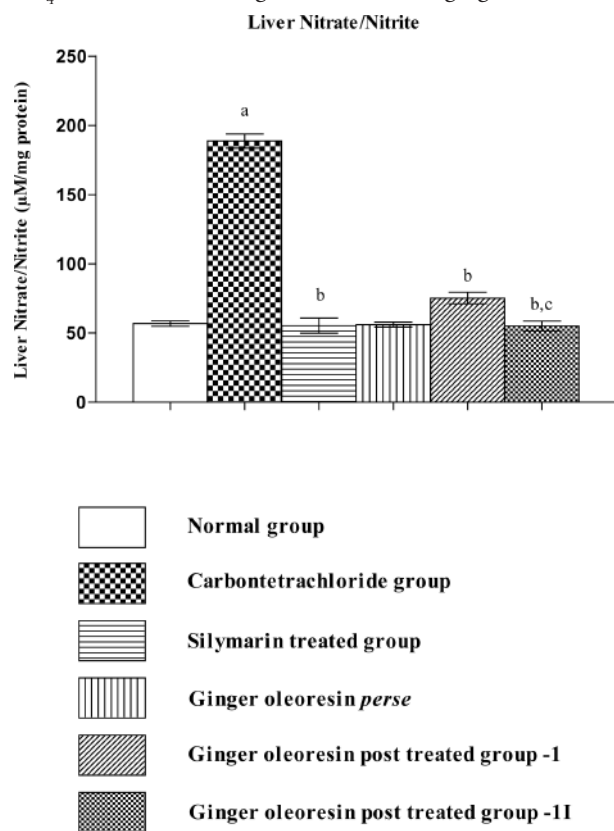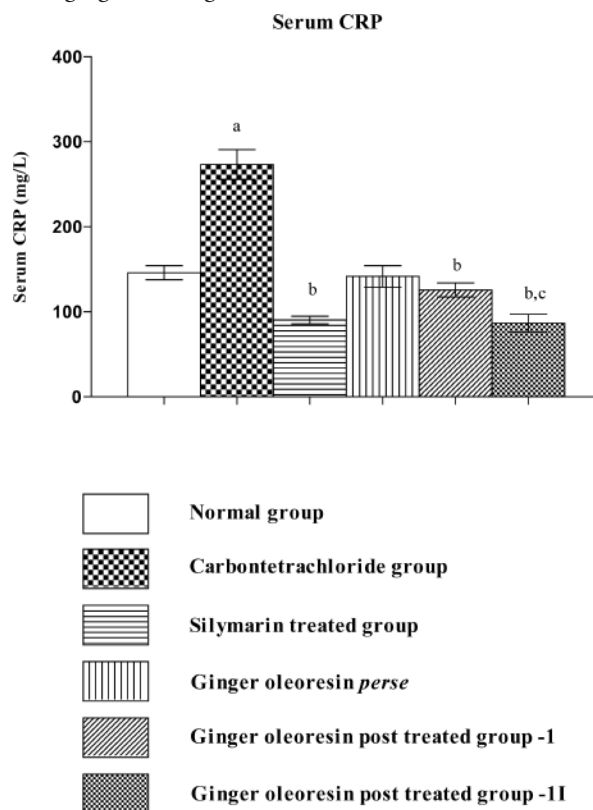### 3.5.1 *Effect of ginger oleoresin on nitrite/ nitrate level in tissue homogenate*

Nitrite accumulation in the supernatant indicates production of nitric oxide. CCl_4 administration significantly increased (p<0.0001) the level of nitrite as compared to normal rats. Post treatment with ginger oleoresin at the dose of 300mg/kg and 600mg/kg showed significant reduction (p<0.0001) in the nitrite levels, as compared with CCl_4 treated group. The dose dependent effects were observed as ginger oleoresin 600mg/kg remarkably reduced (p<0.0001) the levels of inflammatory marker nitrite, as compared to 300mg/kg dose. (Fig. 7)

3.5.2 *Effect of ginger oleoresin on serum C-reactive protein (CRP)*

CCl_4 administration caused inflammation in rats as assessed in term of significantly elevated serum CRP levels, when compared to normal group. CRP was significantly restored

(p<0.0001) in ginger oleoresin post treated at 300mg/kg and 600mg/kg groups respectively, when compared with CCl_4 treated rats. Among treatment doses ginger oleoresin,

600 mg/kg dose was found to be more effective (p<0.0001) in reducing CRP level, when compared with ginger oleoresin 300mg/kg dose. (Fig. 8)

Figure 7: Effect of ginger oleoresin on nitrate/nitrite (μM/mg protein) in tissue homogenate

Values were expressed as mean ± SD. a; P<0.0001 versus Normal group; b P<0.0001 versus Carbontetrachloride group; c p<0.0001 versus Ginger oleoresin post treated group-I

Figure 8: Effect of ginger oleoresin onserum C-reactive protein (CRP) (mg/L)

Values were expressed as mean ± SD. a; P<0.0001 versus Normal group; b P<0.0001 versus Carbontetrachloride group; c p<0.0001 versus Ginger oleoresin post treated group-I

3.6 *Lipid Profile*

Lipid synthesis and degradation imbalance impaired oxidation of lipids, failure of triglycerides to move as VLDL resulted in fat accumulation.

