and increasing the permeability to ATP through cytoplasmic membrane. Similarly, Rath et al.⁹ also reported the anti staphylococcal activity of Juniper and Lime essential oils against methicillin resistant Staphylococcus aureus (MRSA) through inhibition of cell membrane synthesis that corroborates with the findings observed in this investigation. The antibacterial activity of essential oils through membrane inhibition could be attributable to the hydrophobicity of essential oils, enables them to make partitions in the membrane, rendering permeability and leading to leakage of cell contents resulting in death of microbial cells¹⁰-¹².

In conclusion, this investigation amply proved the antibacterial activity and mechanism of action of Jasminum sambac natural oil and its synthetic blends against E. coli MTCC-443 strain.

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Hepatoprotective Activity of Vitex trifolia against Carbon Tetrachloride-induced Hepatic Damage

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Aqueous and ethanol extracts of leaf of Vitex trifolia was investigated for hepatoprotective activity against carbon tetrachloride induced liver damage. To assess the hepatoprotective activity of the extracts, various biochemical parameters viz., total bilirubin, total protein, alanine transaminase, aspartate transaminase and alkaline phosphatase activities were determined. Results of the serum biochemical estimations revealed significant reduction in total bilirubin and serum marker enzymes and increase in total protein in the animals treated with ethanol and aqueous extracts. However, significant rise in these serum enzymes and decrease in total protein level was noticed in CCl4 treated group indicating the hepatic damage. The hepatoprotective activity is also supported by histological studies of liver tissue. Histology of the liver tissue treated with ethanol and aqueous extracts showed normal hepatic architecture with few fatty lobules. Hence the present study revealed that Vitex trifolia could afford significant protection against CCl4 induced hepato cellular injury.

Key words: Vitex trifolia, aqueous and ethanol leaf extracts, CCl4, hepatoprotective activity

The plant Vitex trifolia L., (Verbenaceae) is commonly known as common chaste tree (English), nochi (Kannada) and jalanirgundi (Sanskrit). Leaves are commonly used as poultice for rheumatic pains, in inflammations, sprains and fever. Roots are used to treat febrifuge, painful inflammations, cough and fever. Flowers are used in treating fever and fruits in amenorrhoea. This plant is known to possess various active constituents viz., essential oil, halimane-type diterpenes, vitetrifolins and several pharmacological properties have been studied viz., antipyretic, antibacterial, against asthma and allergic diseases. The plant is used by the local medical practitioners in treating acute jaundice. Literature survey revealed that, this plant has not been subjected to pharmacological screening for its hepatoprotective activity. This paper reports the hepatoprotective activity of Vitex trifolia against CCl4-induced hepatic damage.

Leaves of Vitex trifolia were collected from the Kuduremukha reserve forest of Chikmagalur district, Karnataka State, during December 2003 and a voucher specimen (BKM-1012) is deposited in the Departmental Herbaria, Department of Botany, S. R. N. M. N. College of Applied Sciences, Shimoga, as authentic specimen for future reference.

Leaves were shade dried for a week, powdered mechanically (sieve no. 10/44) and stored in airtight containers. About 250 g of the powdered material was subjected to soxhlet extraction using 70% ethanol for 48 h. The solvent was distilled off at low temperature under reduced pressure using rotary flash evaporator.

The yield was 24.5% w/w. Another 250 g of the powdered material was boiled in distilled water for 30 min, kept for 3 d with intermittent shaking, filtered and concentrated using rotary flash evaporator to get the aqueous extract. The yield was 17.5% w/w. Both the extracts were subjected to preliminary phytochemical tests. Oral suspensions containing 20 mg/ml of ethanol extract and 30 mg/ml of aqueous extract were prepared in 1% w/v gum tragacanth.

Male Wistar rats weighing 150-200 g were procured from the National College of Pharmacy, Shimoga. The animals were housed in polypropylene cages and were maintained at 27±2°C, relative humidity 60±5% and 12 h light/dark cycle, they were fed with commercial diet (Hindustan Lever Ltd., Bangalore) and water ad libitum during the experiment. The study was permitted by the Institutional Animal Ethical Committee with Reg. No. 144 /1999/ CPCSEA/SMG/34.

