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PROTECTIVE EFFECT OF L-ORNITHINE-L-ASPARTATE AND SILYMARIN ON CHEMICALLY INDUCED KIDNEY TOXICITY AND THYROID DYSFUNCTION IN MICE

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ABSTRACT

The present study was designed to reveal the hitherto unknown efficacy of two commonly used hepatoprotective drugs, L-ornithine-L-aspartate (lornit) and silymarin in the regulation of kidney toxicity and thyroid dysfunction in mice. Renal and hepatic lipid peroxidation (LPO) was induced by the administration of carbon tetrachloride (CCl_4) for 2 weeks (2.0 gm/kg twice a week). In two separate groups, along with CCl_4 animals were also treated with either lornit (200 mg/kg) or silymarin (100 mg/kg) every day for the same duration. Other than hepatic and renal LPO, alterations in the concentrations of serum triiodothyronine (T_3), thyroxine (T_4), glucose and insulin and in hepatic type-1 iodothyronine 5'monodeiodinase (5'DI) activity were considered as major parameters. Simultaneously activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphahatase (ALP) and hepatic and renal superoxide dismutase (SOD), catalase (CAT) and reduced glutathione content (GSH) were also studied. Lornit or silymarin administration reversed almost all the toxic effects exhibited by CCl_4 including enhanced tissue LPO, serum ALT, AST and ALP activities and the concentrations of insulin and glucose. Both test drugs also significantly increased hepatic 5'DI activity, cellular antioxidants such as SOD, CAT and GSH and serum levels of both the thyroid hormones.

Keywords: Carbon tetrachloride, L-ornithine-L-aspartate, lipid peroxidation, mice, thyroid hormones, type-1 iodothyronine 5'-monodeiodinase

INTRODUCTION

Liver dysfunction is a clinically relevant problem, which accounts a major population of all the reported cases of acute liver failure (Lee, 2003; Yamazaki et al., 2005). Therefore very often hepatoprotective drugs are recommended to ameliorate liver problems. L-ornithine-L-aspartate (lornit) and silymarin are two such commonly used hepatoprotective drugs which are claimed to be highly effective against chemical-induced toxicity (Maneesh and Jayalekshmi, 2005; Mansour et al., 2006; Poo et al., 2006; Upadhyay et al., 2007; Balderas-Renteria et al., 2007; Jain et al., 2008). Although these two drugs have been reported to provide hepatic protection (Vogels et al., 1997; Rose et al., 1999), their potential to regulate kidney toxicity and thyroid dysfunction, if any, was yet to be established. Therefore, in the present investigation an attempt was made to reveal the relative efficacy of these two drugs to regulate kidney toxicity induced by carbon tetrachloride (CCl_4). In fact, this drug has been used traditionally in rodent models to investigate the therapeutic interventions of hepatoprotective drugs (Paquet and Kamphausen,

1975; Clawson, 1989; Weber et al., 2003; He et al., 2006; Balderas-Renteria et al., 2007). Toxicity generated from its reactive metabolite involves induction of oxidative stress on tissues (Perez Tamayo, 1983; Clawson, 1989; Weber et al., 2003; He et al., 2006) that causes liver injury and invariably alters hepatobiliary functions and very often results in impairment of hormonal metabolism (Goel et al., 1994).

Despite the fact that most biologically active thyroid hormone, triiodothyronine (T_3) is largely produced in liver by the process of mono-deiodination of thyroxine (T_4) with the help of the enzyme type-1 iodothyronine 5'-mondeiodinase (5'DI, Ganong, 2005) and there is every possibility of alteration in thyroid hormones in chemical-induced hepatotoxicity, nothing has been reported so far on the effects of hepatoprotective drugs on the alteration of thyroid function.

In literature, lornit has already been reported to ameliorate acute liver failure (Rose et al., 1999; Vogels et al., 1997), CCl_4-induced liver damage (Gebhardt et al., 1997), and hepatic encephalopathy (Kircheis et al., 2002; Delcker et al., 2002). Similarly, the hepatoprotective role of silymarin on CCl_4-induced liver damage has also been investigated earlier (Balderas-Renteria et al., 2007; Jain et al., 2008). However, to the best of our knowledge no report is available till date on the impact of lornit or silymarin on renal oxidative stress and thyroid metabolism; although thyroid hormones regulate almost all body functions (Ganong, 2005) and chronic medication with some drugs may result in altered levels of thyroid hormones (Vigersky et al., 2006; Isidro et al., 2007).

