COMPARITIVE STUDY OF SOLANUM XANTHOCARTUM AND SILYMARINE IN PARACETAMOL INDUCED LIVER DAMAGE

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ABSTRACT
Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. Therefore, it has an important role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. It is an important organ for detoxification of xenobiotics, environmental toxicants and liver damage is associated with distortion of several metabolic functions; hence liver diseases are of serious health problem. Prolonged drug therapy, excessive use of the some of the commonly used medicines like paracetamol, diclofenac etc., alcoholism, exposure to certain xenobiotic, polutants and certain disease state have been reported to affect liver functioning. Effectively, herbal products are widely used in the treatment of hepatic disorders all over the world. In the absence of reliable liver protective drugs in allopathic medical practices, naturally occurring compounds have been found to have major role in the management of various liver diseases. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional systems of medicine in India.

KEYWORDS: Hepatocytes, Paracetamol, Sillimarin, Sollanum xanthocartum.

INTRODUCTION
The major clinical manifestation of liver disorder is jaundice. Despite of the excellent regenerationat capacity of this organ, a slight injury or toxicity may lead to fatal complications. Therefore damage to the liver inflicted by hepatotoxic agents is of grave consequences.Unavailability of rational therapy in modern medicine and no or very less
positive influence of synthetic drugs in liver damage have urged researchers in this field to look for herbal drugs with better hepatoprotective action. Traditional medicines are effective in certain disease and are based on their age-old use in folklore system of medicine. Natural products of plant origin with hepatoprotective and antioxidants properties play an important role in treatment of liver toxicity. Antioxidants play an important role in inhibiting and scavenging free radicals and thus providing protection against infections and degenerative diseases.

**Silymarin**

Silymarin is a flavonolignan (polyphenolic fraction) obtained from the seeds of silybum marianum (milk thistle), an edible plant that has been used medicinally from ancient times for the treatment of various liver disorders. It is widely prescribed by herbalists and has almost no known side effects.

**Solanum xanthocarpum**

(Family: Solanaceae) commonly known as yellow berried nightshade (synonym: Kantakari), is a prickly diffuse bright throughout 2-3 m height found throughout India, mostly in dry places as a weed on road yellow or white with green veins, surrounded by enlarged calyx.

**MATERIALS AND METHOD**

Paracetamol was purchased from Lupin Ltd., Mumbai, India. 1-chloro2, 4-dinitro benzoic acid (CDNB), 5,5-dithio-bis-2-nitro benzoic acid (DTNB) and reduced glutathione (GSH) were supplied by Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Thiobarbituric acid was purchased from E-Merck, India. All other chemicals used were of analytical grade.
Preparation of plant extract
Fresh and matured fruits were collected from University of Madras, Guindy Campus. The freshly collected fruits (2 kg) of S. xanthocarpum were dried and powdered. The powdered plant material (900g) was macerated with petroleum ether, the mass was exhaustively extracted with of 50% ethanol for three days. The extract was separated by filtration and concentrated on rotavapour and then dried in lyophilizer under reduced pressure. The yield obtained was 198.40 g of solid residue (yield 22.04% w/w). The extract obtained was further subjected to preliminary phytochemical screening and pharmacological investigation.

Collection of Animals
Male Wister Albino rats were purchased from Kings Institute of Technology, Guindy, Chennai. Prior approval by institutional ethical committee was obtained for conduction of this experiment.

Wister Albino rats of male animals weighing 150-200g were used for the studies. They were fed a standard rat pellet and water and maintained under standard laboratory conditions. Animals described as fasted were deprived of food for 18hours but had free access to water.

Procedure for induce hepatotoxicity
250 mg/kg paracetamol administer orally for 14 day. The test drug will administered orally by suspending in water solution. Twenty-four hours after last dose of INH, bloods will obtain from all groups of rats by puncturing retro-orbital plexus. The blood samples will allow coagulating for 45 min at room temperature. Serum will separate by centrifugation at 3000 rpm at room temperature for 20 min and used for the biochemical estimation.

Experimental Design
Rats were divided into five groups, each group consisting of six animals.

Group I: Controls received the vehicle viz. normal saline (2 ml/kg).
Group II: Received paracetamol (750 mg/kg, p.o.) [5] at every 72 h for 21 days.
Group III: Received silymarin 50 mg/kg (p.o.) for 21 days and simultaneously administered paracetamol 750 mg/kg every 72 h.
Group IV: Received methanol extract of S. xanthocarpum 250 mg/kg p.o. for 21 days and simultaneously administered paracetamol 750 mg/kg every 72 h.
Group V: Received methanol extract of S. xanthocarpum 250 mg/kg and Silymarin 50 mg/kg p.o. for 21 days and simultaneously administered paracetamol 750 mg/kg every 72h.

At the end of all experimental methods, all the animals were sacrificed by cervical decapitation. Blood samples were collected, allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters.

