Joint effect of iron overload and carbon tetrachloride on hepatic toxicosis in rats.

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SUMMARY
Forty five rats were divided into 3 groups {carbon tetrachloride (CCl4), iron, and iron + CCl4}. The rats received subcutaneous injection of CCl4 twice weekly with or without intraperitoneal injection of iron dextran once weekly for 8 weeks in addition to absolute ethyl alcohol 5% in the drinking water. In case of iron alone, the liver showed few areas of lytic necrosis, infiltration of mononuclear cells and deposition hemosiderine particles throughout the whole liver, especially in portal tract, midzonal with periportal hydrobic degeneration. However, upon administration of iron with CCl4, lesions were congestion, hemorrhage, hydropic degeneration, necrosis and pseudolobulation, in addition to severe infiltration of inflammatory cells and hemosiderin particles within the portal tracts. In conclusion, iron found to increase the severity of hepatotoxicity induced by CCl4.

Key words: (Iron overload, CCL4, Hepatic toxicosis, Rats).

INTRODUCTION
Iron is the fourth most common element on the earth and it is one of the most studied trace elements in human health. The iron is essential to most, if not all, living organisms; moreover, it is essential for many reactions essential for life in both higher animals and man (Smith, 1965). Excess deposition of iron in the parenchymal tissue of several organs (e.g. liver, heart, pancreas, joints, and endocrine glands) leading to cell injury and functional insufficiency. In liver, the major pathological manifestations of chronic iron overload are fibrosis and ultimately cirrhosis (Bacon and Britton, 1989). Iron may be interacting with other potential liver damaging agents which lead to enhancement of liver injury, and the process of fibrosis (Arezzini et al., 2003). The present study was carried out to estimate the potential interaction of
iron and alcohol as an example of xenobiotics with CCl4.

MATERIAL AND METHODS

Animals

The present study was carried out on 45 male albino rats of 120-150 g body weight and 3 months old. They were housed in metal cages 15 rats each and received the normal chow diet and water ad libitum.

Chemicals

Iron dextran (Fercayl ®) parenteral ampules 100 mg iron/2 ml were registered and manufactured by Laboratories Sterop, S.A., Avenue de Scheut 46-50, b-1070 Brussels- Belgium.

Experimental design:

Rats were randomly divided into 3 equal groups 15 rats each beside the control group. In the first group, CCl4 was injected subcutaneously at a dose of 2ml/kg B.Wt. in liquid paraffin (1:1) twice weekly up to 8 weeks (Matsuda et al., 1991), the second group, iron was injected intraperitoneally at a dose of 200mg /kg B.Wt. once weekly up to 8 weeks (Mackinnon et al., 1995) The third group received the same doses of both CCl4 and iron as previously described in addition to 5% ethyle alcohol in drinking water. Rats from each group were sacrificed after 4 and 8 weeks from the beginning of experiment.

Histopathological studies:

Following necropsy of sacrificed and dead rats, specimens from liver were rapidly fixed in 10% neutral buffered formalin then processed through the conventional paraffin embedding technique. Sections of 5 µ thickness were stained with haematoxylin and eosin (H&E) according to the method described by (Culling, 1983). Perl’s Prussian blue reaction for detection of hemosiderin was performed (Luna, 1968).

RESULTS

Clinical signs:

The clinical signs included depression, loss of appetite and subsequently reduction in weight gain in all the intoxicated groups. Table (1) and figure (1) showed that the weight loss increased by time and it was statistically the highest in CCl4 + iron followed by CCl4 alone then iron. Mortalities occurred only in CCl4 and in CCl4+ iron groups (4 of 15 rats each during the seventh and fifth weeks, respectively).

Histopathological findings:

In CCl4, the encountered lesions during the first month included congestion of portal veins, mild perivascular edema, mononuclear cells infiltration and newly formed bile ducts (Fig. 2). More-
over, variable sized necrotic areas were seen which suffered lysis. In the same time, other areas showed hydropic degeneration which was confined only to the periportal regions (Fig. 3). In addition, there was thickening of portal tracts with congestion of capillaries and aggregation of mononuclear cells (Fig. 4). During the second month, similar lesions were detected in both sacrificed and dead rats in addition to formation of pseudolobules as a result of proliferation of fibrous strands which divided the liver into unorganized pseudolobules.

In iron group, the fundamental lesions during the first and second months were few areas of necrosis which replaced by siderocytes (Fig. 5). Furthermore, the portal tracts were infiltrated with mononuclear cells and hemosiderin particles either free or inside the macrophages (Fig.6). In addition, there was hydropic degeneration appeared together with minute areas of necrosis (Fig.7). Upon staining with Prussian blue, blue iron particles were noticed in necrotic areas, the iron distribution was primarily periportal and midzonal, thereafter, tended to be centrilobular (Fig.8). In the same time, liver showed marked hypertrophy of the Kupffer cells due to overload with dark brown hemosiderin particles.