Acute toxicity study was conducted for both the extracts by stair case method. The LD50 of ethanol

### TABLE 1: EFFECT OF ETHANOLIC AND AQUEOUS LEAF EXTRACTS OF VITEX TRIFOLIA ON CCl4-INDUCED HEPATOTOXICITY IN RATS

<table>
<thead>
<tr>
<th>Group (N)</th>
<th>Total Bilirubin (mg/dl)</th>
<th>Total Protein (g%)</th>
<th>AST (IU/l)</th>
<th>ALT (IU/l)</th>
<th>ALP (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.44±0.01</td>
<td>9.50±0.02</td>
<td>154.49±1.79</td>
<td>53.72±0.81</td>
<td>174.36±1.23</td>
</tr>
<tr>
<td>CCl4</td>
<td>2.50±0.04*</td>
<td>5.93±0.01*</td>
<td>2183.75±21.58*</td>
<td>1322.33±5.85*</td>
<td>442.10±2.07*</td>
</tr>
<tr>
<td>CCl4+silymarin</td>
<td>0.53±0.01**</td>
<td>8.69±0.06**</td>
<td>203.97±1.90**</td>
<td>74.11±1.41**</td>
<td>183.77±1.20**</td>
</tr>
<tr>
<td>CCl4+ethanol extract</td>
<td>0.63±0.01**</td>
<td>8.24±0.03**</td>
<td>217.38±1.08**</td>
<td>106.43±1.49**</td>
<td>202.47±1.72**</td>
</tr>
<tr>
<td>CCl4+aqueous extract</td>
<td>0.93±0.01**</td>
<td>8.05±0.03**</td>
<td>232.60±1.82**</td>
<td>124.17±0.85**</td>
<td>236.91±1.10**</td>
</tr>
</tbody>
</table>

ANOVA

F = 2195, Df = 4,25, 4,25, 4,25, 4,25, 4,25

N=6 animals in each group. Values are expressed as means±SE. *P<0.01 indicates significant when compared to control. **P<0.01 indicates significant when compared to CCl4.
The animals of all the groups were sacrificed on 14th day under light ether anaesthesia. The blood sample of each animal was collected separately by carotid

The ethanol and aqueous extracts were selected for the evaluation of hepatoprotective activity against \( \text{CCl}_4 \) induced hepatic toxicity. The animals were divided into five groups of six rats each. The animals in group I served as control and received the vehicle 1 ml/kg/day of 1% w/v gum tragacanth p.o., for 14 d. All the animals of group II to V received 0.1 ml/kg/day of \( \text{CCl}_4 \) i. p. (E-Merck, Mumbai, India) for 14 d. Group III animals received the standard drug silymarin (Ranbaxy Lab, Dewas) in the dose of 100 mg/kg/day p.o., for 14 d. Ethanol and aqueous leaf extracts of \textit{Vitex trifolia} were administered to the animals of group IV and V in the dose of 20 and 30 mg/kg/day p.o., respectively, for 14 d. The \( \text{CCl}_4 \), silymarin and the extracts were administered concomitantly to the respective groups of animals.

**Fig. 1:** Liver tissue of control animal showing normal histology. Section of normal liver tissue with portal triad showing portal vein (V), portal artery (arrow) and hepatic duct (arrow head). Stain H and E, magnification 100X.

**Fig. 2:** Liver tissue of animal treated with \( \text{CCl}_4 \) showing necrosis. Section of the liver tissue of animal treated with \( \text{CCl}_4 \) showing necrosis (N), fatty vacuole (F) and central vein (V). Stain H and E, magnification 100X.

**Fig. 3:** Liver tissue of silymarin-treated animals showing normal hepatocytes. Section of the liver tissue of silymarin-treated animals showing normal hepatocytes, portal vein (V), portal artery (arrow) and bile duct (arrow head). Stain H and E, magnification 100X.

**Fig. 4:** Liver tissue of aqueous leaf extract-treated animals showing normal arrangement of hepatocytes. Section of the liver tissue of aqueous extract of leaf-treated animals showing normal arrangement of hepatocytes around the portal vein (V), absence of necrosis and moderate accumulation of fatty vacuoles (F). Stain H and E, magnification 100X.

**Fig. 5:** Liver tissue of ethanol extract-treated animals showing normal arrangement of hepatocytes. Section of the liver tissue of ethanol extract of the leaf-treated animals showing normal arrangement of hepatocytes around the portal vein (V), portal artery (arrow), bile duct (arrow head) and absence of necrosis and few fatty vacuoles (F). Stain H and E, magnification 100X.
bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 3000 rpm for 10 min and was subjected to biochemical investigation viz., total bilirubin\textsuperscript{10}, total protein\textsuperscript{11}, serum alanine transaminase, aspartate transaminase\textsuperscript{12} and alkaline phosphatase\textsuperscript{13}. Results of biochemical estimations were reported as mean±SE of six animals in each group. The data was subjected to one way ANOVA followed by Tukey’s multiple comparison tests. P≤0.01 was considered as statistically significant.

The liver samples were excised from the experimental animals of each group and washed with the normal saline. Initially the materials were fixed in 10% buffered neutral formalin for 48 h and processed for paraffin embedding. The sections were taken at 5 µ thickness using microtome, processed in alcohol-xylene series and were stained with alum-haemotoxylin and eosin\textsuperscript{14}. The sections were examined microscopically for the evaluation of histological changes.

