Evaluation of hepatoprotective activity of vasicinone in mice

Chaitali Sarkar\textsuperscript{a}, Sankhadip Bose\textsuperscript{a} & Sugato Banerjee\textsuperscript{b}\textsuperscript{*}

\textsuperscript{a}Gupta College of Technological Sciences, College of Pharmacy, Asansol 713 301, India
\textsuperscript{b}Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi 835 215, India

Received 8 January 2013; revised 4 April 2014

\textit{Justicia adhatoda} (vasaka) leaves have long been used in Indian Ayurvedic system of medicine as antitussive. Its crude extract has been previously reported to have hepatoprotective activity. Vasicinone was isolated from leaves of \textit{J. adhatoda}, column purified and characterized using, TLC UV, FT-IR and \textsuperscript{1}H NMR. The isolated vasicinone was evaluated for hepatoprotective activity using (CCl\textsubscript{4})-induced acute hepatotoxicity model in mice. CCl\textsubscript{4} treatments lead to significant increase in SGOT, SGPT, ALP levels. Pre-treatment with vasicinone and silymarin (25 mg/kg/day for 7 days) significantly decreased these enzyme levels. Histopathology of the livers from vasicinone and silymarin pre-treated animals showed normal hepatic cords and absence of necrotic changes suggesting pronounced recovery from CCl\textsubscript{4} induced liver damage. Both vasicinone and silymarin significantly decrease the CCl\textsubscript{4} mediated increase in pentobarbital induced sleeping time in experimental animals, thus indicating recovery of liver function. Based on the above results it can be concluded that vasicinone may act as hepatoprotective in mice and warrants further investigation on human volunteers.

Keywords: Hepatoprotective, \textit{Justicia adhatoda}, Mice, Vasicinone

Liver is one of the largest organs in human body and the primary site for intense metabolism and excretion. It has a major role in the maintenance, performance and regulation of physiological homeostasis. It is involved in almost all the biochemical pathways involved in growth, immunity, supply of nutrients, energy provision and reproduction\textsuperscript{1}. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, a healthy liver is crucial for overall health and well being. However it is continuously and variedly exposed to environmental toxins, alcohol and prescribed/over-the-counter drugs which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease\textsuperscript{2,3}. Modern medicine has little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for the treatment of liver disorders. CCl\textsubscript{4}-induced hepatotoxicity, which is mainly a free radical mediated cytotoxic model, is widely used for the study of hepatoprotective effects of drugs and plant extracts\textsuperscript{5}.

\textit{Justicia adhatoda} (L) Willd. (Acanthaceae), with the common name ‘vasaka’, is a perennial shrub, 1-2.5 m in height, widespread throughout the tropical regions of Southeast Asia (Nepal, Pakistan, Myanmar) including India, up to an altitude of 1300 m in the sub-himalayan regions. It is commonly known as basak (Bengali), aradusi, adosa (Gujrati), arusa, bansa, adulsa (Hindi), bansa, basuti, bhekkar (Punjabi) swetavasa, vasa and vasaka (Sanskrit) and malabar nut (English)\textsuperscript{5}. The leaves mainly consist of pyrroquanazoline alkaloids like visicine, vasicinone, vasicol, preganine along with other minor constituents like adhatonine, vasicinol and vasicinolone\textsuperscript{6}.

Extract of \textit{J. adhatoda} leaves has been used for the treatment of various diseases and disorders in Ayurved and Unani medicine\textsuperscript{7}. It has been used as an herbal remedy for allergen induced bronchial obstruction\textsuperscript{6}, asthma and tuberculosis\textsuperscript{9}. Vasicinone alone has also been reported to have bronchodilatory, weak cardiac stimulant and antianaphylactic action\textsuperscript{10,11}. Crude vasaka leaf extract is a known antioxidant and has also been reported to possess hepatoprotective activity\textsuperscript{12,13}. The present study has been undertaken to explore the hepatoprotective action of isolated vasicinone from the leaves of \textit{Justicia adhatoda} in mice.

