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Original Research Article

Zinc Sulfate & Curcuma domestica are hepatoprotective agents against acute liver injury model induced by CCl_4

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Abstract: The present study was designed to determine the hepato protective activity of well known agents Zinc Sulfate & aqueous extract of Curcuma domestica in experimental model of acute liver injury induced by carbon tetra chloride. The normal levels of serum hepatic enzymes of AST, ALP, ALT and TSB&TSP, were determined in 28 healthy domestic Rabbits allocated to four groups before CCL4 administration & at two occasions in 24, 120 hr after ALI induction by CCL4 and treatment with one of the following ether Zinc Sulfate or the aqueous extract of curcoma domestica for five successive days. Results revealed a significant reduction of AST, ALP, ALT, TSB, levels with a significant elevation of the TSP level of both tested agents measured in 120 hr compared with the control & their levels in 24 hr after inducation. Both tested agents Proved in having hepatoprotective activity when they were given orally in a dose of 20 mg / kg for zinc sulfate and 1gm/kg for Curcuma domestica. The histopathlogical sections of the liver tissue support the real action of both agents on ALI in Rabbits.
Keywords: Zinc sulfate, Curcuma domestica, ALI, CCL4, Hepatoprotective.

INTRODUCTION

Acute liver injury (ALI) is a clinical condition that results from severe & extensive damage of liver tissue with reduced cell mass &blood flow. It is associated with increase of serum alanine amino transferase (ALT) Aspartate amino transferase (AST) Alkalanine phosphate (ALP) and total serum bilirubin (TSB) [1]. It is a serious condition caused by toxins, viral infections, drugs intoxication & other agents [2]. ALI is characterized by rapid deterioration of liver cell function resulting in hepatic encephalopathy and / or coagulopathy in the liver of normal subjects [3]. Carbon tetra chloride (CCL4) is a famous hepatotoxic agent that was widely used orally or intra-peritoneally in animals to induce. ALI due to formation of free radicals mediated lipid peroxidation. [4] A number of familiar drugs & medicinal herbs has been proved in having hepatoprotective effect [5, 6]. On CCL4 induced model of ALI, therefore, it is interesting to explore the possible hepatoprotective activity of these agents $ZnSo_4$ & aqueous extract of Curcuma domestica in the present study. Zinc is a trace element which is present in chemical structure of $ZnSo_4$ $7H_2o$. It has a biological half-life of 280 day & is highly bound to alpha-2 macro-globulin [7]. It is essential for normal growth, tissue repairs, wound healing. [8] Used in treatment of Kalaazar (Visceral leishmaniasis) [9] Acne & Rheumatoid arthritis [10]. Curcuma domestica (Turmeric) is a tropic plant extensively cultivated in India & Tropic area of Asia & to lesser extent in Africa. Its constituents are curcuminoids (which are mixture of Curcumin), Volatile oil (Tumerone, artumeron, Zungi berene, curlone, Curcumol) and Protein, Starch resins. [11, 12]. It is used in skin disease, Rheumatoid arthritis & cancer therapy. [13] Also used to control hyperlipidemia. [14] Can be used experimentally in renal failure [15].

MATERIALS AND METHODS

Chemicals

ALI the chemicals which are used in the present study of analytical grade. CCL4 was supplied by Merck – Germany as a pure liquid. Zinc sulfate was used as capsule each contained 220 mg and was supplied by AL HAVI – Iran republic. The kits for estimation of serum hepatic enzymes ALP, AST, ALT were purchased from Bio Merieus – France while kits for estimation of TSB& TSP were supplied from Randox – England.

Plant extraction

Turmeric plant was purchased from well Known herbal bureau (AL-Medina) which is present in the center of Baghdad city. The dried rhizomes of turmeric was identified & authenticated by Iraqi National institute for herbs. The rhizomes were cleaned carefully and powdered by an electrical grinder then passed through sieve no.40 to remove the debris. The sieved powder was stored in air tight black container at room temperature. The aqueous extract was prepared by diluting one volume of well grinded powder to ten volume of water at 80c in a stoppered flask after shaking gently. Then, it was allowed to stand for ten minutes to be cold & filtered for practical use. The aqueous extract should be used within 12 hr [16].

