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EFFECTS OF TAURINE AND VITAMIN E ON LIPID PEROXIDATION, ANTIOXIDANT SYSTEM AND HISTOLOGICAL CHANGES IN WISTAR RAT LIVER AND KIDNEY AFTER SHORT-TERM EXPOSURE OF METHIOCARB

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SUMMARY

The present work was designed to examine possible effects of methiocarb on lipid peroxidation, reduced glutathione (GSH), antioxidant enzymes such as superoxide distumtase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) ve glutathione reductase (GSH-Rd) and histological changes in Wistar albino rats after 5-day exposure. Moreover, we evaluated possible protective effects of vitamin E and taurine on methiocarb-induced oxidative damage. Fourty-eight rats were randomly divided into six groups as follows: control; methiocarb; vitamin E; vitamin E+methiocarb; taurine and taurine+methiocarb. 5-day exposure of methiocarb (1/10 of LD_{50}) significantly increased lipid peroxidation in liver and kidney when compared to control group. Levels of GSH and activities of SOD and CAT were found to be decreased, while GSH-Px increased and GSH-Rd remained unchanged in rat tissues treated with methiocarb. Pre-treatment of vitamin E (100 mg/kg b.w.) and taurine (50 mg/kg b.w.) resulted in a significant decrease on lipid peroxidation, alleviating effects on GSH and antioxidant enzymes. Administration of methiocarb caused to the degenerative changes in liver and kidney tissues. Pre-treatment of vitamin E and taurine were partially protected both tissues against methiocarb-induced liver and kidney injury. In conclusion, the study has demonstrated that 5-day methiocarb exposure in Wistar rats caused oxidative damage on liver and kidney which were partly ameliorated by pre-treatment of vitamin E and taurine.

Key words: Methiocarb; Taurine; Vitamin E; Lipid peroxidation; Antioxidant system; Histological changes

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ÖZET

Bu çalışma, Wistar albino sıçanlara 5 günlük methiocarb maruziyetinin lipid peroksidasyonu, indirgenmiş glutatyon (GSH), süperoksid dizmütaz (SOD), katalaz (CAT), glutatyon peroksidaz (GSH-Px) ve glutatyon redüktaz (GSH-Rd) gibi antioksidan enzimler ve histolojik değişiklikler üzerine muhtemel etkilerinin araştırılması için planlandı. Buna ilave olarak, methiocarb indüklü oksidatif hasar üzerine E vitamini ve taurininin muhtemel koruyucu etkileri de araştırıldı. 48 adet sıçan randomize olarak 6 gruba ayrıldı: kontrol; methiocarb; E vitamini; E vitamini+metiocarb; taurin ve taurin+metiyocarb. Kontrol grubuna kıyasla 5 günlük metiyokarb maruziyetinin (1/10 LD_{50}) karaciğer ve böbrekte lipid peroksidasyonunu arttırdı. Metiyokarb maruziyeti sonucunda sıçan dokularında GSH seviyeleri ve SOD ve CAT enzim aktivitelerinde düşüş ve GSH-Px aktivitesinde artış gözlenirken, GSH-Rd aktivitesinde ise bir değişiklik gözlenmedi. E vitamini (100 mg/kg) ve taurin (50 mg/kg) ile tedavi sonrasında lipid peroksidasyonda belirgin bir düşüş ve GSH seviyelerinde ve antioksidan enzim aktivitelerinde de düzelme olduğu gösterildi. Methiocarb maruziyetinin karaciğer ve böbrek dokularında dejeneratif değişikliklere yol açtığı gösterildi ve E vitamini ve taurin ile tedavi sonrasında her iki dokuda da meydana gelen histolojik değişikliklerde kısmen iyileşme olduğu gösterildi. Sonuç olarak, 5 günlük methiocarb maruziyetinin Wistar sıçanların karaciğer ve böbreklerinde oksidatif hasara sebep olduğu ve bu hasarın da E vitamini ve taurin tedavisi ile kısmen iyileşme gösterdiği sonucuna varılmıştır.

