Dose-dependent protective effects of silymarin against ccU-induced liver damage in rats

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Summary:

Background and Objective: *Silymarin, the dried extract of the ripe seeds of Silybum marianum is found to be a powerful protective agent against xenobiotics-induced tissue injury in many organs, including liver. However, the dose-dependent relationship of this effect and tissue availability is not fully explored. So, this project was designed to evaluate the relationship between dose, tissue availability and tissue protection of silymarin against cc^-induced hepatic toxicity in rats.*

Methods: *The tissue protective effects of silymarin were studied through the pre-treatment of rats with various doses (250, 500, and lOOOmg/kg) orally twice daily before the induction of hepatotoxicity of ccl4.*

Malondialdehyde (MDA) and glutathione (GSH) were evaluated in the serum and tissue homogenate. The activities of different enzymes, which are considered as indicators of organ toxicity like alanine amino transaminase (ALT) and aspartate aminotransaminase (AST) were assessed. Histopathological examination of stained tissue sections from the liver were done to evaluate the protective effect at the microscopical levels. In addition, silymarin level in liver tissue homogenate was evaluated using HPLC method.

Results: *The data obtained indicated that, a significant amelioration of oxidative stress experimentally induced in the liver of rats was produced by silymarin, as evidenced by lowering MDA contents and elevation of GSH levels both in the tissues and serum compared with controls. Serum activities of ALT and AST were normalized. Additionally, histologically evident damage in the liver had improved In addition, increasing silymarin dose after oral administration resulted in increased tissue availability of many constituents.*

Conclusion: *There is a dose-dependent relationship in the hepatoprotective effect of silymarin against ccU-induced hepatotoxicity in rats.*

Key words: Silymarin, CcU, hepatotoxicity

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Introduction

Carbon tetrachloride (ccl4) was mainly employed as a spot remover and carpet cleaner, and it is still used in the fumigation of grain and as insecticides (1).

It has been suggested that, free-radical-mediated damage to the hepatocytes play an important role in the development of liver toxicity with. ccl4 [2] The The injury seems to be mediated by a reactive metabolite trichloro methyl free radical (•CCl3)- formed by hemolytic cleavage of ccU or by an even the more reactive species, trichloro methyl peroxy free radical (cl 3coo'), formed by the reaction of »ccl3 with O2.[3] The reaction of free radicals (CCI3 or cl 3coo') with lipids and proteins causes the peroxidation of polyenoic lipids of the endoplasmic reticulum and the generation of secondary free radicals derived from these lipids (a chain reaction). Destructive lipid peroxidation leads to the break-down of membrane structure and functions [4].

Furthermore, ccU causes damage to the mitochondria and this consequently lead to decrease ATP synthesis and the hepatocytes accumulate large droplets of triglycerides in its cytosol as a result of membrane damage .

Silymarin, is a mixture of flavonolignans derived from milk thistle (Silybum marianum), comprised mainly of silybinin (SBN) A and B, isosilybinin (ISBN), Silychristin (SCN), silydianin (SDN), and taxifolin (TXF)[6].

Silymarin has been proven in documented results to protect liver cells against number of toxic substances including alcohol, acetaminophen, and amanita mushroom poison [7,8>9>10].by its anti-oxidant and free-radical scavenging activities[11>12], plasma membrane stabilization and permeability regulating properties[13], as promoter of ribosomal RNA synthesis [1 ,15], as inhibitor of cytochrome P450 detoxifying system [16]. This study was designed to evaluate the possible relationship between dose, tissue availability and hepatoprotective effect of

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silymarin against ccU-induced hepato-toxicity in rats.

Methods:

Thirty rats *{Rattus norvigicus)* of both sexes weighing (180-220gm), were grouped into 5 groups of 6 animals each housed in the animal house-college of pharmacy, University of Baghdad under standard laboratory conditions and had a free access to water and fed a standard rat chow *ad libitum.* Animal groups are treated as follows: Group I: Six rats treated orally with corn oil (vehicle) and injected I.P. with normal saline, served as control. Group II; Six rats received single daily dose of CCl4 (5%) (2.5 ml of a mixture of 1:1 v/v in corn oil/kg/day) for 24 hours by gavage needle[17]. Group III; Six rats received twice daily dose of (250mg/kg silymarin powder dissolved in corn oil) by gavage needle, started 7-days prior to and during the period of treatment with. ccU Group IV and V: Six rats in each group treated with 500 and 1000mg/kg silymarin, respectively, and continued the same procedure as in group III. All rats were sacrificed 24 hours after ccU administration by cervical dislocation Serum and liver tissue homogenate were prepared by standard procedure, and the levels of MDA and GSH were analyzed in both compartments ^ [illegible] ^; in addition to measurement of the serum activities of ALT and AST '. Small pieces of the hepatic tissues were prepared for histopathological examination according to the standard procedure and evaluated by ordinary microscope after staining with hematoxylin and eosin [21]. Finally silymarin levels in the liver tissue homogenate were measured by HPLC according to the method of Zhao and Agarwal [22]and compared with authentic standard purchased for this purpose. Statistical analysis of data was performed utilizing student's t-test. and ANOVA. 95% confidence of data was considered for significance.

