PREVENTIVE AND CURATIVE ROLE OF *SOLANUM NIGRUM* IN LIVER TOXICITY

LAXMI K. YADAV*, NAMRATA VATSA and R. K. ASTHANA

Department of Chemistry, Kuteer P.G. College, Chakkey, JAUNPUR (U.P.) INDIA
*Department of Chemistry, R.S.K.D. (P.G.) College, JAUNPUR (U.P.) INDIA

ABSTRACT

Hepatoprotective effect of *Solanum nigrum* has been studied on CCl₄ induced lipid peroxidation and reduced glutathione content in rat liver homogenate. *In vitro* experiments show significant reduction in the malanodialdehyde (MDA) reduced by 0.1 mM CCl₄. Glutathione content was almost maintained to normal in drug treated rats. Oral treatment of drug upto 3 mL/100 g body weight for 15 days did not show any rise in serum SGOT and SGPT. On similar doses, significant choleratic effect was observed without any adverse effect.

Key words: SGOT, SGPT and MDA.

INTRODUCTION

Liver is the largest physiological gland in human beings. Other factor such as due to the environmental pollution and causing chemicals drugs and contaminated food effect the liver physiology upto a certain extent, which may lead to other secondary physiological changes. Now a days, there are a number of liver protective modern medicines or ayurvedic drugs available for the treatment. Diet quality and life habits are the only remedy. In this situation use of medicinal herbs could be safe and efficient method of treatment. It could be taken as a diet supplement.

Although aqueous extract and active compounds of *S. nigrum* plant is commercially available and is in clinical practice. Since long back, but no scientific study has been performed till date to support the mechanism of action and side effects.

*Author for correspondence; E-mail: laxmikanty6@gmail.com*
EXPERIMENTAL

Material and methods

Rat of same sex age and weighing 100-120 g were procured from central animal house of registered supplier of Varanasi. The animals were housed in PVC cages and allowed free access to standard lab chow supplied by Hindustan Liver Pvt. Ltd., Ghaziabad, India.

The animals were kept one weak in laboratory at controlled animal diet and water before starting the experiments.

Chemicals

Thiobarbituric acid was obtained from Central Drug House, New Delhi. 1,1,3,3 tetraethoxypropane (TEP), CCl₄ and 5,5-dithiobis-nitrobenzoic acid (DTNB) were purchased from Sigma Chemical Co., St. Louis, USA. Other reagents used were of analytical grade.

Drugs

Dry plant of *S. nigrum* were powdered from registered supplier of Varanansi and authenticated in division of taxonomy, Deptt. of Botany, Kutir P.G. College, Chakkey, Jaunpur, U.P.

The dried powder of plant extracts exhaustively by ethyl alcohol for 30 hr. and distilled. Concentrated liquid evaporated on water bath to get the will semi-solid form.

Experimental design

**In vivo study** - Alcoholic extract of 0.1 mM/L (dil) of *S. nigrum* was given orally as per protocol described below.

**Determination of bile flow rate**

24 albino rats were divided in 4 groups having six animals in each 1st group recieved drug vehicle (distilled water) at this dose of 2 mL/100 g body weight for 4 days and served as control.

Similarly group 2nd, 3rd and 4th received drug at the dose of 0.5, 1.5 and 3.0 mL/100 g body weight, respectively for four days and 2 hr. before dissection on 5th days.

In all experiments, overnight fasted animals, were anaesthetised by intraperitonial injection of pentabarbitone (3 mg/100 g body weight).
The common bile duct was surgically exposed by middle line caprotomy and canulated with polyethyline tubing (No. 48). Body temperature of rats was maintained by a heating lamp. Bile collected for first 10 min. was discarded and then it was collected in preweight tubes for 5 min. at the intervals of 10 min. till a constant value was obtained (tubes were changed at 15, 30, 45, 60, 75 and 90 min). Bile flow rate was expressed as mg × 5 min\(^{-1}\) × 100 g\(^{-1}\) body weight.