3.6.1 *Effect of ginger oleoresin on serum high density lipoprotein (HDL)*

Administration of CCl_4 significantly reduced (p<0.0001) serum high density lipoproteins as when compared to normal rats. Reduced HDL cholesterol level was remarkably increased (P<0.0001) by post treatment with ginger oleoresin 300mg/kg and 600mg/kg doses. Dose of 600mg/kg was found to be more effective (p<0.0001), in increasing the HDL levels, when compared with ginger oleoresin 300mg/kg dose. (Fig. 9)
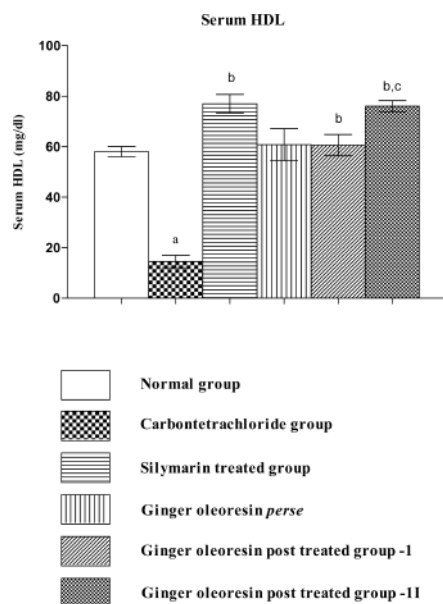
3.6.2 *Effect of ginger oleoresin on serum triglyceride (TG)*

CCl_4 caused significant increase (p<0.0001) in serum triglyceride level, when compared to normal rats. Post treatment with ginger oleoresin at the dose of 300mg/kg and 600mg/kg appreciably (P<0.0001) augmented serum triglycerides, when compared with CCl_4 treated group. Ginger oleoresin 600mg/kg dose significantly reduced (p<0.0001) triglyceride level as compared to 300mg/kg dose. (Fig. 10)

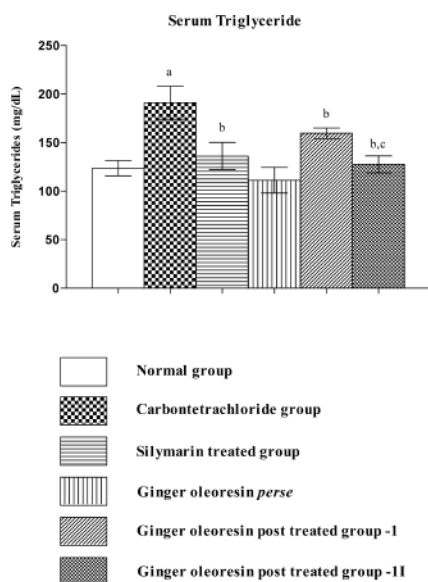### 3.6.3 *Effect of ginger oleoresin on serum total cholesterol (TC)*

After treatment with CCl_4serum total cholesterol level in rat serum wassignificantly increased (p<0.0001), when compared to normal rats. Post treatment with ginger oleoresin at the dose of 300mg/kg and 600mg/kg attenuated

cholesterol level remarkably (P<0.0001), when compared to CCl_4 group. Ginger oleoresin 600mg/kg administration dose dependently reduced (p<0.0001) serum total cholesterol level, when compared to 300mg/kg dose. (Fig.11)

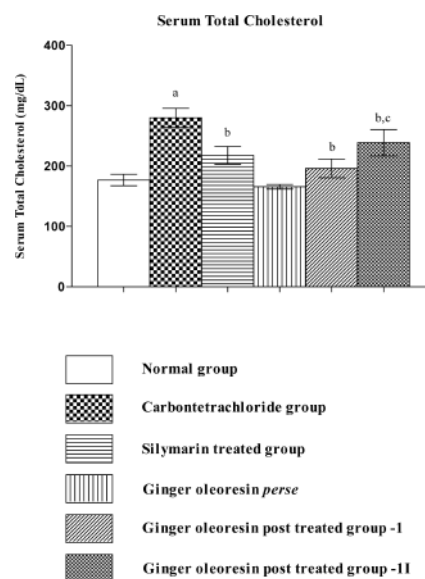**Figure 9:** Effect of ginger oleoresin onserum high density lipoprotein (HDL) (mg/dL)

Values were expressed as mean ± SD. a; P<0.0001 versus Normal group; b P<0.0001 versus Carbontetrachloride group; c p<0.0001 versus Ginger oleoresin post treated group-I

Figure 10: Effect of ginger oleoresin onserum triglyceride (TG) (mg/dL)

Values were expressed as mean ± SD. a; P<0.0001 versus Normal group; b P<0.0001 versus Carbontetrachloride group; c p<0.0001 versus Ginger oleoresin post treated group-I

Figure 11: Effect of ginger oleoresin on serum total cholesterol (TC) (mg/dL)

Values were expressed as mean ± SD. a; P<0.0001 versus Normal group; b P<0.0001 versus Carbontetrachloride group; c p<0.0001 versus Ginger oleoresin post treated group-I

3.7 *Liver marker enzymes*

Increasing oxidative stress accelerated membrane disintegration of liver hepatocytes, which resulted in increased release of cytosolic liver enzymes.