Materials and Methods

\textit{Plant materials}—The leaves of \textit{Justicia adhatoda} (L) Willd were collected from Sripur road, Kulti,
Isolation of phytoconstituents—Leaves of *J. adhatoda* were shed dried in room temperature and ground to powder (500 g). The powder was soaked with ethyl alcohol (600 mL) for 24 h and the extract was filtered and concentrated. The concentrated material was treated with 5% acetic acid (200 mL), warmed for 15 min and filtered. The filtrate was then defatted with hexane and basified with NH$_3$ (pH 9), followed by extraction with chloroform$^{12}$. After qualitative chemical tests, to confirm the presence of alkaloids, thin layer chromatography was performed using chloroform, methanol and ethyl acetate (85:15:10 and 60:20:10) as solvent system. The crude alkaloids were passed through a glass column (Length 32 cm, diameter 2.5 cm and flow rate 35 drops/min) over silica gel by using chloroform: methanol: ethyl acetate (85:15:10 and 60:20:10) with increasing polarity to get pure form of vasicinone$^{14}$.

Characterization—Vasicinone was characterized by comparing UV$^{15}$, FT-IR$^{16}$ and $^1$H NMR spectra of isolated compound with standard vasicinone spectra$^{17}$.

Experimental animals—Male Swiss Albino mice (20-25 g) were obtained from animal house of Gupta College of Technological Sciences. The animals were housed under standard environmental condition (25 $^\circ$C, 12:12 h L: D cycle) and fed with standard diet (Tetragon Chemie Private limited, Bangalore, India) and water *ad libitum*. All animal experiments were carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Forests and Environment, Government of India and the study was approved by the Institutional Animal Ethics Committee.

Acute toxicity studies—The isolated vasicinone was administered at the doses of 200, 400 and 600 mg/kg, orally to different groups of mice, as per OECD test guidelines (425) for oral acute toxicity study and mortality was observed for up to 7 days$^{18}$.

Experimental protocol—Mice (30) were randomly allocated into following five groups of 6. All animals were made to fast for 24 h before the experiment.

Gr I: Control group. The animals received corn oil, po (vol equivalent to vol of corn oil required administration of 25 mg/kg vasicinone; Vijaya Enterprise, Mumbai, India) for 7 days.

Gr II: Hepatotoxic control. The animals received corn oil for 7 days and a single dose of CCl$_4$ (1 mL/kg body weight, po in corn oil, Merck, India Ltd) on day 8$^{18}$.

Gr III: The animals were treated with vasicinone in corn oil (10 mg/kg body weight, po) for 7 days and a single dose of CCl$_4$ on day 8.

Gr IV: The animals were treated with vasicinone in corn oil (25 mg/kg body weight, po) for 7 days and a single dose of CCl$_4$ on day 8.

Gr V: The animals received silymarin (25 mg/kg body weight, po; Micro Lab Inc, Bangalore India) for 7 days$^{17}$ and a single dose of CCl$_4$ on day 8$^{18}$.

On day 9 (24 h after application of CCl$_4$), the animals were used for behavioural experiments (pentobarbital-induced sleeping time) after which they were sacrificed for biochemical and histopathological studies.

Measurement of pentobarbital-induced sleeping time—On day 9 of the experiment, pentobarbital sodium 30 mg/kg, ip (Taj Pharmaceuticals Limited, Mumbai, India) was injected to control (Gr I), CCl$_4$-intoxicated (Gr II), vasicinone pre-treated CCl$_4$-intoxicated (Gr III and IV) and silymarin pre-treated CCl$_4$-intoxicated groups (Gr V). The onset of sleep and total sleeping time were recorded for all the animals of each group$^{20}$.

Biochemical estimation—The animals were anaesthetized using ether and blood was collected by cardiac puncture. The Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP), Transaminase (SGPT), Alkaline Phosphatase (ALP) and total sleeping time were recorded for all the animals of each group$^{20}$.

Histopathological studies—After blood collection for biochemical studies (day 10) the animals were sacrificed by cervical dislocation and livers was excised, washed with phosphate buffer and dried with tissue paper. The histopathological studies were carried out as per Sharma et al$^2$. The liver was weighed by using electronic balance and transferred to a 10% formalin fixative solution for 48 hr. The liver tissues were processed for paraffin embedding and sections of 5 µm thickness were cut in a microtome. After staining with haematoxylin and eosin, slides were examined under (100X) light microscope (Nikon Eclipse E100, Nikon Corporations) for histopathological changes$^{21}$.

Statistical analysis—The data were expressed as mean ± SE. The data were evaluated by one-way ANOVA followed by Tukey's multiple comparison
tests. \( P < 0.05 \) was considered statistically significant. All statistical analysis was carried out using GraphPad Prism 4.0 software (GraphPad Software Inc, San Diego CA, USA).

