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Soy Diet Diminish Oxidative Injure and Early Promotional Events Induced by CCl_4 in Rat Liver

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Abstract: In this study, we have examines the impact of dietary soy on CCl_4 induced oxidative stress, early tumor promoter markers and liver toxicity in male wistar rats. The dietary exposure mimics the repetitive nature of soy intake in human diets. Administration of soy flour (10 and 15% as diet) seven consecutive days as preventive intervention suppresses the lipidperoxidation (LPO), xanthine oxidase (XO) and release of serum toxicity marker enzymes viz., SGOT, LDH and SGPT significantly ($p<0.001$). Hepatic antioxidant status viz., reduced glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPx), quinone reductase (QR), catalase (CAT) was concomitantly restored in soy flour pre-treated groups ($p<0.001$). In addition, soy diet pre-treatment also prevented the CCl_4 enhanced ornithine decarboxylase (ODC) and hepatic DNA synthesis significantly ($p<0.001$). In conclusion, Carbon tetrachloride-induced liver toxicity was markedly attenuated by soy flour pre-treatment and study gives some insight of mechanisms involved in diminution of free radical generating toxicants and enhancement of antioxidant armory, hence preventing further tissue damage, injury and hyper-proliferation.

Key words: Soy bean diet, antioxidants, CCl_4, tumor promotion, ODC

INTRODUCTION

Oxidative stress occurs when there is an imbalance between Reactive Oxygen Species (ROS) formation and scavenging by antioxidants. ROS can cause oxidative damage to biomolecules resulting mutagenesis and carcinogenesis (Aggarwal *et al*., 2009; Rajendran *et al*., 2008; Jahan *et al*., 2011). ROS has been implicated in many degenerative diseases, collectively with aging and cancer (Aggarwal *et al*., 2009; Hamilton, 2007; Morse and Stoner, 1993; Mohamed *et al*., 2011; Avci *et al*., 2005). Carbon tetrachloride induced hepatotoxicity via hepatocellular fatty degeneration and centrilobular necrosis (Ha and Lee, 2003). The widely accepted mechanism of CCl_4^- initiated liver injury involves the bioactivation of CCl_4 by the cytochrome P_{450} mediated reactions to the CCl_3 free radical which is further converted to a peroxy radical, CCl_3O_2. These free radicals readily react with polyunsaturated fatty acids to initiate lipid peroxidation. Peroxy radicals can react with polyunsaturated fatty acids in presence of cellular O_2, to initiate series of self-propagating chain reactions that lead to lipid peroxidation (Ha and Lee, 2003; Das *et al*., 2004; Alisi *et al*., 2011; Dhanasekaran and Ganapathy, 2011).

Antioxidants protect cells from free radical mediated oxidative damage. Cellular damage initiated by free radicals has been implicated in the development of cancer (Khan *et al*., 2006; Khan and Sultana, 2006; Khan and Sultana, 2011; Ramesh *et al*., 2007; Ali *et al*., 2010; Onocha *et al*., 2011). It is essential to identify antioxidant, anti-promoting agents present in human diet. Therefore, we have been constantly working on the identification of chemopreventive plants (Khan *et al*., 2006; Khan and Sultana, 2011, 2004a, 2004b, 2005; Khan *et al*., 2005; Khan and Sultana, 2009). The significance of nutrition in protecting living organisms from the toxic effects of environmental carcinogens has been realized (Aggarwal *et al*., 2009; Onocha *et al*., 2011; Gourineni *et al*., 2010). Herbal products are gaining increasingly attention due to less toxicity and high efficacy against various diseases (Khan *et al*., 2006; Hudecava and Ginter, 1992; Gourineni *et al*., 2010). Soybeans are unique foods, contains complex carbohydrates, protein, dietary fiber, oligosaccharides and isoflavones (Lichtenstein, 1998). Soybean decreases the risk of various diseases and pathological conditions, including various types of cancers, osteoporosis, menopausal symptoms and coronary heart disease