3.7.1 *Effect of ginger oleoresin on serum aspartate aminotransferase (AST)*
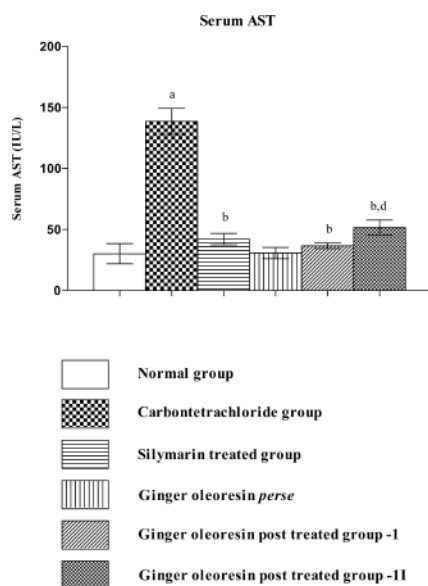
Hepatotoxicity was affirmed by a significant increase (p<0.0001) in AST activity in CCl_4 treated group compared with normal group. In treatment groups, 300mg/kg and 600 mg/kg ginger oleoresin post treatment significantly reduced (p<0.0001) AST level, and attained levels near the normal range. Among treatment doses of ginger oleoresin, 600mg/kg dose exhibited significant reduction (p<0.001) of AST in serum as compared to 300mg/kg dose. (Fig.12)

3.7.2 *Effect of ginger oleoresin on serum alanine aminotransferase (ALT)*

Significant increase in serum ALT (p<0.0001) in toxic group compared to normal group, indicating liver damage. CCl_4 induced elevation of serum enzyme activity, was remarkably prevented (p<0.0001) with post treatment of ginger oleoresin at 300mg/kg and 600mg/kg. Ginger oleoresin 600mg/kg post treatment exhibited significant reduction (p<0.0001) in ALT levels, when compared to ginger oleoresin 300mg/kg post treatment. (Fig.13)
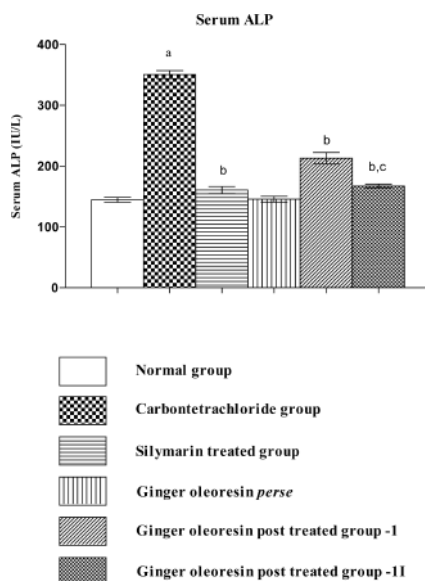
3.7.3 *Effect of ginger oleoresin on serum alkaline phosphatase (ALP)*

Elevated serum ALP level (p<0.0001) in toxic group, when compared to normal group, indicated liver damage. CCl_4

induced elevation of serum ALP activity, was markedly restored (p<0.0001) with post treatment of ginger oleoresin 300mg/kg and 600mg/kg doses. Dose dependent effects were observed with 600mg/kg ginger oleoresin.(Fig.14)

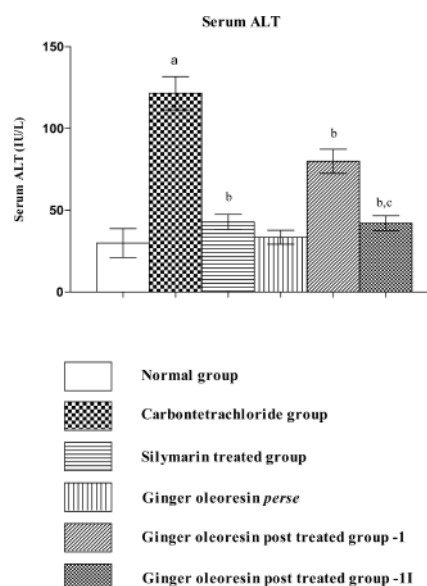**Figure 12:** Effect of ginger oleoresin on serum aspartate aminotransferase (AST) (IU/L)

Values were expressed as mean $\pm$ SD. a; P<0.0001 versus Normal group; b P<0.0001 versus Carbontetrachloride group; d p<0.001 versus Ginger oleoresin post treated group-I

Figure 13: Effect of ginger oleoresin on serum alanine aminotransferase (ALT) (IU/L)

Values were expressed as mean $\pm$ SD. a; P<0.0001 versus Normal group; b P<0.0001 versus Carbontetrachloride group; c p<0.0001 versus Ginger oleoresin post treated group-I

3.8 *Effect of Ginger Oleoresin on Rat Liver Morphology*

Administration of CCl_4 in ratsshowed change in colour of liver as well as enlargement of liver (Fig. 15B), when compared with normal group (Fig. 15A). Post treatment with ginger oleoresin group restored the change in colour and morphology (Fig. 15D, E).Silymarin being standard hepatoprotective drug showed morphologic changes near to normal (Fig. 15C).

3.9 *Histopathological Changes*

3.9.1 *Effect of Ginger Oleoresin on Liver Histopathology*

CCl_4 induced cell injury in livers by was confirmed by histopathological examinations. Photomicrographs of hematoxylin eosin stained liver tissues, normal control hepatocytes had normal architecture (Fig. 16A) as compared in rats treated with CCl_4 where severe hepatocyte necrosis, fatty degeneration, inflammatory cells infilteration and hydropic ballooning were observed (Fig. 16B). Post treatment of ginger oleoresin at 300mg/kg dose showed less marked effects (Fig. 16D). 600mg/kg dose significantly

Figure 14: Effect of ginger oleoresin on serum alkaline phosphatase (ALP) (IU/L)

