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Methanol-Induced Oxidative Stress in Rat Lymphoid Organs

Narayanaperumal J. PARTHASARATHY, Ramasundaram S. KUMAR, Sundaramahalingam MANIKANDAN and Rathinasamy S. DEVI

Immunology Laboratory, Department of Physiology, Dr. ALM. PG. Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai, Tamilnadu, India

Abstract: Methanol-Induced Oxidative Stress In Rat Lymphoid Organs: Narayanaperumal J. PARTHASARATHY, *et al.* Immunology Laboratory, Department of Physiology, Dr. ALM. PG. Institute of Basic Medical Sciences, University of Madras, Taramani Campus, India—Methanol is primarily metabolized by oxidation to formaldehyde and then to formate. These processes are accompanied by formation of superoxide anion and hydrogen peroxide. This paper reports data on the effect of methanol on antioxidant status and lipid peroxidation in lymphoid organs such as the spleen, thymus, lymph nodes and bone marrow of rats. Male Wistar albino rats were intoxicated with methanol (2.37 g/kg b.w intraperitoneally) for detecting toxicity levels for one day, 15 d and 30 d, respectively. Administration of methanol at 15 and 30 d significantly ($p<0.05$) increased lipid peroxidation and decreased the enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic antioxidants (reduced glutathione and vitamin C) in lymphoid organs. However, lipid peroxidation and enzymatic and non-enzymatic antioxidants in the acute methanol exposed group animals were found to be significantly ($p<0.05$) increased. In one day methanol intoxication, the levels of free radicals initially increased, and to remove these free radicals, antioxidants levels were elevated, which generally prevented oxidative cell damage. But in longer periods of intoxication, when the generation of reactive free radicals overwhelmed the antioxidant defense, lipid peroxidation increased. Further, decreased antioxidants in 15 and 30 d methanol intoxication may have been due to overutilization of non-enzymatic and enzymatic antioxidants to scavenge the products of lipid peroxidation. In addition, the liver and kidney markers of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine significantly increased. This study concludes that exposure to methanol causes oxidative stress by altering the oxidant/antioxidant balance in lymphoid organs of the rat.
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Key words: Methanol, Antioxidants, Lipid peroxidation, Lymphoid organs

Methyl alcohol (methanol) is a colorless liquid. This primary alcohol is normally used as an industrial solvent and cleanser. Handling of products that contain methanol exposes the population to the toxic vapours emanated by methanol. Methanol toxicity has been found to be a problem in the drug abuse domain. Accidental intoxication in the human with this compound still takes place since it is mistakenly ingested instead of ethanol. It is often added as an adulterant to illicit liquors manufactured by bootleggers, and its toxic effect afflicts large numbers of people belonging to the lower socioeconomic group. Moreover, aspartame, which is used as an artificial sweetener, forms methanol when the methyl group of aspartame encounters the enzyme chymotrypsin in the small intestine[1]. A relatively small amount of aspartame can significantly increase plasma methanol levels[2]. Accidental or suicidal ingestion can cause severe metabolic acidosis and clinical disturbances such as blindness, serious neurologic sequelae and death[3, 4]. Methanol is being increasingly recognized as a substance that damages the liver cells where it is oxidized to formaldehyde and later to formate[5]. These processes are accompanied by elevation of NADH level and the formation of superoxide anion, which may be involved in lipid peroxidation[6].

Methanol intoxication is associated with mitochondrial damage and increased microsomal proliferation resulting in increased production of oxygen radicals[5, 7]. These factors together with the excess of formaldehyde, formed during acute methanol intoxication, cause significant increases in lipid peroxidation, which is an amplifier for

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Correspondence to: R.S. Devi, Immunology Laboratory, Department of Physiology, Dr. ALM. PG. Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai, Tamilnadu 600 113, India (e-mail: drsheeladevi@yahoo.com)

initially formed reactive aldehydes generated during lipid peroxidation[8, 9]. Products of lipid peroxidation are very harmful to cells ultimately causing their death and can act as 'second toxic messengers' of a complex chain reaction[10].