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(Archer 1988; Barnes, 2010; Nagata, 2010; Steinmetz and Potter, 1991; Pugalendhi and Manoharan, 2010). The chemopreventive effects of soybean and soy containing food may be related to genistein, daidzein and glycitein (Archer, 1988; Nagata, 2010; Messina and Barnes, 1991; Wei *et al*., 1995). These isoflavones may be conjugated with a 7-O-b-glucoside, a 6''-O-malonylglucoside or a 6''-O-acetylglucoside group which play an important role in the prevention atherosclerosis, hypertension (Park *et al*., 2005), breast cancer (DiSilvestro *et al*., 2005) and inflammatory bowel syndrome (Wiseman, 2006). Flavonoids have gained importance as scavengers of free radicals and a potent inhibitor of lipidperoxidation (Khan and Sultana, 2006; Bors *et al*., 1990; Hodgson *et al*., 1996). Isoflavones show DNA topoisomerase activity, synthesis and release of TGF^{β}, modulation of apoptosis and potent inhibitory activity of tyrosine specific protein kinases (Barnes, 2010; Nagata, 2010; Birt *et al*., 2001). It has been reported that population having high intake of isoflavones show lower incidence of cardiovascular diseases, osteoporosis, kidney diseases and cancer risk (Bors *et al*., 1990; Nagata, 2010).

Keeping in view of complex health promoting potential of soybeans and soy foods we have assumed that the soy diet may inhibit oxidative stress and toxicity induced by CCl_4.

MATERIALS AND METHODS

Chemicals: EDTA, Tris, Reduced glutathione (GSH), oxidized glutathione (GSSG), nicotinamide adenine dinucleotide phosphate reduced (NADPH), Bovine Serum Albumin (BSA), 1,2-dithio-bisnitrobenzoic acid (DTNB), 1-chloro-2, 4-dinitro benzene (CDNB), phenyl methylsulfonylfluoride (PMSF), nitrobluetetrazolium (NBT), phenylmethylsulfonylfluoride (PMSF), Brij-35, Pyridoxal-phosphate, 2-mercaptoethanol, dithiothreitol, Tween 80 were obtained from Sigma chemicals Co (St Louis, MO). DL [^{14}C] ornithine and [^{3}H] thymidine were purchased from Amersham Corporation (Little Chalfort, UK). All other chemicals were of the highest purity and commercially available.

Animals: Eight week old adult male wistar rats (150-200 g) were obtained from the Central Animal House Facility of Hamdard University, New Delhi and were housed in a ventilated room at 25±2°C under a 12 h light/dark cycle. The animals were acclimatized for one week before the study and had free access to standard laboratory feed (Hindustan Lever Ltd., Bombay, India) and water ad libitum. All procedures using animals were reviewed and approved by the Institutional Animal Ethical Committee that is fully accredited by the Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA) Chennai, India.

Experimental design: The treatment regimen for soy diet was based on the preliminary studies carried out in our laboratory. To study the biochemical, serological changes 25 male wistar rats were divided into five groups. Group I served as saline treated control. Group II served as negative control and was administered CCl_4 (1 mL kg^{-1} body weight p.o., in corn oil [1:1]). Group III served as positive control and was given higher dose (D_2) of soy diet for seven consecutive days. Groups IV and V were pretreated with soy diet at doses 10 and 15% soy flour as diet for seven consecutive days followed by CCl_4 intoxication on the 7th day. All animals were sacrificed 24 h after CCl_4 intoxication. Serum was separated and stored at 4°C for the estimation of GOT, GPT and LDH. Tissue was processed for the estimation of hepatic ornithine decarboxylase (ODC) activity and other biochemical parameters. For [^{3}H] thymidine incorporation study, same treatment regimen was followed except all the animals were given intraperitoneal [^{3}H] thymidine (25 μCi per animal) 2 h before killing. Time of sacrifice was after 48 h of CCl_4 intoxication; liver sections were quickly excised, rinsed with ice-cold saline, freed of extraneous material and processed for the quantification of [^{3}H] thymidine incorporation into the hepatic DNA.