Values were expressed as mean $\pm$ SD. a; P<0.0001 versus Normal group; b P<0.0001 versus Carbontetrachloride group; c p<0.0001 versus Ginger oleoresin post treated group-I

reduced the severity of injury in hepatocells as indicated in (Fig. 16E). Loss of hepatic architecture, marked necrosis, fatty degeneration, inflammatory cell infilteration and hepatocyte ballooning i.e. vacuolization were reduced to significant extent with 600mg/kg dose, when compared to

CCl_4 group. Post treatment of ginger oleoresin at 600mg/kg dose was exhibited more significant results, when compared to 300mg/kg ginger oleoresin dose. Silymarin being standard hepatoprotective drug showed histological changes near to normal (Fig. 16C).
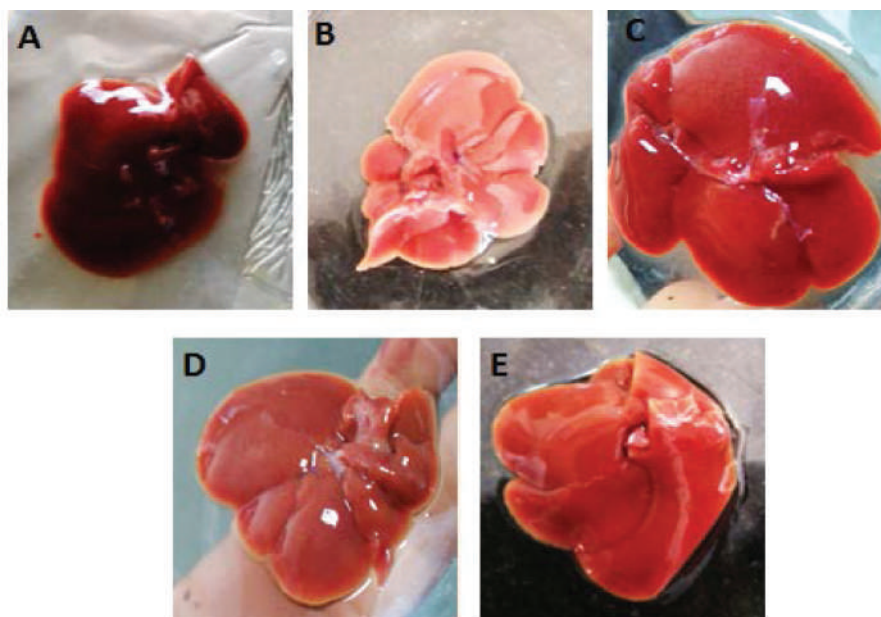
Figure 15: Rat liver morphology
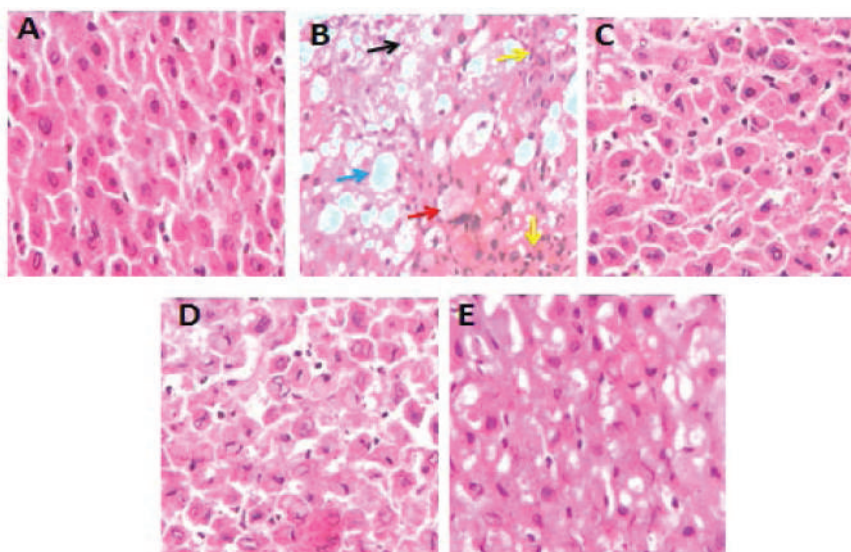
Group I : Normal group

Group III : Silymarin post treated group

Group V : Ginger oleoresin post treated group II

Group II : CCl_4-treated group

Group IV : Ginger oleoresin post treated group I

Figure 16: Effect of Ginger oleoresin on Liver Histology.

(A) Group I (Normal group): normal hepatic architecture and normal hepatocyteswith well-preserved cytoplasm, prominent nucleolus.

(B) Group II (CCl₄-treated group): marked loss of hepatic architecture, marked necrosis ($\longrightarrow$), fatty degeneration ($\longrightarrow$), Inflammatory cell infilteration ($\longrightarrow$), hepatocyte ballooning ($\longrightarrow$).

(C) Group III (CCl₄ + 100mg/kg of Silymarin post treated): showing preserved hepatic architecture.

(D) Group IV (CCl₄ + 300mg/kg of Ginger oleoresin post treated group I): less marked changes

(E) Group V (CCl₄+ 600mg/kg of Ginger oleoresin post treated group II): reversed the changes induced by CCl₄. Necrosis ($\longrightarrow$), fatty degeneration ($\longrightarrow$), inflammatory cells infilteration ($\longrightarrow$) and hydropic ballooning ($\longrightarrow$).