Effect of ethanol and aqueous leaf extracts of \textit{Vitex trifolia} on CCl\textsubscript{4}-induced liver damage in rats with reference to biochemical changes in serum is shown in the Table 1. At the end of 14 d treatment, blood samples of CCl\textsubscript{4}-treated animals showed significant increase in the levels of total bilirubin, alanine transaminase, aspartate transaminase and alkaline phosphatase compared to normal control groups but the total protein level decreased reflecting the liver injury caused by CCl\textsubscript{4}. Whereas blood samples from the animals treated with ethanol and aqueous leaf extracts of \textit{Vitex trifolia} showed significant decrease in the levels of serum markers and significant increase in total protein to the near normal which are comparable to the values registered in the standard drug treated group of animals, indicating the protection of hepatic cells. Among the two extracts ethanol leaf extract showed significant protection against CCl\textsubscript{4} induced hepatic damage.

Histological profile of control animal showed normal hepatocytes (fig. 1). The section of liver of the group II animals exhibited severe intense centrilobular necrosis, vacuolization and macro vesicular fatty changes (fig. 2). The liver sections of silymarin-treated animals showed normal hepatic architecture (fig. 3). The liver sections of the animals treated with aqueous extract exhibited moderate accumulation of fatty lobules (fig. 4). However significant liver protection was observed in the liver sections of ethanol extract treated animals as evident by the presence of normal hepatic cords, absence of necrosis with few fatty lobules (fig. 5).

CCl\textsubscript{4}-induced hepatic injury is the common model used for hepatoprotective drug screening. The extent of hepatic damage is assessed by the elevated level of biochemical parameters which is attributed to the generation of trichloromethyl free radical which in turn causes peroxidation of lipids of cellular membrane\textsuperscript{15}. In the present investigation, preliminary phytochemical analysis of leaf extracts revealed the presence of flavonoids, tannins, saponins, glycosides, steroids and triterpenoids. Flavonoids\textsuperscript{16} and triterpenoids\textsuperscript{17} are well known for their antioxidant and hepatoprotective activities. In this study ethanol extract showed protective effect against toxicity induced by CCl\textsubscript{4}, which may be attributed to the individual or combined effect of antioxidant and hepatoprotective activity of phytoconstituents present in it. Based on the above results of the pharmacological screening, it can be concluded that the ethanol and aqueous leaf extracts of \textit{Vitex trifolia} possesses significant hepatoprotective activity, which provides scientific evidence to the ethnomedicinal value of this rare plant genetic resource used by the tribal group of Western Ghats in treating jaundice.

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Synthesis and Evaluation of L-Glutamic acid Analogs as Potential Anticancer Agents

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Viswanathan, et al.: Synthesis and anticancer activity of L-glutamic amides

Four N- (benzenesulfonyl)-L-glutamic acid bis(p-substituted phenylhydrazides) were synthesized and evaluated for anticancer activity in vitro in DU-145 and PC-3 prostate cancer and in COLO-205 colon cancer cell lines by MTT assay. The analog with the nitro group substitution exhibited potent activity (% Inhibition 84.7 and 72.0 in DU-145 and PC-3 respectively at 80 µg/ml concentration). Another series of substituted 1-(benzenesulfonyl)-5-oxopyrrolidine 2-carboxamides (11a-f) were synthesized and evaluated for anticancer activity in vitro in colon (COLO-205), breast (Zr-75-1) and prostate (PC-3) cancer cell lines by MTT assay using Adriamycin as standard. Test compounds 11a-c showed potent activity (% Inhibition 61.2 to 79.2 at 20 µg/ml and 67.2 to 87.2 at 40 µg/ml) in PC-3 cell line which is superior to the activity of Adriamycin. In comparison compounds 11d-f were less potent. In Zr-75-1 cell line 11a-c showed 67.2 to 87.2 at 40 µg/ml concentration while in COLO-205 cell line 11a-f showed poor activity.

Key words: L-glutamic amides, L-glutamic acid hydrazides, anticancer activity

L-Glutamic acid plays an important role in the biosynthesis of purine and pyrimidine bases of DNA and RNA. It is metabolized to L-glutamine by L-glutamine synthetase and this metabolic process is essential for normal maintenance of cells. The synthesis of L-glutamine is hindered in neoplastic cells due to lower reactivity of L-glutamine synthetase. Thus antagonists of this enzyme can interfere with the metabolic role of L-glutamine and act as anti-cancer agents. Azaserine and 6-diazo-5-oxo-L-norleucine antagonized the metabolic process involving L-glutamine and exhibited antitumor activity in animal models. L-glutamic acid γ-(4-hydroxyanilide) a growth regulatory substance isolated from mushroom Agaricus bisporous was found to inhibit B16 mouse melanoma cells in culture.