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Zinc In CCl_4 Toxicity

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Objective To investigate the protective effect of zinc in CCl_4-induced hepatotoxicity. **Methods** Rats were treated with zinc acetate for four days. The zinc doses were 5 mg Zn/kg and 10 mg Zn/kg body weight respectively. Two groups of the zinc acetate-treated rats were later challenged with a single dose of CCl_4 (1.5 mL/kg body weight). **Results** Compared to control animals, the plasma of rats treated with CCl_4 showed hyperbilirubinaemia, hypoglycaemia, hypercreatinaemia and hypoproteinaemia. When the animals were however supplemented with zinc in form of zinc acetate before being dosed with CCl_4, the 5 mg Zn/kg body weight of zinc acetate reversed the hypoproteinaemia induced by CCl_4, whereas the 10mg Zn/kg body weight of zinc acetate reversed the hypoglycaemia, hyperbilirubinaemia and hypercreatinaemia induced by CCl_4. **Conclusion** The 10mg Zn/kg body weight of zinc acetate is more consistent in protecting against CCl_4 hepatotoxicity. The possible mechanisms of protection are highlighted.

Key words: CCl_4; Zinc; Plasma; Hypoglycaemia; Hyperbilirubinaemia; Hypercreatinaemia

INTRODUCTION

Since the hepatotoxicity of CCl_4 was recognised more than 60 years ago, the halogenated hydrocarbon has been extensively studied as a model of xenobiotic-induced lipid peroxidation and toxicity. CCl_4 is metabolised by the microsomal cytochrome P-450 system to produce the trichloromethyl (CCl_3) and Cl-free radicals which are believed to initiate the biochemical events that are ultimately expressed as liver cell necrosis[1-4]. These reactive intermediates covalently bind to cellular macromolecules, thus producing secondary disturbances of protein, fat and carbohydrate metabolism[2, 5, 6].

Several studies have demonstrated that induction of the cytochrome P-450 system and other mixed-function oxidases by agents such as phenobarbital, polychlorinated biphenyls, ethanol and DDT, increases the extent of CCl_4 metabolism and hence its hepatotoxicity[7-9]. Pretreatments with such agents as chloramphenicol, SKF - 525A, low protein diets, and cobalt chloride decrease CCl_4 bioactivation and the resulting hepatotoxicity, even under prior administration of small doses of CCl_4 which inhibit cytochrome P-450's mediated

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metabolism of CCl_4[10-14].

The metabolism and detoxification of various compounds may be influenced by deficiency or excess of dietary zinc[15]. While deficiency of zinc depresses the activity of several enzymes of drug metabolism[16], supplementation of zinc on the other hand, decreases cytochrome P-450 content[17]. Zinc has also been shown to stabilise biological membranes and provide protection against peroxidative damage[18].

Previous studies have shown that administration of CCl_4 induces hypoglycaemia and hyperbilirubinaemia in the male rat[19, 20]. The authors suggested that these might be due to disruption of hepatic plasma and microsomal membrane systems by CCl_4. Since zinc has been shown to stabilise biological membranes[18] and its supplementation decreases cytochrome P-450 content[17] whose involvement has been demonstrated in CCl_4's bioactivation and hence hepatotoxicity[2, 5], this present work attempted to investigate the *in vivo* effect of zinc acetate on CCl_4 toxicity. Levels of endogenous compounds like glucose, bilirubin, creatinine, protein and glutathione (GSH), along with some other biochemical /functional parameters, were used as indices of the functional integrity of both the liver and the kidney.

MATERIALS AND METHODS

Zinc acetate was a product of Riedel-De Haen AG, Seelze, FRG while CCl_4 was obtained from BDH Chemicals Ltd., Poole, England. All other chemicals used were of the purest grade available and were prepared in appropriate solvents.

Male albino Sprague-Dawley rats (150-200g), bred in the animal holding of the Faculty of Basic Medical Sciences, Ogun State University, Ago-Iwoye, Nigeria, were used for the investigation. They were divided into six groups of 5 rats and housed in metabolic cages for 7 days before the commencement of experiments. Before and during the experiments, the animals had access to food and tap water *ad libitum*.