**Activity of serum transaminases**

Alcoholic extract solution of *S. nigrum* was orally given to rats daily for 15 days (3 mL/100 g body weight). Blood was collected from the branchial artery and glutamate pyruvate transaminase (SGPT) and glutamate oxaloacetate transaminase (SGOT) were determined\(^3,4\) by Kit (J. Mitro Pvt. Ltd., New Delhi) by the Reitman and Frankel method.

**In vitro study** - Alcoholic extract of *S. nigrum* in semi-solid form was selected for this study because of interference of sugar with the estimation of malonaldehyde (MDA)\(^5\).

**Estimation of MDA**

Blood free liver was removed washed and 5% tissue homogenate (containing 130 mg protein/g of liver) was prepared in phosphate buffer saline (pH 7.4) by teflon homogenizer Ohkawa et al.\(^6\) method was used to estimate total amount of lipid peroxidation product (thiobarbituric acid reacting substances) in the homogenate as described earlier\(^7\).

In brief, control and experimental dishes with 3 mL of homogenate and different conc. of drugs were incubated for 20 min. at 37\(^\circ\)C. Then CCl\(_4\) was added to each dish and incubated for 20 min. 0.1 mL aliquot was withdrawn and transferred to 10% TCA (1.5 mL). Lipid peroxidation product in the protein free supernatant was measured with TBA. Value of MDA were evaluated using 1,1,3,3-tetraethoxy propane as standard. Protein was estimated by method of Lowry et al.\(^8\)

**Estimation of reduced glutathione (GSH)**

Reduced glutathione was estimated by Ellman’s method\(^9\). For kinetic study, 10% rat liver homogenate (130 mg protein/g liver tissue, 3 mL) was used. To each plate CCl\(_4\) (0.1 mM) and different conc. of drug (as per protocol) were added simultaneously. While control dish contained only CCl\(_4\) 250 \(\mu\)L aliquots were taken at 5, 10, 20, 30, 40 and 60 min. and mixed with 0.5 mL of 5% TCA-EDTA (1 mM) mixture. Reduced GSH content was measured with DTNB.
Histological evaluation

Liver slices fixed for 48 hr in 10% formosaline were processed for wax embedding. Transverse section (5 μm) were cut and stained with haematoxylin and eosin.

Statistical evaluation

Student’s test was performed to evaluate the degree of significance.

RESULTS AND DISCUSSION

The effect on the rate of bile flow and transaminase activity

*In vivo* experiments indicate its strong choleratic nature which in dose dependent. At the dose of 3 mL/100 g body weight for 4 days. There was 2 fold increase in bile flow rate in comparison to its basal level (21.26 mg / min / 100 g body weight) where only distilled water was given. The result given in Table 1 indicates no rise in SGOT and SGPT activities even at the dose of 3 mL/100 g body weight given for 15 days.

Protective effect of CCl₄ induced lipid peroxidation

Table 2 shows the higher level of MDA in CCl₄ treated rats (510.3 mM/100 mg of protein) than that of drug treated group. The effect was dose dependent⁷,¹⁰.

Table 1: Effect of alcohalic extract of *S. nigrum* on rate of bile flow and enzyme activity in albino rats

[Values are mean ± SE from 6 animals in each group]

<table>
<thead>
<tr>
<th>Dose of treatment mL/g body weight</th>
<th>Bile flow</th>
<th>Enzyme activity (I.U./mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SGOT</td>
</tr>
<tr>
<td>Control</td>
<td>21.26 ± 0.57</td>
<td>74.20 ± 3.35</td>
</tr>
<tr>
<td>0.5</td>
<td>23.72 ± 0.41*</td>
<td>67.10 ± 4.58</td>
</tr>
<tr>
<td>1.5</td>
<td>31.77 ± 1.5*</td>
<td>54.73 ± 1.73*</td>
</tr>
<tr>
<td>3.0</td>
<td>45.50 ± 0.33**</td>
<td>52.64 ± 1.48*</td>
</tr>
</tbody>
</table>

Alcoholic extract of *S. nigrum* are orally given for 4 days in bile flow experiment and for 15 days in SGPT and SGOT experiments as shown in Fig. 1 and 2.