RESULTS

The effects of S. xanthocarpum and Silymarine on biochemical parameters are presented in Table 1 and Table 2. The levels of serum AST, ALT, ALP, total bilirubin, GGTP were markedly elevated and that of Total protein decreased in paracetamol treated animals, indicating liver damage. Administration of Solanum xanthocarpam extract at the doses of 250 mg/kg remarkably prevented paracetamol induced hepatotoxicity whereas Silymarine at the dose of 50 mg/kg also prevented paracetamol induced hepatotoxicity. But combination of both S. xanthocarpum 250 mg/kg and Silymarine 50 mg/kg.

Analysis of LPO levels by thiobarbituric acid reaction showed a significant increase in the paracetamol treated rats. Treatment with S. xanthocarpum 250 mg/kg significantly prevented the increase in LPO level which was brought to near normal. The effect of S. xanthocarpum was comparable with that of standard drug silymarin (Table 3).

Paracetamol treatment caused a significant decrease in the activities of SOD, catalase, GPx and GST in liver tissue when compared with control group (Table 3). The treatment of S. xanthocarpum at the dose of 250 mg/kg resulted in a significant increase in the activities of SOD, catalase, GPx and GST when compared to paracetamol treated rats.

The liver of silymarin treated animals also showed a significant increase in antioxidant enzymes levels compared to paracetamol treated rats. Combination of S. xanthocarpum and Silymarine treated animals showed more significant in antioxidant enzymes levels compared to S. xanthocarpum and Silymarine treated alone.

Morphological observations showed an increased size and enlargement of the liver in paracetamol treated groups. These changes were reversed by treatment with silymarin and also S. xanthocarpum and the combination of these two (Table 4).
Table 1. Effect of Solanum xanthocarpum and silymarine on Total bilirubin, Total protein and ALP levels in paracetamol induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Total bilirubin (mg/dl)</th>
<th>Total Protein (g/dl)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>-</td>
<td>0.8±0.05</td>
<td>8.13±0.46</td>
<td>76.25±1.78</td>
</tr>
<tr>
<td>Group II (Paracetamol)</td>
<td>750</td>
<td>1.42±0.06</td>
<td>6.09±0.32</td>
<td>468.5±0.17</td>
</tr>
<tr>
<td>Group III (Silymarine)</td>
<td>50</td>
<td>0.92±0.04</td>
<td>7.42±0.14</td>
<td>236.19±2.0</td>
</tr>
<tr>
<td>Group IV S. Xanthocarpum</td>
<td>250</td>
<td>1.1±0.07</td>
<td>6.85±0.13</td>
<td>302.4±0.33</td>
</tr>
<tr>
<td>Group V (S.xanthocarpum &amp; Silymarine)</td>
<td>250 &amp; 50</td>
<td>0.79±0.02</td>
<td>8.09±0.47</td>
<td>226.2±2.67</td>
</tr>
</tbody>
</table>

N=6; Values are expressed as mean ± Sd (P ≤ 0.05).

Table 2. Effect of Solanum xanthocarpum and silymarine on AST, ALT and GGTP levels in paracetamol induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>GGTP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>-</td>
<td>132.7±1.18</td>
<td>76.25±1.78</td>
<td>75.34±1.05</td>
</tr>
<tr>
<td>Group II (Paracetamol)</td>
<td>750</td>
<td>269.7±2.45</td>
<td>194.8±43</td>
<td>179.6±0.18</td>
</tr>
<tr>
<td>Group III (Silymarine)</td>
<td>50</td>
<td>193.6±1.32</td>
<td>110.2±1.24</td>
<td>96.4±1.67</td>
</tr>
<tr>
<td>Group IV S. Xanthocarpum</td>
<td>250</td>
<td>224.7±1.15</td>
<td>133.4±1.46</td>
<td>114.2±1.82</td>
</tr>
<tr>
<td>Group V (S.xanthocarpum &amp; Silymarine)</td>
<td>250 &amp; 50</td>
<td>178.4±2.17</td>
<td>96.8±1.93</td>
<td>89.3±1.1</td>
</tr>
</tbody>
</table>

N=6; Values are expressed as mean ± Sd (P ≤ 0.05).