With this concept the present study was performed to ascertain the safe nature of lornit and silymarin in CCl_4-induced hepatotoxic animals with particular reference to thyroid functions and tissue lipid peroxidation (LPO), considering laboratory mouse as working model. We also investigated the alterations in serum glucose and insulin levels; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities and in endogenous antioxidants such as in the activities of superoxide dismutase (SOD), catalase (CAT) and in reduced glutathione (GSH) content, all being related to thyroid functions (Goel et al., 1994; Maiti and Kar, 1998; Kar and Panda, 2005; Jatwa and Kar, 2006a, b; Jatwa and Kar, 2007; Jatwa et al., 2007; Panda and Kar, 2007). As women are known to be more prone to thyroid abnormalities (Fry, 1993; Bülow et al., 2006), for the present investigation only female animals were considered.

MATERIALS AND METHODS

Drugs and Chemicals

The test drugs L-ornithine-L-aspartate (lornit®, Sun Pharma Co., India) and silymarin (Silybon®, Micro Labs. Co, India) were purchased from a registered local medical store, while dithiothreitol (DTT), CCl_4, L-thyroxine (L-T_4) and 2-thiobarbituric acid (TBA) were from Sigma Chemicals Co. Ltd. St. Louis, USA. Pyrogallol, hydrogen peroxide, diethylene triamine penta acetic acid, sodium dodecyl sulphate, ethylene diamine tetra acetic acid (EDTA) and sulphuric acid were purchased from E. Merk Ltd, Mumbai, India. Radioimmunoassay (RIA) kits, for the estimation of different hormones were obtained from Bhabha Atomic Research Centre (BARC), Mumbai, India. All other chemicals were of reagent grade and purchased from Loba Chemie, Mumbai, India.

Animals

Colony bred adult Swiss albino female mice, weighing 30 ± 2 g, were acclimated for a week in a light (14 h light: 10 h dark cycle) and temperature (23 ± 2 °C) controlled room with the provision of laboratory feed (Gold Mohur feed, Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

Experimental design

Forty-two healthy mice were divided into six groups of seven each. Animals of group 1 receiving the vehicle, vegetable oil

(0.1 ml/animal, p.o.) served as control, while those of group 2, 3 and 4 received CCl_4 (2.0 gm/kg, p.o, mixed with vegetable oil at 1:1 ratio) twice a week (Wang et al., 1997; He et al., 2006; Balderas-Renteria et al., 2007) to induce liver damage. After 24 hours of CCl_4 administration animals of group 1 and 2 received vehicle, distilled water (0.1 ml/animal/day, p.o) and those of group 3 and 4 received lornit (200 mg/kg/day, p.o., Maneesh and Jayalekshmi, 2005) or silymarin (100mg/kg/day, p.o., Lee et al., 2007) respectively for 14 days, while animals of group 5 and 6 were administered only with equivalent amount of lornit and silymarin respectively. Drug or vehicle administration was done by gastric intubation method between 1000 and 1100 h of the day to avoid circadian variation, if any.

Animals were maintained as per the guidelines laid down by departmental Ethical Committee for Handling and Maintenance for Experimental Animals and the Committee for the Purpose of Control and Supervision on Experiments in Animals (CPCSEA), Ministry of Environment and Forests, Government of India.

Preparation of serum and tissue samples

On the day of termination (15^{th} day), over-night fasted animals were killed after exposing them to mild ether anesthesia. Blood from each animal was collected and serum was isolated for the estimation of different biochemical and hormonal parameters. After exsanguinations, both the kidneys and liver were removed, quickly freed from blood clots and washed thoroughly with phosphate buffered saline (PBS, 0.1 M, pH 7.4), weighed and processed for the estimations of LPO, SOD and CAT activities and GSH and protein contents.