Biochemical estimations: Tissue processing and preparation of Post Mitochondrial Supernatant (PMS) were done as described by Athar and Iqbal (1998). All the biochemical estimations were completed within 24 h of animal sacrifice.

Estimation of reduced glutathione: Reduced glutathione was determined by the method of Jollow *et al*. (1974). A 1.0 mL sample of PMS was precipitated with 1.0 mL of sulfosalicylic acid (4%). The samples were kept at 4°C for one hour and then centrifuged at 1200 g for 20 min at 4°C. The assay mixture contained 0.4 mL supernatant, 2.6 mL sodium phosphate buffer (0.1 M, pH 7.4) and 0.2 mL DTNB (100 mM) in a total volume of 3.0 mL. The yellow colour developed, was read immediately at 412 nm on a spectrophotometer.

Catalase activity: Catalase activity was measured by the method of Clairborne (1985). The reaction mixture consisted of 2 mL phosphate buffer (0.1 M, pH 7.4), 0.95 mL hydrogen peroxide (0.019 M) and 0.05 mL PMS in a final volume of 3 mL. Changes in absorbance were

recorded at 240 nm. Catalase activity was calculated as nmol H_2O_2 consumed min^{-1} mg^{-1} protein.

Glutathione-S-transferase activity: Glutathione-S-transferase activity was estimated by the method of Habig *et al.* (1974).The reaction mixture consisted of 1.425 mL sodium phosphate buffer (0.1 M, pH 7.4), 0.2 mL reduced glutathione (1 mM), 0.025 mL CDNB (1 mM) and 0.3 mL PMS (10% w/v) in a total volume of 2.0 mL. The changes in the absorbance were recorded at 340 nm and enzymes activity was calculated as nmol CDNB conjugate formed min^{-1} mg^{-1} protein using a molar coefficient of 9.6×10^3 M^{-1} cm^{-1}.

Glutathione reductase activity: Glutathione reductase activity was assayed by the method of Carlberg and Mannervik (1975), as modified by Mohandas *et al.* (1984). The assay system consisted of 0.1 M, pH 7.4 sodium phosphate buffer, 0.5 mM EDTA, 1 mM GSSG, 0.1 mM NADPH and PMS (10% w/v) in a total mixture of 2.0 mL. The enzyme activity was measured at 340 nm and calculated using a molar extinction coefficient of 6.22×10^3 M cm^{-1}.

Glutathione peroxidase activity: Glutathione peroxidase activity was assayed by the method of Mohandas *et al.* (1984), as described by Athar and Iqbal (1998). The assay mixture consisted of 0.1 M, pH 7.4 sodium phosphate buffer, 1 mM EDTA, 0.2 mM NADPH, 1 mM sodium azide, 1 IU mL^{-1} glutathione reductase, 0.25 mM H_2O_2 and PMS (10% w/v) in a total volume of 2.0 mL. The activity was recorded at 340 nm and calculated using a molar extinction coefficient of 6.22×103 M^{-1} cm^{-1}.

Glucose-6-phosphatedehydrogenaseactivity: Theactivity of glucose-6-phosphate dehydrogenase was assayed by the method of Zaheer *et al.* (1965). The reaction mixture consisted of 0.3 mL tris-HCl buffer (0.05 M, pH 7.6), 0.1 mL NADP (0.1 mM), 0.1 mL glucose-6-phosphate (0.8 mM), 0.1 mL $MgCl_2$ (8 mM), 0.3 mL PMS and 2.4 mL distilled water in a total volume of 3 mL. The changes in absorbance were recorded at 340 nm and enzyme activity was calculated as nmol NADPH oxidized min^{-1} mg^{-1} protein using a molar extinction coefficient of $6.22\times10^3M^{-1}$ cm^{-1}.

