SYNTHESIS AND ASSESSMENT OF HEPATOPROTECTIVE ACTIVITY OF SOME NEW 2-ARYL TETRAHYDROQUINOLINE AND SPIROOXYINDOLYL TETRAHYDROQUINOLINE DERIVATIVES

MAHESH ANAND GOUDARI*, JAYADEVAPPA H2, MAHADEVAN K. M2, SHASTRY R. A3, HABBU P. V4, SAYYESWARA H. A5

1DVS College of Arts and Science, Department of Chemistry, Kuvempu University, Shimoga-577202, Karnataka, India, 2Sahyadri Arts and Commerce College, Kuvempu University, Shimoga-577202, Karnataka, India, 3Department of Postgraduate studies and Research in Pharmaceutical chemistry, Kuvempu University P. G. Center, Kadur-577550, Karnataka, India, 4Department of Pharmacognosy, Postgraduate studies and Research center, SETS College of Pharmacy, Dharwad-580002, Karnataka, India, 5Department of Zoology, Sahyadri Science College (Autonomous), Kuvempu University, Shimoga-577202, Karnataka, India.

Email: dvs.mahesh@gmail.com

Received: 27 Nov 2014 Revised and Accepted: 25 Dec 2014

ABSTRACT

Objective: To evaluate the hepatoprotective potential of some newly synthesized tetrahydroquinoline derivatives against carbon tetrachloride induced hepatic damage in wistar rats.

Methods: A series of 2-aryl, 4-N vinyl pyrrolidino/caprolacto and spirooxy indolyl tetrahydroquinolines were synthesized by imino Diels-Alder reaction using Antimony (III) sulfate as catalyst. The titled compounds were characterized by IR, 1HNMR spectroscopy and screened for hepatoprotective activity. Hepatotoxicity was induced in male Wistar rats by intraperitoneal injection of CCl4. Wistar rats weighing 150-200g were randomly assigned in to various groups of six animals each. Group I – Normal control received only 1% Tween in distilled water, Group II – Served as negative control received CCl4 in liquid paraffin 1 ml/kg p. o. at every 72 h for 10 days. Group III – X were intoxicated with CCl4 1 ml/kg p. o. before the administration of Silymarin 100 mg/kg p. o. and suspension of synthetic derivatives in polyethylene glycol-400 at the dose of 25 mg/kg p. o. for 10 days. Different hepatic biochemical parameters viz. SGOT, SGPT, SALP, Total and direct bilirubin were evaluated before and after treatment to evaluate the hepatoprotective activity.

Results: It was observed that in CCl4 intoxicated group; total and direct bilirubin, SGOT, SGPT, SALP levels were significantly increased as compared to control group. Administration of synthesized tetrahydroquinoline derivatives at the dose of 25 mg/kg p. o. reduced these pathological damages caused by CCl4 intoxication compared to normal and Silymarin treated groups.

Conclusion: The present study revealed that synthesized tetrahydroquinoline derivatives, showed hepatoprotective potential against CCl4 induced hepatotoxicity in wistar rats, thus offering a novel synthetic formulation as a hepatoprotective drug.

Keywords: Tetrahydroquinolines, Imino diels-Alder reaction, Antimony (III) sulfate, Hepatoprotective, Silymarin, Liver, Carbon tetrachloride.

INTRODUCTION

Liver plays a major role in metabolism, secretion, storage, detoxification and excretion of many endogenous and exogenous compounds. Liver cell injury caused by systemic drugs, foods, preservatives, agrochemicals, microbial agents and excessive alcohol consumption, leads to many disorders ranging from elevation of liver enzymes to liver failure [1]. Administration of CCl4 causes liver and kidney damage through free radical mediated process. Also CCl4 increases the serum level of marker enzymes SGOT, SGPT, SALP and bilirubin marking the induction of hepatotoxicity. Though the modern medical system as advanced phenomenally there are no potential drugs which can completely cure all liver disorders [2]. Silymarin has been proved to possess hepatoprotective potential by prevention of absorption of toxins in to hepatocytes by occupying binding sites as well as inhibiting many transport proteins at the membrane. These actions along with aniperoxidative property make Silymarin suitable for the treatment of toxic liver disease [3].

