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A STUDY OF FREE RADICAL INJURY TO VITAL ORGANS

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ABSTRACT

The study of toxicities which are caused by free radical inducing chemicals (e.g., carbon tetrachloride), helps to determine the mode of action of those important drugs which act by inhibiting free radicals. Evaluation of toxicities induced by carbon tetrachloride and phenobarbitone administration was carried out in seventy healthy male rabbits. Each animal received CCl_4 0.25-0.5 mL/kg body weight, intraperitoneally, twice weekly for eleven doses. During this period, the only water which was provided for drinking to these animals contained 0.25 g/L pentobarbitone. Examination of these animals revealed that carbon tetrachloride produced severe liver and kidney injuries which could be assessed by physical parameters, liver and kidney function tests, and microscopic examinations of the vital organs of these animals.

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INTRODUCTION

Generally, epidemiological studies help to uncover new etiological leads, quantify the risk associated with different exposures, and assess the efficacy of preventive measures.[1] However, due to simultaneous involvement of a number of factors, these studies fail to provide a clear distinction for the role of different agents. Laboratory studies therefore provide an important mean for testing the distinct role of these agents.[2] They also help to test the hypothetical mechanism of an agent. Some carcinogens have been found to produce free radicals, both metabolically and non-enzymatically during metabolism, which are responsible for carcinogenesis.[3] Therefore carcinogenesis initiated by CCl_4 in laboratory animals provides an important experimental model to confirm the hypothetical mechanism of some drugs which act by inhibiting free radicals. In this study, we have noted the toxicities which are produced by CCL_4 in rabbits grossly, biochemically and histopathologically. The purpose of this study is to observe the changes in different parameters, so that they can be used to determine relative

Table I. Gross toxicities of rabbits.

Toxicity	Interval	Group A	Group B
Average weight variation (gm)	1st	+22*	-94.70**
	2nd	+54	-130.76
	3rd	+11	+13.64
	4th	+79	+43.79
	5th	+15	+185.59
Ulceration	1st	Nil	3.33%***
	2nd	Nil	26.82%
	3rd	Nil	39.28%
	4th	Nil	46.61%
	5th	Nil	Improved
Diarrhea	1st	Nil	Nil
	2nd	Nil	Nil
	3rd	Nil	Nil
	4th	Nil	Nil
	5th	Nil	Nil
Edema	1st	Nil	Nil
	2nd	Nil	Nil
	3rd	Nil	Nil
	4th	Nil	Nil
	5th	Nil	Nil
Hematuria	1st	Nil	Nil
	2nd	Nil	Nil
	3rd	Nil	Nil
	4th	Nil	Nil
	5th	Nil	Nil
Vomiting	1st	Nil	Nil
	2nd	Nil	Nil
	3rd	Nil	Nil
	4th	Nil	Nil
	5th	Nil	Nil
Hair loss	1st	Nil	Nil
	2nd	Nil	Nil
	3rd	Nil	Present
	4th	Nil	Present
	5th	Nil	Improved
Loss of physical activity	1st	Nil	Nil
	2nd	Nil	Nil
	3rd	Nil	Nil
	4th	Nil	Nil
	5th	Nil	Nil

* Average weight gain.
**Average weight loss.
***Percentage of animals showing toxicity.

changes when the free radical producing carcinogen is administered with those drugs which act by counteracting the effect of these free radicals (e.g. methotrexate, mitomycin, etc.) or with other adjuvant compounds (e.g. alpha-tocopherol, vitamin C, ascorbic acid).

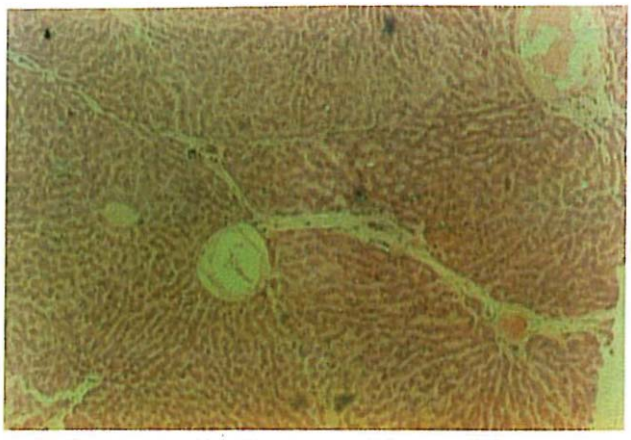

Fig. 1. A section of liver showing the portal vein, portal edema and fibrosis, and congested veins tending to form pseudolobules (Mag. × 10).

MATERIALS AND METHODS

This study was conducted on seventy healthy male rabbits with an average weight of 1250 gm, which were divided into two groups; the first containing ten rabbits (group A) as the control group and the other consisting of sixty rabbits (group B) which were to receive the carcinogen. The rabbits were observed for a week and special attention was given to weight variation, ulceration, diarrhea, edema, hematuria, vomiting, hair loss and loss of physical activity.