Bile flow rate was measured as mg × 5 min⁻¹ × 100 g⁻¹ body weight.

* P < 0.01; ** P < 0.001
Table 2: Effect of alcohalic extract of *S. nigrum* on CCl₄ induced lipid peroxidation in rat liver homogenate

[value expressed in m mole / 100 mg of protein are mean ± SE from 6 animal in each group]

<table>
<thead>
<tr>
<th>Group</th>
<th>CCl₄</th>
<th>Drug (mg / 3 mL)</th>
<th>MDA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>82.84 ± 2.74</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>-</td>
<td>510.32 ± 3.98*</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>0.33</td>
<td>254.92 ± 2.85*</td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>0.66</td>
<td>209.98 ± 2.67*</td>
</tr>
<tr>
<td>5</td>
<td>1.5</td>
<td>1.2</td>
<td>118.87 ± 2.67*</td>
</tr>
</tbody>
</table>

*P < 0.001

**Effect of reduced glutathione (GSH)**

Oxidation rate of reduced glutathione was inhibited by the drug in CCl₄ treated homogenate.

In control there was gradual decline in GSH content upto 40 min., while in CCl₄ treated group it came down sharply by 10 min. and in drug treated group. There was very slow decrease in glutathione contents. It was almost maintained upto 60 min.

The alcohalic extract of *solanum nigrum* are in general not toxic instead presence of balanced alkaloid and terpenoid. Because of this limitation of synthetic drugs majority of people are in favour of taking pure, herbal ayurvedic drugs in the management of certain diseases like liver disorders, kidney diseases etc. Herbal medicinal plants of *S. nigrum* is not 100 popular due to lack of scientific studies specially the toxicological findings. The results indicate serum transaminases activity to remain at normal level even after 15 days treatment. No adverse changes in the histological picture also in support of its non toxic effect at this therapeutic dose is rats.

In a separate study alcohalic extract of *S. nigrum* exhibited quicker recovery of necrosis developed by CCl₄ treatment. Thus it appears that alcohalic extract of *S. nigrum* is a safe, non-toxic liver drug and also possesses healing property.

Bile flow experiments show its true choleratic property. It significantly increases the rate of bile flow in the dose dependent manner. Physico-chemical studies of bile flow
shows the increase in its solid content also besides the age portion\textsuperscript{14}. The findings is similar
to that of \textit{A. paniculata} as reported earlier. It is a case of true choleratic drugs and differs
from hexabarbitonic which belong to the pseudocholeratic group. In the latter case bile flow
increases but the percentage of solid content in the bile goes down. This property supports
its clinical use in Jaundice and hepatotoxicity\textsuperscript{15}.

In a separate \textit{in vitro} study \textit{S. nigrum} prevents CCl\textsubscript{4} induced lipid peroxidation in the
dose dependent manner. This prevention could be because of several reasons such as less
production of free radicals, removal of existing free radicals enhanced superoxide,
dismutase activity, maintained conc. of reduced glutathione content and availability of
another electron acceptor to the on going chain reaction\textsuperscript{14,15}.

Our finding shows that \textit{S. nigrum} prevents the decline of glutathione content in both
normal as well as CCl\textsubscript{4} treated albino rats. Interestingly this response was not dose
dependent, whereas prevention of lipid peroxidation was strictly dose dependent. This shows
that \textit{S. nigrum} may have different action on the lipid peroxidation chain reaction in addition
to the maintenance of GSH content\textsuperscript{16}. On the basis of above results it could be concluded
that aqueous extract, alcoholic extract or its active compounds such as uttronin A, B,
Solasonine, \textalpha{} and \textbeta{} Solamorgine and solasodine are a non-toxic safe hepatostimulant\textsuperscript{16,17}. In
normal condition, it is a strong choleratic and tones up the liver activity. This may improve
the therapeutic value of allopathy medicines.

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