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Research Article

Determination of Reduced Glutathion, Glutathione-S-Transferase and Selenium Levels in Goose Liver Cells with Damage Induced by Carbon Tetrachloride and Ethanol

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Abstract: The pathological effects of different doses of carbon tetrachloride (CCl_4) and ethyl alcohol (C_2H_5OH) on reduced glutathione (GSH) and selenium (Se) levels, and on glutathione-S-transferase (GST) activity in the livers of geese were examined and compared.

The experiments were carried out on 3-week-old clinically healthy geese (*Anser anser*) weighing 200 - 250 g (40 animals), divided into control and experimental groups of 10 animals each. Group I was the control group; groups II and III were given 1 ml/kg CCl_4 and 1.5 ml/kg body weight (bw) CCl_4, respectively; and group IV was given 5 ml/kg bw 50% ethanol. The treatment was administered orally 3 times a week for12 weeks.

The levels of reduced glutathione in the treated groups were significantly higher than those in the control group ($P < 0.01$). Glutathione S-transferase activity and selenium were significantly lower in groups II, III and IV than they were in the control group ($P < 0.01$).

In clinical examinations carried out on the groups dosed with CCl_4 and ethyl alcohol, loss of appetite, diarrhea, coordination defect, difficulty in walking, collapsing of the wings, and torticollis were observed. Under microscopic examination, hydropic and fat degeneration and necrosis formed by cellular infiltration of the liver were observed and radiography revealed that the skeletal system was completely deformed and the bones were twisted in parts.

Key Words: Carbon tetrachloride, ethyl alcohol, free radicals, reduced glutathione, glutathione-S-transferase, selenium, geese

Kaz Karaciğerlerinde Karbon Tetraklorür ve Etil Alkol ile Oluşturulan Doku Hasarlarında Redükte Glutatyon, Glutatyon-S-Taransferaz ve Selenyum Düzeylerinin Araştırılması

Özet: Bu çalışmada, karbon tetraklorürün (CCl_4) farklı iki dozu ile etil alkolün kaz karaciğerlerindeki GSH, GST ve selenyum düzeylerine etkileri ile patolojik değişimleri incelenerek karşılaştırılmıştır. Çalışmada 40 adet 200 - 250 g klinik olarak sağlıklı 3 haftalık *Anse anser* ırkı kaz palazı kullanıldı. Hayvanlar 4 eşit gruba ayrıldı. Birinci grup kontrol grubunu oluşturmuştur. Bu grup standart yem ve su ile ad libutum olarak beslendi. II. gruba canlı ağırlık başına 1 ml/kg CCl_4, III. gruba 1,5 ml/kg CCl_4 ve IV. gruba da %50'lik etil alkolden canlı ağırlık başına 5 ml/kg haftada üç kez olmak üzere 12 hafta boyunca oral yolla verildi.

Karbon tetraklorür uygulan gruplarda redükte glutatyon düzeyleri kontrol grubundan anlamlı derecede yüksek $P < 0, 01$, glutatyon S-transferaz aktivitesi ve selenyum düzeyleri ise düşük bulundu ($P < 0,01$).

Çalışmada CCl_4 ve etil alkol uygulanan gruplarda yapılan klinik incelemelerde iştah kaybı, ishal, koordinasyon bozukluğu, yürüme güçlüğü, kanatlarda düşme, tortikollis ve mikroskobik olarak da karaciğerde hidropik ve yağ dejenerasyonları, sellüler inflitrasyon ve nekroz şekillendiği görüldü. Radyolojik olarak da iskelet sisteminin tamamen deforme olduğu ve yer yer kemiklerde eğilmeler görüldü.

Anahtar Sözcükler: Karbon tetraklorür, etil alkol, serbest radikaller, redükte glutatyon, glutatyon-S-transferaz, selenyum, kaz

Introduction

Experimental evidence indicates that a number of toxic and carcinogenic processes, induced by physical and chemical agents in the liver and other organs, involve the formation of reactive radical species that can induce auto-oxidative changes in biomembranes and other cellular components, resulting eventually in cell death (1). Free radical-mediated peroxidation phenomena play an important role in the mechanism of cellular damage caused by carbon tetrachloride and ethyl alcohol (2).