Group A animals were given distilled water intraperitoneally (I.P.) twice weekly for eleven doses. Group B animals were given CCl_4 0.25-0.5 mL/kg body weight intraperitoneally twice weekly for eleven doses, and phenobarbitone sodium 0.25 g/L orally in drinking water, and this was the only water available to these animals during the study period.

Gross toxicities were observed at an interval of each three doses. At the end of the experiment, blood samples were drawn by cardiac puncture. γ-GT, SGPT, SGOT, alkaline phosphatase, bilirubin, total lipid, cholesterol, total protein, albumin and globul' levels in these samples were determined by specific reagent kits with a spectrophotometer (Jasco-CRT 400, Bausch and Lomb Spectronic 20). The fibrinogen level was also determined. These animals were sacrificed after three months and all organs were examined for the presence of gross changes. Samples from the liver, kidney and spleen were collected for histological examination. Student's t-test was applied for the statistical analysis of data.

Table II. Mortality of rabbits during the experiment.

Interval	Group A	Group B
After two doses	Nil	19/60(31.66%)*
After five doses	Nil	13/41(31.70%)
After eight doses	Nil	7/28(25.00%)
After eleven doses	Nil	6/21(28.57%)
After three months	Nil	4/15(26.66%)

* No. of animals expired/total animals (percentage).

RESULTS

Table I shows the general toxicities noted during the experiments. No animal of any group developed diarrhea, edema, hematuria, vomiting or loss of physical activity. An average weight gain was noted in animals of group A throughout the experiment, while an average weight loss was observed in animals of group B during the first and second interval and an average weight gain during the third, fourth and fifth interval. Similarly, no ulceration was noted in any animal of group A while it was seen in variable percentages in group B animals. Hair loss was not seen in any group A animal while it was variably present in animals of group B during the third and fourth intervals of the experiment. Ulceration and hair loss improved in these animals after the fifth interval, i.e. after the drug-free period.

Table II shows the mortality rate during the period of experiments. The mortality rate was very high in group B, in which 31.66% mortality was noted after the first, 31.70% after the second, 25.00% after the third, 28.57% after the fourth and 26.66% after the fifth interval of the experiment. Survival rates following dosing were 100% in animals of group A and 18.33% in animals of group B.

Table III shows important liver parauneters. No significant difference ($P<0.05$) was found in γ-GT, total protein, albumin, glohulin, and bilirubin levels of both groups. However, significant differences ($P<0.05$) were present in SGPT, SGOT and alkaline phosphatase levels in these groups. Fibrinogen levels of group B animals were significantly lower ($P<0.005$) than those of group A. Total lipid and cholesterol levels were significantly higher ($P<0.05$) in group B than in group A animals (Table IV).

Histopathological exaninations of the liver, kidneys and spleen in hoth groups of animals revealed significant differences. Fatty change, hepatitis, and pre-cirrhotic changes in the liver, glomerulonephritis and degenerative changes in the kidneys, and hyperplasia of lymphoid follicles and fibrosis of the spleen were found in animals of group B only. These changes are shown in Figs. 1-5.

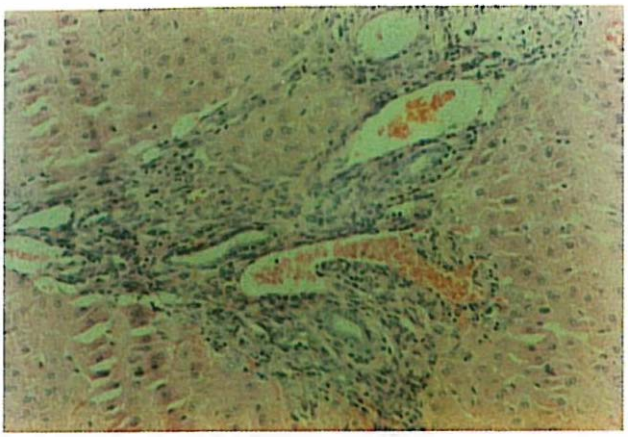

Fig. 2. A section of liver showing heavy infiltration in the portal area, extension of fibrous tissue in the parenchyma, and a small pseudonodule (Mag. × 10).

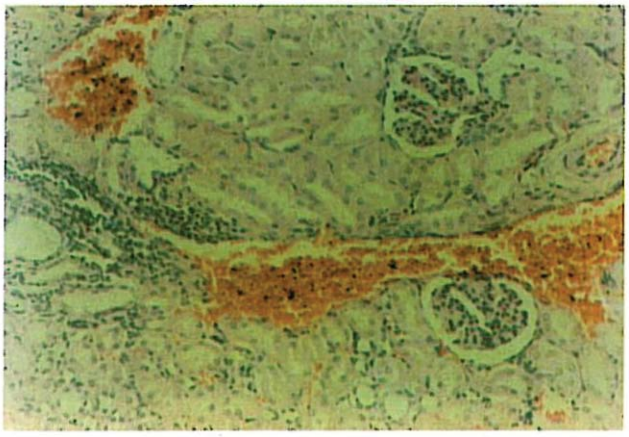

Fig. 3. A section of kidney showing congested veins (Mag. × 5).