Animals

Twenty eight healthy domestic rabbits (850-950) gm were used in the present study. They were supplied by animal house of Al-Nahrain collage of medicine. Animals were house under good conditions at 28c in separated cages which provided with a wide wire mesh floor. Animals were fed standard oxoid pellets & were given water ad libitum. The rabbits were randomly allocated to four groups (each contains seven rabbits). They were given a single daily dose of the followings for five successive days:

- Group-1 (Control) received distilled water 3ml orally.
- Group-2 (Drug control) Received distilled water 3ml orally.
- Group-3 Received Zinc sulfate 20 mg / kg orally.
- Group-4 Received extract of turmeric 1gm/ kg orally.

The doses of Zinc sulfate & turmeric had been chosen by using many doses in pilot study. At 10:00 am of the first day, CCL4 was given in a dose of 1.25 ml / kg orally to the animals of groups 2, 3, & 4 for induction of ALI. Blood Samples were collected from marginal ear vein of the rabbits of all groups for biochemical analysis of serum AST, ALT, ALP and TSP & TSP at two occasions 24&120hr. using spectrophotometer method for comparison between values of these results [17]. Later on, all the rabbits were sacrificed under light anesthesia of ether to take liver specimen. The histo- pathological examination was performed to check the microscopic changes of the liver tissue using polarized microscope after fixating the sections in 10% formalin for 48hr and staining with hematoxylin & eosin [18].

Statistical analysis

All the results were expressed as mean SEM. The differences among means had been analyzed by student test using SPSS version 12 p values < 0.05 consider to be statistically significant.

RESULTS

Table-1: Effect of Zinc sulfate &Extract of turmeric on CCL4 induced acute liver injury in rabbits

Groups	Dose	Duration (hours)	SALT U/ L	SAST U/L	SALP U/L	T.S.B U mol/L	T.S.P g/dl
1.Control	– – –	-------	26.33± 2.34	25.37± 4.50	45.61± 7.53	11.34± 0.76	5.5± o.36
2. CCL4	1.25 ml/kg	24 hour	132.11± 1.87	130.23± 4.5	189± 2.61	29.17± 0.60	48.66± 0.49
		120 hour	121.92± 1.61	103.8± 1.99	173± 7.89	25.66± 0.34	45± 1.81
3.Zinc sulfate + CCL4	20mg / kg	24 hour	*56.0±0.96	*72.68± 140	*102±1.17	*11.16± 0.47	*58.5± 0.42
		120 hour	45.65±1.14	*17.66± 1.05	*77.83±1.13	*9.83± 0.36	*52.5± 0.47
4.Tumeric + CCL4	1gm / kg	24 hour	*83± 1.86	*80.66± 1.19	*131.15±3.40	*11.67 ±0.80	*53.5± 1.23
		120 hour	*39.34±4.71	*42.83± 7.23	*37.85±0.59	*10.50 ±1.20	*52.66 ±1.89

* Significant lowering& rising effect at P<0.05

The obtained results showed a marked elevation of serum AST, ALT, ALP and TSB levels with a decrease of TSP level after administration of CCL4 compared with control group. Both of Zinc sulfate &extract of turmeric (group 3, 4) demonstrated a significant reduction in ALT, AST, ALP, and TSB levels measured after 120hr compared with control group and to their levels measured in 24hr. The effect of zinc sulfate was more potent in than extract of turmeric in lowering significantly AST & TSB levels the values of 17.66±1.05 & 9.83±0.30 respectively versus 42.83±7.23 & 10.5±1.20 respectively for turmeric extract measured in 120hr compared with control group and their levels in 24hr. The effect of turmeric was more potent than Zinc sulfate to in lowering significantly ALT, ALP of levels with values 39.34±4.71 & 37.85±0.59 respectively versus 45.65±1.14 & 77.83±1.13 for Zinc sulfate compared

