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Hepatoprotective effect of Zincovit drop against Carbon Tetrachloride Induced Liver damage in Wistar rats

Satyam SM[1], Bairy KL[2]*, Rao SS[3]

[1]*Department of Pharmacology, Melaka Manipal Medical College, Manipal University, Manipal-576104, Karnataka, INDIA.*
** [2]Department of Pharmacology, Kasturba Medical College, Manipal University, Manipal-576104, Karnataka, INDIA.*
[3]*Department of Anatomy, Melaka Manipal Medical College, Manipal University, Manipal-576104, Karnataka, INDIA.*

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ABSTRACT

The present study was aimed to investigate hepatoprotective activity of Zincovit drop on the CCl_4 induced hepatotoxicity in Wistar rats. Hepatotoxicity was induced by administering CCl_4 (1 ml/kg body weight; 1:1 CCl_4 and olive oil). Zincovit drop at three different doses (25, 50 and 100 mg/kg/day, orally) were administered to the respective group of Wistar rats. Reference drug silymarin (50 mg/kg/day, orally) was given to one group of rats. The treatment duration was seven days. Biochemical parameters like serum alanine transaminase, aspartate transaminase, alkaline phosphatase, total bilirubin and direct bilirubin were estimated to assess the liver function. Biochemical observations were also supplemented with histopathological examination of liver section. Oral treatment with Zincovit drop reversed CCl4-induced alterations in serum aspartate transaminase ($p<0.01$) compared to untreated hepatotoxic control animals. In comparison with carbon tetrachloride induced hepatotoxic control group serum total bilirubin level was significantly decreased among Zincovit drop treated animals at all the three doses 25 mg/kg ($p<0.05$), 50 mg/kg ($p<0.01$) and 100 mg/kg ($p<0.001$). There was also a significant decrease in aspartate transaminase (AST) level in hepatotoxic rats treated with 50 mg/kg of Zincovit drop when compared with silymarin 50 mg/kg/day) treated hepatotoxic rats ($p<0.05$). Profound fatty degeneration, fibrosis, and necrosis observed in the hepatic architecture of CCl4 intoxicated control animals were found to be normal in the animals treated with Zincovit drop. The present findings support a hepatoprotective potential of Zincovit drop against CCl_4 induced liver injury in rats.

Keywords: *Zincovit drop, flaxseed oil, hepatotoxicity, liver function test, hepatotoxicity, carbon tetrachloride.*

INTRODUCTION

The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. Inspite of tremendous scientific advancement in the field of hepatology in recent years, liver disorders are on the peak. Administration of single dose of CCl_4 to a rat produces a centrilobular necrosis and fatty changes. The poison reaches its maximum concentration in the liver within 3 h of administration. The development of necrosis is associated with leakage of hepatic enzymes into serum [2, 3]. Effect of antioxidant or free radical scavenging has been widely tested for the prevention and treatment of acute and chronic liver injuries [4, 5]. The 21st century is ushering in a new era of nutritional science, demonstrating the astonishing power of nutrition to benefit human health. It is well documented that treatment with antioxidants such as vitamins C and E can ameliorate the toxic effects of CCl_4 on liver and kidneys [6]. Zincovit drop is an advanced formulation of high concentration of vitamins, minerals, flaxseed oil and lysine. Zincovit drop releases a stream of anti-oxidant benefits. In previous studies, we had reported the hepatoprotective potential of combined formulation of grape seed extract and Zincovit tablets [7, 8]. Consequently, the aim of the present study was to investigate the hepatoprotective effect Zincovit drop against carbon tetrachloride induced liver damage in Wistar rats.

***Corresponding author:**
Dr. Laxminarayana Bairy K
Professor of Pharmacology
Kasturba Medical College, Manipal University
Manipal-576104, Karnataka, INDIA.
Fax: 0820-2922083; Phone: 0820-2922365
**E-Mail: kl.bairy@manipal.edu*

MATERIALS AND METHODS

Drugs and Reagents:

Zincovit drop was procured from Apex Laboratories Private Ltd., Chennai (India). The diagnostic kits for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin (TB) and direct bilirubin (DB) were obtained from Aspen Laboratories, New Delhi (India). Carbon tetrachloride (CCl4), sodium chloride and all other chemicals were obtained from Merck Chemicals, Mumbai (India). The reagents were equilibrated at room temperature for 30 minutes before use, either at the start of analysis or when
reagent containers were refilled.