Assay of thyroid hormones

Total circulating T_3 and T_4 were estimated by RIA in serum samples following the protocols provided in the kits as routinely followed in our laboratory (Jatwa and Kar, 2006a, b; Jatwa and Kar, 2007; Jatwa et al., 2007; Panda and Kar, 2007). In brief, RIA was performed using tris hydroxymethyl amino methane buffer (0.14 M, containing 0.1 % gelatin; pH 8.6). The antisera, specific hormone standards, radio labeled hormones (I^{125} T_4 and I^{125} T_3) and the control sera were reconstituted with assay buffer/double distilled water. The reaction mixture comprised of standard/sample, buffer, radio labeled hormone and the respective antibody was incubated at 37 °C (30 min. for T_4 and 45 min. for T_3). Incubation was terminated by the addition of polyethylene glycol. Tubes were then centrifuged at 2000 X g for 20 min. After decanting the supernatant, traces of liquid were removed with the help of filter paper wicks without disturbing the precipitate. Finally the tubes were subjected to radioactivity counting for one minute (CPM) using an I^{125} gamma counter. A set of quality control sera was also used with each assay.

Hormone assay of insulin

Assay of total serum insulin was also done following the protocol provided in RIA kit as routinely followed in our laboratory (Jatwa and Kar, 2006a; Jatwa and Kar, 2007; Jatwa et al., 2007). In brief, 200 µl of assay buffer and 100 µl of serum sample/standard were mixed and then 100 µl of primary antibodies (anti-porcine guinea pig IgG) were added and the mixture was incubated at 4 °C for overnight. Following the incubation, 100 µl of I^{125}-labeled insulin was added. After 3 hours of incubation at room temperature, 100 µl of secondary antibodies (anti-guinea pig-rabbit IgG) were added followed by addition of 1 ml polyethylene glycol. After gentle mixing, tubes were incubated at room temperature for 20 min and then centrifuged at 1500 X g for 20 min at room temperature. After decanting the supernatant, traces of liquid were removed with the help of filter paper wicks without disturbing the precipitate. Finally tubes were subjected to radioactivity counting for one minute (CPM) using an I^{125} gamma counter. A set of quality control sera of mice was also used with each assay.

Study of hepatic 5'DI activity

For the evaluation of hepatic 5'DI enzyme activity, an established method (Kahl et al., 1987) was followed as commonly used in our laboratory (Maiti and Kar, 1998; Jatwa and Kar, 2007; Panda and Kar, 2007). In brief, the liver was homogenized in 3 volumes of ice-cold phosphate buffer (0.15 M, pH 7.4) containing 5 mM EDTA. The homogenate was centrifuged at 2000 X g for 30 min at 4 °C, then the supernatant was incubated with assay buffer, L-T_4 (4 μM) and DTT for an hour at 37 °C. Finally the incubation was terminated with the addition of absolute ethanol and the estimation of T_3 was done using RIA.

Estimations of ALT, AST and ALP activities

For the estimation of ALT and AST activities in serum samples, commercially available enzymatic kits, based on the reaction of 2, 4 dinitro phenyl hydrazine with pyruvate and/or oxaloacetate to yield a brown colored complex in alkaline medium were used (Reitman and Frankel, 1957). Serum ALP activity was evaluated using the spectrophotometric method of King (1965), as followed earlier in our laboratory (Jatwa and Kar, 2006b; Jatwa et al., 2007).

Serum glucose estimation

Fasting serum glucose concentration was estimated by glucose oxidase/peroxidase method of Trinder (1969), where 4-aminoantipyrine and phenol react with glucose to yield a red colored complex (Jatwa and Kar, 2007; Panda and Kar, 2007).

Biochemical estimations of LPO and GSH content

For the evaluation of LPO, the liver and kidney tissues were homogenized in 10 % (w/v) ice-cold phosphate buffered saline (0.1M, pH 7.4), centrifuged at 2,000 X g for 30 min and the supernatant was used for the assay (Ohkawa et al., 1979; Jatwa and Kar, 2006a, b; Jatwa and Kar, 2007; Jatwa et al., 2007; Panda and Kar, 2007). In brief, LPO was determined by the reaction of 2-thiobarbuturic acid with malondialdehyde (MDA), one of the major products formed by peroxidation of lipids, in acidic medium. Amount of MDA was measured by taking the absorbance at 532 nm (extinction coefficient, E =1.56 $x10^5$), using a Shimadzu UV-1700 spectrophotometer. While tissue reduced glutathione content was measured by taking the absorbance of the product formed by the reaction of Ellman's reagent with GSH at 412 nm (Extinction coefficient, E= $1.36x10^4$) following the method of Ellman (1959), as done earlier in our laboratory (Jatwa and Kar, 2007; Jatwa et al., 2007).