Four groups of rats were injected with zinc in the form of zinc acetate for 3 days and left free of any treatment on the 4th day. The zinc doses were 5 and 10mg Zn/kg body weight respectively. On the 5th day, the zinc administration was repeated. From the four groups of the zinc supplemented animals, two groups were later challenged with a single dose of CCl_4 (1.5mL/kg body weight) about 1 h after the last zinc injection. On this same day, another set of rats received CCl_4 alone (1.5mL/kg body weight). Control animals received 0.9% sodium chloride solution. The weight of rats in all the groups was taken daily before treatment so as to calculate the volume of solution to be injected. All injections were given by the subcutaneous route of administration.

Removed 24 h after the last injection, the animals were killed under ether anaesthesia by blood collection from the abdominal aorta by means of a heparinised Teflon tubing. The blood samples collected were immediately centrifuged to separate plasma and red blood cells. The plasma samples were then stored frozen at -30℃ until the following day before being analysed for bilirubin[21], glucose[22], creatinine[23] and protein[24].

The liver and kidney were also removed, rinsed in ice-cold normal saline, mopped dry and weighed. A weighed portion of the liver was immediately transferred into the oven in a petri dish for water content determination as described earlier[25]. The remaining tissues were stored frozen in aliquots at -30℃ until the analysis of protein[24] and glutathione[26].

Results were expressed as $\bar{x} \pm s$ and statistical analysis was done using analysis of variance (ANOVA) with $P < 0.05$ considered as being significant.

RESULTS

Fig.1A shows the plasma protein profile of the animals under different treatment conditions. Administration of CCl_4 to the animals resulted in a significant decrease in the plasma protein content. When the animals were however treated with zinc acetate before being given CCl_4, the plasma protein of rats given the 5mg $Zn+CCl_4$ rose significantly above the control, whereas the animals given 10mg $Zn+CCl_4$ responded with a further decrease in their plasma protein. The plasma of the animals treated with zinc acetate alone also responded with a slight dose-dependent increase in the protein content, although the increase only in the animals given 10mg Zn was statistically significant.

Fig. 1B also shows the plasma creatinine pattern of the animals. All the animals treated with CCl_4 alone and zinc alone exhibited a significant increase in their plasma creatinine. Compared to control animals, hypercreatinaemia was more severe in the animals treated with CCl_4 alone, attaining a 3-fold increase over that in the control. Animals pre-treated with zinc acetate before being challenged with CCl_4 however exhibited a different pattern. In the case of animals given the 5mg $Zn+CCl_4$, a further increase in their creatinine content over that of CCl_4 alone was observed, whereas animals given the 10mg $Zn+CCl_4$ had their creatinine content not significantly different from controls.