Quinone reductase activity: Quinone reductase activity was measured by the method of Benson *et al.* (1980). The assay mixture consisted of 0.1 mL cytosolic fraction (10%), 0.7 mL of BSA (0.1%), 0.02 mL tween-20 (1%), 0.1 mL of FAD (150 M), 0.02 mL of NADPH (0.2 mM), 0.05 mL of 2,6, DCIP (0.29%) and 2 mL of tris-HCl buffer (25 mM, pH 7.4) with a final volume of 3 mL and the optical density was read at 600 nm for 3 min. The enzyme activity was calculated as nmol 2,6, DCIP reduced min^{-1} mg^{-1} protein.

Xanthine oxidase activity: The activity of xanthine oxidase was assayed by the method of Stirpe and Corte (1969). The reaction mixture consisted of 0.2 mL PMS which was incubated for five minutes at room temperature with 0.8 mL phosphate buffer (0.1 M, pH 7.4). Then 0.1 mL xanthine (9 mM) was added to the reaction mixture and kept at 37°C for 20 min which was followed by the addition of 0.5 mL of 10% perchloric acid and 2.4 mL of double distilled water in a total volume of 4 mL. After 10 min, the mixture so obtained was centrifuged at 4000-rev min^{-1} for 10 min and µg uric acid formed min^{-1} mg^{-1} protein was recorded at 290 nm.

Estimation of lipid peroxidation: The assay of microsomal lipid peroxidation was done according to the method of Wright *et al.* (1981). The reaction mixture consisted of 0.58 mL phosphate buffer (0.1 M, pH 7.4), 0.2 mL microsome, 0.2 mL ascorbic acid (1 mM) and 0.02 mL ferric chloride (100 mM) in a total volume of 1 mL. The mixture was incubated at 37°C in a shaking water bath for 1 h. Then 1 mL 10% trichloroacetic acid and 1 mL 0.67% TBA was added. All the tubes were placed in a boiling water bath for 20 min. The tubes were placed in an ice bath and then centrifuged at 2500 g for 10 min. The amount of malanodialdehyde (MDA) formed in each of the samples was assayed by measuring the optical density of the supernatant at 535 nm.The results were expressed as nmol MDA formed h^{-1} g^{-1} tissue at 37°C using a molar extinction coefficient of $1.56\times10^5M^{-1}$ cm^{-1}.

Lactate dehydrogenase (LDH) activity: Lactate dehydrogenase activity was estimated in serum by the method of Kornberg (1955). The reaction mixture consisted of serum, NADH (0.02 M), Sodium pyruvate (0.01 M), sodium phosphate buffer (0.1 M, pH 7.4) and distilled water in a total volume of 3.0 mL. The changes in the absorbance were recorded at 340 nm and enzyme activity was calculated as nmol NADH oxidized min^{-1} mg^{-1} protein.

Determination of serum oxaloacetate and pyruvate transaminases (GOT and GPT): Serum GOT and GPT were determined by the method of Reitman and Frankel (1957). Ach substrate (0.5 mL) (2 mM α-ketoglutarate and either 200 mM α L-Alamine or L-Aspartate was incubated for 5 min at 37°C in a water bath. Serum (0.1 mL) was then added and the volume was adjusted to 1.0 mL with sodium

phosphate buffer. The reaction mixture was incubated for exactly 30 and 60 min for GPT and GOT, respectively. Then to the reaction mixture, 0.5 mL of DNPH (1 mM) was added and left for another 30 min at room temperature. Finally, the colour was developed by addition of 5.0 mL of NaOH (0.4 N) and product read at 505 nm.