Estimations of SOD and CAT activities and protein content

The endogenous SOD activity was determined using the pyrogallol autoxidation inhibition assay following the protocol of Marklund and Marklund (1974). The rate of autoxidation was determined by recording the increase in the absorption at 420 nm (Jatwa and Kar, 2006a, b; Jatwa and Kar, 2007; Jatwa et al., 2007; Panda and Kar, 2007). CAT activity was estimated by considering the method of Aebi (1983), based on the estimation of amount of hydrogen peroxide decomposed as routinely done in our laboratory (Jatwa and Kar, 2006a, b; Jatwa et al., 2007; Panda and Kar, 2007). Tissue protein estimation was done by the routine method of Lowry et al. (1951) using bovine serum albumin as standard.

Statistical analysis

Data are expressed as mean ± S.E.M. For statistical evaluation of the data, analysis of variance (ANOVA) and the Student's t-test were used.

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RESULTS

Effects on LPO and antioxidants

Administration of CCl_4 to mice on one hand increased hepatic and renal LPO (Fig. 1), on the other hand it decreased GSH, SOD, and CAT activities. While treatment with either lornit or silymarin to CCl_4 intoxicated animals reduced the LPO, there was a significant increase in the activities of SOD, CAT and GSH. In normal animals also both the drugs reduced tissue LPO with a significant increase in these three antioxidants (Table 1).

Effects on serum thyroid hormones and on hepatic 5'DI enzyme activity

A significant decease in serum T_3 concentration and hepatic 5'DI enzyme activity was observed following CCl_4 administration. However, treatment with either lornit or silymarin reversed these abnormalities as both drugs increased 5'DI activity and serum T_3 concentration (Fig. 2). No significant alteration in serum T_4 level was noticed.

Fig. 1

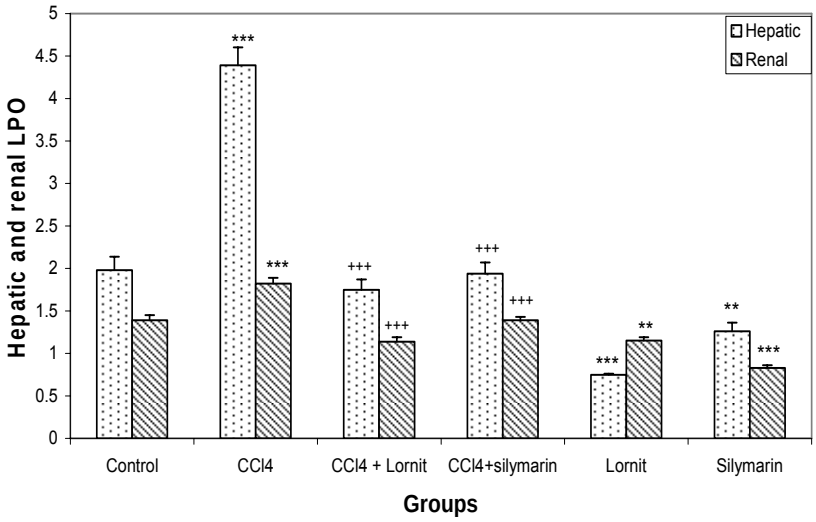

Figure 1: Effects of lornit or silymarin on the changes in hepatic and renal LPO (nM MDA formed / mg protein / hr) in CCl_4-induced hepatotoxic and control female mice. Each vertical bar represents the mean ± S.E.M. (n=7). ***, $P < 0.001$; **, $P < 0.01$ as compared to the respective control values and $^{+++}$, $P < 0.001$ as compared to the respective values of the CCl_4-treated group.