It is well known that there are protective systems which can trap or inactivate toxic metabolites, thereby preventing their accumulation within tissues and subsequent toxicity. Cells of the immune system are particularly sensitive to changes in the antioxidant status because they carry out important functions through the generation of a high number of oxygen free radicals[11]. This antioxidant-oxidant balance is an important determinant of immune cell function, including maintenance of the integrity and functionality of membrane lipids, cellular proteins, and nucleic acids and control of signal transduction of gene expression in immune cells[12]. Moreover, the cells of the immune system have a high percentage of polyunsaturated fatty acids in their plasma membranes, and therefore it is not surprising that these cells usually contain higher concentrations of antioxidant nutrients than to other cells[13].

Methanol has been proposed as an alternative motor fuel, both as pure methanol and as methanol/gasoline blends. This proposed use may increase airborne concentrations of methanol as well as formaldehyde, a combustion product of methanol. The resulting potential for increased human exposure to methanol has raised concerns over possible human health effects[14]. Even so, attention has not focused on the lymphoid system and its antioxidant levels. Therefore, the present study was designed to investigate lipid peroxidation and antioxidant status in lymphoid organs upon methanol intoxication.

Materials and Methods

Materials

Methanol (HPLC grade) was obtained from SRL Chemicals, Mumbai, India. Thiobarbituric acid and glutathione (reduced) were purchased from Sigma Chemicals, St. Louis, USA. All other chemicals and solvents used were of analytical grade.

Animal model

Animal experiments were carried out after getting clearance from the Institutional Animal Ethical Committee (IAEC No: 08/010/03) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The experimental animals were healthy, inbred adult male Wistar albino rats, weighing approximately 180–200 g (11 wk of age). All the animals were maintained under standard laboratory conditions and were allowed to have food (standard rat feed pellets supplied by M/s. Hindustan Lever Ltd., India) and water *ad libitum*.

Experimental protocol

Experimental animals were divided into 4 groups consisting of 6 animals each. The LD_{50} of methanol in rats is 9.5 g/kg b.wt.[15]. Methanol was administered intraperitoneally (i.p.) to avoid the uncertainties of gastrointestinal absorption. The methanol dosage used for this study was one fourth of the LD_{50} (2.37 g/kg b.wt) mixed 1:1 with 0.9% saline. The dosage of methanol used in this study did not affect survival rate of the rats, while higher dosages markedly decreased the survival rate (Fig.1). Group I animals received 1 ml of saline daily for 30 d through i.p. injection. Group II animals received 2.37 g/kg b.wt/d of methanol for only one day via i.p injection, and Group III animals received 2.37 g/kg b.wt/d of methanol via i.p injected daily for 15 d. Group IV animals received 2.37 g/kg b.wt/d of methanol for 30 d via i.p. injection.

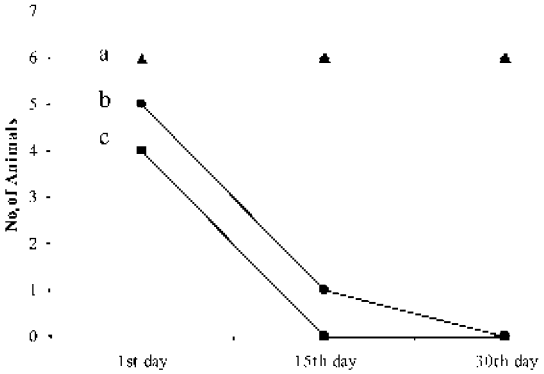

Fig. 1. Survival rate and sublethal dosage of methanol. Values are expressed as Mean ± SD of six animals. Methanol intoxication was as follows: a—Group animals received one fourth of the LD_{50} (2.37 g/kg b.wt). b—Group animals received one third of the LD_{50} (3.16 g/kg b.wt). c—Group animals received half of the LD_{50} (4.74 g/kg b.wt).

