ABSTRACT
The purpose of this study was to evaluate the protective and curative effect of Justicia tranquebariensis leaf extract (JTLE) using acetaminophen - induced liver injury in mice. The leaf extract at dosage of 500 and 1000 mg/kg exhibited significant protective effect against acetaminophen induced hepatotoxicity. Level of serum markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin (TB) were significantly increased in acetaminophen treated mice. Simultaneously, Justicia tranquebariensis leaf extract significantly suppressed mainly the increase in plasma activities of AST, ALT, ALP and TB concentration, which are considered as markers of liver functional state. The results of this study confirmed the protective and curative effect of the aqueous leaf extract of Justicia tranquebariensis.

Key words: Justicia tranquebariensis, hepatoprotective activity, acetaminophen, liver enzymes.

INTRODUCTION
Many indigenous plants have been in the use of men since time immemorial for curing various ailments without the actual knowledge of their toxic potential(s). Recently an interest in medicinal plants has increased scientific scrutiny of their therapeutic potentials and safety thereby providing physicians with data to help patients make wise decision on their usage.[1] The ethnobotanical uses of plants are diverse in both traditional and veterinary medical practices [2] and the use of plants for medicinal purposes dates back to antiquity.

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles[3]. It is also concerned with regulation of internal chemical environment and an organ of paramount importance as it plays an essential role in maintaining the biological equilibrium of vertebrates. The spectrum of its functions includes, metabolism of lipids, carbohydrates and proteins, blood coagulation and immunomodulation[4]. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequence. There is an ever increasing need for an agent which could protect liver from damages especially of one which facilitates regeneration by proliferation of parenchymal cells after damage arrest growth of fibrous tissue[5].

In spite of tremendous strides in modern medicine, there are not much drugs available for the treatment of liver disorders [6]. Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Hence there is a worldwide trend to go back to traditional medicinal plants. Many natural products of herbal origin are in use for the treatment of liver ailments [7].

Justicia tranquebariensis (Acanthaceae) is a small shrub, which is widely distributed in the southern parts of India. Some species of the genus Justicia have been used in the traditional system of medicine for the treatment of fever [8], pain, inflammation [9], diabetes [10], diarrhea [11] and liver diseases [12]. They also possess anti
tumoral, anti viral, analgesic and anti-inflammatory activities. In this genus about 20 species have been chemically investigated and the major secondary metabolites isolated were lignans, flavonoids, steroids and triterpenes. The juice of small and somewhat fleshy leaves of this species of Justicia is considered by the natives of India as cooling and aperients, and is prescribed for the children in the smallpox, in the doses of a spoonful or two, twice daily; bruised leaves are also applied to blows and other external injuries.

As far as our literature survey could ascertain, no information was available on the protective and curative effect of Justicia tranquebariensis leaf extract. Therefore the aim of this study was to investigate the protective and curative effect of Justicia tranquebariensis from India.

MATERIAL AND METHODS
Preparation of plant extract
Justicia tranquebariensis leaves were collected at Raspipuram, Namakkal Dt, Tamilnadu and was identified at The Rapinant Herbarium of St. Joseph’s College, Trichy, Tamilnadu. The leaves of the plant were shade dried and powdered. Dried powder (200g) was taken and extracted using Soxhlet apparatus. The extract obtained was concentrated to dry residue under reduced pressure at room temperature. Concentrated residue was stored at 4°C and used for this study. All the chemicals used were obtained from Himedia Private Limited.

Phytochemical study:
The leaf extract of Justicia tranquebariensis was already subjected for phytochemical study by us.

Experimental animals:
Swiss Albino mice of both sex, weighing about 55-70g were used for the study. They were fed with standard pelleted diet (M/s Hindustan Lever Foods, Bangalore, India) and water ad libitum. They were housed in polypropylene cages maintained under standard conditions (12 hour light - dark cycle; 25 ± 3°C; 35–60% humidity). The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee, and was cleared by same before beginning the experiment.