Fig. 2

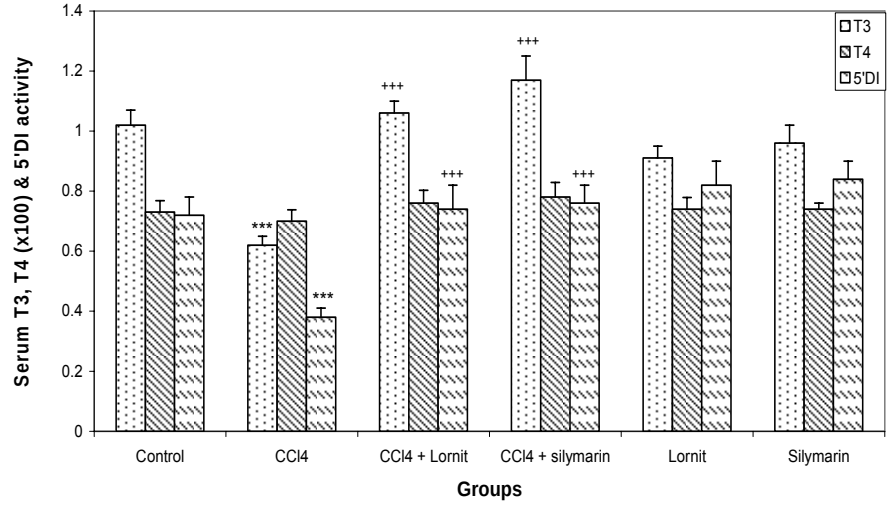

Figure 2: Effects of lornit or silymarin on the changes in concentrations of serum T_3 and T_4 (ng/ml) and hepatic 5'DI (ng of T_3 generated/mg protein/hr) in CCl_4-induced hepatotoxic and normal female mice. Each vertical bar represents the mean ± S.E.M. (n=7). ***, $P < 0.001$ as compared to the respective control values and $^{+++}$, $P < 0.001$ as compared to the respective values of the CCl_4-treated group.

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Table 1: Effect of Lornit (200 mg/kg/day, p.o.) or Silymarin (100 mg/kg/day, p.o.) administration for 14 days on the activities of hepatic and renal SOD (U/mg protein) and CAT (μM H_2O_2 decomposed/min/ mg protein) and GSH content (μM GSH/mg protein) on control and CCl_4 (2.0 g/kg, twice a week) treated female mice.

Parameters	Control	CCl_4	CCl_4 + Lornit	CCl_4 + silymarin	Lornit	silymarin
Hepatic						
SOD	2.53 ±0.10	1.05^{***} ±0.09	3.65^{+++} ±0.12	3.93^{+++} ±0.13	3.68^{***} ±0.18	3.10^{***} ±0.07
CAT	42.79 ±2.12	27.62^{***} ±1.01	48.50^{+++} ±2.13	42.61^{+++} ±2.21	49.22^{*} ±2.01	50.64^{*} ±2.63
GSH	3.50 ±0.21	2.07^{***} ±0.10	4.23^{+++} ±0.31	5.07^{+++} ±0.42	4.09^{*} ±0.42	4.72^{*} ±0.41
Renal						
SOD	4.72 ±0.32	3.36^{**} ±0.22	5.50^{+++} ±0.35	5.21^{+++} ±0.36	7.10^{**} ±0.6	8.12^{***} ±0.7
CAT	40.31 ±2.08	30.72^{**} ±2.04	42.61^{++} ±2.48	43.66^{++} ±2.61	49.06^{*} ±3.01	49.05^{*} ±2.01
GSH	5.73 ±0.28	4.53^{*} ±0.31	8.80^{+++} ±0.39	8.63^{+++} ±0.51	8.81^{***} ±0.29	8.13^{***} ±0.31

Data are mean ± S.E.M. (n=7). $^{+++}$, $P < 0.001$ and $^{++}$ $P < 0.01$ as compared to the respective values of CCl_4 treated group. ***, $P < 0.001$; **, $P < 0.01$ and *, $P < 0.05$ as compared to the respective control values.

Serum ALT, AST and ALP activities and on the concentration of insulin and glucose

CCl_4 administration significantly increased serum ALT, AST and ALP activities as well as insulin and glucose concentrations. However, either lornit or silymarin administration to CCl_4 treated animals reversed all these changes bringing down the values to nearly normal levels (Table 2).

DISCUSSION

Results revealed that administration of CCl_4 to mice increased the LPO in both hepatic and renal tissues; serum AST, ALT and ALP activities; fasting glucose and insulin concentrations, but lowered serum level of T_3 and hepatic 5'DI activity, indicating a peroxidative, hyperglycemic and hypothyroidic conditions. While the CCl_4 induced alterations in most of these parameters including hypothyroidism, hyperinsulinemia and increase in hepatic LPO are in accordance with the earlier observations made by some other workers (Meyer-Alber et al., 1992; Goel et al., 1994; Castilla-Cortazar et al., 1997), practically nothing was known in relation to hepatic activity of 5'DI, the enzyme responsible for major amount of T_3 production (Ganong, 1995) and on renal peroxidative system which have been evaluated in the present study.