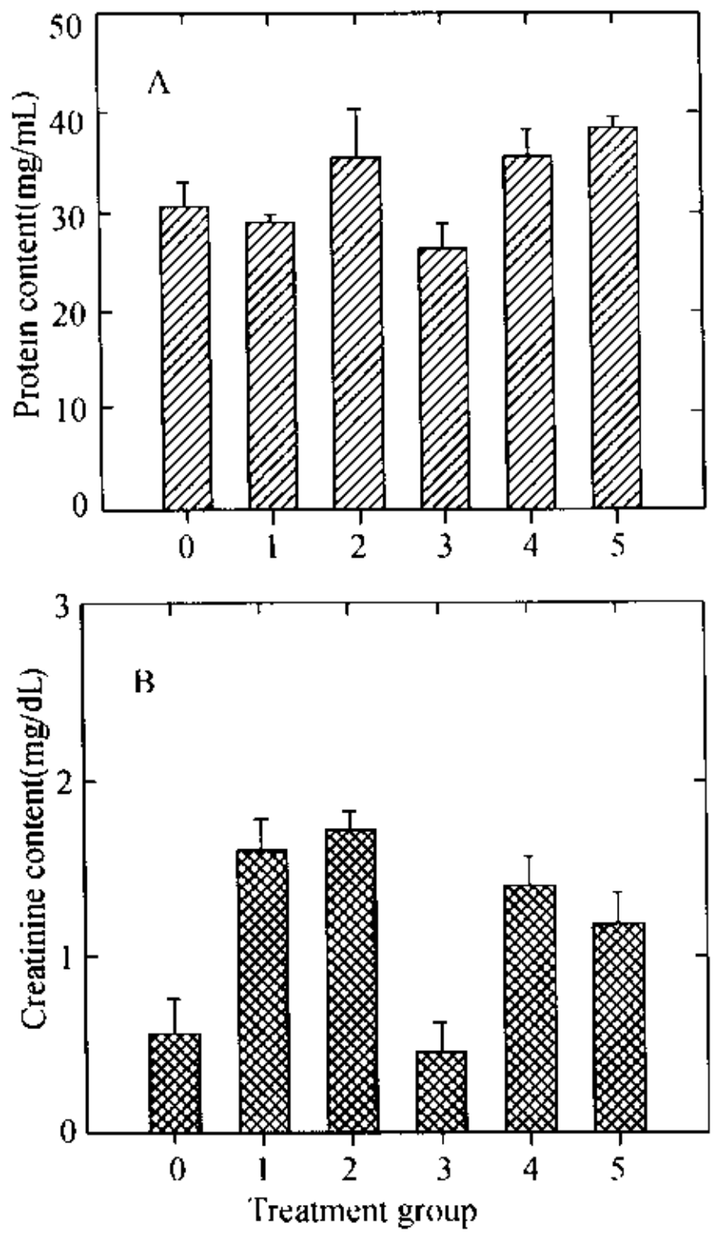

FIG.1. Effect of CCl_4, zinc acetate, and zinc acetate + CCl_4 on plasma protein and creatinine contents of rats. Values are $\bar{x} \pm s$ and are expressed as mg/mL (protein) and mg/dL (creatinine); $n = 5$.
0: Control, 1: CCl_4 alone, 2: zinc acetate (5mg Zn/kg body weight) + CCl_4, 3: zinc acetate (10mg Zn/kg body weight) + CCl_4, 4: zinc acetate alone (5mg Zn/kg body weight), 5: zinc acetate alone (10mg Zn/kg body weight).

The plasma bilirubin profile is depicted in Fig. 2A. Except for the animals treated with 10mg Zn+CCl_4 whose bilirubin level was not significantly different from controls, all the animals in other groups showed varying degrees of hyperbilirubinaemia. As expected, the hyperbilirubinaemia was more pronounced in the animals treated with CCl_4 alone.

Fig. 2B depicts the plasma glucose levels of the animals. Whereas administration of CCl_4 alone resulted in a marked hypoglycaemia, administration of the zinc compound alone resulted in hyperglycaemia in the animals. A dose-dependent increase in this phenomenon was however not observed. The hypoglycaemia induced by CCl_4 was reversed by zinc acetate and the reversal tended to be dose-dependent. In all, it appears that the 10mg Zn/kg body weight of zinc acetate is more effective in preventing changes brought about by CCl_4 in the plasma contents of bilirubin, creatinine, glucose and protein.