Tissues preparation

At the ends of the experimental periods (1 d, 15 d and 30 d), all the animals were sacrificed under deep anesthesia using pentothal sodium (40 mg/kg). Lymphoid organs (spleen, thymus, lymph nodes and bone marrow) were removed immediately and placed in ice cold 0.15 M NaCl solution, perfused with the same solution to remove blood cells, blotted on filter paper, quickly weighed and homogenized by using Teflon glass homogenizers in (1/10 weight per volume) ice cold Tris-HCl buffer (0.1 M, pH 7.4) and centrifuged. The supernatant was used for estimation of lipid peroxidation and various enzymatic and non-enzymatic antioxidants.

Assay of lipid peroxidation

Lipid peroxidation (LPO) was determined by the procedure of Ohkawa *et al.*[16] Malondialdehyde (MDA) forms as an intermediate product of the peroxidation of

lipids and serves as an index of the intensity of oxidative stress. MDA reacts with thiobarbituric acid to generate a colored product which absorbs light at 532 nm. Protein estimations were carried out according to the method of Lowry *et al.*[17]

Determination of the activities of enzymatic antioxidants

The activity of catalase (CAT) was assayed by the method of Sinha[18]. In this method, dichromate in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2), with the formation of perchloric acid as an unstable intermediate. The chromic acetate thus produced is measured colorimetrically at 610 nm. Superoxide dismutase (SOD) was assayed according to the method of Marklund and Marklund[19]. The unit of enzyme activity is defined as the enzyme required for 50% inhibition of pyrogallol autooxidation. Glutathione peroxidase (GPx) activity was estimated by the method of Rotruck *et al.*[20], which is based on the reaction between glutathione remaining after the action of GPx and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to give a compound that absorbs light at 412 nm.

Estimation of non-enzymatic antioxidants

Reduced glutathione (GSH) in the lymphoid organs was estimated by the method of Moron *et al.*[21], which is based on the reaction of GSH with DTNB that gives a compound which absorbs light at 412 nm. Ascorbic acid was assayed by the method of Omaye *et al.*[22] Ascorbic acid (Vit C) was oxidized by copper to form dehydroascorbic acid and diketoglutaric acid, and was treated with 2,4-dinitrophenyl hydrazine to form the derivative of bis-2,4-dinitrophenyl hydrazine. This compound in sulphuric acid undergoes a rearrangement to form a product which absorbs light at 520 nm.

Estimation of liver and kidney functions

Serum collected from the blood samples was subjected to biochemical estimations of aspartate aminotransferase[23] (AST), alanine aminotransferase[23] (ALT), urea[24] and creatinine[25], which reflect the liver and kidney functions.

Statistical analysis

All data were analyzed with the SPSS statistical package for Windows (version 10.0, SPSS Institute Inc., Cary, North Carolina). Data are expressed as mean ± standard deviation (SD). Statistical significance between the different groups was determined by one way-analysis of variance (ANOVA). If the groups showed significant differences, Tukey's multiple comparison test was performed. The significance level was fixed at $p<0.05$.

Results

Table 1 depicts the liver and kidney function test in the experimental animals. Methanol intoxicated rats of 15 and 30 d showed a significant increase in their serum AST, ALT, urea and creatinine when compared with control rats. The duration of methanol exposure significantly enhanced the serum AST, ALT, urea and creatinine when compared within the 1-, 15- and 30-d methanol-exposed rats.

The results of lipid peroxidation in the spleen, thymus, lymph node and bone marrow are summarized in Table 2. In rats exposed to 1-, 15- and 30-d methanol intoxication, the LPO level was significantly ($p<0.05$) increased when compared to the control animals. There was a significant ($p<0.05$) increase in the LPO level in the 15- and 30-d methanol-injected groups when compared to the 1-d group. Moreover the LPO level was found to be significantly ($p<0.05$) increased in the 30-d exposure group when compared to the 15-d methanol exposure animals.

