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

Nada Naji Al-Shawi*  
Saad Abdel-Rehman Hussain  
Husam Hasson Ali*  
Yahia Zeki Rashid**  
Nawfal Abdel-Munim Numan*

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 ccU-induced hepatotoxicity in rats.

Methods: The tissue protective effects of silymarin were studied through the pre-treatment of rats with various doses (250, 500, and 1000 mg/kg) orally twice daily before the induction of hepatotoxicity of ccU. 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 microscopic 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, CeU, hepatotoxicity

Introduction

Carbon tetrachloride (ccU) 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, ccU (2). 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 (CCl3 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 breakdown 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), silyidian (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 (5,6,9-10) 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 (10). This study was designed to evaluate the possible relationship between dose, tissue availability and hepatoprotective effect of
silymarin against ccU-induced hepatotoxicity in rats.

Methods:
Thirty rats (Rattus norvegicus) of both sexes weighing (180-220gm), were grouped into 5 groups of 6 animals each housed in the animal house-cell 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. 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 and 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. Finally, silymarin levels in the liver tissue homogenate were measured by HPLC according to the method of Zhao and Agarwal 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.005). While a significant increase in both serum and liver GSH levels by about 6, and 12 times, respectively compared to control animals (P<.005).

Animals pre-treated with twice daily doses of silymarin (250, 500, and 1000 mg/kg) 7-days before ccU 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, silybin A, silybin B, and isosilybinin, sequenced according to their appearance in the chromatogram, compared with previous reports established by Jose, in 2002 with the retention time 1.3, 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 tissue according to their appearance from the eluent, compared to standard (figures 6 and 7), while silymarin administered in a dose of 1000mg/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 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, associated with the formation of protein-lipid cross linkage, and among these also the binding of wcc13 adducts with DNA proteins and lipids.

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.

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 severe depletion of GSH levels in both sites (tables 1 and 2); these results are consistent with those observed by others. 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(29).
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 2 and 3).
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 ccU-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 study can 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 (35). 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 ccU 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 (30).
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 countered 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 (4,5,6,7).
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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