Anahtar Kelimeler: Methiocarb; Taurin; E Vitamini; Lipid peroksidasyonu; Antioksidan enzimler; Histolojik değişiklikler

INTRODUCTION

N-methylcarbamate pesticide, methiocarb (4-methylthio-3,5-xylylmethylcarbamate) has a wide range of use in agriculture and public health applications. However, the unregulated use and its aerial application over large agricultural areas have caused environmental pollution and potential health hazards. Residual amounts of methiocarb have been reported in the water, soil, honey, plants, fruits and vegetables [1-9]. Moreover, the first clear report on a fatal poisoning of a human with methiocarb has been reported recently [10]. Methiocarb is a non-systemic

acaricide with contact and stomach action, molluscicide with neurotoxic action and a bird repellent when used as a seed treatment. It is classified as a highly hazardous compound (Class 1B) by World Health Organization [11] with acute oral lethal dose (LD_{50}) of 100 mg/kg for male rats [12]. In 2007, methiocarb was included in an endocrine disrupter screening program of the US Environmental Protection Agency due to its multiple exposure pathways and high production volume [13]. The main toxic mechanism of methiocarb is inhibition of acetylcholinesterase enzyme, which causes the accumulation of acetylcholine and subsequent activation of cholinergic system. However, in recent years, the possible role of oxidative stress in carbamate toxicity has also been highlighted [14]. In our previous study we have reported that oral administration of methiocarb at its acute and subacute concentrations induced lipid peroxidation in Wistar rats [15]. However, no attention has been paid whether there were any effects of different doses and treatment time on methiocarb induced oxidative damage in rat tissues. Further, the protective role of known antioxidant such as vitamin E on pesticide-induced toxicity also needs to be studied. Vitamin E is a lipid soluble, chain-breaking antioxidant playing a major protective role against oxidative stress. Several studies reported that administering Vitamin E may be useful in controlling the toxic effect of pesticides [16-21]. Taurine (2-aminoethanesulfonic acid), a ubiquitous free amino acid is present in most mammalian tissues. It is known to have various physiologic functions in cell metabolism including osmoregulation, membrane stabilization, detoxification and regulation of cellular calcium homeostasis [22]. In addition, taurine has been demonstrated to be an antioxidant by scavenging reactive oxygen radicals, inhibition of lipid peroxidation and to protect cells against toxic injury of oxidant compounds [23-25]. In view of this, in the present study the aims were to highlight the effects of methiocarb on lipid peroxidation, GSH and antioxidant enzyme systems such as SOD, CAT, GSH-Px and GSH-Rd and to investigate the ensuing histological changes after 5 days exposure. Moreover, we investigated the putative protective effects of taurine comparison with vitamin E against methiocarb induced hepatotoxicity and nephrotoxicity.

MATERIALS AND METHODS

Materials

Chemicals

Methiocarb (also known by the trade name Mesurol), technical purity 99.8%, was obtained from Bayer (Istanbul, Turkey). Vitamin E (Evigen, 300 mg DL-a-tocopherol acetate) was purchased from Aksu Farma (Istanbul, Turkey). Taurine and all other chemicals were obtained from Sigma-Aldrich Chemicals Co (St. Louis, MO, USA), and Merck (Darmstadt, Germany).

Animals

Male Wistar Albino rats, aged 12-13 weeks and weighed 160 ± 20 g were used in this study. Animals were kept in clean polypropylene cages under standard laboratory conditions (temperature at 25 ± 2 °C, relative humidity of 50 ± 15% and normal photoperiod of 12-12 h light-dark cycle) with free access to standard dry pellet diet and water *ad libitum*. All treatments were started after almost 1 week of stabilization from arrival. The experiments reported here complied with the current laws and regulations of the Turkish Republic on the care and handling of experimental animals and the Animal Ethical Committee of Cerrahpasa Faculty of Medicine.