3.10 *Effect of Ginger Oleoresin on Genotoxicity Studies*

3.10.1 *Effect of ginger oleoresin on liver agnor count*

Administraion of CCl₄ significantly increased the region of AgNORs (Fig. 17B). Preventive efficacy of ginger oleoresin against CCl₄ administration in rat on AgNORs region is as shown in (Fig. 17 D and E). Ginger oleoresin treatment reduced the region of AgNOR. It also reduces the cell proliferation. Thus higher dose i.e. 600mg/kg ginger oleoresin lessens the AgNOR region and the irregularity of AgNORs when compared to 300mg/kg ginger oleoresin dose. Silymarin being standard hepatoprotective drug showed histological changes near to normal (Fig. 17C).
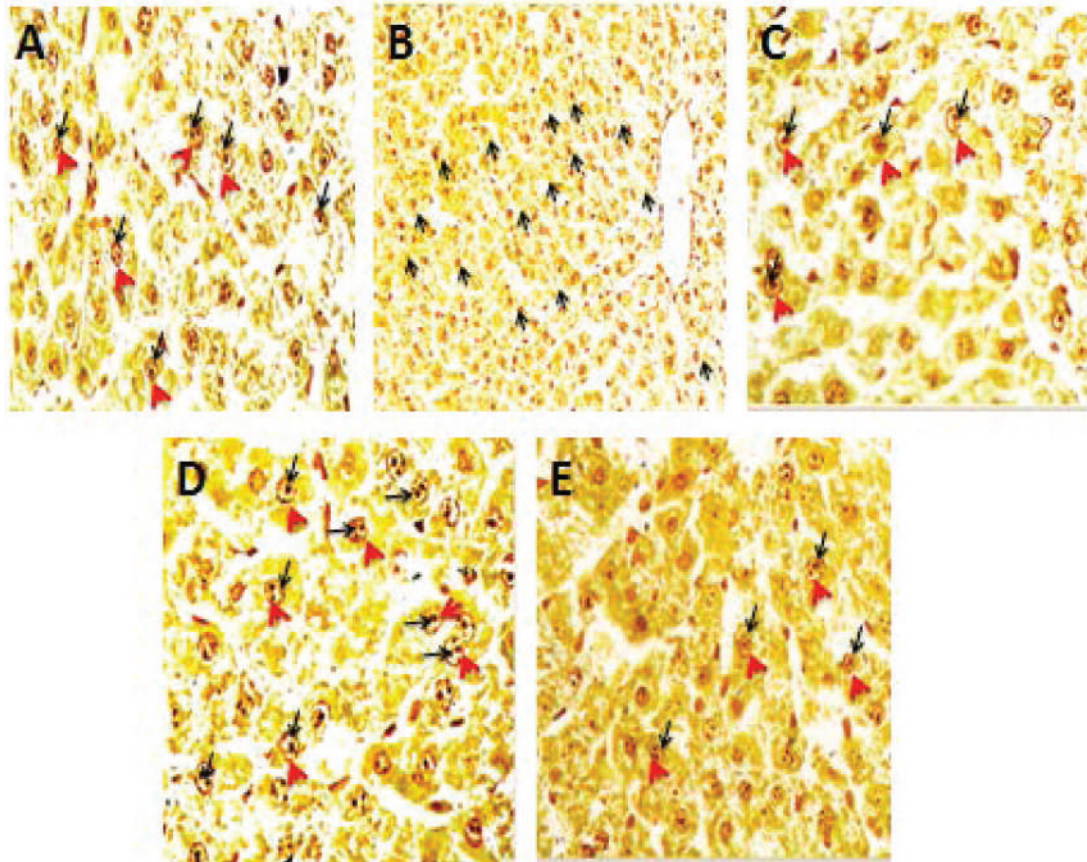
Figure 17: Effect of Ginger oleoresin on AgNORs Count.

(A) Group I (Normal group): Normal hepatic cells with nucleus ($\longrightarrow$) and AgNOR ($\longrightarrow$)

(B) Group II (CCl₄-treated group): Proliferation of cells and increase AgNORs region (dark spots)

(C) Group III (CCl₄ + 100mg/kg of Silymarin post treated): showing preserved hepatic nucleus and AgNOR

(D) Group IV (CCl₄ + 300mg/kg of Ginger oleoresin post treated group I): less marked changes

(E) Group V (CCl₄+ 600mg/kg of Ginger oleoresin post treated group II): decrease the region of AgNORs that induced by CCl₄
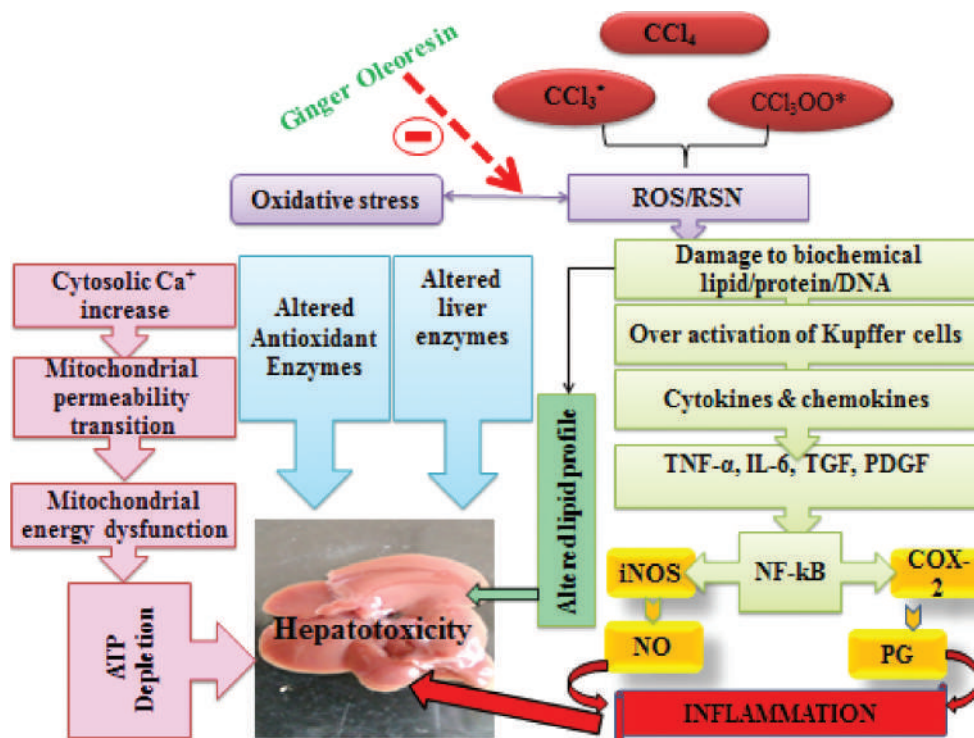
Figure 18 : Effect of ginger oleoresin on CCl4 induced hepatotoxicity