**Results**

*Isolation of phytoconstituents*—Crude alkaloids (4.98 g) were obtained from the 500 g leaves of *J. adhatoda*. As the \( R_f \) value (0.61) of isolated crude alkaloid was same as that of standard vasicinone, it can be concluded that the isolated alkaloid may be vasicinone. After collecting all the eluents of all 85 test tubes from column, test tube number 1-28 and 46-81 gave a sharp single spot in TLC with \( R_f \) value 0.61. Next, this compound was used for further characterization. Melting point of isolated vasicinone was determined and it was found to be 110-112 °C, which was comparable to standard vasicinone.

*Characterization of isolated vasicinone*—The structure of isolated compound was confirmed by interpretation of UV, FT-IR spectra and \(^1\)H-NMR. \( \lambda_{\text{max}} \) of the isolated vasicinone was found to be 236 nm (Fig. 1) and the IR (KBr) spectrum of the compound (Fig. 2) showed the characteristic absorption at 1636 cm\(^{-1}\) (due to C=N bond); 1656, 1684 cm\(^{-1}\) (due to stretching vibration of C=O); 2343 cm\(^{-1}\) (due to C≡N); 3411 cm\(^{-1}\) (due to stretching vibration of O-H group). The proton nuclear magnetic resonance (\(^1\)H NMR) spectra of vasicinone in CDCl\(_3\) exhibited four singlets between \( \delta \) 7.53-8.11 resonances which were attributed to the four aromatic protons. One proton triplet at 5.033 due to methane. Two multiplet at \( \delta \) 2.32 and 2.65 due to methylene and two multiplet at \( \delta \) 3.92 and 4.93 due to methylene. By comparing with values of standard vasicinone it was concluded that the above product was vasicinone.
Acute toxicity studies—The isolated vasicinone did not show any mortality up to a dose of 600 mg/kg po for 7 days in mice.

Pharmacological studies—Crude vasaka leaf extract has been reported to possess hepatoprotective activity at 50 mg/kg in rats\(^1\), in the present study the hepatoprotective potential of isolated vasicinone was explored at lower doses of 10 and 25 mg/kg in CCl\(_4\) induced acute liver damage.

Measurement of pentobarbital-induced sleeping time—Pentobarbital-induced sleeping time is an established indicator for functional integrity of the liver\(^2\). Both vasicinone (\(P<0.01\)) and potent hepatoprotective, silymarin (\(P<0.001\)) were found to significantly decrease the CCl\(_4\) mediated increase in pentobarbital-induced sleeping time (Fig. 3) thus providing evidence for restoration of liver function by vasicinone.

Estimation of SGOT, SGPT and ALP level—Single dose of CCl\(_4\) treatment led to elevated levels of serum SGOT, SGPT and ALP (Table 1, \(P<0.001\)) which were significantly reduced upon both vasicinone (25 mg/kg \(P<0.05\)) and silymarin pre-treatment (25 mg/kg \(P<0.01\)). The above biochemical results show hepatoprotective action of vasicinone.

Histopathological observation—Histology of liver section of control animal (Fig. 4A) exhibited normal hepatic cells each with well defined cytoplasm, prominent nucleus and well brought out central vein, whereas that of CCl\(_4\) treated group (Fig. 4B) showed total loss of hepatic architecture with centrilobular hepatic necrosis, inflammatory changes and crowding of central vein. Liver sections of the mice pre-treated with vasicinone (25 mg/kg) for 7 days followed by CCl\(_4\) treatment (Fig. 4C) showed minimal inflammation, the normal cellular architecture was retained which was comparable with the potent hepatoprotective agent silymarin (7 day pre-treatment at 25 mg/kg/day) + CCl\(_4\) treated group (Fig. 4D). This suggests the reparative quality and maintenance of structural integrity of hepatocytic cell membrane by vasicinone.

Discussion

In the present study vasicinone was isolated from leaves of \(J.\) adhatoda, characterized using UV, FT-IR and \(^1\)H-NMR spectra. The alkaloid vasicine is known to be the main active constituent of \(J.\) adhatoda, however it may be easily oxidized in presence of light and moisture\(^2\) to its more stable form vasicinone which may account for more than 30% of crude vasaka extract\(^2,21\). Some studies even claim vasicinone to be more efficacious than vasicine\(^2\).