In an attempt to expand the spectrum of hepatoprotective agents, the present study was carried out to synthesize some new tetrahydroquinoline and spirooxyindolyl tetrahydroquinoline derivatives and evaluate their hepatoprotective potential against CCl4 induced toxicity in wistar rats. Biological importance of tetrahydroquinolines has been demonstrated by recent studies as hundreds of them bearing various simple or complex substituents [4-8]. Further the presence of spirooxy indole core in number of natural products has evolved significant interest in synthesis of spirooxy indole derivatives [9]. In the present work, a series of 2-aryl, 4-N vinyl pyrrolidino/caprolacto and spirooxy indolyl tetrahydroquinolines were synthesized by imino Diels-Alder reaction in the presence of Antimony (III) sulfate as catalyst and were screened for hepatoprotective activity against CCl4 induced hepatotoxicity in wistar rats.

MATERIALS AND METHODS

Synthesis and characterization of compounds (1-7)

All melting points were recorded in open capillaries and were uncorrected. The purity of the compounds was monitored by TLC and they were purified by column chromatography. 1H NMR spectra were recorded on a Bruker-500 Hz spectrometer using TMS as an internal standard. IR spectra were obtained using a FTS-135 spectrometer. The identity of compounds (1-7) was established by means of IR, 1HNMR, mass spectral study and elemental analysis.

Procedure for synthesis of compounds

(1) and (2): Antimony (III) sulfate (0.2m. mol) was added to a mixture of 1.0 m. mol N-benzylidene and 2.3 dihydrofuran in 5 cm3 acetonitrile. The reaction mixture was stirred at 50°C for 4 hrs. After completion of the reaction (as indicated by TLC) the mixture was quenched with saturated NaHCO3, extracted with ethyl acetate, dried over anhydrates Na2SO4 and purified by column chromatography on SiO2 with an ethyl acetate and petroleum ether as eluent (Scheme-1).

(3) and (4): A mixture of aromatic amine (1m. mol) and N-vinyl pyrrolidone and N-vinyl caprolactum and Antimony (III) sulfate (0.28 m. mol) in 10 cm3 acetonitrile was stirred at room temperature. After completion of reaction (as indicated by TLC), the reaction mixture was quenched in water, extracted with ethyl ether, dried, concentrated, and separated by column chromatography (Scheme-2).
(5) and (6): Isatin amine was made to react with 2,3 dihydrofuran/3,4 dihydro 2H-pyran in presence of Antimony (III) sulfate (20 mol%) in acetonitrile, mixed for 2 hours at room temperature to give corresponding Spirooxy indolyl tetrahydroquinolines (Scheme-3).

(7): Isatin Schiff base was made to react with N-vinyl pyrrolidine in presence of Antimony (III) sulfate catalyst (20 mol %) in acetonitrile, mixed for 1 hour at room temperature to get the target Spirooxy indolyl tetrahydroquinolines (Scheme-4).

Physical and analytical data of newly synthesized compounds were reported in table 1.

Table 1: Physical and analytical data of synthesized components

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>MP(°C)</th>
<th>Yield</th>
<th>Molecular formula</th>
<th>Mol. Wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>92-94°C</td>
<td>40</td>
<td>C_{26}H_{21}O</td>
<td>265</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>94-96°C</td>
<td>40</td>
<td>C_{26}H_{21}O</td>
<td>281</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>105-152°C</td>
<td>93</td>
<td>C_{15}H_{15}C_{6}N_{2}O</td>
<td>265</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>138-140°C</td>
<td>93</td>
<td>C_{21}H_{17}FN_{2}O</td>
<td>277</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5.png" alt="Structure" /></td>
<td>150°C</td>
<td>90</td>
<td>C_{16}H_{19}N_{2}O_{3}</td>
<td>321</td>
</tr>
<tr>
<td>6</td>
<td><img src="image6.png" alt="Structure" /></td>
<td>120°C</td>
<td>91</td>
<td>C_{26}H_{21}N_{2}O_{3}</td>
<td>333</td>
</tr>
<tr>
<td>7</td>
<td><img src="image7.png" alt="Structure" /></td>
<td>290-294°C</td>
<td>91</td>
<td>C_{21}H_{20}N_{2}O_{3}</td>
<td>364</td>
</tr>
</tbody>
</table>
Spectral data

