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IMMUNOTOXIC ACTION OF POSTORAL ADMINISTRATION OF CARBON TETRACHLORIDE IN MALE ALBINO RAT

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ABSTRACT

Carbon tetrachloride (CCl4) was administered (p.o.) every other day for one month to male Sprague Dawely rats. At the first day after the treatment period, leucocytes (WBCs), and lymphocytes (LYM) numbers, and serum IgM level were significantly higher than those of the control values. At day 15 after CCl4 treatment period WBCs and LYM numbers were below the control levels, whereas, IgM was still above the control level, but lower than the value recorded at day one after CCl4 treatment period. The same dose level and treatment period of CCl4 induced significant increases in serum IL-4 and IL-6 levels that were lasted for 15 days post the administration period. Late administration (i.p.) of DMSO (0.5 ml/100 g b.wt.) at 30 minutes post CCl4 treatment period selectively prevented changes of WBCs and LYM.Tthese results indicate that the toxic effect of CCl4 under the present experimental conditions, could induce changes in both cellular and humoral immune response of rats and these changes could be selectively reversed with time and late administration of DMSO.

INTRODUCTION

Carbon tetrachloride (CCl_4) is a clear, colourless, heavy, and non-flammable liquid [(1)]. The average daily intake of CCl_4 for the general population is estimated to be 0.1 μg. Once exposed to this toxic compound by ingestion, inhalation or skin absorption, it is distribution throughout the body, with highest concentrations in the liver, brain, kidney, muscle, fat and blood [(1)]. In spite of the its obvious deleterious effects, CCl_4 is still used as a solvent for oils, fats, lacquers, varnishes rubber waxes and resins [(2,1)]. CCl_4 is a well known hepatotoxin and nephrotoxin [(3,4,5)] . It has also been identified as a probable human carcinogen based on evidence of tumors in animals [(16)]. Furthermore, CCl_4 has been demonstrated as a potent immunosuppressive agent [(7,8,9,10,11)] . Several agents have been reported to reduce or erase the toxic effect of CCl_4, these include propolis extract [(12)] , ginkobiloba extract [(13,14,15)] , black tea extract [(15)] , and caffeic acid phenthyl ester [(4)] .

The immunoglobulins are proteins with anti-body activity; i.e., they combine specifically with the substance that elicite their formation (immunogen or antigen), and they make up the humoral arm of the immune response. With the possible exception of "natural" antibody, antibodies arise in response to foreign substances introduced into the body. They are therefore products of induced responses. The immunoglobulins, which circulate in body fluids, comprise a heterogeneous family of proteins, they account for approximately 20% of the total plasma proteins (**Goodman, 1991**).

The two hallmarks of immunoglobulins are the specificity of each for one particular antigenic structure and their diversity as a group, which meets the challenge of a vast array of antigenic structures in the environment. In addition to specifically binding antigens, the immunoglobulins express secondary biologic activities, which are important in defense against disease, e.g., transplacental passage, and facilitation of phagocytosis (**Goodman, 1991**).

IgM is secreted by plasma cells **(Goldsby et al., 2003)**. It constitutes approximately 10% of normal immunoglobulins and normally exists as a pentamer with a molecular weight of approximately 900,000(19S) (**Goodman, 1991**)

IgM antibody is prominent in early immune responses to most antigens and predominates in certain antibody responses such as "natural" blood group antibodies. IgM is the major immunoglobulin expressed on the surface of B cells **(Goodman, 1991).**

Cytokines are soluble proteins that affect an array of biological processes including inflammation and immunity. Almost every mammalian cell type has the capability to produce and respond to cytokines **(Dinarello, 2007)**.

Interleukin-4 (IL-4) is a cytokine with multiple activities that is mainly produced by the helper CD4 T-lymphocytes of the TH2 subset. It is both an early and late acting growth and differentiation factor for B-lymphocytes which increases cell surface expression of both class II histocompatibility antigens **(Noelle et al., 1984; Roehm et al., 1984)** and the low affinity receptor for IgE **(DeFrance et al., 1987)**.