4. Discussion

Hepatotoxicity indicates damage to the cells, tissues, and structure or liver functions. Extraction of therapeutically effective agents from natural products may reduce the risk of hepatotoxicity. The present work showed the hepatoprotective effect of 300 mg/kg and 600mg/kg ginger oleoresin administered by oral route against CCl_4 induced liver injury in rats.

Currently millions of people are suffering from hepatic damage induced by alcohol, chemicals, drugs, and immune response. Metabolic activation is required for hepatotoxicants such as CCl_4, nitrosamines, and polycyclic aromatic hydrocarbons, by liver CYP P450 enzymes to form reactive toxic metabolites, which resulted in liver injury in experimental animals and human(Gonzalez *et al.,* 1988)To investigate the hepatotoxicity in animals by initating lipid Peroxidation , Liver injury induction by CCl_4 is a well-known hepatotoxin or classic model used extensively.Indeed in the present study, intraperitoneal administration of CCl_4 at the dose 2ml/kg produced significant hepatic damage in rats as assessed by observing body weight, liver weight, oxidative stress and inflammatory markers, lipid profile, liver marker enzymes, genotoxicity and histological changes. Ginger oleoresin in dose dependent manner was found successful in attenuating these changes. The efficacy of ginger oleoresin might be the contributory effects of 6-gingerol, 6-shogaol

and zingerone, which wielded intense hepatoprotective activity as suggested by reported studies (Flynn *et al.,* 1986; Alqasouami *et al.,* 2011; Hikino *et al.,* 1985).

After CCl_4administration, severe body weight loss and liver swelling revealed hepatic damage. Decreased food intake, appetite weight loss is an indirect indication of the declining hepatic function and also increase in the liver weight of animals is attributed to the formation of nodules. In addition, apathetic condition with emaciation and the rising of liver index (liver weight as a percentage of body weight) in rats was mostly exhibited in CCl_4 treated group. In present study, CCl_4-treated rats showed a marked obvious decrease in body weight, increase (p< 0.0001) in liver weight and liver index, when compared with the normal control group. However, treatment with ginger oleoresin resulted in improvement of the body weight and reduction in liver swelling as well as notable reduction in liver index (p<0.0001). The action might be due the bioactive ingredient 6-gingerol present in ginger oleoresin, which significantly decreased liver weight index as reported by (Tzeng *et al.,* 2015).

Generally, CCl_4 accumulated in parenchyma cells is metabolized by reductive dehalogenation through the microsomal cytochrome P450-dependent mono-oxygenase system, in the ER of hepatic cells to form trichloromethyl free radical (CCl_3*). CCl_3* which reacts rapidly with molecular oxygen to produce the trichloromethyl peroxyl

radical (CCl_3O_2*)(Weber *et al.,* 2003; Feng *et al.,* 2010). These radicals bind to proteins, lipids or abstract a hydrogen atom from an unsaturated lipid to initiate lipid peroxidation and thus liver damage, thereby contributing majorly to the pathogenesis of diseases (in fig.18)(Weber *et al.,* 2003; Recknagel *et al.,* 1989). Cyp2E1capacity to generate ROS and lipid peroxides it playsan vital role in the pathogenesis of liver injury (Gumieniczek, 2005).

Harmful modifications in DNA and proteins are the result of productsobtained from phase 1 reaction due electrophilic and highly reactive nature. Total protein is a common laboratory test to evaluate the effect of various toxic chemicals. Decrease in total protein content can be deemed as a useful index of severity of hepatocellular damage (Nevin and Vijayammal, 2005). The lowered level of total protein in the liver of CCl_4 treated rats revealed the severity of hepatopathy, which was in agreement with our study.(Taniyama and Griendling, 2003) Post treatment with ginger oleoresin at dose dependent manner increases the level of total protein. Stimulation of protein synthesis has been advanced as a contributory self-healing mechanism, which accelerates liver regeneration process, i.e. the production of liver cells (Rip *et al.,* 1985; Tadeusz *et al.,* 2001). Oxidative stress associated with increase in the formation of TBARS is indicative of lipid peroxidation. Oxidative stress has been adduced to be a crucial step in CCl_4 toxicity. CCl_3OO* is far more likely than CCl_3* to abstract a hydrogen from polyunsaturated fatty acids (PUFA)(Janero, 1990), thereby initiating the process of lipid peroxidation, which caused an increase in MDA levels (Manibusan *et al.,* 2007; Halliwell and Chirico, 1993). A major reactive aldehyde MDA appears during the peroxidation of biological membrane polyunsaturated fatty acid (Ohkawa *et al.,* 1979; Vaca *et al.,* 1988). The reactive species mediated hepatotoxicity with a rise in MDA level was effectively managed by administered ginger oleoresin, which possess anti-oxidant, free radical scavenger and anti-lipid peroxidant activities (Bellik, 2014; Verma *et al.,* 1983). The effects of ginger oleoresin can be due to bioactive constitutents such as 6-gingerol, 6-shogaol and zingerone. 6-gingerol increased the expression of antioxidant enzyme by free radical scavenging, lipid peroxidation inhibition, DNA protection and reducing power abilities indicating strong antioxidant properties (Singh *et al.,* 2009; Siddaraju and Dharmesh, 2007). 6-Shogoal was comparatively more significant in decreasing the hepatic MDA than 6 gingerol possibly due to its dominant antioxidant property(Dugasani *et al.,* 2010). Zingerone pretreatment significantly lowered the radiation-induced lipid peroxidation in terms of MDA production (Reddy and Lokesh, 1992).