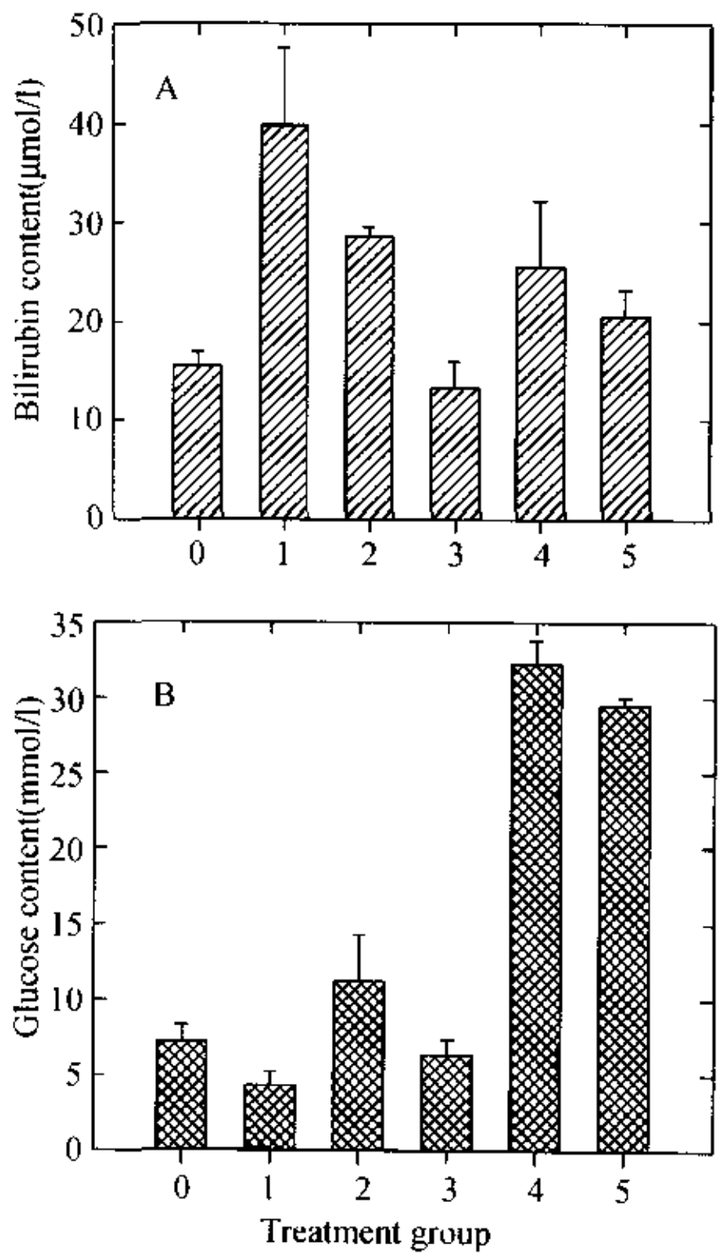

FIG.2. Effect of CCl_4, zinc acetate, and zinc acetate + CCl_4 on plasma bilirubin and glucose contents of rats. Values are $\bar{x}\pm s$ and are expressed as mg/dL; $n = 5$.
0: Control, 1: CCl_4 alone, 2: zinc acetate (5mg Zn/kg body weight) + CCl_4, 3: zinc acetate (10mg Zn/kg body weight) + CCl_4, 4: zinc acetate alone (5mg Zn/kg body weight), 5: zinc acetate alone (10mg Zn/kg body weight).

The biochemical parameters determined in the kidney are shown in Fig.3. While relative kidney weight was not affected by any of the treatments (Fig. 3A), administration of zinc acetate (5mg Zn/kg body weight) along with CCl_4 resulted in a significant increase in the renal protein content, whereas the 10mg Zn/kg body weight of zinc acetate along with CCl_4 did not have any effect (Fig. 3B). Renal GSH was not affected by CCl_4 alone or zinc acetate (10mg Zn/kg body weight) + CCl_4, whereas the animals in other treatment groups had their renal GSH reduced (Fig. 3C).