Experimental Design

The animals were randomly divided into 6 experimental groups each containing eight rats. The substances were administered in the morning (between 09:00 and 10:00 h) to non-fasted rats. The dose of methiocarb (10 mg/kg b.w.; 1/10 of LD_{50}) used in present study were selected based on data from previous study which showed that this dose induce lipid peroxidation in rat tissues [15] Dose selection of vitamin E (100 mg/kg b.w.) was based on the published studies which showed that this dose was effective against the toxicity of pesticides (methiocarb, fenvalerate, deltamethrin) [18, 26-27]. Dose selection of taurine (50 mg/kg b.w.) was also based on the published studies which showed that this dose was effective against the toxicity induced by various xenobiotics [26, 28-31]. The pre-treatment of vitamin E and taurine was provided in order to build antioxidant pool in animal body before pesticide exposure.

Control group (C): Rats received i.g. the equivalent volumes of corn oil (0.5 mL per animal) instead of methiocarb for 5 day.

Methiocarb-treated group (MC): Methiocarb at the dose of 10 mg/kg

b.w. (1/10 of LD_{50}) in corn oil was applied to rats i.g. for 5 day.

Vitamin E- treated group (Vit E): Commercial available vitamin E at the dose of 100 mg/kg b.w in olive oil (0.5 mL per animal) was applied to rats i.p. once a day for 25 days.

Vitamin E plus methiocarb-treated group (Vit E + MC): Before methiocarb treatment, rats were pretreated i.p. with vitamin E (100 mg/kg b.w.) once a day for 20 days and then methiocarb at the dose mentioned above was applied along with vitamin E for 5 days.

Taurine-treated group (T): Taurine at the dose of 50 mg/kg b.w. in distilled water (0.5 mL per animal) was applied to rats i.p. once a day for 25 days.

Taurine plus methiocarb-treated group (T + MC): Before methiocarb treatment, rats were pretreated i.p. with taurine (50 mg/kg b.w.) once a day for 20 days and then methiocarb at the dose mentioned above was applied along with taurine for 5 days.

At the end of the treatments, the rats were sacrificed by cervical dislocation. Liver and kidney samples were dissected out and washed immediately with ice-cold physiological saline (0.9% NaCl) and one parts of the both tissues immediately stored at -80°C until analysis. The other parts of the both tissues were taken from the rats for histological analysis.

Preparation of Tissue Samples

The tissues were homogenized in 0.9% NaCl using an Ultra Turrax tissue homogenizer to make up the 10% tissue homogenate (w/v) and then, one parts of tissue homogenate (10%) centrifuged at 10000 × g at 4°C for 20 min to obtain cytosolic fraction. Tissue homogenates (10%) were used to determine levels of GSH and MDA. Cytosolic fractions were used to determine activities of antioxidant enzymes.

Methods

Lipid Peroxidation Assay

Quantitative measurement of lipid peroxidation was performed in liver and kidney homogenates (10%) according to the method of Buege and Aust [32] based on the formation of thiobarbituric acid reactive substances (TBARS) and expressed as the extent of malondialdehyde (MDA) production. The amount of TBARS was determined in tissue homogenates by using reversed-

phase high-performance liquid chromatography (HPLC) with UV diode-array detection at 532 nm according to the modification of the HPLC method of Draper and Hadley [33]. The levels of TBARS were calculated using 1,1,3,3-tetraethoxypropane as the standard and expressed as nmol MDA per mg of protein [26].

GSH Assay

Levels of GSH were determined in the liver and kidney homogenates (10%) according to the method of Beutler [34] by using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) reagent. DTNB was reduced by free -SH groups of GSH to form 5-mercapto-2-nitrobenzoate and its absorbance were measured by spectrophotometric means at 412 nm. Results were expressed as μmol GSH per mg of protein using standard calibration curve.

Measurement of Antioxidant Enzymes

Activities of antioxidant enzymes were determined in the cytosolic fractions of liver and kidney homogenates. SOD activity was determined according to the method of Sun et al. [35] based on the inhibition of nitroblue tetrazolium (NBT) reduction by using the xanthine-xanthine oxidase system as a superoxide generator. CAT activity was assayed by the decomposition of hydrogen peroxide according to the method of Aebi [36]. GSH-Px activity was determined according to the method of Pleban et al. [37] based on the decrease in the absorbance of reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm. GSH-Rd activity was performed by monitoring the oxidation of NADPH in the presence of oxidized glutathione according to the method of Beutler [38]. Specific activities were expressed as U/mg protein for SOD and GSH-Px, mU/mg protein for GSH-Rd and k/mg protein for CAT using standard calibration curves.