Interleukin-6 (IL-6) is a poleiotropic cytokine with both pro and anti-inflammatory properties **(Barton, 1996)**. This cytokine is expressed by a variety of cells, including lymphocytes, monocytes, macrophages, neutrophils, endothelial cells, epithelial cells, and fibroblasts **(Biffl et al., 1996; van der Boll and van Deventer, 1998)**.

Dimethyl sulfoxide (DMSO), an amphipathic solvent soluble in both aqueous and organic media if often used to dissolve hydrophobic substances employed in biological research **(Basile et al., 1996; Szonyi et al. 2001; Harris et al., 2005)**. In experimental studies, DMSO has been shown to effectively prevent the chemically-induced liver or kidney damage **(Lind & Grandolfi, 1997, 1999; Gilot et al., 2002; Kishioka et al., 2007)**. The objective of this study was to further delineate the effects of CCl_4 on the immune system in male albino rats and the possible protective action of late administration of DMSO against these effects.

MATERIAL AND METHODS

Male Sprague-Dawely rats weiging 150-200 g were used in this study. They were obtained from the animal house of the National Research Center, doki, Giza. Rats were housed under standard laboratory conditions. They had free access to a standard food and

provided with tap water *ad libitum*. All animals were acclimatized to these conditions for 10 days before being used.

Carbon tetrachloride and dimethyl sulfoxide were applied in the present study as commercial solutions. CCl_4 was obtained from Merck (Germany) DMSO City, Egypt was obtained from NOURESH'SHARK Co. 10th of Ramadan

The acute post oral LD_{50} of CCl_4 for male Sprague-Dawely rats was determined according to the method of **Reed and Meuench** [32] and was found to be 0.9 ml/100 g b.wt., (1.431 g/100 g b.wt.).

Animals were randomly allocated into 3 groups (10 rats in each group).

Rats of group 1: were not treated and used as controls.

Rats of group 2: received postorally CCl_4 at the dose level of 1/10 LD_{50}/100 g b.wt. every other day for one month.

Rats of group 3: were injected subcutaneously with DMSO at the dose level of 0.05 ml/100 g b.wt 30 minutes after each CCl4 treatment, the desired dose of DMSO was diluted by distilled water to a volume of 0.5 ml for each rat.

From each group, 5 rats were deprived of food for overnight before the 1st day and 15 days following termination of each treatment period.

These rats were slightly anaesthetized with ether and blood samples were collected through heart puncture into plain tubes and centrifuged at 3000 rpm for 15 minutes. Serum was separated in eppendorfs and kept at -80°C till the immunological analysis were started. Other blood samples were collected into vaccutainer tubes containing EDTA for haematological analysis.

Determination of serum immunoglobulin IgM:

IgM in serum samples of control and treated rats were assayed by means of immunonephelometry on the BN ProSpec System manufactured by Dade Behring GnbH, Marburg , Germany.

Determination of the serum cytokines interleukin-4 (IL-4) and interleukin-6 (IL-6):

IL-4 and IL-6 concentrations in serum samples of control and treated rats were measured using AviBion Enzyme-Linked Immunosorbent Assay (ELISA) kits (Orgenium Laboratories, Helsinki, Finland),according to the manufacturer's instructions.

Determination of haematological parameters:

Total white blood cells (WBCs) number and lymphocyte (Lym) number, were measured following the methods described by **Brown** [33] using the automated haematology analyzer, Sysmex KX-21N manufactured by Sysmex Co. Kobe, Japan. Statistical analysis.

RESULTS

Data presented in table (1) and illustrated graphically in figure (1) indicate that subchronic postoral administration of CCl_4 (0.09 ml/100 g. wt.) every other day for one month caused a significant increase in total WBCs and lymphocyte (LYM) numbers above the control at the first day after the end of treatment, and were decreased but not significantly below the control at the day 15 post treatment.

Table (2) and figure (2) demonstrate a significant elevation of serum IgM above the control level after the termination of CCl_4 treatment. This elevation was more pronounced at the first day that observed at the fifteenth day of the post treatment period.