Isolated vasicinone was evaluated for hepatoprotective activity in CCl\(_4\) induced liver injury. Vasicinone pre-treatment significantly reversed CCl\(_4\) mediated liver damage in mice. CCl\(_4\) is one of the most commonly used hepatotoxin in animal models of liver diseases. CCl\(_4\) like other halogenated alkanes (i.e., chloroform, dichloromethane, bromotrichloromethane, etc.) undergoes cytochrome P-450 catalyzed reductive dehalogenation and liberates trichloromethyl (CCl\(_3\))\(^5\). Reaction of CCl\(_3\) with poly-unsaturated fatty acids in membrane lipids

---

### Table 1—Effect of Vasicinone on serum SGOT, SGPT and ALP levels in CCl\(_4\) induced hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Corn oil</td>
<td>40±3.21</td>
<td>50±2.88</td>
<td>81±1.6</td>
</tr>
<tr>
<td>II</td>
<td>CCl(_4) (1 ml/kg)</td>
<td>99.3±6.96***</td>
<td>96.6±4.40***</td>
<td>151.3±4.48***</td>
</tr>
<tr>
<td>III</td>
<td>Vasicinone (10 mg/kg) + CCl(_4)</td>
<td>87±1.15</td>
<td>89±2.08</td>
<td>138.4±4.41</td>
</tr>
<tr>
<td>IV</td>
<td>Vasicinone (25 mg/kg) + CCl(_4)</td>
<td>76.6±4.05*</td>
<td>74±5.78*</td>
<td>115.7±2.3*</td>
</tr>
<tr>
<td>V</td>
<td>Silymarin (25 mg/kg) + CCl(_4)</td>
<td>52.3±4.33**</td>
<td>61.6±0.88**</td>
<td>98.5±1.6**</td>
</tr>
</tbody>
</table>

\(P\) values: ***\(<0.001\); **\(<0.01\), *\(<0.05\)
releases lipid free radicals (R) which along with reactive oxygen species (ROS) initiates the process of lipid peroxidation as indicated by increased levels of malondialdehyde. The process is exacerbated by reduction in the levels of antioxidant enzymes like superoxide dismutase, catalase, glutathione and glutathione peroxidase. Free radicals lead to auto-oxidation of the fatty acids present in the cytoplasmic membrane phospholipids and cause functional and morphological changes in the hepatocyte membrane leading to its loss of integrity. This was evidenced in the present study with an increase in the levels of serum hepatic enzymes SGOT, SGPT and ALP in CCl₄ treated animals. Elevation of liver enzymes especially SGPT is a clear indication of liver injury. Vasicinone pre-treatment led to a significant decrease in the levels of CCl₄ mediated elevated liver enzymes.

Increase in pentobarbital induced sleeping time is another indication of reduced liver function in experimental animals. Pentobarbital is metabolized by the hepatic microsomal drug metabolizing enzymes (MDME) to inactive metabolites and any drug or chemical which inhibits MDME or cause hepatic damage is likely to prolong pentobarbital-induced sleeping time. The duration of pentobarbital-induced sleeping time in intact animal is considered to be a reliable index for the activity of hepatic MDME. In the present study we show significant decrease in CCl₄ mediated increase in pentobarbital-induced sleeping time in vasicinone pre-treated animals indicating normalization of liver function. Histopathological studies provided additional evidence for the hepatoprotective actions of vasicinone.

CCl₄ induced liver damage being a highly oxidative process is usually reversed by naturally occurring antioxidants. This may be due to stabilization of plasma membrane and or repair of hepatic tissue damage by these compounds. Various studies have previously shown the hepatoprotective actions of alkaloids due to their antioxidant properties. Crude vasaka extract as well as extract containing 30% vasicinone are known for...
their antioxidant activity. Thus antioxidant character of vasicinone may be responsible for its hepatoprotective function.

To conclude vasicinone was successfully isolated and characterized from the leaves of *J. adhatoda*. Significant decrease in pentobarbital induced sleeping time, SGOT, SGPT, ALP levels and protection of hepatic cells from CCl₄ induced liver damage as evidenced by histopathological observation provide strong evidence that vasicinone may act as hepatoprotective in mice. Since vasaka extract which mainly contain vasicine and vasicinone is known for its pharmacological activities against cough, asthma and tuberculosis its hepatoprotective action may add to the benefits of individuals on vasaka therapy.

**Acknowledgement**

Thanks are due to Dr. Kalyan Kumar Sen, Principal, Gupta College of Technological Sciences, Asansol, Prof. Debesh Chandra Majumdar, Chairman, Trinity Trust and all the faculty members of Gupta College of Technological Sciences, Asansol for constant support and encouragement and to Dr. Arun Chandra Karmakar Sr, Vice President, IPCA Laboratories Ltd for NMR studies.

**References**

28. Hiraganahalli BD, Chinampudur VC, Dethe S, Mundkinajeddu D, Pandre MK, Balachandran J & Agarwal A,