Experimental design:
Hepatotoxin (acetaminophen) was induced in the mice by oral administration of 500 mg/kg of body weight for one day after pretreatment with Justicia tranquebariensis for 15 days. The mice were divided into following five groups of six mice each.

- **Group-I** Mice served as normal control
- **Group-II** Mice served as an induced experimental control (acetaminophen - 500 mg/kg of body weight)
- **Group-III** Mice pretreated with Justicia tranquebariensis leaf extract alone (500 mg/kg of body weight).
- **Group-IV** Mice pretreated with Justicia tranquebariensis leaf extract (500 mg/kg and acetaminophen induced.
- **Group-V** Mice pretreated with Justicia tranquebariensis leaf extract 1000 mg/kg and acetaminophen (500 mg/kg of body weight) induced.

The pretreatment of the mice with Justicia tranquebariensis leaf extract belonging to group III, IV and V were carried out for 15 days and the dosage of the leaf extract fed was 500 mg/kg for groups III and IV and 1000 mg/kg for group V. The mice in group I and II were fed with normal pellet diet during the pretreatment period. The mice of groups IV and V were orally administered with a single dose of acetaminophen. After 4 hours, the mice of all the groups were sacrificed by cervical decapitation. Blood was collected and serum was separated for studying various biochemical parameters.

Biochemical parameters i.e., aspartate amino transferase (AST) and alanine amino transferase (ALT), acid and alkaline phosphatases (ALP), γ-glutamyl transferase (GGT), liver glycogen, triglycerides, total bilirubin, tissue and serum cholesterol, tissue and serum protein were analyzed according to the respective methods, mentioned in the parenthesis.

Histopathology:
All the sacrificed mice were necropsied. Liver was collected from different groups and fixed in 10% neutral buffer formalin. Paraffin sections (6-8 microns) were prepared and stained with Harris haematoxylin and eosin for microscopic examination.

Statistical analysis:
All results were expressed as mean ± S.D. Statistical evaluation was done using one-way analysis of variance (ANOVA), followed by Student’s t-test.

RESULTS
The results clearly depict the damage caused by acetaminophen to the hepatic tissue. The protective effect of the aqueous extract of Justicia tranquebariensis from India.
The marker enzymes and serum bilirubin levels elevated in Group II were restored to normal in the drug treated groups. The dysfunction of the liver was evident from the lowered serum & tissue protein and liver glycogen levels in Group II animals. The protein and liver glycogen levels were restored to normalcy in the plant drug treated groups.

Table 1. Effect of *Justicia tranquebariensis* leaf extract in serum and liver biochemical parameters in albino mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I Control</th>
<th>Group II Acetaminophen induced</th>
<th>Group III JTLE (500 mg)</th>
<th>Group IV Acetaminophen induced + JTLE (500 mg)</th>
<th>Group V Acetaminophen induced + JTLE (1000 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Protein (mg/dl)</td>
<td>9.25±0.12</td>
<td>6.10±0.35</td>
<td>7.80±0.52*</td>
<td>7.5±0.40*</td>
<td>8.79±0.10*</td>
</tr>
<tr>
<td>Total Cholesterol(mg/dl)</td>
<td>72.8±0.30</td>
<td>88.9±1.20</td>
<td>74.30±0.90*</td>
<td>76.20±0.60*</td>
<td>73.30±0.70**</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>14.60±1.00</td>
<td>42.90±8.70</td>
<td>15.50±0.50*</td>
<td>16.70±0.70*</td>
<td>15.80±0.50**</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>70.20±1.60</td>
<td>52.20±0.30</td>
<td>63.40±0.20*</td>
<td>61.30±0.20*</td>
<td>66.80±0.60*</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Protein (mg/g)</td>
<td>0.85±0.04</td>
<td>0.61±0.03</td>
<td>0.82±0.09*</td>
<td>0.81±0.08*</td>
<td>0.83±0.01*</td>
</tr>
<tr>
<td>Total Cholesterol(mg/g)</td>
<td>82.40±3.70</td>
<td>102.10±1.9</td>
<td>84.50±3.50*</td>
<td>92.4±3.20*</td>
<td>88.70±4.00*</td>
</tr>
<tr>
<td>Glycogen (mg/g)</td>
<td>42.01±0.30</td>
<td>20.41±0.11</td>
<td>36.80±0.20*</td>
<td>28.51±0.42*</td>
<td>32.01±0.02*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six animals in each group. Comparisons are made between: group I and groups, III, IV, V. The symbols * represent statistical significance: at $P < 0.01$ and ** - Not significant at $p < 0.01$.