Table 1. Effect of methanol intoxication on serum alanine aminotransferase, aspartate aminotransferase, urea and creatinine in control and experimental groups

Parameters	Group I	Group II	Group III	Group IV
ALT (μ moles of pyruvate liberated/ min/mg protein)	29.0 ± 2.5	31.4 ± 3.3	53.1 ± 2.3*[a]	60.4 ± 2.8*[ab]
AST (μ moles of pyruvate liberated/ min/mg protein)	5.8 ± 0.4	6.4 ± 0.3	9.0 ± 1.2*[a]	13.7 ± 1.2*[a]
Urea (mg/dl)	30.1 ± 3.6	33.2 ± 2.9	43.6 ± 3.5*[a]	51.3 ± 3.2*[ab]
Creatinine (mg/dl)	0.46 ± 0.03	0.48 ± 0.03	0.56 ± 0.02*[a]	0.70 ± 0.04*[ab]

Values are expressed as Mean ± SD of six animals. Methanol intoxication was as follows: Group I—control; Group II—intoxication for one day; Group III—intoxication for 15 d; Group IV—intoxication for 30 d. Comparisons were made between: *All intoxicated groups with Group I; [a]Group III & Group IV with Group II; [b]Group III with Group IV. The symbols represent statistical significance: *, a, b=$p<0.05$

Table 2. Effect of methanol intoxication on lipid peroxidation (nmols of MDA/mg protein) in the lymphoid organs in control and experimental groups

Lymphoid Organs	Group I	Group II	Group III	Group IV
Spleen	2.62 ± 0.19	4.14 ± 0.25*	7.22 ± 0.31*[a]	9.72 ± 0.52*[ab]
Thymus	3.58 ± 0.35	5.76 ± 0.36*	9.23 ± 0.57*[a]	11.6 ± 0.33*[ab]
Lymph nodes	3.15 ± 0.25	5.08 ± 0.24*	8.77 ± 0.57*[a]	9.17 ± 0.67*[ab]
Bone marrow	3.14 ± 0.33	4.47 ± 0.18 *	7.20 ± 0.42*[a]	9.75 ± 0.56*[ab]

Values are expressed as Mean ± SD of six animals. Methanol intoxication was as follows: Group I—control; Group II—Intoxication for one day; Group III—Intoxication for 15 d; Group IV—Intoxication for 30 d. Comparisons were made between: *All intoxicated groups with Group I; [a]Group III & Group IV with Group II; [b]Group III with Group IV. The symbols represent statistical significance: *, a, b=$p<0.05$

Table 3. Effect of methanol on spleen enzymatic and non-enzymatic antioxidants in control and experimental animals

Parameters	Group I	Group II	Group III	Group IV
SOD (units/mg protein)	2.40 ± 0.16	4.06 ± 0.19*	1.76 ± 0.09*[a]	1.00 ± 0.07*[ab]
Catalase (μ moles of H_2O_2 consumed/ min/mg protein)	35.8 ± 2.77	52.5 ± 3.86*	19.1 ± 1.55*[a]	10.8 ± 1.10*[ab]
GPx (μg of GSH consumed/ min/mg protein)	11.2 ± 0.60	20.0 ± 1.00*	7.07 ± 0.83*[a]	5.18 ± 0.45*[ab]
GSH (μg/mg protein)	2.11 ± 0.11	3.75 ± 0.15*	1.66 ± 0.09*[a]	0.89 ± 0.04*[ab]
Vitamin C (μg/mg protein)	0.45 ± 0.04	0.73 ± 0.05*	0.34 ± 0.18[a]	0.11 ± 0.03*[ab]

Values are expressed as Mean ± SD of six animals. Methanol intoxication was as follows: Group I—control; Group II—intoxication for one day; Group III—intoxication for 15 d; Group IV—intoxication for 30 d. Comparisons were made between: *All intoxicated groups with Group I; [a]Group III & Group IV with Group II; [b]Group III with Group IV. The symbols represent statistical significance: *, a, b=$p<0.05$