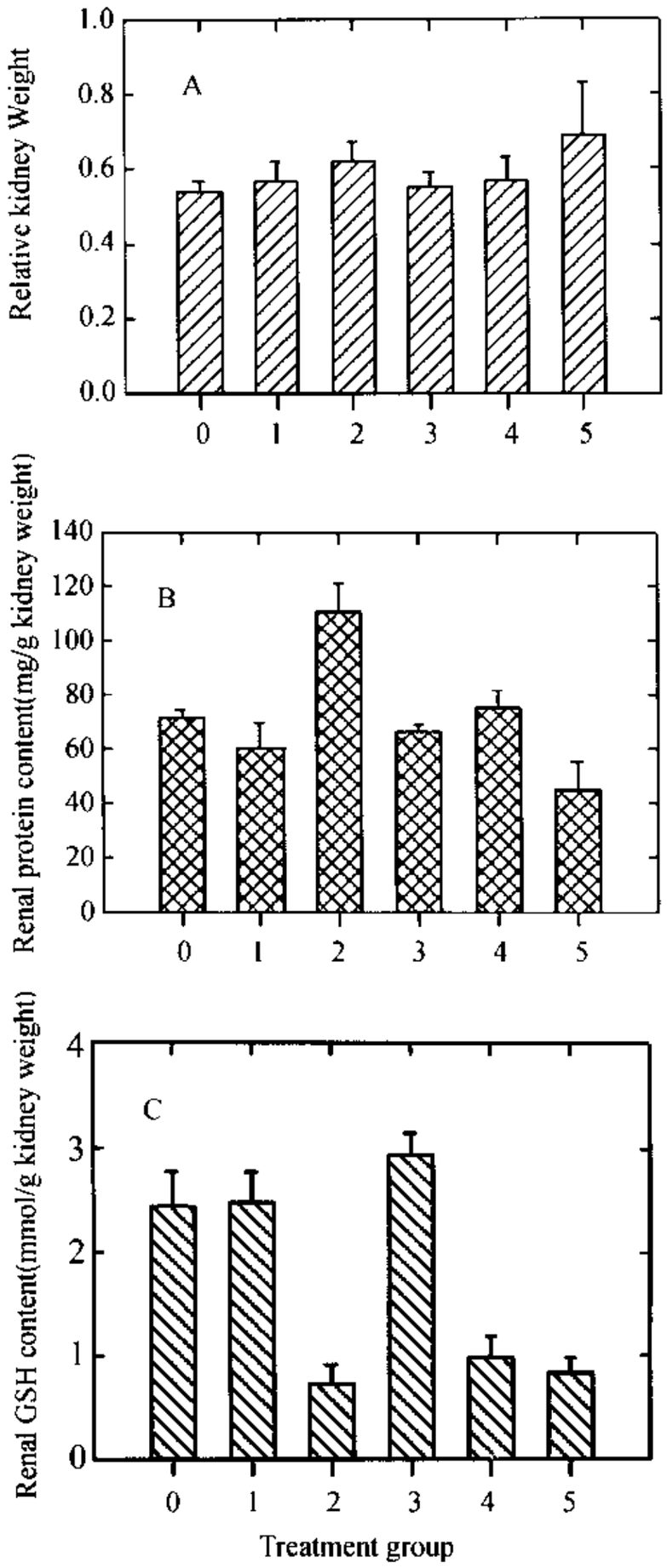

FIG.3. Effect of CCl_4, zinc acetate, and zinc acetate + CCl_4 on relative kidney weight, renal protein and renal GSH. Values are $\bar{x} \pm s$ and $n = 5$. Relative kidney weight is expressed as g/100g body weight; renal protein as mg/g kidney weight and renal GSH as mmol/g kidney weight.
0: Control, 1: CCl_4 alone, 2: zinc acetate (5mg Zn/kg body weight) + CCl_4, 3: zinc acetate (10mg Zn/kg body weight) + CCl_4, 4: zinc acetate alone (5mg Zn/kg body weight), 5: zinc acetate alone (10mg Zn/kg body weight).

The biochemical parameters determined in the liver are depicted in Figs.4 and 5. Relative liver weight, as well as the hepatic water contents, were not affected by any of the treatments (Fig.5). While administration of CCl_4 did not affect hepatic protein content, administration of zinc along with CCl_4 resulted in an increase in hepatic protein over that in the control and in the group with CCl_4 alone. This increase also showed a dose-dependence (Fig. 4A).

Hepatic GSH profile is shown in Fig. 4B. All the treatments resulted in a significant decrease in the hepatic GSH of the animals.

