Induction of Microsomal Liver Enzymes by Silymarin in Experimental Animals

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Summary:

Background: previous researches showed that the hepatoprotective effect of silymarin was through inhibition of cytochrome p450- system (e.g. protection against Amanita toxin), in addition to protection from free radicals generated by this enzymatic system. In contrast to that, many evidences clarified that silymarin may increase first pass metabolism of e.g. cyclosporine and benzodiazepenes.
Objective: Our aim from this animal experiment was to relieve this confusion and detect that this herbal extract is absolutely hepatoprotective or induce hepatotoxicity in other conditions.
Materials and Methods: this study was performed on 15 healthy male rats randomized into two treatment groups; first group (5 rats) pretreated with phenobarbital (I.P) then given acetaminophen (I.P) and the second group (5 rats) pretreated with silymarin (orally) then given acetaminophen (I.P.); while the last five rats were considered as control group for comparison . Activities of SGOT, SGPT and histopathological sections were detected for both groups and compared with that of control.
Results: for both treatment groups, the activities of transaminase enzymes were significantly higher than control group. Meanwhile; activity of these enzymes and severity of hepatic damage were significantly higher for Phenobarbital group compared with silymarin.
Conclusion: we can conclude from this experimental study that, even though silymarin act as hepatoprotective by multifactorial mechanisms (antioxidant, increase glutathione level, antifibrotic, anti-inflammatory and enzyme inhibitor), it may act as enhancer for some hepatotoxic agents (like acetaminophen) by cytochrome P-450 induction mechanism.
Key word: Acetaminophen, Phenobarbital, Silymarin, P450-system.

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Introduction:

Silymarin is a flavonolignan that has been introduced recently as a hepatoprotective agent. It's most well known compound of flavonoids, thanks to it's well defined therapeutic properties. Silymarin extracted from the seeds and fruits of *Silybum marianum* and in reality is a mixture of three structural components: silibinin, silydianine and silychristine (1). From a medical point of view, silymarin has been found to provide cytoprotection and, above all, hepatoprotection.It's used for the treatment of numerous liver disorders characterized by degenerative necrosis and functional impairment (2). Furthermore, silymarin is able to antagonize the toxicity of *Amanita phalloides* and provide hepatoprotection against the damage produced by ethanol, galactosamine, halothane and CCL_4(3).It also protect hepatocyte from injury caused by ischemia, radiation, iron overload and viral hepatitis(4).The hepatoprotective mechanism of silymarin is multifactorial and involve: stabilization of hepatocyte membrane and block receptor – binding of various toxins, free radical scavenging activity and antioxidant effect, protect against glutathione depletion and increase protein synthesis, also it reverse hepatic fibrosis due to CCL_4 – induced damage (5,6).

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The anti-inflammatory effects of silymarin include mast cell stabilization, inhibition of neutrophil migration, kuppfer cell inhibition and inhibition of leukotriene and prostaglandin formation (7). It has been shown in mice that silibinin is able to inhibit numerous hepatic cytochrome p-450 enzymatic activities, whereas other researchers did not detect any effects of silymarin on this enzymatic system; this effect could explain some of the hepatoprotective properties of silymarin especially against the intoxication induced by *Amanita phalloides* which is bioactivited to it's toxic metabolite by cytochrome p-450(8). Many clinically important drug interactions results from enzyme induction. Rifampicin, for example, given for three days reduces the effectiveness of warfarin as an anticoagulant. Conversely, enzyme induction can increase toxicity of the second drug whose toxic effects are mediated via a metabolite (9). Acetaminophen is a case in point: it's due to N-acetyl- p- benzoquinone imine (NAPBQI) which is formed by cytochrome p-450. Consequently, the risk of serious hepatic injury following acetaminophen overdose in patients whose cytochrome p-450 system has been induced, for example by Phenobarbital (9,10). Previous researches showed that the hepatoprotective effect of silymarin was through inhibition of cytochrome p450- system (e.g. protection against Amanita toxin), in addition to protection from free radicals generated by this

enzymatic system. In contrast to that, many evidences clarified that silymarin may increase first pass metabolism of e.g. cyclosporine and benzodiazepenes. Our aim from this animal experiment was to relieve this confusion and detect that this herbal extract is absolutely hepatoprotective or induce hepatotoxicity in other conditions.