Table (3) and figure (3) show that CCl_4 treatment induced significant increases in IL-6 that were last significantly for 15 days after CCl_4 administration was terminated.

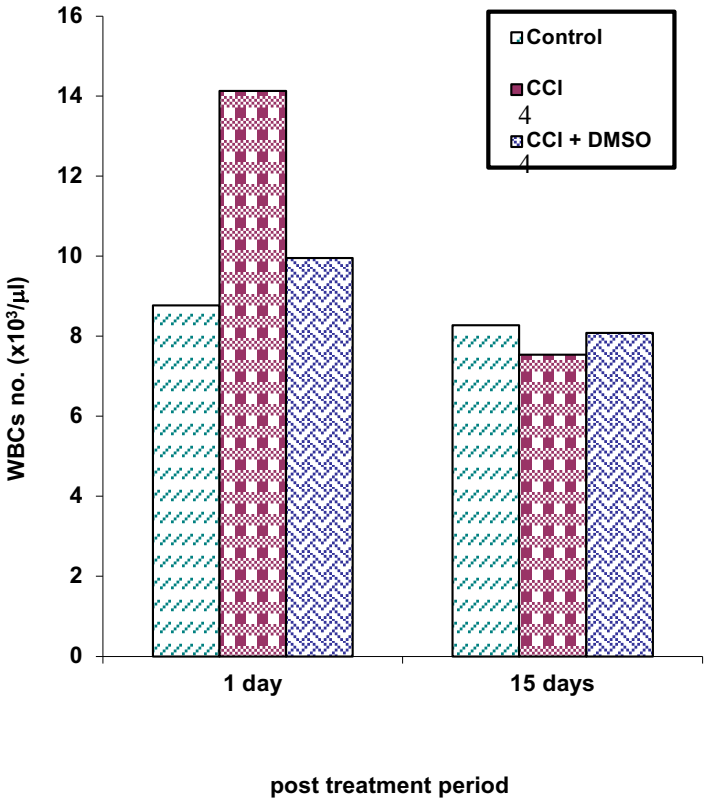
Administration of DMSO (0.5 ml/100 g b.wt. ip) at 30 minutes after each CCl_4 treatment selectively prevented the CCl_4-induced changes in WBCs and LYM numbers (Table 1 and Fig. 1), but had no effects on CCl_4-induced changes in IgM (table 2 and Fig. 2) and in IL-4 and IL-6 as seen in table (3) and figure (3).

Table (1): The effects of postoral administration of CCl_4 (0.09 ml /100 g b.wt.) and that of DMSO (0.05 ml /100 g b.wt.) administered intraperitoneally 30 minutes post each CCl_4 administration every other day for one month on total leukocytes (WBCs) and lymphocytes (LYM) numbers in male albino rats at the 1st day and 15 days following termination of treatment period.