Table 2. Effect of *Justicia tranquebariensis* leaf extract in serum marker enzyme parameters in albino mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I Control</th>
<th>Group II Acetaminophen induced</th>
<th>Group III JTLE (500 mg)</th>
<th>Group IV Acetaminophen induced + JTLE (500 mg)</th>
<th>Group V Acetaminophen induced + JTLE (1000 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Glutamate Oxaloacetate Transaminase(SGOT) (IU/L)</td>
<td>36.30±0.40</td>
<td>73.40±7.90</td>
<td>40.60±2.30*</td>
<td>56.20±3.40*</td>
<td>44.60±1.20*</td>
</tr>
<tr>
<td>Serum Glutamate Pyruvate Transaminase (SGPT) (IU/L)</td>
<td>29.10±0.30</td>
<td>50.60±2.40</td>
<td>33.20±0.50*</td>
<td>48.40±0.40*</td>
<td>36.50±0.50*</td>
</tr>
<tr>
<td>Gamma Glutamyl Transferase (GGT) (IU/L)</td>
<td>47.23±1.40</td>
<td>64.50±3.20</td>
<td>50.10±2.10*</td>
<td>52.70±1.60*</td>
<td>49.00±1.80**</td>
</tr>
<tr>
<td>Alkaline Phosphatase(ALP) (IU/L)</td>
<td>13.60±0.060</td>
<td>37.50±0.12</td>
<td>15.20±0.04*</td>
<td>26.30±0.17*</td>
<td>20.10±0.15*</td>
</tr>
<tr>
<td>Acid Phosphatase (ACP)(IU/L)</td>
<td>30.10±0.16</td>
<td>54.80±0.54</td>
<td>32.40±0.18*</td>
<td>44.60±0.69*</td>
<td>34.20±0.66*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six animals in each group. Comparisons are made between: group I and groups, III, IV, V. The symbols * represent statistical significance: at $P < 0.01$ and ** - Not significant at $p < 0.01$. 

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DISCUSSIONS
Medicinal plants have traditionally used for treating liver diseases since centuries. There is growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Herbal drugs or their extracts are prescribed widely, even when their biological active components are unknown[32]. Therefore, studies with plant extracts are useful to know their efficacy and mechanism of action and safety. Natural remedies from plants are considered to be effective and safe alternative treatment for hepatotoxicity.

Acetaminophen (Paracetamol) is widely used over the country as drug for analgesic and antipyretic effects. Its use in overdose (Suicidal or accidental) or with chronic alcohol abuse causes fulminant liver failure and contributes significantly to intensive care unit admissions and cost of hospitalization[33,34]. Acetaminophen induced hepatic failure is the second leading cause of liver transplantation and accounts to considerable level of morbidity and mortality[35].

Acetaminophen is safe in recommended doses but produces hepatic necrosis when ingested in very large doses. It is established that at these relatively large doses, paracetamol is bio-transferred in to a reactive metabolite N-acetyl P-benzoquinone imine (NAPQI) by cytochrome P-450 mixed function oxidase[36].