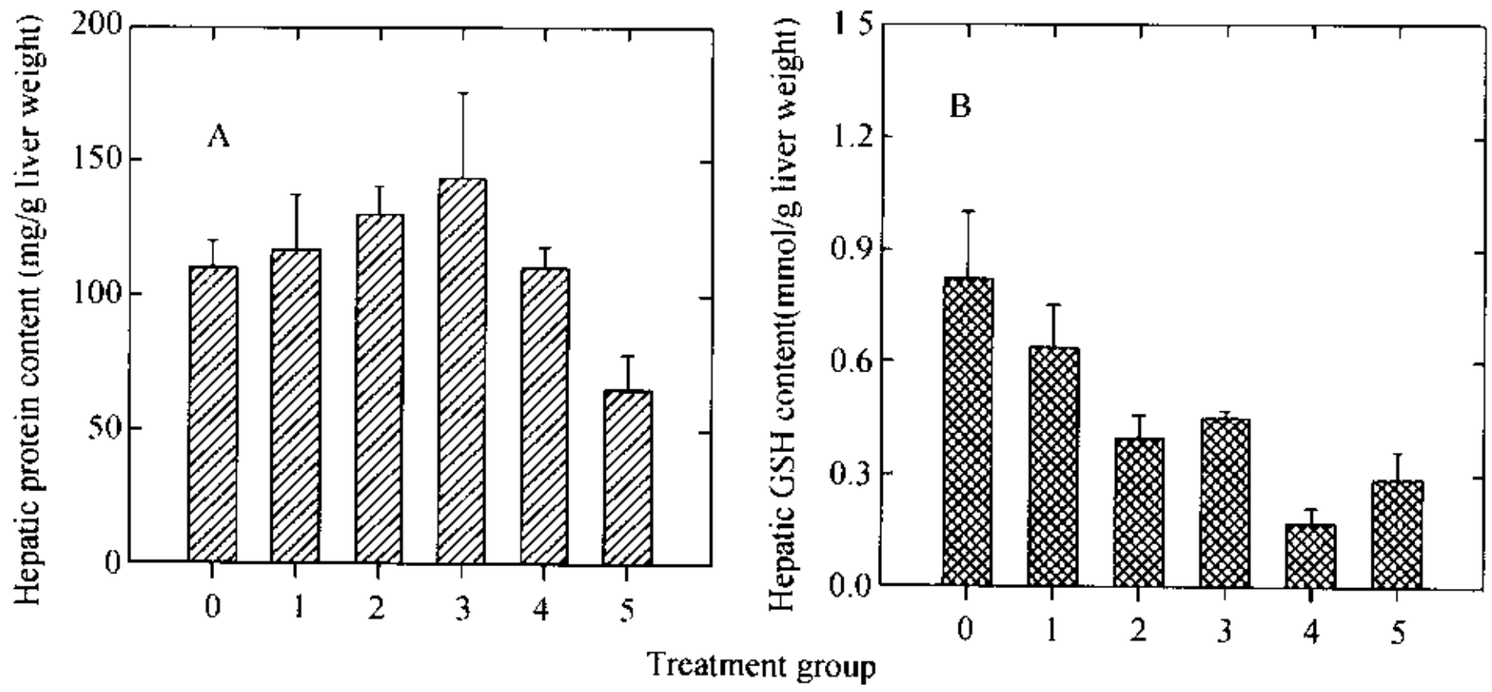

FIG.4. Effect of CCl_4, zinc acetate, and zinc acetate + CCl_4 on hepatic protein and hepatic GSH. Values are $\bar{x} \pm s$ and $n = 5$. Hepatic protein is expressed as mg/g liver weight while hepatic GSH is expressed as mmol/g liver weight.
0: Control, 1: CCl_4 alone, 2: zinc acetate (5mg Zn/kg body weight) + CCl_4, 3: zinc acetate (10mg Zn/kg body weight) + CCl_4, 4: zinc acetate alone (5mg Zn/kg body weight), 5: zinc acetate alone (10mg Zn/kg body weight).

DISCUSSION

Standard liver function and kidney function tests provide some information about the numerous functional relationships of these organs. Determination of plasma proteins, blood glucose and bile pigments in plasma may provide some evidence about possible hepatic and renal disorders caused by hepatotoxic and nephrotoxic agents. It is known, however, that damage to a particular organ by a toxicant depends upon its mode of association with that organ and the nature of the damage caused. It is well established that lipid peroxidative degradation of biomembranes occurs with CCl_4-induced liver injury and is one of the principal causes of hepatotoxicity[1, 27-29].