Protein Assay

Contents of proteins were measured in the liver and kidney homogenates (10%) and cytosolic fractions of liver and kidney homogenates according to the method of Lowry et al. [39] using bovine serum albumin as standard.

Histological Examinations

Liver and kidney tissues were cut into small pieces and fixed in Bouin's solution. Following dehydration in an ascending series of ethanol, tissue samples were cleared in xylene and embedded in paraffin and sliced in 5 μm sections. Slides were stained with Masson's triple dyes (Masson). All sections were examined under a light microscope (Olympus-CX 41).

Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Data were analyzed by ANOVA test using "SPSS version 13.0 for Windows", statistical program and the individual comparisons of groups were obtained by using Scheffe`s multiple comparison procedure. p values of less than 0.05 and 0.001 were selected as the levels of significance.

RESULTS

Biochemical evaluations

None of the rats treated with methiocarb at the dose of 10 mg/kg b.w. (1/10 LD_{50}) showed sign of morbidity or mortality during the studies. As shown in Table 1-2, treatment of rats with vitamin E and taurine alone did not affect the levels of MDA and GSH and activities of SOD, CAT, GSH-Px and GSH-Rd in liver and kidney when compared to the vehicle control groups.

As shown in Table 1, content of MDA in the rats treated with methiocarb was 1.35-fold increased in liver ($p<0.05$) and 1.5-fold in kidney ($p<0.001$) when compared to the control group. Pretreatment with vitamin E and taurine resulted in a significant decrease in the content of hepatic ($p<0.05$) and renal ($p<0.05$, $p<0.001$, respectively) MDA when compared to the methiocarb group. Conversely, as depicted in Table 1, the level of GSH in the rats treated with methiocarb was significantly decreased in liver (29%, $p<0.001$) and kidney (35%, $p<0.001$) when compared to the vehicle control group. However, pretreatment with vitamin E and taurine resulted in a significant increase in the level of hepatic ($p<0.05$) and renal ($p<0.05$) GSH in comparison with that of the methiocarb group.

Table 1. The effects of vitamin E and taurine on MDA and GSH levels in methiocarb treated rat liver and kidney. Values are expressed as Mean ± SD; n=8 for each treatment group.

	C	MC	Vit E	Vit E + MC	T	T + MC
MDA (nmol/mg protein)						
Liver	3.86 ± 0.58	5.23 ± 0.66 [a]	3.73 ± 0.46	4.08 ± 0.42 [b]	3.80 ± 0.44	4.16 ± 0.19 [b]
Kidney	5.26 ± 0.9	7.89 ± 0.94 [c]	5.26 ± 0.49	5.69 ± 0.61 [d]	5.36 ± 0.24	6.07 ± 0.36 [b]
GSH (μmol/mg protein)						
Liver	0.17 ± 0.01	0.15 ± 0.01 [c]	0.18 ± 0.01	0.17 ± 0.01 [b]	0.17 ± 0.01	0.17 ± 0.01 [b]
Kidney	0.1 ± 0.014	0.08 ± 0.01 [c]	0.1 ± 0.003	0.1 ± 0.003 [b]	0.1 ± 0.003	0.1 ± 0.01 [b]

[a]Significantly different from control group ($p<0.05$). [b]Significantly different from methiocarb group ($p<0.05$). [c]Significantly different from control group ($p<0.001$). [d]Significantly different from methiocarb treated group ($p<0.001$).