Results:

The data presented in tables 1 and 2 showed that ccU produces highly significant increase in the serum MDA and tissue lipid peroxides. The levels being 2, and 4 fold, respectively compared to controls (PO.0005). While a significant decrease in both serum and liver GSH levels by about 6, and 12 times, respectively compared to control animals (P<0.005).

Animals pre-treated with twice daily doses of silymarin (250, 500, and 1000 mg/kg) 7-days before ccLt treatment, exhibited a significant reduction in ccU-induced increase in the serum and liver tissue homogenate lipid peroxides levels compared to controls, while a significant increase in the level of GSH in both sites (PO.005) compared to controls. (Tables 1 and 2).

In table 3, treatment with CcU resulted in highly significant increase in serum activities of ALT and AST compared to saline injected group (control). Pre-treatment with various doses of silymarin (250, 500 and 1000 mg/kg) for seven days resulted in significant reduction in serum activities of ALT and AST compared to vehicle-treated group (Table 3). Liver tissue histology showed CcU hepatic degeneration and necrotic changes, congestion and hemorrhage in the portal tract, fatty changes affecting mainly zone 2 with centrilobular necrosis as a result of treatment with ccU Bile stasis in biliary tracts in scattered hepatocytes was seen, in comparison with the control liver. (Figures 1 and 2). Increasing doses of silymarin produce a dose-dependent improvement in the degree of fatty degeneration in the scattered hepatocytes, previously induced by ccU administration, associated with the attenuation of the degree of congestion and hemorrhage. The extent of inflammatory cell infiltration was decreased as seen in figures 3 and 4. In figure 5, the HPLC profile of standard silymarin was noted to contain six major constituents of the total extract, which are supposed to be taxifolin, Silychristin, silydianin, silybinin A, silybinin B, and isosilybinin, sequenced according to their appearance in the chromatogram, compared with previous reports established by Jose, in 2002 [23] with the retention time 1.5, 2, 2.5, 3, 4.8, and 6 minutes, respectively. the hepatic tissue distribution of orally-administered silymarin in doses 250 and 500mg/kg, showed the appearance of a single peak, which is expected to belong to taxifolin in a concentration of 570.3 u.g/g tissue according to their appearance from the eluent, compared to standard (figures 6 and 7), while silymarin administered in a dose of lOOOmg/kg, showed the appearance of two peaks in the liver homogenate, which may belong to taxifolin, and Silychristin and in a concentrations of 567 and 509.35 u.g/g tissue, respectively (figure 8) compared to standard.

Discussion:

CcU exerts its toxicity through its metabolites, including the free radical (*Ccl3) and (Cl 3C00*), both of these free radicals injure the hepatocytes by causing lipid peroxidation and binding covalently to cell systems, assoiated with the formation of protein-lipid cross linkage, and among these also the binding of »ccl3 adducts with DNA proteins and lipids. [24,25]

The most serious consequences of free-radical induced liver injury is lipid peroxidation, and it has been found that, free radical can cause oxidative damage to cellular proteins and alter cellular function[26]

The biochemical and histological evidences presented in this study, clearly demonstrated the state of oxidative stress induced-hepatic tissue damage by ccU treatment, manifested by the elevation of MDA contents in serum and tissue homogenate, which is associated with sever depletion of GSH levels in both sites (tables 1 and 2); these results are consistent with those observed by others[27] The observed decline in GSH levels, the

important water-soluble anti-oxidant, which can directly scavenge reactive species and are extensively produced during the metabolism of ccU in the hepatocytes, worsen the state of oxidative stress, and as much as more GSH were consumed for conjugation of metabolites, the redox potential of the tissue was impaired[28]

The present work showed that, silymarin reduces the increased content of MDA in both liver homogenate and serum of rats treated with ccU. Moreover it increase the levels of GSH in both sites (tables land 2).