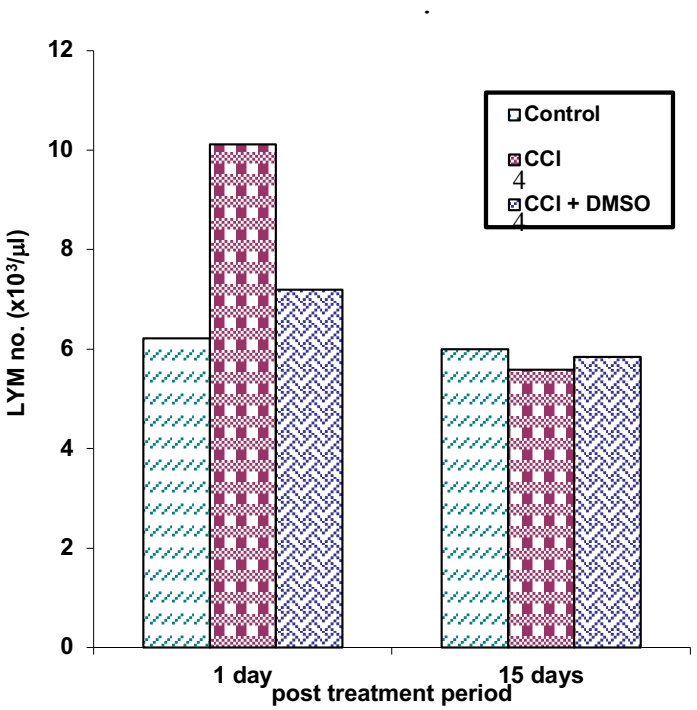
.Measured parameters	Treatment	Post-treatment period	Control	Treated	% difference
WBCs ($x10^3/\mu l$)	CCl_4	1 day	8.78±0.6398	14.14±1.3163^{a+}	61.05
		15 days	8.28±0.8108	7.54±1.1474^{a-}	-8.94
	CCl_4 + DMSO	1 day	8.78±0.6398	9.95±0.4904^{a-}	13.33
		15 days	8.28±0.8108	8.08±0.8772^{a-}	-2.42
LYM ($x10^3/\mu l$)	CCl_4	1 day	6.22±0.6003	10.12±0.8963^{a+}	62.70
		15 days	6.00±0.6261	5.58±0.9682^{a-}	-7.00
	CCl_4 + DMSO	1 day	6.22±0.6003	7.20±0.4626^{a-}	15.76
		15 days	6.00±0.6261	5.84±0.4331^{a-}	-2.67

a+: Significant when compared with the control value where $P \leq 0.05$ (t-test)

a- : Non significant when compared with the control value where $P \leq 0.05$ (t-test)



Fig(1): The effects of postoral administration of CCl_4 (0.09 ml /100 g b.wt.) and that of DMSO (0.05 ml /100 g b.wt.) administered intraperitoneally 30 minutes post each CCl_4 administration every other day for one month on total leukocytes (WBCs) number in male albino rats at the 1st day and 15 days following termination of treatment period. Each value represents the mean±SE of five separate experiments.

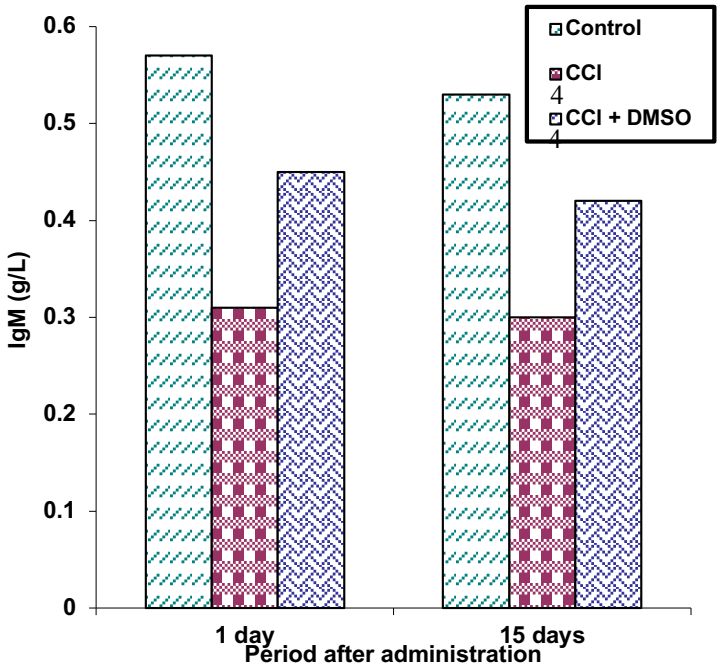


Fig(2): The effects of postoral administration of CCl_4 (0.09 ml /100 g b.wt.) and that of DMSO (0.05 ml /100 g b.wt.) administered intraperitoneally 30 minutes post each CCl_4 administration every other day for one month on lymphocytes (LYM) number in male albino rats at the 1st day and 15 days following termination of treatment period. . Each value represents the mean±SE of five separate experiments.