Materials and Methods:

Fifteen healthy male rats with weight range (150-200g), age range (10-14) weeks and Norway albino strains (*Rattus norvegicus*) obtained from the college of pharmacy / Baghdad university and involved in this study. They were kept in plastic cages with constant temperature, humidity, lighting and feeding on distilled water and standard pills diet. Rats were randomized into two treatment groups: Group(I): five rats received Phenobarbital by I.P route (100mg/kg/day) for three days, then given acetaminophen I.P. (600 mg/kg) as a single dose at the fourth day .Both drugs supplied by Syrian Ibn-Hayyan company. Group (II): five rats received silymarin orally (250 mg/kg/ day) for one week supplied as standard powder from Egyptian Luna company, then given acetaminophen I.P (600 mg/kg) as a single dose at the eighth day.The last five rats were considered as a control group. After anaesthetizing each animal by ether for 90 second, we collect blood specimen via intracardiac aspiration, each sample was transferred to plastic centrifuge tube and left at room temperature for complete clotting of blood. Serum was aspirated after centrifugation at 1000 rpm for 10 minutes and kept in the freeze to be ready for doing assay of SGOT and SGPT activities spectrophotometrically (11). Concerning histological morphometry to detect hepatic microscopical appearance, the fixed tissue specimens were processed for routine paraffin-wax embedding and stained by haematoxylin and eosin stain (12).

Statistical analysis: data were expressed as mean ± SD and the student's "t" test was used for statistical evaluation of significant difference between both therapeutic groups and compared with that of control. P-value of < 0.5 was regarded as significant.

Results:

Data presented in the following table showed that the activities of SGOT and SGPT for both group I (phenobarbital group) and group II (silymarin group) were significantly higher than that of control ($p < 0.05$). Meanwhile; activities of these enzymes were significantly higher in rats received phenobarbital compared with those received silymarin ($p < 0.05$). Regarding histopathological results: Control group slides showed normal liver architecture without focal fatty changes. Phenobarbital group slides showed focal liver necrosis with focal mixed inflammatory cell infiltrations around central venules. Portal tract clarified moderate mixed inflammatory cell infiltration. Silymarin group slides showed focal liver necrosis within liver paranchyma with mild inflammatory cells.However, the light microscopical appearance reported that the hepatotoxic effects within group I (Phenobarbital group) were more than of group II (silymarin group).

Table (1): Animal Groups and Hepatic Transaminase Levels.

Animal group	SGOT(U/L)	SGPT(U/L)
Control (N=5)	30.0 ± 2.33	23.0 ± 1.52
Gr. I (N=5)	377.0 ± 81.34 ^{*a}	130.4 ± 45.25 ^{*a}
Gr. II (N=5)	278.5 ± 88.0 ^{*b}	70.6 ± 46.98 ^{*b}

Data expressed as Mean ± SD.
Gr. I : received phenobarbital + acetaminophen.
Gr. II : received silymarin + acetaminophen.
Results with non identical superscript (a,b) were considered significantly different ($p < 0.05$).
***= significant difference from control ($p < 0.05$)**
N= number of rats .

Discussion:

Acetaminophen hepatotoxicity is well known by previous studies (5) and our results consist with this fact. With toxic dose of acetaminophen the enzymes that catalyzing the normal conjugation reaction are saturated and cytochrome p-450 system convert the drug to it's reactive metabolite (NAPBQI) (13). Acetaminophen hepatotoxicity increased in patients in whom cytochrome p-450 enzymes have been induced, for instance by chronic excessive consumption of alcohol and barbiturates. NAPBQI initiate several of the covalent and non covalent interactions and the oxidative stress process due to glutathione depletion is an important leader for cell death or degeneration (14). The significantly higher activities of hepatic transaminases and sever necrosis in microscopical slides for rats receiving Phenobarbital compared with those receiving silymarin may be attributed to the powerful enzymatic induction properties of barbiturates in addition to the hepatoprotective role of silymarin (2,14). Regarding silymarin – receiving group, our biochemical and histological results illustrated that silymarin act as enzyme inducer by enhancing hepatotoxicty. These results were inconsist with the previous experimental data which fixed that this herbal extract act as cytochrome p450- inhibitor so protect hepatic tissues from *Amanita phalloides* via decreasing free radical generation (15)in addition to its role by increasing cellular contents of glutathione, so act as a membrane stabilizer by inhibiting lipid peroxidation and oxidative stress complications (16).

Conclusion:

We can conclude from this experimental study that, even though silymarin act as hepatoprotective by multifactorial mechanisms (antioxidant, increase glutathione level, antifibrotic, anti-inflammatory and enzyme inhibitor activities), it may act as enhancer for some hepatotoxic agents (like acetaminophen) by cytochrome P-450 induction mechanism.

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