Twenty four hours following subcutaneous injection of a single dose of CCl_4 (1.5mL/kg body weight), there was a 2.5-fold increase in plasma total bilirubin. CCl_4 administration also resulted in hypercreatinaemia, hypoglycaemia and decrease in protein concentration in the plasma. Hyperbilirubinaemia and hypoglycaemia are indications of hepatic dysfunction. When the animals were pre-treated with zinc acetate and later challenged with CCl_4, the 5mg Zn/kg body weight of zinc acetate reversed the hypoproteinaemia induced by CCl_4 whereas the 10mg Zn/kg body weight of zinc acetate reversed the hyperbilirubinaemia, hypoglycaemia and hypercreatinaemia induced by CCl_4. Hepatic protein also increased in the animals pretreated with zinc before being challenged with CCl_4. These results indicate that depending on the dose of zinc, the pathologic changes induced by CCl_4 could be reversed by zinc, indicating that zinc acetate affords protection against hepatotoxicity induced by CCl_4.

The underlying biochemical basis of the protective effect of zinc acetate in this study could be viewed from the nature of damage caused by CCl_4. The structural, functional and biochemical changes provoked in the liver following CCl_4 poisoning are diverse[30]. Included among these changes are the collapse of the membrane of smooth endoplasmic reticulum