Table (2): The effects of postoral administration of CCl_4 (0.09 ml /100 g b.wt.) and that of DMSO (0.05 ml /100 g b.wt.) administered intraperitoneally 30 minutes post each CCl_4 administration every other day for one month on serum IgM concentration in male albino rats at the 1st day and 15 days following termination of treatment period.

Measured parameters	Treatment	Post-treatment period	Control	Treated	% difference
IgM (g/L)	CCL_4	1 day	0.54±0.0457	0.78±0.0560[a+]	44.44
		15 days	0.56±0.0286	0.72±0.0466[a+]	28.57
	CCL_4 + DMSO	1 day	0.54±0.0457	0.81±0.0567[a+]	50.00
		15 days	0.56±0.0286	0.82±0.0638[a+]	46.43

a+: Significant when compared with the control value where P≤0.05 (t-test)



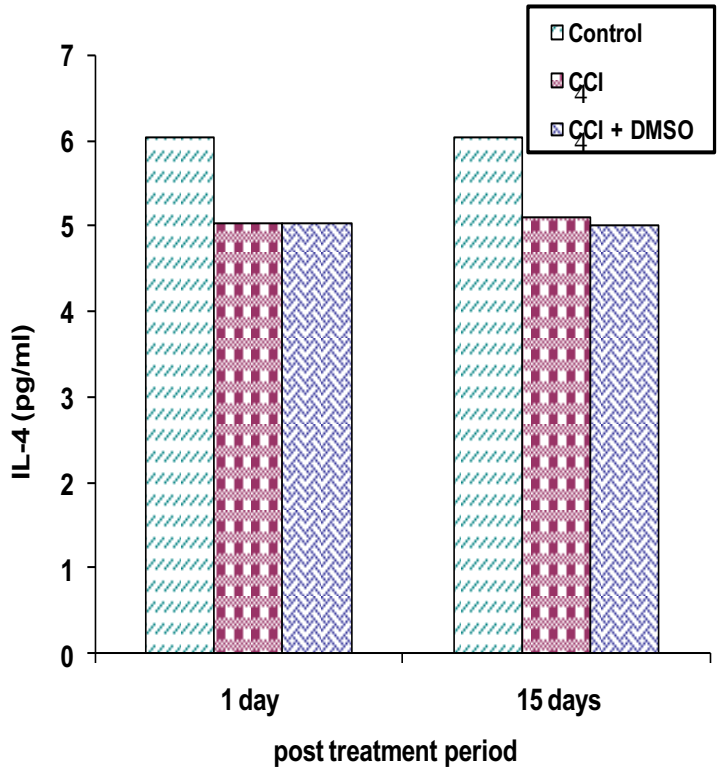
Fig(3): The effects of postoral administration of CCl_4 (0.09 ml /100 g b.wt.) and that of DMSO (0.05 ml /100 g b.wt.) administered intraperitoneally 30 minutes post each CCl_4 administration every other day for one month on serum IgM concentration in male albino rats at the 1st day and 15 days following termination of treatment period. Each value represents the mean±SE of five separate experiments.

Table (3): The effects of postoral administration of CCl_4 (0.09 ml /100 g b.wt.) and that of DMSO (0.05 ml /100 g b.wt.) administered intraperitoneally 30 minutes post each CCl_4 administration every other day for one month on serum IL-4 and IL-6 concentrations in male albino rats at the 1st day and 15 days following termination of treatment period.