As presented in Table 2, the activity of SOD in the methiocarb group was significantly decreased in liver (28%, $p<0.001$) and kidney (19%, $p<0.001$) when compared to the control group. However, pretreatment with vitamin E and taurine resulted in a significant increase in the activity of SOD in liver ($p<0.05$) and kidney ($p<0.05$) in comparison with that of the methiocarb group. As depicted in Table 2, the activity of CAT in the methiocarb group was significantly decreased in liver (16%, $p<0.001$) and kidney (21%, $p<0.05$) when compared to the vehicle control group. However, the activity of CAT was significantly increased by vitamin E and taurine pretreatment in liver ($p<0.05$) and kidney ($p<0.05$) when compared to the methiocarb group. As shown in Table 2, the activity of GSH-Px in the methiocarb group was significantly increased in liver (36%, $p<0.001$) and kidney (29%, $p<0.05$) when compared to the vehicle control group. However, pretreatment with vitamin E and taurine resulted in a significant decrease in the activity of GSH-Px in liver ($p<0.05$) and kidney ($p<0.05$) in comparison with that of the methiocarb group. As shown in Table 2, the activity of GSH-Rd in the methiocarb group in nonsignificantly increased in liver (8%) and kidney (6%) when compared to the control group. Pretreatment with vitamin E and taurine showed no change in the activity of GSH-Rd in liver and kidney when compared to the methiocarb group.

Table 2. The effects of vitamin E and taurine on the activities of SOD, CAT, GSH-Px, GSH-Rd in methiocarb treated rat liver and kidney. Values are expressed as Mean ± SD; n=8 for each treatment group.

	C	MC	Vit E	Vit E + MC	T	T + MC
SOD (U/mg protein)						
Liver	137.1 ± 16	98.31 ± 16 [c]	138.7 ± 6.57	130.3 ± 7.1 [b]	136.1 ± 9.8	123.4 ± 9.3 [b]
Kidney	204.9 ± 19	166.1 ± 7.8 [c]	199.6 ± 6.59	196.3 ± 9.7 [b]	198.4 ± 7.7	189.7 ± 6.7 [b]
CAT (k/mg protein)						
Liver	555 ± 39.2	467 ± 53.3 [c]	559 ± 18.7	549 ± 19.8 [b]	556 ± 7.73	533 ± 11.0 [b]
Kidney	548 ± 94.8	431 ± 42.1 [a]	552 ± 10.3	539 ± 13.8 [b]	545 ± 12.8	527 ± 31.4 [b]
GSH-Px (U/mg protein)						
Liver	15.36 ± 2.4	20.89 ± 3.7 [c]	15.11 ± 0.64	15.66 ± 0.8 [b]	14.62 ± 1.1	16.12 ± 2.2 [b]
Kidney	19.53 ± 1.9	25.15 ± 3.3 [a]	19.64 ± ± 1.7	20.19 ± 0.9 [b]	19.78 ± 0.6	20.98 ± 1.1 [b]
GSH-Rd (mU/mg protein)						
Liver	6.11 ± 0.68	6.60 ± 0.93	6.42 ± 1.2	6.44 ± 0.36	6.27 ± 0.39	6.81 ± 0.40
Kidney	8.41 ± 0.56	8.88 ± 0.52	8.16 ± 0.37	8.23 ± 0.22	7.96 ± 0.65	8.11 ± 0.47

[a]Significantly different from control group ($p<0.05$). [b]Significantly different from methiocarb group ($p<0.05$). [c]Significantly different from control group ($p<0.001$).

Histological Examinations

Light microscopic appearances of liver for all groups are presented in Fig. 1. Some histological changes were noticed in liver tissues of control groups given especially only vitamin E or taurine. In the liver tissue after 5-day methiocarb treatment, moderate degenerative changes such as vacuolization and hyperemia, accumulation of collagen fibres in connective tissue around veins of some animals, few picnotic nuclei and mild sinusoidal dilatations were observed. While, these degenerative changes were decreased except for mild vacuolization and hyperemia by vitamin E and except for mild hyperemia by taurine in 5-day methiocarb treated groups. The most improvement was noticed in periferal area of central veins in the liver tissues.