It is well documented that CCl_4 triggers hepatic and renal changes in animals and humans. Activation of CCl_4 by the cytochrome p-450 is a prerequisite for CCl_4-induced hepatic injury (3). It is known that the hepatic cytochrome p-450 content decreases following starvation (4), but that starvation enhances CCl_4-induced hepatic injury. There are reports that the increase in CCl_4-induced hepatic injury caused by starvation results from a decrease in GSH content (5,6). On the other hand, it has been reported that ethanol and its major metabolite, acetaldehyde, are able to stimulate lipid peroxidation (LP), possibly through the formation of free radicals or via depletion of antioxidant substances (7). Heavy, long-term alcohol consumption clearly plays a major role in the development of alcohol-related liver damage (2,8,9). Much of the direct cell damage that occurs during alcoholic liver disease is thought to be caused by free radicals. These fragments are quickly scavenged by natural protective molecules in the cell, called antioxidants (e.g., reduced glutathione (GSH) and glutathione-S-transferase (GST)). Inhibition of LP is commonly used in analysis to determine antioxidative activity (10,11).

Hepatic GSH, GST and Se play an important role in the detoxification of xenobiotics, including CCl_4 and alcohol (12). GSH and its related enzymes such as GSH-Px, GSH reductase and GST are involved in cellular detoxification (13). Due to the sensitivity of these enzymes to environmental pollutants and drugs, they have been used as markers of the toxic effects of ingestion or inhalation of various xenobiotics (6,10,11). GST is thought to play a physiological role in initiating the detoxification of potential alkylating agents (14,15). These enzymes catalyze the reaction of such compounds with the SH-group of glutathione, creating products that are more water soluble (15).

Se is an essential trace element in the nutrition of mammalian and domestic animals. It is a structural component of GSH-Px (16). Se functions as a cofactor of 2 functionally distinct enzymes: GSH-Px and 52 deiodinase typ I cthyroxine deiodinase (17,18). Se dependent GSH-Px and other antioxidants are involved in the elimination of free radicals and reactive oxygen species (ROS). Highly reduced nutritional intake of Se may impair enzyme activity and thus have deleterious effects on not only on cells and organs but also on the whole organism (11).

In this study pathological effects of different doses of CCl_4 and ethyl alcohol on GSH and Se levels, and GST activities in the livers of geese were examined and compared.

Materials and Methods

Animals and Treatment

Forty, 3-week-old geese (*Anser anser*), weighing 200-250 g, were divided randomly into 4 equal groups and fed a standard pellet diet providing 2910 kcal kg^{-1} (19). (Tables 1 and 2). They were kept in a room with controlled temperature (23 ± 2 °C) and relative humidity (60 ± 10%). The light was controlled by an automatic timer on a 12 h light / 12 h dark cycle.

Group I was the control group. Groups II and III were administered orally 1 ml/kg bw CCl_4 and 1.5 ml/kg bw CCl_4, respectively, and group IV was given 5 ml/kg bw ethanol 1:1 w/w (5 ml/kg b.w) 3 times a week for 12 weeks. The body weights all of the geese were recorded at the end of each week.

Table 1. Diet composition (Part 1, 200 kg).

Corn	61.20%
Soybean meal	26.20%
ACK	10.00%
Limestone	1.20%
Salt	0. 25%
Dicalcium phosphate	0.80%
Vit + Min.*	0.35%
CP	20.00%
Kcal/DM	2910

Table 2. Diet composition (Part 2, 400 kg).

Corn	60.00%
Wheat	15.40%
Soybean meal	16.00%
Limsestone	1.20%
Salt	0.25%
Dicalcium phosphate	0.80%
Vit + Min.*	0.35%
Wheat bran	6.00%
CP	20.00%
Kcal/DM	2910

* Vit.+Min. Promix: Supplementary mix per kg ration: Vitamin A, 21,000 IU; Vitamin D3, 42.00 IU; Vitamine E, 52.5 mg; Vitamin K3, 4.38 mg; Vitamin B1, 5.25 mg; Vitamin B2, 12.25 mg; Vitamin B6, 7 mg; Vitamin B12 0.03 mg; Folic acid, 1.75 mg; D-Biotin, 0.08 mg; Vitamin C, 87.5 mg; Niacin, 70 mg; Cal-D-pantothenate, 14 mg; Choline chloride 218.75 mg; Fe, 140 mg; Zn, 105 mg; Cu, 14 mg; Co, 0.35 mg; I, 1.75 mg; Se, 0.26 mg; Mn, 140 mg.

Biochemical Analysis of Tissues

The geese were euthanized under ether anesthesia after 12 weeks. The liver was immediately excised. The tissues were weighed, rinsed with ice-cold deionized water, cut into small pieces, then dried with filter paper and homogenized using the appropriate buffer depending upon the variable to be measured. The homogenates were centrifuged at 700 g for 10 min and recentrifuged at 16,500 g for 20 min at 4 °C to obtain a postnuclear homogenate and postmitochondrial supernatant fractions. The liver and kidneys were either used immediately to study mitochondrial function or stored at –70 °C for subsequent assays.