Estimation for tumor markers

Ornithine decarboxylase activity: ODC activity was determined using 0.4 mL hepatic 105,000 g supernatant fraction per assay tube by measuring the release of CO_2 from DL-[^{14}C] ornithine by the method of O'Brien *et al.* (1975). The liver was homogenized in Tris-HCl buffer (pH 7.5, 50 mM) containing EDTA (0.4 mM), pyridoxal phosphate (0.32 mM), PMSF (0.1 mM), 2-mercaptoethanol (1.0 mM), dithiothreitol (4.0 mM) and Tween 80 (0.1%) at 4°C using a Teflon-glass homogenizer. In brief, the reaction mixture contained 400 µL enzymes and 0.095 mL co-factor mixture containing pyridoxal phosphate (0.32 mM), EDTA (0.4 mM), dithiothreitol (4.0 mM), ornithine (0.4 mM), Brij 35 (0.02%) and DL-[^{14}C] ornithine (0.05 µCi) in total volume of 0.495 mL. After adding buffer and cofactor mixture to blank and others tubes, the tubes were closed immediately with a rubber stopper containing 0.2 mL ethanolamine and methoxyethanol mixture (2:1) in the central well and kept in water-bath at 37°C. After 1 h of incubation, injecting 1.0 mL citric arrested the enzyme activity acid solution (2.0 M) along the sides of glass tubes and the solution was continued for 1 h to ensure complete absorption of CO_2. Finally, the central well was transferred to a vial containing 2 mL ethanol and 10 mL toluene based scintillation fluid. Radioactivity was counted in liquid scintillation counter (LKB Wallace-1410). ODC activity was expressed as pmol CO_2 released h^{-1} mg^{-1} protein.

Hepatic DNA synthesis: The isolation of hepatic DNA and incorporation of [^{3}H] thymidine in DNA was done by the method of Smart *et al.* (1986). Liver was quickly removed cleaned free of extraneous material and homogenate (10% w/v) was prepared in ice-cold water. The precipitate thus obtained was washed with cold trichloroacetic acid (TCA) (5%) and incubated with cold perchloric acid (PCA) (10%) at 4°C for overnight. After the incubation it was centrifuged and the precipitate was washed with cold PCA (5%). The precipitate was dissolved in warm PCA (10%) followed by incubation in boiling water bath for 30 min and filtered through Whatman 50. The filtrate was used for [^{3}H] thymidine counting in liquid scintillation counter (LKB-Wallace-1410) by adding the scintillation fluid. The amount of DNA in the filtrate was estimated by diphenylamine method of Giles and Myers (1965). The amount of [^{3}H] thymidine incorporated was expressed as DPM μg^{-1} DNA.

Protein estimation: Protein content in all samples was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Statistical analysis: The level of significance between different groups is based on ANOVA test followed by Dunnett's t test.

RESULTS

CCl_4 intoxication leads to depletion of hepatic glutathione, its metabolizing enzymes GST and GR and antioxidant enzymes CAT, GPx, QR and G6PD by ($p<0.001$), respectively as compared with the saline treated control group. CCl_4 also caused elevation in the activity of XO and H_2O_2 content and increase in the levels of MDA formation and hepatic toxicity markers SGOT and SGPT by ($p<0.001$). Pretreatment with soy diet (10 and 15%) restored hepatic glutathione content and its dependent enzymes GST and GR significantly ($p<0.001$) as shown in Table 1. Other antioxidant enzymes like CAT GPx, G6PD and QR were significantly restored at ($p<0.001$) as shown in Table 2.