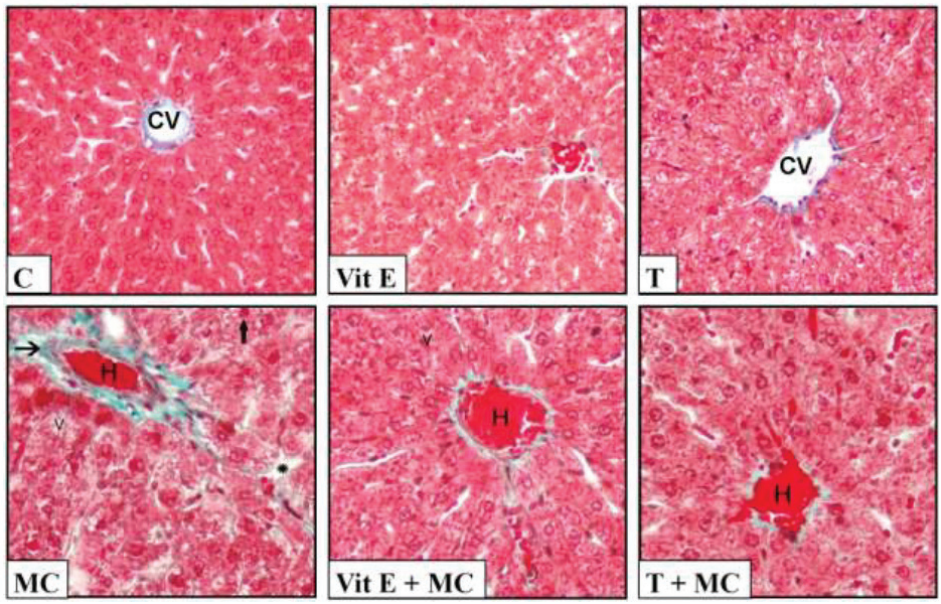

Fig. 1. The effects of vitamin E and taurine on histological changes in methiocarb-treated rat liver. Central vein (CV), vacuolization (V), hyperemia (H), picnotic nuclei (²), sinusoidal dilatation (*), collagen accumulation in connective tissue (g). C: Control, Vit E: Vitamin E-treated group, T: Taurine-treated group; M: Methiocarb-treated group, VitE + MC: Vitamin E and Methiocarb-treated group, T + MC: Taurine and Methiocarb-treated group. Masson, X 270.

Light microscopic appearances of kidney for all groups are presented in Fig. 2. Partly histological changes were observed in the kidney tissue of the control animals. Methiocarb treatment caused marked alterations such as widening between parietal and visseral leaves of bowman capsules, shortening at the brush border, cytoplasmic debris and desquamated nuclei in the widened lumens of proximal tubules, accumulation of collagen fibers in necrotic areas, a mild hyperemia and few mononuclear cell infiltration. On the other hand, treatment of vitamin E and taurine was partially decreased kidney damage in methiocarb-administered rats.