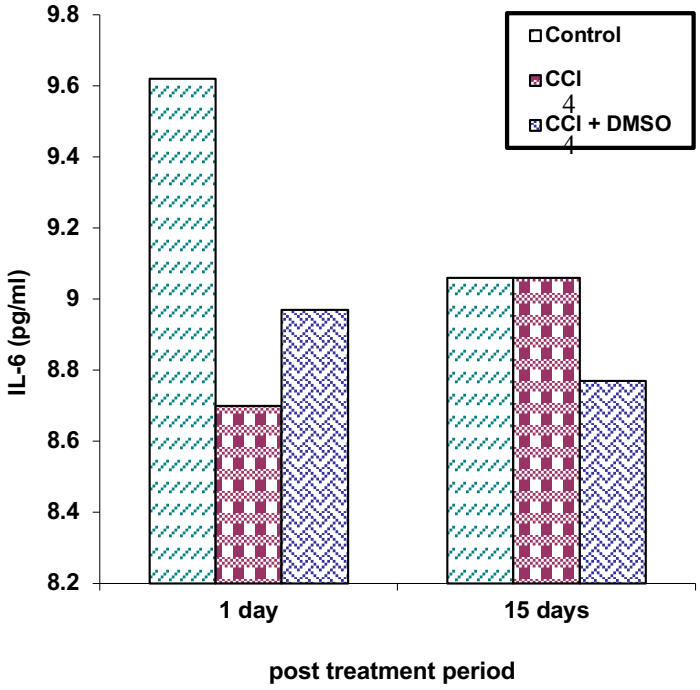
Measured parameters	Treatment	Post-treatment period	Control	Treated	% difference
IL-4 (pg/ml)	CCl_4	1 day	6.20 ±0.1445	5.02±0.0048^{a+}	-19.03
		15 days	6.20 ±0.1445	5.09±0.1669^{a+}	-17.90
	CCl_4 + DMSO	1 day	6.20 ±0.1445	5.03±0.0588^{a+}	-18.87
		15 days	6.20 ±0.1445	4.99±0.0563^{a-}	-19.52
IL-6 (pg/ml)	CCl_4	1 day	9.62±0.1207	8.70±0.1114^{a+}	-9.60
		15 days	9.62±0.1207	9.06±0.1489^{a+}	-5.86
	CCl_4 + DMSO	1 day	9.62±0.1207	8.97±0.2144^{a+}	-6.72
		15 days	9.62±0.1207	8.77±0.0773^{a+}	-8.88

a+: Significant when compared with the control value where $P \leq 0.05$ (t-test)

a- : Non significant when compared with the control value where $P \leq 0.05$ (t-test)



Fig(4): The effects of postoral administration of CCl_4 (0.09 ml /100 g b.wt.) and that of DMSO (0.05 ml /100 g b.wt.) administered intraperitoneally 30 minutes post each CCl_4 administration every other day for one month on serum IL-4 concentration in male albino rats at the 1st day and 15 days following termination of treatment period. Each value represents the mean±SE of five separate experiments.



Fig(5): The effects of postoral administration of CCl_4 (0.09 ml /100 g b.wt.) and that of DMSO (0.05 ml /100 g b.wt.) administered intraperitoneally 30 minutes post each CCl_4 administration every other day for one month on serum IL-6 concentration in male albino rats at the 1st day and 15 days following termination of treatment period. Each value represents the mean±SE of five separate experiments.

DISCUSSION

The organic solvent and environmental contaminant CCl_4 **(ATSDR, 2003)**, has been identified as an immunotoxicant **(Jeon *et al.*, 1997; Ladics *et al.*, 1998)** in addition to its hepatotoxic, nephrotoxic and a probable carcinogenic effects **(Sundori *et al.*, 1997; Thrall *et al.*, 2000; Ogeturk *et al.*, 2005a,b)**.

The results obtained in the present investigation indicate that postoral (po) administration every other day for one month of CCl_4 at a dose of 1/10 LD_{50}, 0.09 ml/100 g b.wt. caused significant increases in total leucocytes (WBCs), lymphocytes (LYM) count and serum immunoglobulin IgM at the first day following the treatment period. These changes were reversed in case of WBCs and LYM but decreased in case of IgM at day 15 post termination of CCl_4 treatment period. **Farombi *et al.* (1997)** have observed significant decrease in total WBCs number in male Wister rats as a result of oral CCl_4 (0.5 ml/kg b.wt) administration. Causes of leucopenia have been reported to be the depression of the blood forming elements and/or destruction and degeneration of these cells as a result of chemical agents **(Tirkey *et al.*, 2005)**. Therefore, the present simultaneous increase in WBCs and LYM could be speculated to be a result of CCl_4-induced activation of the haematopoiesis process in the bone marrow.