(1): Colorless crystalline solid, IR (KBr): ν = 3401 cm⁻¹; 1H NMR (CDCl₃): δ = 7.26-7.46 (6H, m), 6.99 (d, J = 7.4 Hz, 1H), 6.72 (t, J = 7.5 Hz, 1H), 4.55 (d, J = 4.9 Hz, 1H), 4.00-4.08 (m, 2H), 3.75-3.84 (m, 2H), 2.40-2.47 (m, 1H), 2.08 (s, 3H), 1.95 (m, 1H), 1.62-1.69 (m, 1H) ppm.

(2): Colorless crystalline solid, IR (KBr): ν = 3298 cm⁻¹; 1H NMR (CDCl₃): δ = 7.39-7.46 (m, 5H), 6.99 (d, J = 2.8 Hz, 1H), 6.80 (dd, J = 8.1, 2.8 Hz, 1H), 6.61 (d, J = 8.1 Hz, 1H), 4.63 (d, J = 5.3 Hz, 1H), 4.06 (m, 1H), 3.78 (s, 3H), 3.73-3.87 (2H, m), 2.49 (1H, br), 1.98-2.04 (m, 1H), 1.68-1.73 (m, 1H), 1.18-1.24 (m, 1H) ppm.

(3): Colourless crystalline solid, IR (KBr): ν = 3493 cm⁻¹; 1H NMR (400 MHz, CDCl₃): δ = 1.24 (d, 3H, J = 6.2 Hz), 1.75 (dd, 1H, J = 12.3, 5.5, 2.2 Hz), 1.95 (ddd, 1H, J = 11.6, 5.9, 2.4 Hz), 1.99-2.15 (m, 2H), 2.42-2.59 (m, 2H), 3.11-3.30 (m, 2H), 3.47-3.61 (m, 1H), 4.5 (brs, 1H, NH), 5.5 (dd, 1H, J = 11.9, 5.9 Hz), 6.48 (d, 1H, J = 8.5 Hz), 6.78 (s, 1H), 6.96 (dd, 1H, J = 8.6 Hz, 2.0 Hz) ppm.

(4): White crystalline solid; mp=139-140 °C; 1H NMR (400 MHz, DMSO-d₆): δ = 1.13 (d, J = 6.2 Hz, 3H), 1.23-1.29 (m, 1H), 1.40-1.51 (m, 3H), 1.77-1.82 (m, 4H), 2.37-2.43 (m, 1H), 2.72 (t, J = 12.4 Hz, 1H), 2.83-2.88 (m, 1H), 3.14-3.20 (m, 1H), 3.37-3.41 (m, 1H), 5.61 (s, 1H), 5.74 (dd, J = 11.6, 5.0 Hz, 1H), 6.47 (m, 2H), 6.72-6.77 (m, 1H) ppm.

(5 and 6): Crystalline yellow solid; 1H NMR (400 MHz, DMSO-d₆): 10.4 (br, s), 7.2 (t, J=5.4 Hz, 1H), 6.7 (d, J=2.3 Hz, 1H), 6.6 (s, J=3.7 Hz, 1H), 6.5 (d, J=13.1 Hz, 1H), 5.01 (s, 1H), 3.6 (t, J=4.5 Hz, 1H), 2.4 (t, J=6 Hz, 1H), 2.2 (d, J=4.2 Hz, 1H), 1.9 (t, J=9 Hz, 1H), 1.8 (d, J=5.8 Hz, 1H).

(7): Crystalline yellow solid; 1H NMR (400 MHz, DMSO-d₆): 10.2 (br, s, NH), 7.3 (d, J=5.49 Hz, 1H), 7.2 (t, J=7.64 Hz, 1H), 7.0 (t, J=7.44 Hz, 1H), 6.8 (d, J=7.72 Hz, 1H), 6.6 (d, J=2.36 Hz, 1H), 6.5 (d, J=8.64, 1H), 6.2 (s, 1H), 6.1 (s, 1H), 3.6 (s, 3H), 3.3 (t, J=7.16 Hz, 1H), 3.1 (d, J=5.6 Hz, 1H), 2.3 (m, 3H), 1.9 (q, J=4.68, 2H), 1.6 (s, 1H).