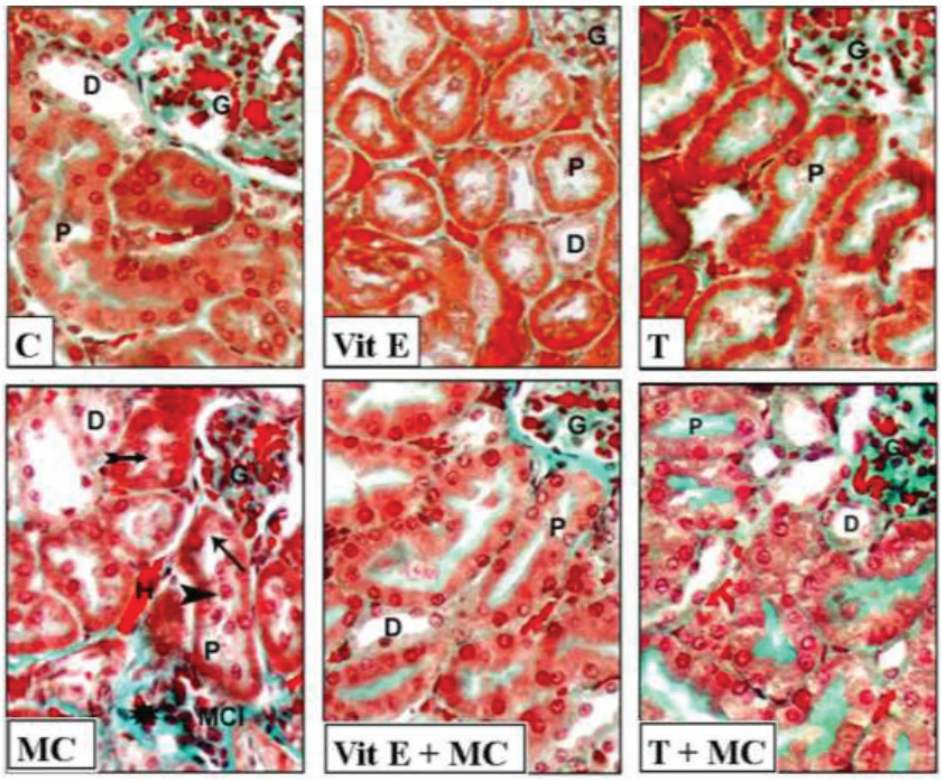

Fig. 2. The effects of vitamin E and taurine on histological changes in the kidney of methiocarb-treated rats. The shortening at the brush border (à), desquamated nuclei (➤) in the proximal tubules (P) cytoplasmic debris (▯) in the proximal tubules (P), accumulation of collagen fibers in necrotic areas (), hyperemia (H) and few mononuclear cell infiltration (MCI) can be seen in the kidney of Methiocarb-treated rats. C: Control, Vit E: Vitamin E-treated group, T: Taurine-treated group; M: Methiocarb-treated group, VitE + MC: Vitamin E and Methiocarb-treated group, T + MC: Taurine and Methiocarb-treated group. Masson, X270.

DISCUSSION

Toxic manifestations induced by carbamates may be associated with induction of oxidative stress through the generation of free radicals and alteration in antioxidant systems [14, 40-50]. The aims of the present work were to highlight 5-day exposure of methiocarb on antioxidant defense

mechanisms and histological alterations in rat liver and kidney in order to study effects of methiocarb on oxidative damage.

The most widely used marker of lipid peroxidation is MDA formation often assayed with the thiobarbituric acid assay. We observed that 5-day oral dose of methiocarb resulted in significant increase in the hepatic and renal content of MDA, indicating increased lipid peroxidation caused by administration of methiocarb. Radicals may be generated during the metabolism of methiocarb by the cytochrome P450-oxidase and FMOs enzyme systems and induce lipid peroxidation [15]. Recent study indicated that oral administration of carbaryl at a dose of 225 mg/kg b.w. for 21 days increased the levels of MDA in rat blood and tissues [14]. Another study reported by Eraslan et al. demonstrated that 14-day exposure of propoxur at a dose of 20 mg/kg b.w. increased the levels of MDA in rat blood and tissues [41]. Pre-treatment with vitamin E resulted in higher decrease in the content of MDA in liver (22%) and kidney (28%) than taurine (20% in liver and 23% in kidney) when compared to the methiocarb group. Garg *et al*. also reported that pre-treatment with vitamin E decreased levels of MDA after single dose of methomyl administration in rat erythrocytes [51].

GSH, one of the most potent biological molecules, play a key role in the detoxification of the reactive toxic metabolites. A considerable decline in GSH levels in liver and kidney after 5-day exposure of methiocarb may have been due to its utilization to challenge the common oxidative stress in the influence of ROS generated from methiocarb metabolism [15]. The decrease of renal GSH level (35%) was more than the decrease of hepatic GSH level (29%), confirming that liver has more antioxidant potential then kidney. Maran et al. reported that glutathione plays a central role in the wide range of cellular functions of CHO-K1 cells including protection and detoxification of carbamate pesticides such as aldicarb and propoxur [52]. Our findings are in accord with those of Seth et al. who have reported the decreasing of GSH levels by propoxur in rat blood after 30-day exposure [48], and Banks et al. showed that 7-day exposure of benomyl at a dose of 200 mg/kg b.w. decreased the GSH levels in rat liver [40]. However, pre-treatment with vitamin E and taurine normalised the decreased levels of GSH after 5-day exposure of methiocarb in liver and kidney.