Table 4. Effect of methanol on thymus enzymatic and non enzymatic antioxidants in control and experimental animals

Parameters	Group I	Group II	Group III	Group IV
SOD (Units/mg protein)	2.31 ± 0.10	3.98 ± 0.28*	1.57 ± 0.15* [a]	0.90 ± 0.10*[ab]
Catalase (μ moles of H_2O_2 consumed / /min/mg protein)	38.7 ± 1.58	49.0 ± 1.66*	19.1 ± 1.17*[a]	10.2 ± 0.87*[ab]
GPx (μg of GSH Consumed/ min/mg protein)	8.52 ± 0.35	13.5 ± 0.28*	6.01 ± 0.11*[a]	2.99 ± 0.15*[ab]
GSH (μg/mg protein)	1.65 ± 0.05	2.51 ± 0.21*	1.08 ± 0.07*[a]	0.62 ± 0.0*[ab]
Vitamin C (μg/mg protein)	0.60 ± 0.07	0.95 ± 0.05*	0.34 ± 0.04*[a]	0.15 ± 0.04*[ab]

Values are expressed as Mean ± SD of six animals. Methanol intoxication was as follows: Group I—control; Group II—intoxication for one day; Group III—intoxication for 15 d; Group IV—intoxication for 30 d. Comparisons were made between: *All intoxicated groups with Group I; [a]Group III & Group IV with Group II; [b]Group III with Group IV. The symbols represent statistical significance: *, a, b=$p<0.05$

Tables 3, 4, 5 and 6 depict the enzymatic and non-enzymatic antioxidant levels in lymphoid organs of control and experimental animals. All enzymatic (SOD, CAT and GPx) and non-enzymatic antioxidants (GSH and Vit C) were significantly ($p<0.05$) increased in 1-d methanol-exposed animals when compared with control animals. However, the enzymatic and non-enzymatic antioxidant levels were significantly ($p<0.05$) decreased

Table 5. Effect of methanol on lymph node enzymatic and non-enzymatic antioxidants in control and experimental animals

Parameters	Group I	Group II	Group III	Group IV
SOD (units/mg protein)	2.59 ± 0.07	4.06 ± 0.35*	1.43 ± 0.15*[a]	0.82 ± 0.04*[ab]
Catalase (μ moles of H_2O_2 consumed /min/mg protein)	40.4 ± 1.44	64.9 ± 2.84*	21.8 ± 1.00*[a]	16.9 ± 0.44*[ab]
GPx (μg of GSH consumed/ min/mg protein)	9.23 ± 0.40	17.6 ± 0.55*	6.73 ± 0.12*[a]	4.50 ± 0.58*[ab]
GSH (μg/mg protein)	2.45 ± 0.18	4.70 ± 0.18*	1.42 ± 0.07*[a]	0.94 ± 0.09*[ab]
Vitamin C (μg/mg protein)	0.51 ± 0.01	0.81 ± 0.04*	0.30 ± 0.01*[a]	0.20 ± 0.02*[ab]

Values are expressed as Mean ± SD of six animals. Methanol intoxication was as follows: Group I—control; Group II—intoxication for one day; Group III—intoxication for 15 d; Group IV—intoxication for 30 d. Comparisons were made between: *All intoxicated groups with Group I; [a]Group III & Group IV with Group II; [b]Group III with Group IV. The symbols represent statistical significance: *, a, b=$p<0.05$

Table 6. Effect of methanol on bone marrow enzymatic and non-enzymatic antioxidants in control and experimental animals