Animals:

Adult male albino Wistar rats weighing 150-200 g were housed in separate polypropylene cages, maintained under standard conditions with temperature (22–24^{0}C), 12-h light/12-h dark cycle and relative air humidity 40–60%. The animals were acclimatized to the laboratory conditions for one week before the start of the experiment. The animals were provided with a normal pellet diet (VRK Nutritional Solutions, Pune, India) and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics.

Committee and experiments were conducted according to the ethical norms of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Experimental design; [8]

In the experiment, 36 adult male Wistar rats (150-200 g) were used. The rats were divided into 6 groups containing 6 rats in each group. Treatment was done for seven days as follows-

Group I: Normal control rats were given 2% gum acacia (1ml/kg/day; *p.o*).

Group II: CCl4 intoxicated control rats + 2% gum acacia (1ml/kg/day; *p.o*) and simultaneously administered CCl4: olive oil (1:1); (1ml/kg; *i.p.* every 72 h).

Group III: CCl4 intoxicated rats + Silymarin (50 mg/kg/day; *p.o*) and simultaneously administered CCl4: olive oil (1:1); (1ml/kg; *i.p.* every 72 h).

Group IV: CCl4 intoxicated rats + Zincovit drop (25 mg/kg/day; *p.o*) and simultaneously administered CCl4: olive oil (1:1); (1 ml/kg; *i.p.* every 72 h).

Group V: CCl4 intoxicated rats + Zincovit drop (50 mg/kg/day; *p.o*) and simultaneously administered CCl4: olive oil (1:1); (1 ml/kg; *i.p.* every 72 h).

Group VI: CCl4 intoxicated rats + Zincovit drop (100 mg/kg/day; *p.o*) and simultaneously administered CCl_4: olive oil (1:1); (1 ml/kg; *i.p.* every 72 h).

Collection of blood sample and isolation of liver:

After 7 days of above treatment rats were anesthetized anesthetized with ketamine (80 mg/kg; i.p) and blood samples were collected by retro-orbital puncture. Following collection of blood in micro-centrifuge tubes and its clot formation, serum was obtained by centrifugation of blood at 3,000 rpm for 20 min at 4ºC using a refrigerated centrifuge (MIKRO 22R, Andreas Hettich GmbH & Co. KG, Germany). The resulting supernatant (serum) was stored at -20˚C. Serum separated was used for all biochemical estimations. Animals were autopsied and liver was excised carefully and washed in saline.

Biochemical estimations:

Serum was analyzed for assay of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin (TB) and direct bilirubin (DB) using commercially available kit (ASPEN laboratories, New Delhi, India).

Qualitative histopathological examination:

The liver tissue samples were taken randomly from the 1-2 animals of each group and fixed in 10% phosphate buffered formalin. A small part of liver was cut and dehydrated in ascending grades of alcohol, defatted in xylene, and embedded in paraffin. 24 hours after block preparation, 6 µm (micron) thick paraffin sections were obtained using microtome and mounted on albumenized glass slides followed by their respective labeling. Tissues were then de-waxed in xylene for 10 minutes and further hydrated through descending grades of alcohol to water. The sections were stained with hematoxylin and eosin (H&E). At the end 2-3 drops of DPX mountant was put on the glass slides and the cover slips were placed gently to avoid drying of tissue and then observed for any morphological changes under a light microscope (Magnus, Olympus Private Ltd., New Delhi, India) 40X. Later the microscopic slides of the liver cells were photographed.

Statistical analysis:

Using Statistical Package for Social Sciences (SPSS version 20.0;SPSS Inc., Chicago, USA), data were expressed as Mean ± SEM (Standard Error of Mean) and analyzed by one way analysis of variance (ANOVA) followed by post hoc Tukey test. A level for $P \leq 0{\cdot}05$ was considered to be statistically significant.

RESULTS

Effect on biochemical parameters:

In the present study, CCl4 caused significant increase in serum alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (TB) and direct bilirubin (DB) levels when compared to normal control rats levels ($p<0.01$). Oral treatment with Zincovit drop reversed CCl4-induced alterations in serum aspartate transaminase ($p<0.01$) compared to untreated hepatotoxic control animals. In comparison with carbon tetrachloride induced hepatotoxic control group serum total bilirubin level was significantly decreased among Zincovit drop treated animals at all the three doses 25 mg/kg ($p<0.05$), 50 mg/kg ($p<0.01$) and 100 mg/kg ($p<0.001$). There was also a significant decrease in aspartate transaminase (AST) level in hepatotoxic rats treated with 50 mg/kg of Zincovit drop when compared with silymarin 50 mg/kg) treated hepatotoxic rats ($p<0.05$) (Table 1 and 2).