GSH-S-transferase (EC 2.5.1.18) was determined according to the procedure described by Habig et al. (15). The specific activity of GST was expressed as µmol GSH-CDNB (1-chloro-2.4-dinitrobenzene) conjugate formed/min/mg protein using an extinction coefficient of 9.6 mM^{-1} cm^{-1}.

The reduced GSH levels of the tissue homogenates were measured spectrophotometrically using Elman's reagent (20).

The activity of the enzymes and GSH were calculated to 1 g protein content of the 10,000 g supernatant fraction, which was determined by Folin-phenol reagent with bovine serum albumin as the standard (21).

The Se levels of the tissue homogenates were measured spectrofluorometrically (22).

Statistical Analysis

Groups II, III and IV were compared with the controls (group I).

The results of the experiment were evaluated statistically by Student's t-test.

The data means were considered different at $P < 0.05$ and $P < 0.01$.

Results

The results are presented in Table 3. After 12 weeks of a diet supplemented with 1.5 ml/kg CCl_4, the GSH levels in the liver of group III were significantly increased in comparison with those of the control group ($P < 0.01$), at 4.513 µmol/g compared to 5.372 µmol/g. When compared with the control group value of 956.696 nmol/min mg prot wet tissue, there was a decrease in GST activity in the liver of group III to 825.486 nmol/min mg protein wet tissue. The liver Se levels in this group decreased to 184.27 ng/ml compared to the control value of 210.52 ng/ml.

GST activity in the liver tissues of group IV, the alcohol group, decreased to 804.376 nmol/min mg prot compared to the control value of 956.696 nmol/min mg prot. However, the GSH level in the liver of this group increased compared to that of the control group ($P < 0.01$), to 7.055 µmol/g as against 5.376 µmol/g. The level of Se in the liver of the alcohol group decreased to 196.131 ng/g tissue.

Discussion

In oxidative metabolism, much of the oxygen consumed is bound to hydrogen during oxidative phosphorylation, thus forming water. However, it has been estimated that 4-5% of the oxygen consumed during respiration is not completely reduced to water and instead forms free radicals. Thus, as oxygen consumption increases during exercise a concomitant increase occurs in free radical production and LP (10).

The formation of highly reactive oxygen-containing molecular species is a normal consequence of a variety of essential biochemical reactions. If a reactive molecule contains one or more unpaired electrons, the molecule is

Table 3. GSH, GST and Se levels of all groups.

GROUPS Number	GSH (mmol/g tissue)	GST (nmol/min mg protein)	Se (ng/mg tissue)
Control Group I (n = 0)	Mean ± S.E 5.37 ± 0.14	Mean ± S.E 956.70 ± 33.97	Mean ± S.E 210.53 ± 6.98
1 ml/kg CCl_4 Group II (n = 10)	6.89 ± 0.14**	831.11 ± 18.32**	183.54 ± 4.02**
1.5 ml/kg CCl_4 Group III (n = 10)	7.51 ± 0.11**	825.49 ± 14.11**	184..27 ± 3.71**
50% Ethyl alcohol Group IV (n = 10)	7.06 ± 0.09**	804.38 ± 14.60**	196.13 ± 2.87*

** $P < 0.01$, * $P < 0.05$

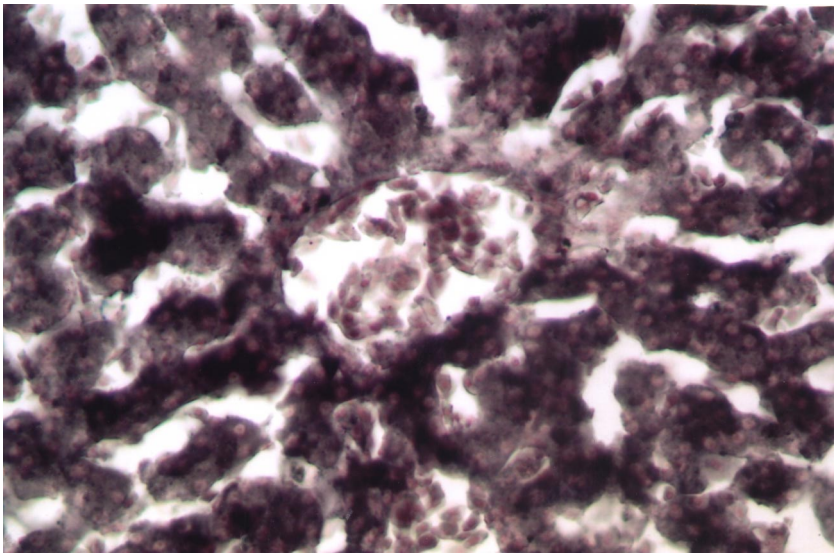

Figure 1. Lipid diffusion of liver and lymphocytic plasmacytic cell infiltration in periportal parts. Sudan X.

termed a free radical (23,24). As a result of the relative instability of free radicals and their potential to damage cells and tissues, there are both enzymes and small-molecular-weight molecules with antioxidant capabilities that can protect against the adverse effects of free radical reactions (10). There is, therefore, a critical balance between free radical generation and antioxidant defenses.