Parameters	Group I	Group II	Group III	Group IV
SOD (units/mg protein)	2.81 ± 0.14	4.65 ± 0.05*	1.63 ± 0.14*[a]	1.09 ± 0.06*[ab]
Catalase (μ moles of H_2O_2 consumed /min/mg protein)	34.9 ± 2.74	50.5 ± 3.22*	21.3 ± 1.43*[a]	14.9 ± 0.47*[ab]
GPx (μg of GSH consumed/ min/mg protein)	8.59 ± 0.32	16.1 ± 0.16*	4.59 ± 0.18*[a]	1.99 ± 0.17*[ab]
GSH (μg/mg protein)	1.81 ± 0.03	2.43 ± 0.05*	1.03 ± 0.07*[a]	0.70 ± 0.04*[ab]
Vitamin C (μg/mg protein)	0.33 ± 0.01	0.58 ± 0.04*	0.17 ± 0.01*[a]	0.08 ± 0.02*[ab]

Values are expressed as Mean ± SD of six animals. Methanol intoxication was as follows: Group I—control; Group II—intoxication for one day; Group III—intoxication for 15 d; Group IV—intoxication for 30 d. Comparisons were made between: *All intoxicated groups with Group I; [a]Group III & Group IV with Group II; [b]Group III with Group IV. The symbols represent statistical significance: *, a, b=$p<0.05$

in 15- and 30-d methanol-exposed animals when compared to the control animals. Also, all enzymatic and non-enzymatic antioxidant levels were significantly ($p<0.05$) decreased in the 15 and 30 d methanol exposure groupts when compared to the 1 day methanol-exposed animals. Furthermore, all enzymatic and non-enzymatic antioxidant levels were significantly ($p<0.05$) decreased in 30-d methanol-exposured animals when compared to the 15-d methanol-exposed animals. The depletion of antioxidant molecules indicates that methanol can induce oxidative damage on the immune cells of lymphoid organs.

Discussion

Methanol is known to be oxidized via three main oxidative pathways among which the alcohol dehydrogenase (folate dependent) and catalase peroxidative system have been extensively studied[26]. In rats, the oxidation of methanol is performed primarily by catalase. This enzyme forms the catalase-hydrogen peroxide (H_2O_2) system in the presence of H_2O_2, which intermediates the oxidizing of various alcohols into corresponding aldehydes. There are two known systems in which formate undergoes catalytic oxidation into carbon dioxide: the system that depends on folic acid and the system that depends on the catalase-peroxidase complex. Low activity of the enzymes that are responsible for the generation of hydrogen peroxide means that *in vivo*, formate is probably oxidized exclusively in the system that depends on tetrahydrofolate[27]. It has been found that consumption of methanol provokes changes in the activity of antioxidant enzymes, i.e. an increase in the activity of catalase[28].

A significant loss in body weight was observed in methanol-exposed rats from day 15 onwards[29]. Estimating the activities of serum marker enzymes, like AST and ALT, makes assessment of liver function

possible. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the blood stream. Therefore, their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage[30]. The enhanced activities of these serum marker enzymes was due to methanol intoxication, altered oxidant/antioxidant balance and surface charge density, which caused leakage of ALT and AST[31]. Significant increases in serum urea and creatinine levels were also seen. Creatinine has been reported to be a chemical marker of renal function with elevated concentration often taken as an indication of muscular dystrophy or atrophy[32, 33]. Methanol-induced free radicals and an imbalanced antioxidant system may damage the kidney functions and probably contributed to the increased serum urea and creatinine concentrations seen in this study.