Severe lesions were noticed in CCl4 + alcohol + iron group in comparison to CCl4 and iron groups (table 2). Moreover, lesions noticed during the second month were more severe than those of first month besides areas of hemorrhage (Fig. 9). The most prominent lesion during the second month was more developed pseudolobulation, particularly in dead rats (Fig. 10).
Figure (1): Body weight curve of four groups of rats throughout the interval of experiment CCl4 (Carbon tetrachloride), FE (Iron), CCLFE (Carbon tetrachloride and Iron), C (Control).

**Descriptions of Figures**

Fig. (2): Liver of CCl4 intoxicated rat after 4 weeks showing congestion of portal vein with mild perivascular edema (A) together with infiltration of mononuclear cells (B) and newly formed bile ducts (arrows). H, E. (X100).

Fig. (3): Liver of CCl4 intoxicated rat after 4 weeks showing midzonal and periportal hydropic degeneration. H, E. (X100).

Fig. (4): Liver of CCl4 intoxicated rat after 4 weeks showing thickened portal area with congested capillaries and aggregates of mononuclear cells (A). H, E. (X400).

Fig. (5): Liver of iron intoxicated rat after 4 weeks showing area of necrosis replaced by hemosiderin particles (A). H, E. (X400).

Fig. (6): Liver of iron intoxicated rat after 4 weeks showing infiltration of portal tract with mononuclear Cells and dark brown hemosiderin particles either free or inside the macrophages. H, E. (X400).

Fig. (7): Liver of iron intoxicated rat after 4 weeks showing hydropic degeneration with minute areas of necrosis (arrows). H, E. (X100).

Fig. (8): Liver of iron intoxicated rat after 8 weeks showing hemosiderin particles. Perl's Prussian blue reaction. (X100).

Fig. (9): Liver of iron + CCl4 intoxicated rat after 4 weeks showing extensive areas of hepatic hemorrhage. H, E. (X100).

Fig. (10): Liver of iron + CCl4 intoxicated dead rat after 5 weeks showing fibrous strands extended to separate the hepatic parenchyma into pseudolobules together with hydropic degeneration. H, E. (X100).


DISCUSSION

Iron may interact with other potential liver damaging agents as CCl4 and ethanol which lead to enhancement of liver injury and accelerate the process of fibrosis (Arezzini et al., 2003).

In the present study, the clinical signs were mainly depression and significant loss of body weight in all groups. The statistical analysis revealed that the final losses were 13.07, 33.75, and 44 g losses in case of iron, CCl4 or both, respectively. Furthermore, the losses began earlier in CCl4+ iron (third week) followed by CCl4 (fifth week) and later iron (seventh week). These losses may be attributed to depressed appetite. Mackinnon et al., (1995) and Arezzini et al., (2003) supported the joint or synergistic effect of CCl4, iron and alcohol in induction of hepatotoxicity. Generally, the microscopic lesions in the liver...
of all intoxicated groups were nearly similar, but were much more in severity and distribution in case of the simultaneous administration of the three hepatotoxins (CCl4, iron and alcohol). A possible explanation of this is that CCl4, iron and alcohol are hepatotoxins. CCl4 interferes the lipid peroxidation (Cheville, 1988), while iron liberates free radicals and deplets of endogenous antioxidant (Bacon et al., 1983). Therefore, degeneration and necrosis were permanent lesions. Pseudolobulation was noticed in all groups, especially in CCl4 + iron group but during the second month because fibroplasia is a feature of chronicity. Similar lesions were reported by (Mackinnon et al., 1995; Arezzini et al., 2003; Lebda, 2006 and Sundram et al., 2007).

REFERENCES


The effect of iron overdose in rats on the kidney.

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Iron deficiency and iron overload are common disorders that affect various tissues and organs in the body. Iron deficiency is characterized by a decrease in the amount of iron in the body, while iron overload is characterized by an excess of iron in the body. Both conditions can lead to serious health problems if left untreated.

In this study, the effect of iron overdose in rats on the kidney was investigated. The study was conducted on rats divided into three groups: control, treatment, and experimental. The control group received normal diet, while the treatment group received a diet enriched with iron, and the experimental group received an iron overload diet.

The results showed that iron overdose in rats caused significant changes in the kidney, including changes in the structure and function of the renal tubules. These changes were characterized by an increase in the number of tubular casts and a decrease in the number of glomeruli. The results also showed that the treatment group had better kidney function than the control group.

These findings suggest that iron overdose in rats can cause significant damage to the kidney, and that efforts should be made to prevent iron overdose in the population.