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Table 2: Effect of Lornit (200 mg/kg/day, p.o.) or Silymarin (100 mg/kg/day, p.o.) administration for 14 days on the activities of serum AST (U/l); ALT (U/l) and ALP (KA units/l) and insulin (IU/l) and glucose (mg/dl) concentrations in normal and CCl_4 (2.0 g/kg, twice a week) treated female mice.

Parameters	Control	CCl_4	CCl_4 + Lornit	CCl_4 + Silymarin	Lornit	Silymarin
ALT	38.25 ±1.57	120.2*** ±5.11	44.0^{+++} ±2.03	45.12^{+++} ±2.40	40.2 ±2.11	41.0 ±2.03
AST	13.80 ±0.61	30.8*** ±1.50	15.0^{+++} ±0.54	15.80^{+++} ±0.61	14.80 ±0.80	15.0 ±0.94
ALP	15.69 ±0.98	19.74* ±1.21	10.18^{+++} ±0.81	11.07^{+++} ±0.82	12.94 ±0.87	16.46 ±0.81
Insulin	21.0 ±0.93	42.0*** ±1.2	20.0^{+++} ±0.89	18.75^{+++} ±0.80	20.33 ±1.2	20.25 ±0.79
Glucose	84.84 ±2.89	118.34*** ±4.03	90.34^{+++} ±2.31	93.75^{+++} ±2.85	80.83 ±3.89	79.83 ±4.89

Data are mean ± S.E.M. (n=7). $^{+++}$, $P < 0.001$ as compared to the respective values of CCl_4 treated group. ***; $P < 0.001$ and *, $P < 0.05$ as compared to the respective control values.

While carbon tetrachloride induced increase in hepatic LPO, observed in the present study indicating a condition of liver damage is well documented in experimental models (Weber et al., 2003; Yamazaki et al., 2005; He et al., 2006; Lee et al., 2007; Tu et al., 2007), the additional findings on the increased levels of serum ALT, AST and ALP activities in CCl_4 treated group further corroborated its hepatotoxic nature. This could be an outcome of loss of membrane integrity and increased oxidative stress as suggested earlier (He et al., 2006; Lee et al., 2007). It is well understood that CCl_4 is activated by phase 1-cytochrome P450 enzymes to trichloromethyl (CCl_3) free radicals (Weber et al., 2003; Aleksunes et al., 2005) which initiate a chain of reactions by abstracting hydrogen ion from nearby polyunsaturated fatty acids (PUFA) and result in increased production of thiobarbituric acid reactive substances (TBARS), the major products of lipid peroxidation (He et al., 2006). In fact, peroxidation of lipids, particularly those containing PUFA can dramatically change the properties of biological membranes and some times result in severe cell damage (Weber et al., 2003; Aleksunes et al., 2005). Therefore, in the present investigation, elevation in hepatic LPO could be an outcome of CCl_4-induced cellular damage. It could also be due to the decreased activities of endogenous antioxidants such as SOD, CAT and GSH (Yamazaki et al., 2005; He et al., 2006; Lee et al., 2007) as all these have an inverse relationship with LPO.

The interesting findings are that the administration of either of the test drugs to CCl_4 treated animals reversed most of these adverse effects including CCl_4 induced hypothyroidism and oxidative stress. To the best of our knowledge, for the first time we report the impact of lornit and silymarin therapy on thyroid metabolism of an animal model. Therefore, our findings can be compared to that of some recent clinical reports suggesting an altered thyroid hormone metabolism due to chronic medication with some other drugs (Vigersky et al., 2006; Isidro et al., 2007). In fact, lornit administration stimulated the hepatic 5'DI activity and the serum T_3 level, but reduced the tissue LPO and serum glucose level. While