The distribution of methanol by the blood to all organs and tissues is proportionate to their water content[7]. Exposures of tissue to free radicals in a variety of experimental systems have documented the ability of free radicals to produce damage. The detection and measurement of LPO is the evidence most frequently cited in support of the involvement of free radicals in toxicology and human diseases[34]. LPO is initiated by the abstraction of a hydrogen atom from the side chain of polyunsaturated fatty acids in the membrane[35]. LPO in cellular membranes damages polyunsaturated fatty acids tending to reduce membrane fluidity, which is essential for proper functioning of the cell. The increase in the MDA level observed in this study, which is an index of LPO, indicated lymphoid organ cell membrane damage after methanol administration. The present work also showed that the changes in LPO were accompanied by a concomitant decrease in the activities of antioxidant enzymes, namely SOD, CAT and GPx, in the 15- and 30-d methanol exposure groups. SOD, CAT and GPx constitute a mutually supportive team of enzymes which provide a defense against the intermediates of dioxygen. SOD constitutes an important link in the biological defense mechanism through dismutation of endogenous cytotoxic superoxide radicals to H_2O_2 and O_2^-, which are deleterious to polyunsaturated fatty acids and proteins[36]. Methanol administration to rats induces free radical generation and hence the first line defense comes to the rescue as shown by the significant increases in SOD in methanol-intoxicated animals[37]. In the presence of inadequate CAT or GPx activity to degrade H_2O_2, more H_2O_2 could be converted to toxic hydroxyl radicals that may contribute to oxidative stress due to methanol intoxication. A decline in the activities of these enzymes might be due to their inactivation caused by excess ROS production[38]. The antioxidant enzymes CAT and GPx protect SOD against inactivation by H_2O_2. Reciprocally, SOD protects CAT and GPx against superoxide anion. Thus, the balance of this enzyme system is essential to dispose of superoxide anion and peroxides generated in the lymphoid organs. The reduction in the activities of these enzymes and increase in LPO could reflect the adverse effects of methanol on this finely balanced antioxidant system in the lymphoid organ tissues.

After the acute methanol intoxication, there was increase in both the enzymatic and non-enzymatic antioxidants status. Free radicals initially increase due to induction of methanol intoxication. To remove the continuously generated free radicals, the endogenous antioxidant enzymes increase and act to prevent oxidative cell damage[39]. Hence, when the generation of reactive free radicals overwhelms the antioxidant defense, LPO of the cell membrane occurs. This would cause disturbances in cell integrity leading to cell damage. The increase in SOD levels with the increase in the severity of poisoning showed that the greater the stress, the more the free radicals generated. This is additionally corroborated by the increased LPO levels. The involvement of free radicals other than superoxide anions like hydroxyl radical cannot be ruled out, since the increased SOD levels were only partially effective in combating the oxidative damage[40]. This calls for the investigation of the involvement of other antioxidant enzymes (CAT, GPx) and non-enzymatic antioxidants in conditions such as oxidative stress due to methanol intoxication.

Glutathione plays a unique role in the cellular defense system against toxic chemicals of endogenous and exogenous origin[41]. In addition, GSH has been reported to be involved in protein and DNA synthesis, in the maintenance of cell membrane integrity, and the regulation of enzyme activity[42]. Depletion of GSH increases vulnerability to free radical induced damage. The decrease in GSH concentration observed in the present study seems to have been caused by methanol intoxication because methanol metabolism depends upon GSH[43]. In addition, a decrease in GSH concentration would also be caused by its rapid reaction with the highly reactive compound, formaldehyde, which is generated during methanol metabolism, forming nucleophilic adducts and/or LPO products[44, 45].

GSH reduction can also explain the decreased concentration of Vit C, which enters the cell mainly in its oxidized form where it is reduced by GSH[46]. The diminution of this vitamin has serious consequences as, in addition to its antioxidant function, it also plays a role in other regenerating antioxidants[47]. Vit C is a hydrophilic reducing agent which directly reacts with superoxides, hydroxyls, and various lipid hydro peroxides more effectively than any other water soluble antioxidant[48]. Vit C is a nutrient that regulates the immune system, and because of its antiviral and antioxidant properties, it plays a role in the phagocytic function[49]. The results of the

present study indicate that a decrease in the antioxidant status is one of the main factors contributing to methanol intoxication of the lymphoid organs. The observed significant increase in the LPO level in the immune tissues of methanol-exposed animals suggests that the tissues are subjected to increased oxidative stress. Methanol affects the antioxidant capacity of lymphoid organs, a result that could lead to impaired immune functions. The antioxidant functions were more affected in the 30-d methanol exposure group than the 1-d and 15-d exposure groups, which clearly indicates that repeated methanol intoxication plays an important role in the suppression of the lymphoid organ functions.

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