Hepatoprotective activity

Chemicals

All the solvents and chemicals used were of analytical grade. Standard kits for SGOT, SGPT and Bilirubin (Teco Diagnostic, USA), Standard drug Silymarin (Micro laboratory, India), were used in the present study.

Animals

Adult mice (25-30g) and wister rats (180-200g) were used in the present study. The animals were procured from disease free animal house, National Institute of Pharmacy, Shivamogga, Karnataka, India. All the animals were kept in quarantine for 10 days under standard husbandry conditions with temperature (25°± 2°C), 12-h light/12-h dark cycle and relative air humidity 40-60% and were given water adlibitum.

Assessment of Hepatoprotective activity

In the experiment wister rats weighing 150-200 g were used. The rats were divided into ten groups of six animals in each group.

**Group I:** Served as normal control and received 1% Tween 80 in distilled water.

**Group II:** Served as negative control and received CCl₄ in liquid paraffin (1:1), 1 ml/kg, i. p. Intraperitoneally on 3rd, 6th and 10th day.

**Group III:** Treated with Standard drug Silymarin at the dose of 100 mg/kg p.o. for ten days.

**Group IV, V, VI, VII, VIII, IX and X** received suspensions of synthetic derivatives (1-7) in polyethylene glycol-400 at the dose of 25 mg/kg p.o. for 10 days [11].

**Group III – X** were intoxicated with CCl₄ 1h before the administration of Silymarin or synthetic compounds for ten days.

On the eleventh day after administration of last dose of synthetic derivatives, the rats were anesthetized by light ether anesthesia and blood was collected from the retro-orbital plexus. It was allowed to coagulate for 30 minutes and serum was separated by cold centrifugation at 3000 rpm. The centrifugate was used to estimate the serum glutamate pyruvate transminase (SGPT), Serum glutamate oxaloacetate (SGOT) [12]and serum alkaline phosphatase (SALP) [13]. Total and direct bilirubin levels [14] were also determined.

Statistical analysis

The data obtained from this study were expressed as mean value ± SEM (n=6) for each parameter. The data was analyzed using one-way ANOVA followed by Dunnet's multiple comparison tests. A probability level of less than 5% (p<0.05) was considered statistically significant [15].

RESULTS

Synthetic derivatives (1-7) did not show any toxicity and behavioral changes in mice up to dose level of 50mg/kg. Hence, the doses selected were 25 mg/kg, p. o.

Hepatoprotective activity

Rats treated with CCl₄ (1.0 ml/kg in liquid paraffin, 1:1, i. p.) suffered from hepatotoxicity. The serum levels of SGOT, SGPT, SALP, bilirubin (Total and direct) levels were significantly elevated. Newly synthesized compounds (2-7) (25 mg/kg p.c. o.) exhibited significant hepatoprotective activity (p<0.01) by decreasing the elevated enzyme levels when compared with compound 1 against CCl₄ induced hepatotoxicity (Table-2 and Figs. 1-2). The higher activity of enzyme levels when compared with compound 1 against CCl₄ induced hepatotoxicity (Table-2 and Figs. 1-2). The higher activity of enzyme levels when compared with compound 1 against CCl₄ induced hepatotoxicity (Table-2 and Figs. 1-2).