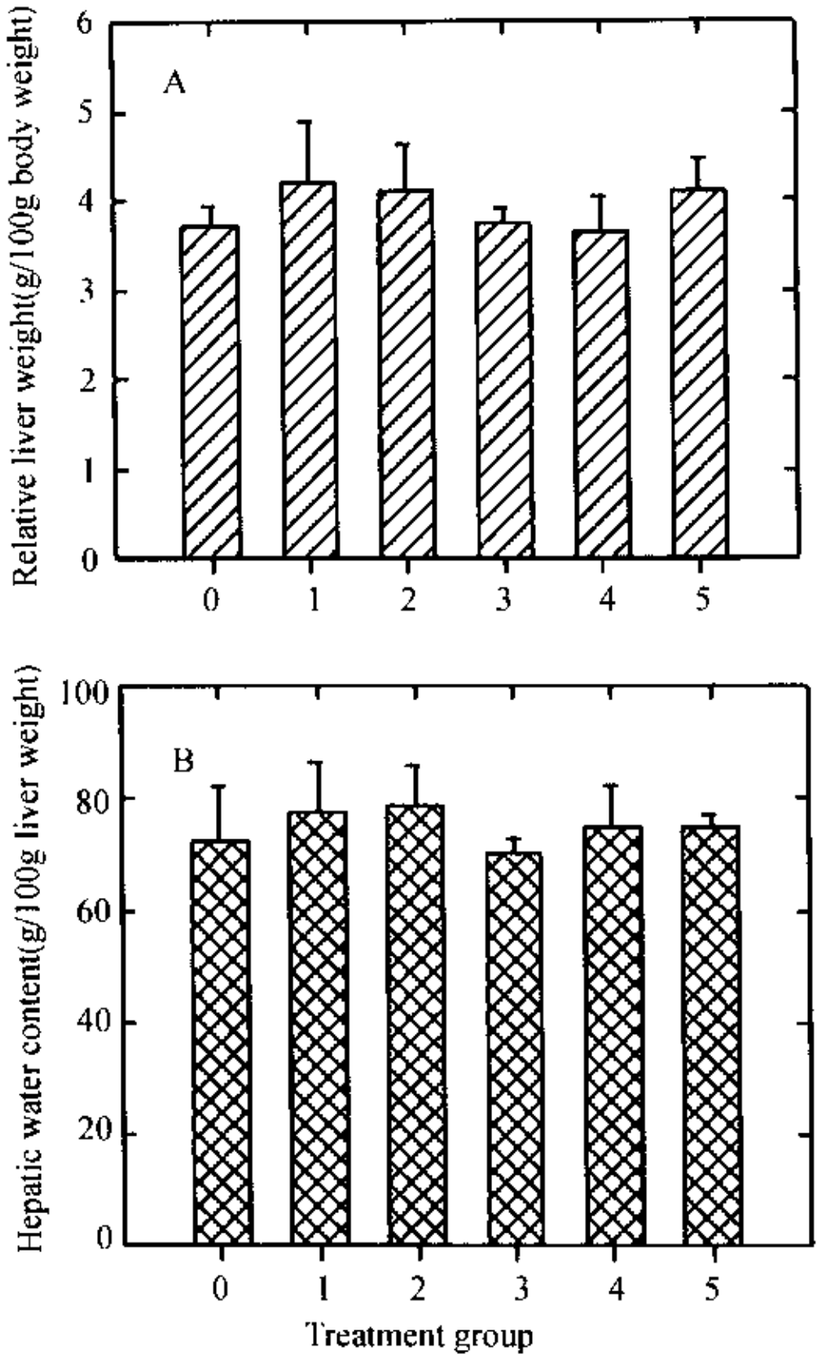

FIG.5. Effect of CCl_4, zinc acetate, and zinc acetate + CCl_4 on relative liver weight, hepatic water content. Values are $\bar{x} \pm s$ and $n = 5$. Relative liver weight is expressed as g/100g body weight while hepatic water content is expressed as g/100g liver weight.
0: Control, 1: CCl_4 alone, 2: zinc acetate (5mg Zn/kg body weight) + CCl_4, 3: zinc acetate (10mg Zn/kg body weight) + CCl_4, 4: zinc acetate alone (5mg Zn/kg body weight), 5: zinc acetate alone (10mg Zn/kg body weight).

and shedding of ribosomal rough endoplasmic reticulum[31], decreased *in vivo* protein synthesis[32], decreased glucuronide conjugation of bilirubin[33] and decreased glucose metabolism[19, 20, 34]. Both UDP-glucuronyltransferase (the enzyme responsible for conjugation of bilirubin) and Glucose-6-phosphatase (a key enzyme in glucose metabolism), are associated with the endoplasmic reticulum and depression of their activities specifically reflects injury to the endoplasmic reticulum[1]. In addition, the functional integrity of the two enzymes is dependent on the presence of phospholipids; and peroxidative decomposition of microsomal lipids, as occurs with CCl_4, results in a significant loss of the activities of these two enzymes[1, 30, 35]. Since zinc has been shown to interact *in vivo* with phospholipid components of the various membrane systems, thereby stabilising them[36, 37], it is tempting to speculate that the protection afforded by zinc in this study in CCl_4 hepatotoxicity could be due to the following:

1. Zinc may interact with the membrane phospholipids of the endoplasmic reticulum and thus prevent interaction of CCl_4 with these lipids. Such a competitive binding of zinc to membrane phospholipids may result in conformational changes of the endoplasmic reticulum

membrane or may have some effects on the enzymes controlling the integrity of the endoplasmic reticulum membrane and this would reduce the amount of CCl_4 interacting with this membrane system that otherwise would have led to toxic manifestations.

2. Zinc, being a powerful reducing agent, may also interact with the trichloromethyl free radical (CCl^{3+}) arising from CCl_4 metabolism, thereby destroying them.

3. Zinc may inhibit the cytochrome P-450 and thus prevent the bioactivation of CCl_4 to the trichloromethyl radical.

Since zinc reverses hyperbilirubinaemia, hypoglycaemia and hypoproteinaemia induced by CCl_4, this suggests that zinc not only prevents the events involved in the initiation of hepatic injury induced by CCl_4, but also prevents the processes that might be involved in the progression of the hepatic injury.

Data in Figs. 3B and 4A also suggest that zinc induces the synthesis of protein in both the liver and the kidney. This action of zinc is well established[38, 39]. Zinc administration induces the synthesis of metallothionein. It remains to be established, however, the role of this protein in the protection afforded by zinc against CCl_4-induced hepatotoxicity.

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