There was a marked depletion in levels of XO and MDA formation at ($p<0.001$) and concomitant down regulation of release of SGOT and SGPT in serum at ($p<0.001$). Figure 1 and 2 show the significant inhibition of early markers of tumor promotion like ODC activity ($p<0.001$) and hepatic DNA synthesis ($p<0.005$) in rat. The prophylactic treatment of animals with soy diet against CCl_4 induced elevation in XO level, H_2O_2 and MDA formation is shown in Table 3. Prophylactic administration of soy diet prior to CCl_4 intoxication significantly decreased the MDA levels at significance ($p<0.001$),

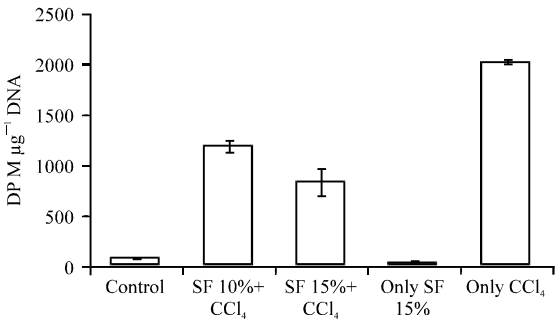

Fig. 1: Effect of pretreatment of SF on [^{3}H] thymidine incorporation in hepatic DNA

Table 1: Effect of pretreatment of soy diet on the CCl_4 mediated depletion in the activity of glutathione content and its metabolizing enzymes in the liver of wistar rats

Treatment groups	Reduced glutathione (μ moles GSH reduced/hr/gm tissue)	Glutathione reductase (n moles NADPH oxidized/min/mg protein)	Glutathione-S-transferase (nmol CDNB conjugate formed/min/mg protein)
Group I normal pellet diet	0.461±0.0028	194.87±6.26	210.85±9.84
Group II Only CCl_4 (1 mL kg^{-1} b.wt)	0.202±0.0045***	98.60±5.21***	97.21±5.11***
Group III 15% Soy flour	0.5194±0.0063	199.99±6.52	213.56±11.05
Group IV 10% Soy flour+CCl_4 (1 mL kg^{-1} b.wt)	0.257±0.0029$^{\#\#}$	125.53±3.14$^{\#\#}$	147.17±4.68$^{\#\#\#}$
Group V 15% Soy flour+CCl_4 (1 mL kg^{-1} b.wt)	0.316±0.0037$^{\#\#}$	160.57±7.50$^{\#\#}$	170.84±3.31$^{\#\#\#}$

*p≤0.05, **p≤0.01 and ***p≤0.001 on comparison of normal control with CCl_4 treated group and only soy group. $^{\#}$p≤0.05 $^{\#\#}$p≤0.01 and $^{\#\#\#}$p≤0.001 on comparison of soy diet treated groups with only CCl_4 treated group

Table 2: Effect of pretreatment of soy diet on the CCl_4 mediated depletion in the activity of antioxidant enzymes in the liver of wistar rats

Treatment groups	Catalase (n moles H_2O_2 consumed/min /mg protein)	Glutathione peroxidase (n moles NADPH oxidized/min/mg protein)	Glucose-6-phosphate Dehydrogenase (nmoles NADP reduced/min/mg protein)	Quinone reductase (nmolesdichloroindophenol reduced/min/mg protein)
Group I normal pellet diet	187.83±1.88	249.04±4.87	63.62±2.37	273.20±1.98
Group II only CCl_4 (1 mL kg^{-1} b.wt)	82.87±0.77***	130.75±6.94***	19.91±0.63***	124.75±1.47***
Group III 15% soy flour	188.86±3.29	255.41±2.44	68.55±1.57	271.42±5.33
Group IV 10% soy flour+CCl_4 (1 mL kg^{-1} b.wt)	118.41±1.58$^{\#\#\#}$	163.54±4.80$^{\#\#}$	27.71±0.99$^{\#\#\#}$	188.98±2.24 $^{\#\#\#}$
Group V 15% soy flour+CCl_4 (1 mL kg^{-1} b.wt)	143.26±2.29$^{\#\#\#}$	181.78±3.62 $^{\#\#\#}$	38.65±1.36$^{\#\#\#}$	229.34±1.73$^{\#\#\#}$

*p≤0.05, **p≤0.01 and ***p≤0.001 on comparison of normal control with CCl_4 treated group and only soy group. $^{\#}$p≤0.05, $^{\#\#}$p≤0.01 and $^{\#\#\#}$p≤0.001 on comparison of soy diet treated groups with only CCl_4 treated group