Table No. 1: Effect of Zincovit drop on serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase levels (ALP) in U/L

Groups (n=6)	ALT	AST	ALP
I- Normal control (2% gum acacia;1 ml/kg/day)	48.43±2.70	150.00±12.34	340.00±17.98
II-HT control (2% gum acacia; 1 ml/kg/day)	140.00±12.00^{**a}	342.22±12.20^{**a}	702.14±14.42^{**a}
III- HT + Silymarin (50 mg/kg/day)	111.14±12.22	330.42±12.24	590.22±15.20
IV- HT + ZVT drop (25 mg/kg/day)	105.73±1.77	371.60±28.71	729.70±17.27
V-HT + ZVT drop (50 mg/kg/day)	102.67±3.95	224.56±29.41$^{**b, *c}$	500.86±44.51
VI- HT + ZVT drop (100 mg/kg/day)	115.30±2.18	230.78±33.16^{**b}	697.21±35.04^{**b}

n, number of rats in each group; ALT-alanine transaminase; AST-aspartate transaminase; ALP- alkaline phosphatase, HT- hepatotoxic, ZVT- Zincovit. Values are expressed as mean ± standard error of mean a, b, c - Significant as compared to Normal control, Hepatotoxic negative control, Hepatotoxic positive control-treated with Silymarin; level of significance- ***p< 0.001, **p< 0.01, *p< 0.05

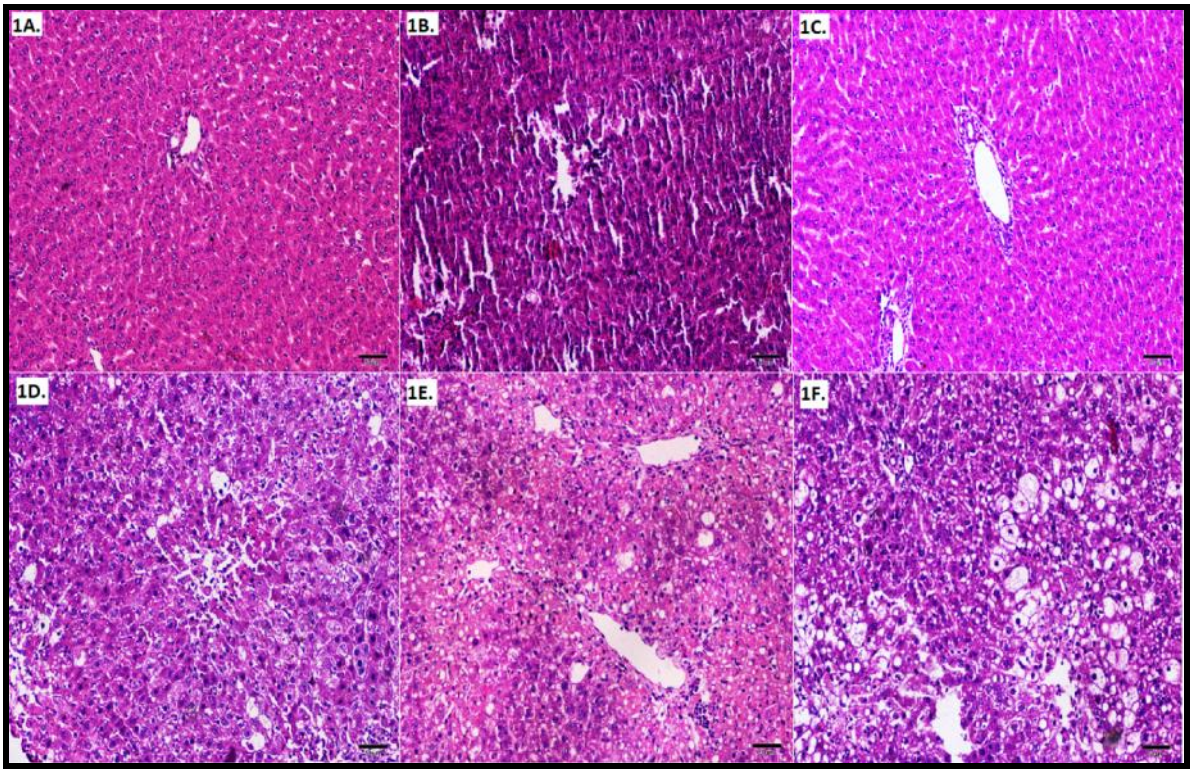
Table No. 2: Effect of Zincovit drop on serum total bilirubin and direct bilirubin levels (mg/dl)