MDA increased level in the liver of CCl_4-treated rats, further suggests thatto scavenge excessive free radicals, natural antioxidant defense mechanism has been compromised(Joseph *et al.,* 2014; Suresh and MIshra, 2008). Therefore, ROS affects the antioxidant defense mechanisms, reduced GSH, NADPH and antioxidant enzymes such as SOD, CAT. GR promotes the NADPH-dependent reduction of GSSG to GSH(Maritim *et al.,* 2003). Free radical scavengers and chain reaction terminators, enzymes such as SOD, CAT, and GPx systems helps in defending biological systems against the damaging effects of ROS by several means, including (Proctor and McGinness, 1986; Skrzydlewska and Farbiszewski, 1997; Huang *et al.,* 2012). Depletion of GSH pool by reactions catalysed by phase 2 enzymes, which promote the conjugation of phase 1 products with various hydrophilic moieties such as GSH and glucuronic acid initiated hepatic necrosis (Williams and Burk, 1990; Kobayashi and Yamamoto, 2006). CCl_4 led to significantreduction in the levels of GSH and NADPH, and in the activity of the GR (Moreira *et al.,* 2014). SOD and CATboth enzymatic antioxidant systems play a vital role in protection against the unwanted effects of lipid peroxidation andhydrogen peroxide in diseases related to oxidative stress (Webb and Twedt, 2008; Zhu *et al.,* 2012). It is well documented that the disturbance of the pro-oxidant/ antioxidant balance induced by CCl_4 is attributed to the massive amount of reactive free radicals generated during its metabolism.In present study results obtained were in agreement with above studies that treatment with CCl_4 reduced the level of NADPH, SOD, CAT and GSH in hepatic tissue (fig.18). Post treatment with ginger oleoresin protected the liver from damage in dose dependent manner by significantly increasing the hepatic content of NADPH, SOD, CAT and GSH. The action of ginger oleoresin was might be due to 6-gingerol and zingerone. 6-gingerol reduced blood sugar and ease oxidative stress due to stimulation of SOD, CAT, GPx and GSH antioxidant activities (Chakraborty *et al.,* 2012; Dugasani *et al.,* 2010). Zingerone that inhibited the decline in stable component of ginger oleoresin which increase the intracellular antioxidant enzyme levels viz., SOD, CAT, GSH and GST (Kumar *et al.,* 2014; Shin *et al.,* 2005).

Hepatotoxicity induced by CCl_4 lead to disruption of calcium homeostasis. CCl_4-intoxicated livers were found to have increased Ca^{2+} content and ROS production. Impaired calcium homeostasis activate many membrane damaging enzymes, like ATPases, phospholipases, proteases and endonuclease, and disruption of mitochondrial metabolism, decreased ATP synthesis and damage of micro-filaments that support cell structure (Deepa *et al.,* 2013; Hemmings *et al.,* 2002). Continued CCl_4 exposure, led to depletion in ATP supply and inactivates plasma membrane Na+, K+ - and Ca^{2+} ATPase activities; as Ca^{2+} rushes into the cell from outside, cytosolic concentrations rise in an uncontrolled manner (Fig.18). The involvement of microsomal Ca^{2+}-

ATPase in the regulation of cytosolic Ca^{2+} concentration and hormonal mechanisms is interconnected to other biochemical pathways responsible for the physiological well-being of the organisms. Therefore, reduction of Ca^{2+} ATPase activity in the present study by ginger oleoresin in dose dependent manner was highly significant, crucial and critical in preventing cytosolic calcium overload, alteration of calcium homeostasis and ultimately mitochondrial-mediated cell death inducible by CCl_4 toxicity.