Antioxidant enzymes, mainly SOD, CAT and GSH-Px are the first line of defense against free radical induced oxidative stress. SOD catalyses the

destruction of the superoxide radicals and protects oxygen-metabolizing cells against harmful effects of superoxide radicals [53]. CAT is responsible for the catalytic decomposition of hydrogen peroxide to molecular oxygen and water [36]. The decreasing in the activities of SOD and CAT in liver and kidney after 5-day exposure of methiocarb appears to be due to increased generation of reactive oxygen species. These findings are in accordance with those of Eraslan *et al*. who reported that 14-day exposure of propoxur caused a significant decrease in the activities of SOD and CAT in rat liver [41]. GSH-Px, responsible for enzymatic defence against hydrogen peroxide or other lipid hydroperoxides, is strictly linked with the concentration of GSH because it catalyses the reaction between glutathione and hydrogen peroxide, resulting in the formation of glutathione disulphide [54]. Furthermore, GSH-Px activity appears to be linked to the activity of GR which supplies reducing equivalent for GSH-Px function. While methiocarb caused a significant increase in activity of GSH-Px, GSH-Rd remained unchanged in rat liver and kidney after 5-day exposure of methiocarb. The obtained results indicated that increased production of reactive oxygen species may cause an adaptive increase in the activity of GSH-Px which confirms the reduction of GSH levels. However, no difference in the activities of GSH-Rd shows disturbances in the activities of the enzyme regulating glutathione metabolism. These findings are compatible with study of El-Sharkawy et al. who have reported no difference of GSH-Rd activity in mice liver following 1- and 5-day periods of carbaryl treatment [55]. Pre-treatment of vitamin E and taurine attenuated the activities of SOD, CAT and GSH-Px to the similar values noted in normal control rats which indicate that vitamin E and taurine provide protection against methiocarb-induced oxidative stress.

As clearly demonstrated in present study, pretreatment with vitamin E and taurine decreased the levels of lipid peroxidation and attenuated the levels of GSH and the activities of antioxidant enzymes in liver and kidney. Vitamin E is a chain-breaking antioxidant playing a major protective role against oxidative stress Biochemical investigations of the present study was demonstrated that taurine has a prominent protective effect against pesticide toxicity as well. The protective effects taurine in biological systems resulted from antioxidant activity including stabilization in the intracellular defense systems, reductions in the lipid peroxidation products and the production of reactive oxygen species [22].

In the present study, light microscopic observations showed that 5-day exposure of methiocarb leads degenerative changes in the morphology of rat liver and kidney. We previously observed that 1-day and 28-day exposures of methiocarb showed morphological changes in rat liver and kidney [26, unpublished data]. Hepatic damage after 5-day exposure of methiocarb was more than both of 1-day and 28-day exposures [26, unpublished data]. In addition, pre-treatment of vitamin E and taurine showed the most evident improvement of the histological liver damage after 5-day exposure of methiocarb according to the other our earlier findings [26, unpublished data]. We observed renal injury after 5-day exposure of methiocarb was less than both of 1-day and 28-day exposures [26, unpublished data]. Also, pre-treatment of vitamin E and taurine partly decreased methiocarb-induced degenerative damage in kidney after 5-day exposure. We might say that pre-treatment of vitamin E and taurine showed more protective effect on the liver comparing to the kidney against 5-day methiocarb-induced injury. The regeneration capacity can be held liable for the response differences of these tissues. The liver is well known as one of the few organs in the body that has the ability to regenerate of its tissue. Injury of liver can induce the proliferation of the remained hepatocytes until the lost mass is restored [56]. As a contrary, regenerative capacity of the kidney remains largely unexplored. Following an injury, the proximal tubular epithelial cells are more damaged and the surviving epithelial cells undergo proliferation to replenish the epithelial lining of the proximal tubule, however, the mechanisms remain controversial [57]. Briefly, we might suggest that seriously damaged kidney tissue could not regenerate itself completely because of its lower regeneration capacity when comparing to liver tissue after pre-treatment of vitamin E and taurine.

In conclusion, present study shows that oxidative stress may play a key role in methiocarb-induced liver and kidney toxicity as demonstrated by induction of lipid peroxidation, altered antioxidant status of cells and histological damages in liver and kidney. Furthermore, vitamin E and taurine may partially modulate the toxic responses resulting from 5-day exposure of methiocarb.

Declaration of interest*:* The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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