Groups (n=6)	Total bilirubin	Direct bilirubin
I- Normal control (2% gum acacia;1 ml/kg/day)	2.00±0.30	0.82±0.11
II-HT control (2% gum acacia; 1 ml/kg/day)	4.12±0.60^{**a}	0.40±0.10^{**a}
III- HT + Silymarin (50 mg/kg/day)	2.98±0.20^{*b}	0.44±0.14
IV- HT + ZVT drop (25 mg/kg/day)	3.11±0.21^{*b}	0.35±0.10
V-HT + ZVT drop (50 mg/kg/day)	1.89±0.15^{**b}	0.42±0.12
VI- HT + ZVT drop (100 mg/kg/day)	1.29±0.09^{***b}	0.40±0.12

n, number of rats in each group; HT- Hepatotoxic, ZVT- Zincovit. Values are expressed as mean ± standard error of mean; a, b- Significant as compared to Normal control, Hepatotoxic negative control; level of significance- ***p< 0.001, **p< 0.01, *p< 0.05

Effect on histopathology of liver sections:

Histology of liver of normal control animals displayed normal liver architecture. The hepatic cords and the sinusoids were well visible (Figure 1A). In the CCl_4 intoxicated control (untreated) group, classical centrizonal necrosis was seen. The hepatocytes around the central vein were necrosed with no distinguishable nuclei (Figure 1B). Silymarin treated group revealed very mild signs of liver injury. Only difference from the normal control group was the presence of inflammatory cells and constricted sinusoids indicating apparent hepatocyte swelling (Figure 1C). The group treated with Zincovit drop in a dose of 25 mg/kg for 7 days showed feathery degeneration in the centrizonal area which was the predominant histological feature of this group (Figure 1D). The primary feature in the groups treated with 50 mg/kg of Zincovit drop for 7 days was the presence of fatty changes in the centrizonal area. Necrosis was mild (Figure 1E). Almost normal hepatic lobule architecture was seen in the group treated with 100 mg/kg of Zincovit drop for 7 days. Necrosis or inflammation was absent (Figure 1F).



1A- Normal control (2% gum acacia; 1 ml/kg/day); **1B**- Toxic control (CCl_4 + 2% gum acacia; 1 ml/kg/day); **1C**- Positive control (CCl_4 + Silymarin; 50 mg/kg/day); **1D**- CCl_4 + Zincovit drop; 25 mg/kg/day; **1E**- CCl_4 + Zincovit drop; 50 mg/kg/day; **1F**- CCl_4 + Zincovit drop; 100 mg/kg/day

Fig. 1: Microphotographs of hematoxylin and eosin stained sections of liver seen under 40X

DISCUSSION

The results showed that oral treatment of rats with Zincovit drop for seven days effectively protected the animals against CCl_4-induced hepatic injury. Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. CCl_4-induced hypofunctions of the hepatic cell membrane due to hepatic injury are associated with the peroxidation of lipids and reduced antioxidant levels through the production of toxic species-trichloromethyl free radical (CCl_3 •) or/and trichloromethyl peroxy radical (CCl_3OO•) which in turn alter the hepatic metabolism via oxidative stress leading to hepatotoxicity [9, 10]. The lipid peroxidative degradation of biomembranes is one of the principle causes of hepatotoxicity induced by CCl_4 [11, 12]. In the present study, silymarin was considered as standard drug (positive control) because studies suggest the protective role of silymarin in hepatic oxidative stress [13, 14]. The reversal of increased serum enzymes in CCl_4-induced liver damage by Zincovit drop may be due to the prevention of the leakage of intracellular enzymes by its antioxidant and membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [15]. Effective control of ALP, and bilirubin levels indicates towards an early improvement in the secretary mechanism of the hepatic cells.

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. Both silymarin and Zincovit drop decreased CCl4 induced elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells. The most suggested mechanism for hepatoprotective activity of Zincovit drop might be due to its antioxidant property, on account of which it may exert an inhibitory effect on lipid peroxidation and a stimulatory effect on hepatic regeneration as well. In the current study taken together all the above facts, the ability of the Zincovit drop (nutritional food supplement) to ameliorate the hepatic injury induced by carbon tetrachloride might be attributed to synergistic interplay of chemical constituents of Zincovit drop, such as- Vitamins C, E, folic acid, biotin and minerals like zinc, copper, selenium, magnesium, manganese, chromium and molybdenum, flaxseed oil and lysine mainly, which are promoters of antioxidant activity.

CONCLUSION

The present study revealed that Zincovit drop is the potential dietary supplement that has offered a novel therapeutic option as hepatoprotective agent against carbon tetrachloride-induced hepatic injury in Wistar rats.

Conflict of interest: The authors unveil that they do not have any conflict of interest.

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