### Table 2: Effect of some tetrahydroquinoline derivatives on CCl₄ induced hepatotoxicity

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>SALP (mg/dl)</th>
<th>Total bilirubin (mg/dl)</th>
<th>Direct bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>140±3</td>
<td>65±1.6</td>
<td>142.74</td>
<td>1.01±2</td>
<td>0.20±0.01</td>
</tr>
<tr>
<td>CCl₄</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1m1/kg p.o)</td>
<td>43±5.46</td>
<td>296±27.2</td>
<td>44±26.1</td>
<td>3.66±0.38</td>
<td>0.96±0.24</td>
</tr>
<tr>
<td>Silymarin+CCl₄</td>
<td>159±22</td>
<td>79.34±2200</td>
<td>167.81</td>
<td>0.96±0.05</td>
<td>0.24±0.01</td>
</tr>
<tr>
<td>(10mg/kg p.o)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1)+CCl₄</td>
<td>30±9.46</td>
<td>238±28</td>
<td>402±32.5+542</td>
<td>2.62±0.29</td>
<td>1.28±0.08</td>
</tr>
<tr>
<td>(2)+CCl₄</td>
<td>25±6.34</td>
<td>228±2</td>
<td>35.5±4</td>
<td>0.88±0.98</td>
<td>0.98±0.47</td>
</tr>
<tr>
<td>(25 mg/kg p.o)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3)+CCl₄(25mg/kg p.o)</td>
<td>26±5.3</td>
<td>230±35</td>
<td>36±5.34</td>
<td>2.86±1.29</td>
<td>1.29±0.47</td>
</tr>
</tbody>
</table>
Carbon tetrachloride has been used as a tool to induce hepatotoxicity in experimental rats. The hepatotoxic effects of CCl₄ is due to its hepatic conversion, catalyzed by cytochrome P-450 which gives trichloromethyl peroxide radical (Cl₃COO⁻) by reaction with oxygen. These activated radicals bond covalently with sulfohydroxyl group of several membrane molecules like glutathione, which is considered as the initial step in the chain of events leading to lipid peroxidation and hepatic tissue destruction [16-19]. The degree of hepatotoxicity developed by CCl₄ can be observed by elevated levels of biochemical parameters SGOT, SGPT, SALP and bilirubin which are attributed to the generation of trichloromethyl free radical during metabolism by hepatic microsomes which in turn cause peroxidation of lipids of cellular membrane [20]. Hepatocellular necrosis lead to the very high level of SGOT, SGPT released from liver in the blood. Among the two, SGPT is a better index of liver injury, as liver SGPT activity represents 90% of total enzyme present in the body [21]. SALP activities on the other hand are related to the functioning of the hepatocytes. Increase in SALP level is due to increased synthesis in presence of increased biliary pressure [22]. Reduction in levels of SGOT and SGPT towards the respective normal value in synthetic compounds treated groups of rats was an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by carbon tetra chloride. The serum levels of transaminase bond on 6-indole. Biorg Med Chem Lett 2008;18(12):3504-8.

Fig. 1: Effect of synthetic derivatives on SGOT, SGPT and SALP in CCl₄ induced hepatotoxicity

Fig. 2: Effect of synthetic derivatives on total and direct bilirubin in CCl₄ induced hepatotoxicity

DISCUSSION

CONCLUSION

The results suggest that synthetic compounds (2-7) treated groups exhibited significant hepatoprotective activity when compared with the compound (1) treated groups. The possible hepatoprotective mechanism of synthetic tetrahydroquinolines may be through inhibition of the cytochrome P-450 activity which prevents the process of lipid peroxidation leading to stabilization of hepatocellular membrane. The higher liver protective effect of synthetic derivatives (2-7) may be due to the presence of methoxy, fluoro and Chloro groups as substituents. The present study leads to conclude that synthetic derivatives (2-7) may be employed in the management of hepatic disorders.

ACKNOWLEDGEMENT

Authors are thankful to the President and Principal, National College of Pharmacy, Shivamogga for providing necessary facilities to carry out the experimental work.

Ethical clearance

The research work was approved by Institutional Animal Ethics Committee (NCP/IAEC/CLEAR/25/02/2009-10, dated 09/03/2010)

CONFLICT OF INTERESTS

Declared None

REFERENCES


The text includes a table with data comparing different groups, represented as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>SALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>259.46</td>
<td>379.28</td>
<td>1.58</td>
</tr>
<tr>
<td>2</td>
<td>238.21</td>
<td>2.68</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>± 3.26</td>
<td>± 4.26</td>
<td>± 0.26</td>
</tr>
<tr>
<td>4</td>
<td>± 4.89</td>
<td>± 0.26</td>
<td>± 0.56</td>
</tr>
</tbody>
</table>

a p<0.05 as compared to normal group. b p<0.01 as compared to normal and CCl₄ group.