In previous study on male CD rats, oral administration of 12.5 and 25 mg/kg b.wt. CCl_4 for 30 days decreased sRBC-specific serum IgM level, while administration of 25 mg/kg b.wt CCl_4 for 90 days increased sRBC specific IgM level **(Ladics et al., 1998)**. **Delaney *et al.*[9]** found that CCl_4-induced suppression of the humoral immune response to the T-cell dependent antigen sRBC was due to the induction of a serum-borne immunosuppressive factor, i.e transforming growth factor-β1 (TGF-β1) by CCl_4. In contrast to the finding of **Ladics and co-workers[11]**, **Smialowicz *et al.*[36]** reported that exposure of Fisher 344 rats to 5-40 mg/kg b.wt CCl_4 for 10 days did not alter the primary humoral immune response to sRBC. Differences in the effect of CCl_4 have been suggested to be attributed to the use of different rat strains **(Ladics *et al.*, 1998)**.

In the present study, it has been observed that repeated po administration of CCl_4 into male rats every other day for 30 days

induced significant increases in serum IL-4 and IL-6 levels that were lasted for 15 days post administration. A possible indirect role of TGF-β1 in that long lasting decrease of IL-4 is supported by previous observation that direct addition of TGF-β1 to naïve splenocytes culture produced marked and dose-related inhibition of the anti-sRBC IgM antibody forming cell response coincided with a decrease in T-helper cytokine IL-4 **(Jeon et al., 1997)**.

It has been reported that IL-6 production is induced by many proinflammatory factors such as IL-1 and tumor necrosis factor (TNF-α) cytokines, histamine, and prostaglandin E_2 (PGE_2) in various cells **(Barton, 1996)**. These inflammatory cytokines were detected in the peritoneal cavity of rats immediately after ip CCl_4 administration and that they increase IL-6 production in culture mesothelial cells **(Yamaji *et al.*, 2008)**. This IL-6 production process could explain the high level of IL-6 production which peaked at 4 hr in the peritoneal cavities of rat administered CCl_4 intraperitoneally **(Zuinen *et al.*, 2007)**.

A possible inhibition of these proinflammatory production could be speculated as a cause of the decrease in IL-6 concentration recorded in the present investigation.

The present study demonstrated that i.p. administration of DMSO (0.5 ml/100 g b.wt.) 30 minutes following each po CCl_4 treatment to rats succeeded in preventing selectively the toxic effects on total WBCs and LYM numbers recorded at one day after CCl_4 treatment period, but has no effect on changes in serum IgM, IL-4 and IL-6 induced by CCl_4 administration. It has been previously reported that in the chemically induced liver injury or kidney damage, DMSO was administered prior to or concomitant with the toxicants **(Lind & Gandolfi, 1997; 1999; Bruck *et al.*, 1999)**.

The protective action of DMSO when administered prior to or concomitant with the toxicants has been attributed to DMSO-induced inhibition of bio-activation of the compounds to toxic intermediates **(Lin & Gandolfic, 1997)**. In evaluating a number of known hepatoprotective agents for their late (hours after toxicant exposure) protective effects, it was found that DMSO was a highly efficacious protective agent **(Lind *et al.*, 1990, 1992, 1994)**. The potential

mechanisms of the protective action of late administration of DMSO in the guinea pig model of halothane hepatotoxicity were investigated by observing its effect on halothane biotransformation, covalent binding by reactive intermediates to liver protein, and hepatic glutathione (GSH) concentration **(Lind *et al.*, 1997)**. They compared their results with those obtained with the structurally analogous cytochrome P-450 2E1 CYPeE1) inhibitor diallyl sulfide. DAS, which provided evidence for mechanism of late DMSO protection being unlike and not due to inhibition of biotransformation. In more recent study, **Kishioka *et al.*[31]** reported that the necrosis of the liver induced by the hepatotoxin thioacetamide in rats was totally prevented by co-administration of DMSO 18 and 1h before and 8 h after each administration of thioacetamide. They suggested that the protective effect of DMSO was related to its antioxidative property.

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