with control group & their levels in 24hr. Turmeric extract showed no significant increase in rising TSP level as compared to that of Zinc sulfate with value of 52.66±1.89 versus 52.5±1.89 for Zinc sulfate in 120hr of turmeric extract & Zinc sulfate were significantly changed as compared with control & their levels in 24hr. The histo-pathological examination of the liver in both agents showed a marked improvement in the hepatocytes congestion infiltration of lymphocytes, fatty changes necrosis. These improvement support the hepatoprotective effect of these agents against CCL4 induced ALI (Figures 1, 2, 3, & 4).

Fig-1: Normal rabbit liver, showing normal hepatocytes with normal lobular appearance (10X, H&E stain)

Fig-2: Rabbit liver section after CCL4 administration showing massive necrosis, Fatty changes, lymphocyte inflammation congestion

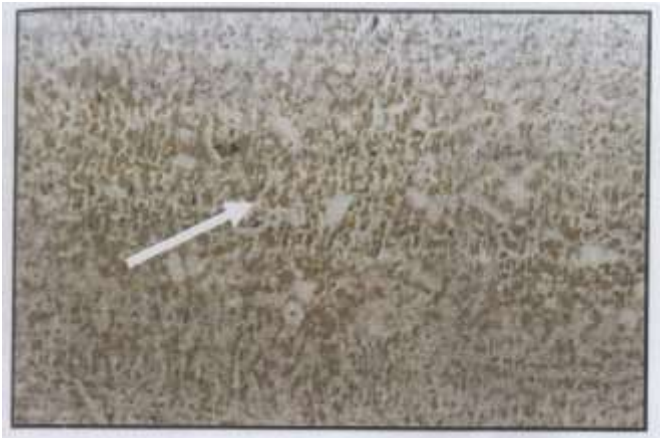

Fig-3: Rabbit liver treated with zinc sulfate after CCL4 necrosis, mild fatty changes, mild inflammatory infiltration and mild congestion (10X, H&E stain)

Fig-4: Rabbit liver treated with turmeric extract after CCL4 administration, Showing mild necrosis, mild fatty change, mild inflammatory infiltration and mild congestion (10X, H &E stain)

DISCUSSION

CCL4 is a well-known hepato-toxic agent to be used orally or peritoneally for induction of ALI in experimental animal model. it is biotransformed in the cytochrome P450 system to its metabolite (trichloro methyl free radical (CClI3) which is in the presence of oxygen forms trichloro methyl peroxyl – free radical (CCI3O2) that attacks lipid of endoplasmic reticulum eliciting lipid Peroxidation with leakage of hepatocellular enzymes like sALT, sALP, SAS in the serum and causing an increase in TSB level and a decrease in TSP level [19]. The results of the treated control (Group2) in the present study are compatible with results of others [20]. Who demonstrated that changes of CCL4 after day one in correspondance with hepatic intoxication and also to changes in day seven which are related to the effect of hepatic regeneration after CCL4 induce ALI.

Zinc is essential for DNA synthesis & liver protein metabolism & may be of importance for repairing process following cell injury in liver [21]. The hepatoprotective effect of zinc sulfate (Group3) is related to reduce release of hepatic enzyme through inhibiting lipid peroxidation stabilizing cell membrane [22]. The scavenging effect of zinc as a part of superoxide dismutase (SOD) which is related to anti-inflammatory anti-oxidant enzymes present in all oxygen metabolizing cells [23].

The hepatoprotective effect of turmeric extract may be attributed to its anti-phlogistic effect, and to powerful antioxidant & cytoprotective property of tetra hydrocurcumin [24]. Which is derived from curcumin (the active component of turmeric) by hydrogenration?

CONCLUSION

Both zinc sulfate &turmeric extract possess hepatoprotective activity restoring the normal hepatic function, enhancing the bio defense of the liver against oxidative damage that occur by CCL4 administration.

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