DISCUSSION

Cancer is an important field of research. Although a lot of research has been conducted so far, unfortunately no satisfactory treatment has been discovered as yet. Among the obstacles which exist in the path of developing new strategies, the absence of a suitable model for the trials of newly investigated compounds is a major problem. Our study provides a suitable model for conforming the hypothetical mechanisms of those anticancer agents which act via interacting free radicals or those carcinogens which act via releasing those radicals.[3]

Carbon tetrachloride releases free radicals after interaction with the electrons available as a result of oxidation of heavy metals in hepatic tissue. These radicals cause

Fig. 4. A section of spleen showing an area of fibrosis (Mag. × 5).

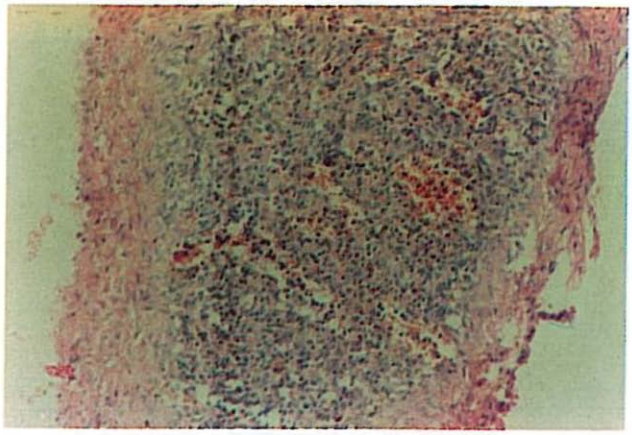

Fig. 5. A section of spleen showing mild fibrosis and a thickened capsule (Mag. × 10).

oxidation of polygenic fatty acids, present within the membrane phospholipids. They react rapidly with other molecules and form additional free radicals, leading to chain reactions. This in turn may cause membrane damage and breakdown the structure and impair the functions of the endoplasmic reticulum.[4] The synthesis of DNA and RNA is impaired, and the hepatocytes undergo necrosis and karyolysis.[5,6] Parenchymatous nodules in the liver of mice are regenerated after long-term treatment.[7] Liver cirrhosis was also noted after administering carbon tetrachloride in rats,[8-26] dogs[27,28] and rabbits.[29-31]

In our study, a rise in serum GPT and GOT levels revealed persistent functional damage of the hepatic parenchyma, and the development of chronic active hepatitis and cirrhosis. This is in accordance with the results obtained from a number of other workers.[32-43] In our study fibrinogen levels were decreased, further demonstrating the presence of hepatitis and cirrhosis. This finding was also supported by previous studies.[44,45] Increased total serum lipid and cholesterol levels in the study group

Table III. Liver function tests.

Parameters	Group A	Group B
γ-GT (U/L)	15.63±6.44 (8)*	8.41±1.65 (9)$^{\#}$
Transaminases (U/L)		
SGPT	55.30±20.60 (10)	143.90±39.00 (9)$^{\#\#}$
SGOT	47.80±17.80 (10)	130.30±32.60 (7)$^{\#\#\#}$
GOT/GPT.	0.86 (10)	1.831 (7)
ALP (U/L)	80.21±7.98 (7)	54.76±7.84 (8)$^{\#\#}$
Bilirubin (mg/dL)	0.45±0.04 (9)	0.58±0.23 (9)$^{\#}$
Protein (g/dL)		
Total	7.19±0.59 (10)	7.08±0.55 (10)$^{\#}$
Albumin	3.81±0.20 (8)	3.74±0.17 (10)$^{\#}$
Globulin	4.21±0.51 (8)	3.35±0.47 (10)$^{\#}$
A/G ratio	1.00±0.12 (8)	1.33±0.24 (10)$^{\#}$
Fibrinogen (mg/mL)	0.22±0.0009(8)	0.09±0.004 (8)$^{\#\#\#}$

* average value ±S.E.M. (no. of animals)
$^{\#}$P>0.05, $^{\#\#}$P<0.05, $^{\#\#\#}$P<0.005

Table IV. Serum lipid concentrations.

Parameters	Group A	Group B
Total lipid (mg/dl)	439.88±42.97(10)*	737.09±105.67(11)$^{\#\#}$
Cholesterol (mg/dl)	64.22±8.44 (9)	138.98±21.07 (11)$^{\#\#}$

* average value ±S.E.M. (no. of animals)
$^{\#\#}$P<0.05.

may be due to enhanced hepatocyte permeability.[46]

Microscopic examination of hepatic and renal tissues of the animals revealed ultrastructural changes consistent with the biochemical changes. It confirms the cirrhotic changes in the liver and toxic glomerular abberations in the kidneys. Previous studies also confirm these changes.[12,29-31]

Our study reveals the changes which take place during free radical mediated injuries in almost all biochemical parameters or microscopic details. Previous studies on this topic have not encircled so many parameters and markers simultaneously. This may be helpful in designing further studies involving drugs such as methotrexate or agents like alpha-tocopherol which induce their effects by interacting with free radicals. The relative changes of different parameters with such drugs or agents may help to determine their probable mechanisms.

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