silymarin was earlier known to be effective against chemically induced tissue LPO, hyperinsulinemia and hyperglycemia (Soto et al., 2004; Huseini et al., 2006), practically nothing was reported on the impact of lornit on these aspects. Interestingly, simultaneous administration of either lornit or silymarin to these animals reversed the CCl_4-induced hepatic LPO. These observations are somewhat similar to the earlier reports which too indicated the amelioration of chemical-induced tissue LPO and oxidative stress following the administration of test hepatoprotective drugs (Maneesh and Jayalekshmi, 2005; Mansour et al., 2006; Poo et al., 2006; Lee et al., 2007; Upadhyay et al., 2007). The tissue protective nature of both the evaluated drugs was further supported by the increased activities of endogenous antioxidants such as SOD, CAT and GSH, as also observed by some other workers (Mansour et al., 2006; Balderas-Renteria et al., 2007). However, lornit exhibited a better efficacy in the reduction of CCl_4-induced hepatic LPO (60.13 %) than that of silymarin (55.58 %) and some what similar inhibition was observed when the tested dugs were administered to normal healthy animals (62.12 % and 36.86 % for lornit and silymarin, respectively).

Observations on fasting serum glucose following CCl_4 administration indicated a hyperglycemic condition as reported by earlier workers (Meyer-Alber et al., 1992; Castilla-Cortazar et al., 1997). However, CCl_4 also elevated serum insulin concentration, indicating a condition of insulin resistance. This observed elevation in fasting serum glucose (39.48 %) could be an outcome of CCl_4-induced decrease in GLUT2 mediated absorption of circulating sugar as suggested earlier (Meyer-Alber et al., 1992; Castilla-Cortazar et al., 1997). Interestingly, simultaneous administration of either lornit or silymarin and CCl_4 reversed these abnormalities suggesting the potency of both the test drugs, in the amelioration of CCl_4-induced hyperglycemia and insulin resistance. Of course, the percent decrease of fasting serum glucose in case of lornit was little higher (23.66) as compared to silymarin (19.08) suggesting a relatively better efficacy of the former one over the latter drug in ameliorating hyperglycemia. These favorable effects of both the test drugs on glucose metabolism could be the outcome of a decrease in oxidative stress (discussed latter) on hepatic tissues, which might have facilitated the glycogen synthesis and reduced insulin resistance (Szkudelski, 2001).

For the first time an increase in renal LPO (23.62 %) following CCl_4 administration was also observed, which was nearly normalized by the administration of lornit or silymarin, suggesting the beneficial role of the test drugs with respect to renal functions, too. This kidney protective effect could either be the direct free radical scavenging activity of two drugs or could indirectly be the result of an elevation in the levels of endogenous antioxidants such as SOD, CAT and GSH, those were reduced following CCl_4 treatment.

With respect to the changes in the level of thyroid hormones, there was a decrease in T_3 concentration following CCl_4 administration as reported by earlier workers (Goel et al., 1994), which was reversed on simultaneous administration with either lornit or silymarin. It is quite possible that this CCl_4 induced decrease in thyroid function is an outcome of CCl_4-induced hepatotoxicity, as out of the two major circulating thyroid hormones, T_4 is synthesized only in thyroid gland, while the major amount of T_3 (85-90 %) is produced by the mono-deiodination of T_4, primarily in hepatic and renal tissues with the help of enzyme 5'DI (Ganong, 2005). This fact was further supported by the decreased hepatic 5'DI enzyme activity (47.22 %) in the animals of CCl_4 treated group, suggesting that chronic administration of hepatotoxic chemicals may reduce hepatic 5'DI enzyme activity (Maiti and Kar, 1998). On the other hand, lornit or silymarin administration to CCl_4-induced hepatotoxic animals resulted in the reversal in the serum T_3 concentration. This was further supported by increased 5'DI enzyme activity in hepatic tissues (94.73 % and 100 % for lornit and silymarin, respectively). As in present study, following lornit

or silymarin administration only the level of T_3 and 5'DI activity were increased, it seems that the test drugs regulate thyroid function only at the level of peripheral conversion of T_4 to T_3. The possible mechanism could be that the tissue protective effects of both the test drugs might have reduced the oxidative stress and increased the liver function indicating 5'DI activity and subsequently the T_3, as suggested earlier (Maiti and Kar, 1998; Kar and Panda, 2005; Jatwa and Kar, 2007).

In summary, both lornit and silymarin appear to have the potential to protect hepatic and renal toxicity induced by xenobiotics, with an additional benefit of ameliorating hyperglycemia and thyroid dysfunction. It is further suggested that both the test drugs may prove to be safe for chronic medication with particular reference to antioxidant defense system and thyroid functions. However, lornit appears to be more potent than silymarin in regulating the tissue toxicity, both in liver and kidney.

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