High doses of acetaminophen have been demonstrated to increase the serum levels of SGOT and SGPT[37,38]. A marked increase in blood aminotransferase enzyme is related with ischemic or toxic liver injury[39]. In group II animals the extent of increase in the SGOT level was nearly 102.2% and SGPT was increased to 73.8%. When there is gross cellular necrosis, as in paracetamol poisoning, the level of SGOT and SGPT are raised. This is because ALT levels is increased in the serum solely due to conditions where cells of the liver have been inflamed or undergo cell death, and is specific for the liver cells[40] but the AST levels can be triggered on other conditions such as myocardial infarction apart from hepatocellular damage[40]. The increased levels of SGOT and SGPT in animals treated with toxicant only were a clear indication of a kind of injury or the other caused by toxicity[41] (Table-2).

Pre-treatment with aqueous leaf extract of *Justicia tranquebariensis* reduced the enhanced level of SGOT and SGPT, ACP, ALP which seems to offer the protection and maintain the functional...
The integrity of hepatic cells\[^{42,43}\] is maintained during acetaminophen (APAP) induced hepatotoxicity. The pre-treatment with the JTLE showed marked recovery in group IV and Group V animals and the extent of recovery when compared to the acetaminophen induced group II were 10.2% decrease in the mean SGPT value of group IV and 65.5% decrease with group V. Similar trend was exhibited in SGOT the recovery was 35.5% in case of group IV and 77.6% in case of group V (Table-2). This clearly showed the dose dependent effect of JTLE. 

Increase in the concentration of the drug decreased the activity of these marker enzymes conferring protection to the hepatocytes.

Reduction in the levels of SGOT and SGPT towards the normal value in the pretreated groups is an indication of generation process. Reduction in ACP and ALP levels suggests the sterility of the biliary function during injury with acetaminophen. The pre-treatment with the JTLE showed marked recovery in group IV and Group V animals and the extent of recovery when compared to the acetaminophen induced group II were 10.2% decrease in the mean SGPT value of group IV and 65.5% decrease with group V. Similar trend was exhibited in SGOT the recovery was 35.5% in case of group IV and 77.6% in case of group V (Table-2). This clearly showed the dose dependent effect of JTLE. 

Marked recovery in the levels of marker enzymes (SGPT, SGOT, ACP, ALP) was observed in the pretreated groups. The levels of SGPT were decreased to normal values in group IV and group V (65.5% and 77.6% decrease respectively) compared to the control group. The levels of SGOT were also decreased to normal values in group IV and group V (35.5% and 77.6% decrease respectively) compared to the control group. The levels of ACP and ALP were also decreased to normal values in group IV and group V (82% and 83.4% decrease respectively) compared to the control group.

Assessment of liver function can be made by estimating the activity of GGT, which is the enzyme originally present in higher concentration in cytoplasm. When there is hepatotoxicity, this enzyme leak in to the blood stream in conformity with the extent of liver damage\[^{46,47}\]. In the animals belonging to group II the percentage of increase in the levels of GGT when compared to the control animals were nearly 36.5% and in the pre-treated group with JTLE the percentage of decrease in the enzyme levels were 41.2% with group IV and 83.4% in group V. Approximate doubling of the activity was noted in this case.

Attachment of near normal in bilirubin level in acetaminophen intoxicated and Justicia tranquebariensis leaf extract treated animals confirms the hepatoprotective effect of the plant extract\[^{51}\].

The levels of total proteins were reduced due to acetaminophen (35.05%) induced hepatotoxicity. The reduction is attributed to the initial damage produced and localized in the endoplasmic reticulum, which results in the loss of P450 leading to functional failure with a decrease in protein synthesis and accumulation of triglyceride leading to fully liver (Table 1).\[^{44}\]

After the treatment plant extract (Justicia tranquebariensis leaf extract) the protein levels are raised (12.6% in group IV and 85% in group V) suggesting the stabilization of endoplasmic reticulum leading to protein synthesis.\[^{44}\]

**CONCLUSION**

The results obtained have demonstrated the protective effect of Justicia tranquebariensis and thus supports its use in indigenous medicinal systems of India. Further investigations are required to fully elucidate the mechanisms of action of the extract and its dose dependent effect.

**ACKNOWLEDGEMENT**

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