Table 3: Effect of pretreatment of soy diet on the CCl_4 mediated alteration in the activities of xanthine oxidase and enhancement of lipid peroxidation in the liver of wistar rats

Treatment groups	Xanthine oxidase (μg uric acid formed/min/mg protein)	Lipid peroxidation (n moles MDA formed/min/mg protein)
Group I normal pellet diet	0.273±0.0290	3.86±0.153
Group II only CCl_4 (1 mL kg^{-1} b.wt)	0.543±0.0009***	15.05±0.078***
Group III 15% soy flour	0.270±0.0057	4.30±0.194
Group IV 10% Soy flour+CCl_4 (1mL kg^{-1} b.wt)	0.415±0.0026$^{\#\#}$	11.74±0.113$^{\#\#}$
Group V 15% Soy flour+CCl_4 (1mL kg^{-1} b.wt)	0.373±0.0028$^{\#\#}$	9.83±0.109$^{\#\#}$

*p≤0.05, **p≤0.01 and ***p≤0.001 on comparison of normal control with CCl_4 treated group and only soy group. $^{\#}$p≤0.05, $^{\#\#}$p≤0.01 and $^{\#\#\#}$p≤0.001 on comparison of soy diet treated groups with only CCl_4 treated group

Table 4: Effect of pretreatment of soy diet on the CCl_4 mediated enhancement of liver markers in wistar rats

Treatment groups	SGPT (I.U/lt)	SGOT (I.U/lt)	LDH (nmol NADH oxidized/min/mg protein)
Group I normal pellet diet	18.63±0.453	25.67±0.334	369.05±4.90
Group II only CCl_4 (1 mL kg^{-1} b.wt)	69.59±0.319***	78.86±1.199***	577.39±6.44***
Group III 15% Soy flour	20.02±0.479	26.01±0.598	357.15±3.75
Group IV 10% Soy flour+CCl_4 (1mL kg^{-1} b.wt)	34.60±0.172$^{\#\#}$	58.93±0.549$^{\#\#}$	395.72±1.87$^{\#\#}$
Group V 15% Soy flour+CCl_4 (1 mL kg^{-1} b.wt)	28.48±0.319$^{\#\#}$	45.12±0.317$^{\#\#}$	387.86±2.75$^{\#\#}$

*p≤0.05, **p≤0.01 and ***p≤0.001 on comparison of normal control with CCl_4 treated group and only soy group. $^{\#}$p≤ 0.05, $^{\#\#}$p≤ 0.01 and $^{\#\#\#}$p≤0.001 on comparison of soy diet treated groups with only CCl_4 treated group

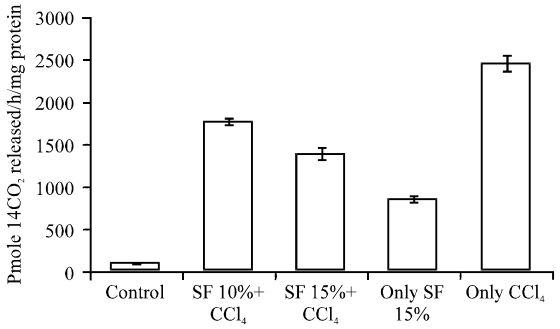

Fig. 2: Effect of pretreatment of SF on ODC activity in liver

respectively in a dose dependent manner. However, soy diet alone group produced results near to saline control values. Soy diet showed a significant protection of GSH content by p<0.001 in a dose dependent manner is shown in Table 1. The effect of prophylactic administration of soy diet on CCl_4 mediated leakage of liver marker enzymes in serum is shown in Table 4. Marked inhibition was recorded in serum toxicity markers in soy diet treated groups at (p<0.001). The effect of prophylactic treatment of rats with soy diet on CCl_4 induced rate of [^{3}H] thymidine incorporation into hepatic DNA has been shown in Fig. 2. Intoxication with CCl_4 resulted in significant (p<0.005) increase in the rate of [^{3}H] thymidine incorporation into hepatic DNA and significant (p<0.001) increase in ODC activity which is an early marker of tumor promotion. The prophylactic treatment of