Garrido *et al,* in 1991[29] suggested that, silybinin, the active constituent of silymarin, inhibits cytochrome P450 system, therefore reducing the reactive toxic metabolites of acetaminophen (namely benzoquinone, which act as a potent free radical, that produces GSH depletion in the liver).This suggestion may be employed for the hepatoprotective effect of silymarin against the hepatotoxicity produced by cc^-treatment, which is observed during our study and in the reports of others. But, Dvorak et al in 2003 [30] observed that silymarin extract at lower doses than those used in our studycan not afford protection against acetaminophen- or D-galactosamine-induced hepatotoxicity, which was attributed to failure of active components (silydianin and Silychristin) to achieve effective tissue levels at the given doses.

Silymarin has also been shown to increase serum levels of GSH and GSH-Px activity in many other patients [31-32]. These results indirectly indicated that, free radical scavenging activity of silymarin is able to improve the whole anti-oxidant profile of tissues which consequently improve the protection of the cell against toxic insults by ameliorating the deleterious effects of free radical reactions.

Our results showed that, an increased in the serum activities of ALT and AST in ccU-treated rats compared to control (table 3); as a result of increased lipid peroxidation , permeability of plasma membrane was severely affected and may lead to leakage to the plasma of ALT and AST, and this is consistent with those obtained by Jayasekhan *et al,* in 1997 [33]. These high values of serum activities of both enzymes may be attributed to the alteration in the structure and functions of the hepatocellular membrane as a result of binding of toxic metabolites of ccLj to the lipid and protein components of the membrane [34].

silymarin attenuates the increase in the serum activities of ALT and AST after intoxication by ccU. (Table 3), and this may be attributed to that, silymarin may stabilize hepatocytes plasma membrane and prevent delivery of ALT and AST to the extra cellular fluid[35].

Centrilobular hepatocytes are the richest sites in cytochrome P450 activity; ccU is metabolites by cytochrome P450 into toxic free radicals and these reactive radicals being available at highest levels at the centrilobular zone, that why necrosis manifested clearly and evidently there and is associated with diffused fatty infiltration[36].

When liver tissues sustain free radical damage, fatty acids are released, which can cause inflammation and set up the production of damaging leukotriens. Alcohol or ccU can cause this fatty infiltration and its inflammatory consequences in the liver. Silymarin helps to neutralize this reaction by inhibiting lipid breakdown, which decreases the presence of fatty acids. Interestingly, while fatty production is counteracted by silymarin, protein synthesis is stimulated and this action causes an increase in the regeneration process which repair the damaged hepatocytes as a result of production of new liver cell, which are so vital if the liver is damaged [14-7]

In conclusion, this study clearly demonstrated that tissue availability of silymarin is dose-dependent, and can be positively correlated with its hepatoprotective effect.

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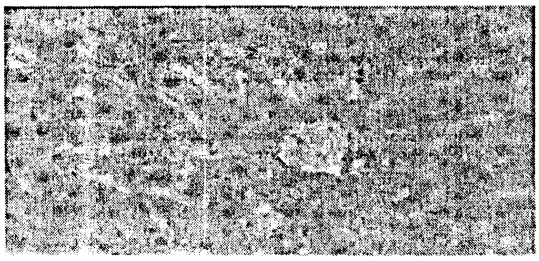

Fig.1: Section showing normal rat's liver.

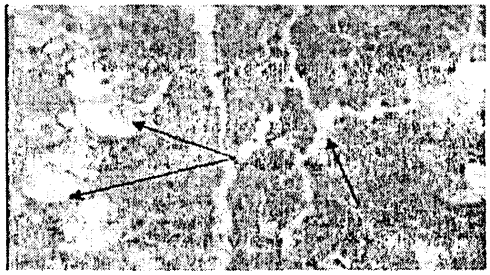

Fig. 2: Section showing morphological alteration of livers from CeI_4-treated rats. Black arrow represents fatty changes.

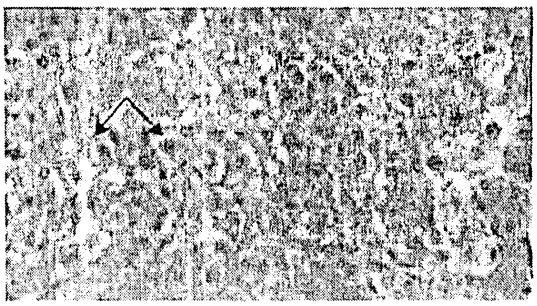

Fig. 3: Section showing the administration of 250mg/kg silymarin improved CeI_4-induced hepatic damage.