Inflammatory processes contribute to a number of pathological events after exposure to various hepatotoxins as liver is a major inflammatory organ. Activation of Kupffer cells, the resident macrophages of liver by CCl_4, release pro-inflammatory cytokines such as TNFα, NO, TGF-ß, and CRP, IL-1, IL-6, and IL-10 (Mohamed *et al.,* 2014; Badger *et al.,* 1996).TNF-α accelerates the release of cytokines from other macrophages and induces a phagocyte oxidative metabolism and NO production (Morio *et al.,* 2001; Leist *et al.,* 1995). NO can exacerbate oxidative stress by reacting with ROS, particularly with the superoxide anion, and cause the formation of peroxynitrite. Moreover, overproduction of NO by iNOS may mediate CCl_4-induced acute hepatotoxicity through up-regulation of inflammatory responses (Li and Billiar, 1999). Inflammatory factorsgeneration is associated with NF-κB pathway in CCl_4-induced acute liver injury (Fig.18). In present study, administration of CCl_4 significantly caused increase in nitrite/nitrate level and CRP level, characterizing massive nitrosative stress due to hepato intoxication. However using ginger oleoresin dose dependently on lab animals with CCl_4-induced hepatopathy determined a decrease in nitrate/nitrite and CRP levels, suggesting a suppression of inflammatory response. Anti-inflammatory action observed by ginger oleoresin might be due to 6-Gingerol and 6-Shogaol which have been reported to inhibit NO production and reduce iNOS. 6-Gingerol scavenges peroxynitrite-derived radicals and inhibits peroxynitrite-induced oxidation and nitration reactions(Ippoushi *et al.,* 2005). 6-shogaol has been reported to inhibit inflammation in diclofenac induced hepatotoxicity (Flynn *et al.,* 1986; Pan *et al.,* 2008; Mascolo *et al.,* 1989).

Significant increase of lipid profile indicated a severe lipid peroxidation and physiological damage to organs after administering CCl_4. CCl_4 increases denovo synthesis of fatty acids and triglycerides from acetate and also the rate of lipid esterification (Boll *et al.,* 2001). Andrițoiu *et al.,* showed that an increase of the parameters of the lipid profile was observed in the animals with CCl_4-induced hepatopathy, concerning total cholesterol, triglycerides, VLDL and also lowering HDL.[12] Similarly the present study also showed, an increase of the parameters of the lipid profile was observed in the animals induced with CCl_4.There was significant increase

in the cholesterol and triglyceride levels, whereas HDL was reduced due to hepatic injuries caused by their free radicals. Treatment with ginger oleoresin determined a regulation of these parameters. Increased HDL, decreased triglycerides and cholesterol levels were noticed in lab animals. Post treatment with 600mg/kg dose of ginger oleoresin was of more potential. Again the action might be due to its active constituent 6-gingerol 6-gingerol significantly decreased liver weight index and restored the levels of triglycerides, total cholesterol and free fatty acid in the plasma and livers which may be the primary protective mechanism exerted by the compound in high fat diet fed hamsters (Tzeng *et al.,* 2015).

Increased serum levels of ALT, AST and ALP in livers induced with CCl_4 toxicity is an indication of damaged structural and functional integrity of the liver cell membranes since, these cytosolic enzymes are only released into circulation after hepatic cellular damage (Zhou *et al.,* 2010). CCl_4 could result in defective excretion of bile by hepatocytes and so increased ALP levels could reflect a pathological alteration in biliary flow (Rajesh and Latha, 2004). In this study, a significant increase in serum ALT, AST and ALP activity was observed after CCl_4 administration. However, the serum ALT, AST and ALP activities significantly declined by treatment with ginger oleoresin in dose dependent manner and silymarin, implying that the ginger oleoresin can rise up stabilization of plasma membrane and ameliorate biliary dysfunction effectively there by preserving the structural integrity of cells. Again the contribution of 6-Shogoal and 6- gingerol cannot be ruled out as 6-Gingerol-rich fraction help in restoring the biomarkers ALT, AST, ALP, and GGT, sensitive indicators of hepatocellular damage and dysfunction (Salihu *et al.,* 2016; Sabina *et al.,* 2011), whereas 6-Shogoal, exhibited a significant hepatoprotective activity by reducing serum activities of AST, ALT and ALP, when compared to diclofenac sodium intoxicated rats(Alqasouami *et al.,* 2011).

Histopathology is an important clinical standard for the diagnosis of hepatic damage. Thus, CCl_4-treatment caused massive histopathological changes such as necrosis, fatty degeneration, inflammatory cells infilteration and hydropic ballooning (Moreira *et al.,* 2014; Al-Sayed *et al.,* 2014). Biochemical improvements after ginger oleoresin treatment were paralleled by histopathological findings. According to microscopic examinations, severe liver damage induced by CCl_4 was markedly reduced by the administration of ginger oleoresin, which was good correlation with the results of serum enzymes activities, hepatic antioxidant enzyme activities, anti-inflammatory activities, hypolipidemic and hepatic lipid peroxidation. It was evident from the absence of cellular necrosis, hydropic ballooning, fatty degeneration and inflammatory cells infilteration, normalization of

cellular structures in the liver section of treatment doses especially in 600mg/kg post treated group. Silymarin drug at the dose of 100mg/kg; *p.o.* for 14 days produced significant histological changes in rat liver. Present results suggest that the activities of ginger oleoresin were responsible for the normalization of hepatic function at the biochemical and structural level. Silymarin, an established antihepatotoxic drug at the dose of 300mg/kg; *p.o.* for 14 days produced significant biochemical and histological changes.

5. Conclusion

Present study demonstrated that hepatoprotective effects of ginger oleoresin dueto its ability to reduce the rate of lipid peroxidation, increased antioxidant defense status, anti-inflammatory, hypolipidemic activity and to guard against the pathological changes of the liver induced by CCl_4 intoxication. The hepatoprotective activity of Ginger oleoresin is concluded to be partly mediated by its active constituent's 6-gingerol, shogaol and zingerone which showed superior effect on boosting the antioxidant capacity, anti-inflammatory and hypolipidemic activity. Thus the study demonstrated experimental evidences and clearly justifies the traditional claims and use of Ginger oleoresin in the treatment of liver diseases.

Conflict of Interest

The authors declare no potential conflict of interest.

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