rats with soy diet showed a marked ($p<0.001$) suppressing effect on the rate [^{3}H] thymidine incorporation into hepatic DNA of treated control and simultaneous and significant ($p<0.001$) inhibition of ODC activity was recorded in a dose dependent manner as shown in Fig. 1 and 2.

DISCUSSION

Single oral CCl_4 dose induced significant depletion in the hepatic GSH content and its metabolizing enzymes. Glutathione is often considered as first line of defense against oxidative damage (Ali-Osman, 1989; Patrick-Iwuanyanwu and Wegwu, 2008). There was significant restoration of glutathione and dependent enzymes in soy diet pretreated groups. The restoration of depleted GSH and GST on pretreatment of animals with soy diet indicate its role as chemopreventive measure. CCl_4 causes increased formation of pro-oxidants and a concomitant decrease in the antioxidant status of the cells. Hepatotoxic effects of CCl_4 depend on the major unstable radical trichloromethyl radical which reacts with oxygen molecule and form trichloromethylperoxy radical which is reported to be highly reactive. These free radicals covalently bind to macromolecules and proteins and thus initiating a site for secondary biochemical process which ultimately leads to several pathological conditions of CCl_4 metabolism (Singh *et al*., 2005; Hassan *et al*., 2008). It has been suggested that Soy diet protects by suppressing CCl_4 mediated toxicity through decrease in levels of MDA formation and inhibition in hepatic XO levels. GST is responsible for GSH conjugation with various electrophiles and plays an important role in detoxification of various xenobiotics (Touliatos *et al*., 2000). Induction of GST by Soy diet could be one possible mechanism to combat hepatic oxidative stress (Singh *et al*., 2000; Dhanasekaran and Ganapathy, 2011). QR catalyzes hydroquinone formation from quinones, preventing cyt P_{450} dependent activation to toxic semiquinones and ultimate generation of Reactive Oxygen Species (ROS) (Khan and Sultana, 2005). Well documented correlation exists between its modulation and chemoprevention of cancer Substantial depression in hepatic GSH with concomitant decrease in GR, GPx, QR, GST and G6PD levels on CCl_4 administration; however, dose dependent marked restoration was observed with prophylactic treatment of Soy diet.

The mechanisms by which Soy diet attenuates hepatic damage induced by CCl_4 can be attributed to recovery of antioxidant status. Previous studies have shown that Soy potential to increase cellular antioxidants like GSH and other phase II metabolizing enzymes (Khan and Sultana, 2004a, b; Khan *et al*., 2005; Borras *et al*., 2010). Soy diet prior to CCl_4 intoxication restored the levels of serum toxicity markers viz., SGOT and SGPT as compared to negative control. CCl_4 administration resulted in significant induction in hepatic ODC and [^{3}H] thymidine uptake into hepatic DNA, There is enough evidence that indicates that the polyamines play essential role in the regulation of various cellular and metabolic functions including DNA synthesis, modulation of membranous functions and numerous enzyme activities (Wallon and O'Brien, 2005). Prophylactic treatment with Soy diet prior to CCl_4 intoxication showed profound suppression ODC activity and rate of [^{3}H] thymidine incorporation.

CONCLUSION

Modulatory action of Soy diet is observed by several mechanisms, it reduces the risk of acute hepatic injury induced by CCl_4 and thus protecting against post necrotic hepatic injury. In conclusion present study give some insight into mechanisms involved in modulatory action of Soy diet against CCl_4 induced hepatic toxicity in rats. Thus, we suggest it may further be used as potent cancer chemopreventive agent.

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