Animal cells produce different reactive oxygen species: peroxides, singlet oxygen and free radicals such as superoxide, hydroxyl and peroxyl radicals (23). Free-radical-mediated peroxidation phenomena play an important role in the mechanism of cellular damage caused by CCl_4 and ethyl alcohol (6,11).

It is well known that CCl_4 injures hepatic cells through free-radical-induced LP and/or direct free radical attack (6). Data have also been presented to suggest that the primary event in the development of ethanol-induced fatty liver, as well as in liver injuries induced by other chemicals, is the formation of lipoperoxides at selective subcellular sites because of the alteration in the

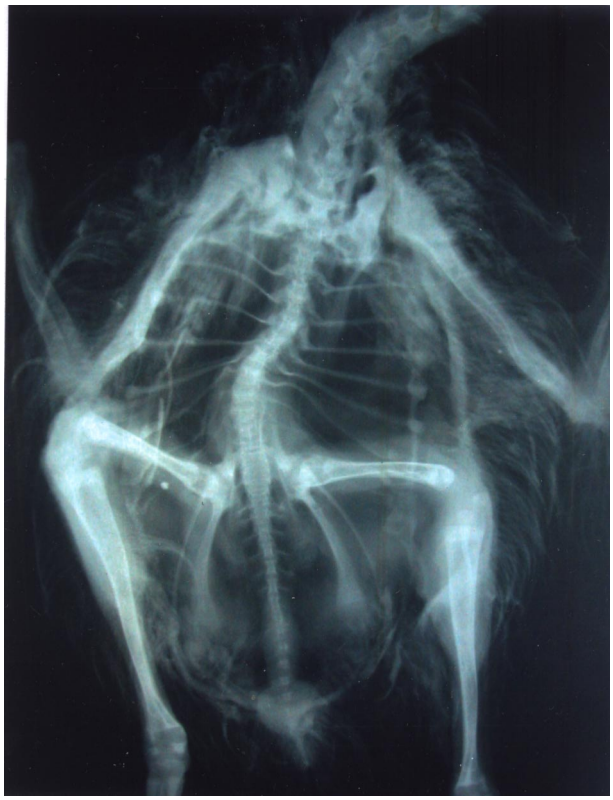

Figure 2. Appearance of the skeletal system on X ray.

antioxidant activity of the hepatic cells (6,25,26). In fact, there are reports that the increase in CCl_4-induced hepatic injury caused by starvation results from the decrease in GSH content (27).

The elevation of plasma and tissue lipid peroxide levels is an indicator of membrane disruption in various tissues and organ cells and it is positively correlated with the gravity of the disease. GSH, GST and Se are among the major antioxidant defense systems that eliminate lipid peroxidase and toxic oxygen radicals (18).

Ethanol consumption is able to induce oxidative stress in the liver and in extrahepatic tissues, linked to an imbalance between the pro-oxidant and the antioxidant systems in favour of the former. Peroxidation of lipids leads to by-products that have been shown to promote collagen production and to form adducts with proteins (28,29).

The decrease in GST and Se levels and the increase in GSH levels in the liver of the CCl_4 and ethanol groups when compared with the control group can be explained by the role of the toxic effects of CCl_4 and ethanol on peroxidation on membranes. Our study also shows that the changes in lipid peroxidation were also accompanied by a decrease in the activities of enzymes involved in the disposal of superoxide anions and peroxides, namely SOD and CAT, as well as the levels of GSH and its related enzymes (GST, GSH-Px and GRase). From these findings, it appears that the initial changes induced by alcohol and CCl_4 are due to the formation of LP and toxicity is mediated through antioxidant enzymes as well as GSH metabolism.

In clinical examinations carried out on the groups dosed with CCl_4 and ethanol, loss of appetite, diarrhea, coordination defect, difficulty in walking, collapsing of the wings, and torticollis were observed. Under microscopic examination hydropic and fat degeneration of the liver were observed and in radiography the skeletal system was seen to be completely deformed with the bones twisted in parts (Figures 1 and 2).

In conclusion, in *Anser anser* geese with induced liver degeneration, the levels in liver tissue of the antioxidant defense system constituents GSH, GST and the closely interlinked Se are reported here in detail for the first time.

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