Table 3. Effect of Solanum xanthocarpum and Silymarine on lipid peroxidation levels and antioxidant enzyme activities in paracetamol induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>LPO</th>
<th>SOD</th>
<th>Catalase</th>
<th>GPX</th>
<th>GST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (control)</td>
<td>---</td>
<td>7.85±0.92</td>
<td>46.7±1.12</td>
<td>78.0±3.1</td>
<td>28.45±1.16</td>
<td>2.04±0.06</td>
</tr>
<tr>
<td>Group II (Paracetamol)</td>
<td>750</td>
<td>19.8±2.1</td>
<td>15.60±1.1</td>
<td>32±2.36</td>
<td>11.18±1.45</td>
<td>1.16±0.07</td>
</tr>
<tr>
<td>Group III (Silymarine)</td>
<td>50</td>
<td>12.4±0.32</td>
<td>31.2±1.32</td>
<td>49±1.86</td>
<td>23.65±1.36</td>
<td>1.79±0.03</td>
</tr>
<tr>
<td>Group IV S. Xanthocarpum</td>
<td>250</td>
<td>15.9±1.14</td>
<td>25.6±0.37</td>
<td>37±1.35</td>
<td>16.64±1.18</td>
<td>1.36±0.05</td>
</tr>
<tr>
<td>Group V (S. Xanthocarpum and Silymarine)</td>
<td>250 &amp; 50</td>
<td>10.01±0.87</td>
<td>36.6±1.27</td>
<td>59±1.87</td>
<td>26.42±1.25</td>
<td>1.92±0.05</td>
</tr>
</tbody>
</table>

N=6; Each value is expressed as mean ± Sd (P ≤ 0.05).

Units: LPO = µ moles of MDA/min/mg protein; SOD = Units/min/mg protein; CAT = µ mole of H2O2 consumed/min/mg protein; GPX = µ moles of GSH oxidized/min/mg protein; GST = µ moles of chloro-dinitro benzene (CDNB) conjugation formed/min/mg protein.
### Table 4. Effect of Solanum xanthocarpum and Silymarine on comparison of liver weight in all groups.

<table>
<thead>
<tr>
<th>Design of treatment</th>
<th>Dose (mg/kg)</th>
<th>Liver wt per 100g body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (control)</td>
<td>2 ml/kg</td>
<td>4.12 ±0.18</td>
</tr>
<tr>
<td>Group II (paracetamol)</td>
<td>750</td>
<td>7.16 ±0.16</td>
</tr>
<tr>
<td>Group III (Silymarine)</td>
<td>50</td>
<td>5.54±0.12</td>
</tr>
<tr>
<td>Group IV (S. Xanthocarpum)</td>
<td>250</td>
<td>6.13±0.06</td>
</tr>
<tr>
<td>Group V (S. Xanthocarpum and Silymarine)</td>
<td>250 &amp; 50</td>
<td>4.88±0.1</td>
</tr>
</tbody>
</table>

N=6; values are expressed as mean ± Sd (P ≤ 0.05).

### DISCUSSION

Paracetamol, a widely used antipyretic-analgesic drug, produces acute hepatic damage on accidental overdosage. It is established that, a fraction of paracetamol is converted via the cytochrome P450 pathway to a highly toxic metabolite, N–acetyl–p–benzoquinamine (NAPQI), which is normally conjugated with glutathione and excreted in urine.

Overdose of paracetamol depletes glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction, and development of acute hepatic necrosis. Several P450 enzymes are known to play an important role in N-acetyl-p-aminophenol (APAP) bioactivation to NAPQI. P450 2E1 (CYP2E1) have been suggested to be primary enzymes for paracetamol bioactivation in liver microsomes. Studies demonstrated that paracetamol induced hepatotoxicity can be modulated by substances that influence P450 activity.

In the assessment of liver damage by paracetamol the determination of levels of enzymes such as AST, ALT is largely used. Necrosis or membrane damage releases the intracellular enzymes into circulation and hence it can be measured in the serum. Increased levels of AST indicates liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury, ALT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore ALT is more specific to the liver and is a better parameter for detecting liver injury.

Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. Serum ALP, bilirubin and total protein levels are also related to the function of the hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure. Administration of Paracetamol caused a
significant elevation of enzyme levels such as AST, ALT, ALP, GGTP, total bilirubin and decrease in total protein when compared to control. There was a significant restoration of these enzyme levels on administration of S.xanthocarpum extract at dose of 250 mg/kg and also by silymarin at a dose of 50 mg/kg but the combination of S.xanthocarpum and Silymarine in the dose of 250 mg/kg and 50 mg/kg respectively (Table 1).

The reversal of increased serum enzymes in paracetamol induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its 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.

Decrease in enzyme activity of SOD is a sensitive index in hepatocellular damage. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. Combination of *S.xanthocarpum and Silymarine* causes a significant increase in hepatic SOD activity and thus reduces reactive free radical induced oxidative damage to liver.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals. Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. Combination of *S.xanthocarpum and Silymarine* increases the level of CAT as produced by silymarin, the standard hepatoprotective drug.

Glutathione (GSH) is one of the most abundant tripeptide, non-enzymatic biological antioxidants present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Decreased level of GSH is associated with an enhanced lipid peroxidation in paracetamol treated rats. Administration of Combination of *S.xanthocarpum and Silymarine* significantly increased the level of GPx and GST in a dose dependent manner.
REFERENCE


