ETHANOL EXTRACT OF MOMORDICA TUBEROSA TUBERS PROTECTS LIVER IN PARACETAMOL-INDUCED DAMAGE

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Abstract - The study assessed the in vivo antioxidant and hepatoprotective activity of an ethanol (70%) extract of Momordica tuberosa Cogn. (Cucurbitaceae) (TMT) tubers in experimentally induced liver damage by paracetamol (2 g/kg, po.) in albino rats. The degree of protection was ascertained by estimating the levels of biochemical markers like SGPT, SGOT, bilirubin (total and direct), ALP, and triglycerides. Tissue GSH and lipid peroxidation were also determined. The ethanol (70%) extract of tubers in an oral administration of 20 and 40 mg/kg doses produced significant protection by decreasing the activity of serum enzymes, bilirubin, cholesterol, triglycerides and tissue lipid peroxidation, while it increased tissue GSH at 40 mg/kg dose. The effects of the extract were comparable to the standard drugs silymarin (100 mg/kg). Results suggested that an ethanol (70%) extract of the tubers of the plant at 40 mg/kg possesses potential hepatoprotective activity against paracetamol-induced hepatic damage and significant antioxidant activity in rats.

Key words: Biochemical markers, GSH, lipid peroxidation, liver, Saponins, SGPT, SGOT.

INTRODUCTION

Liver diseases are a worldwide problem. The management of liver disease has become a critical concern in medical science. Drugs available in the present system of medicine are associated with toxic effects (Premila, 1995). Hence, herbs and traditional medicinal plants are regaining their lost glory. The interest in herbal drugs also stems from the fact that modern medicine does not have a suitable answer for many conditions such as liver disorders, heart diseases, and chronic conditions such as arthritis, asthma and many skin conditions (Premila, 1995). Herbs play a major role in the treatment of liver diseases (Swati and Saluja, 2002) and recent progress in the study of such plants has resulted in the isolation of about 170 different phytoconstituents from plants belonging to about 55 families which exhibit hepatoprotective activity (Sharma et al., 1991).
plant. A literature survey suggests limited phytochemical as well as pharmacological profiles of the plant. Since the fruits are reported to contain vitamin C, it was hypothesized that the tubers of the plant may also contain antioxidants and hence were selected for evaluation of their hepatoprotective nature. We have earlier reported on the hepatoprotective and antioxidant nature of the tubers of this plant in a carbon tetrachloride (CCl4) model in rats (Kumar et al., 2008). Paracetamol (N-acetyl-p-aminophenol) is a widely used analgesic and antipyretic drug and is safe when used in therapeutic doses. However, an overdose of paracetamol is hepatotoxic and nephrotoxic in human beings and animals (Parmar et al., 1995). Paracetamol is a direct hepatotoxin, intoxication being dose-dependent and reproducible (Tygstrup et al., 1996). The exposure of animals to higher doses produces centrilobular or massive hepatic necroses which eventually culminate in sinusoidal erythrocytic congestion, cellular damage and death. The hepatic necrosis is associated with damage to subcellular organelle including mitochondria. CCl4 is an industrial solvent, causing occupational toxicity and hepatotoxic above the threshold limit. Since, hepatotoxicity caused by CCl4 and paracetamol follow different mechanisms, CCl4 by releasing the trichloromethyl radical (CCl3•) (Beuge and Steven Aust, 1978) and paracetamol through the N-acetyl-p-benzoquinoneimine (NAPQI) metabolite (Sing and Reen, 1999), it is essential to confirm the protective effect of the plant on toxicity induced by both agents. Furthermore, the claim of the hepatoprotective effect of a preparation needs to be confirmed on a minimum of two to three experimentally induced hepatic injury models. Hence, paracetamol was used as a hepatotoxin in the present study to induce hepatic injury.

MATERIALS AND METHODS

Plant material

The tubers were collected from the suburban fields of Raichur, India in the month of January, 2005, and were identified and authenticated by Prof. Srivatsa, Retired Professor, Dept of Botany, L.V.D. College, Raichur, India. A herbarium specimen (VLCP-02/05) is deposited in the Dept. of Pharmacognosy, V.L. College of Pharmacy, Raichur, India.

Preparation of extracts

A coarse powder of shade-dried tubers of M. tuberosa was extracted successively with petroleum ether (60-80), chloroform, ethanol and water (Kokate, 1996). Similarly, an ethanol (70%) extract of the tubers of M. tuberosa (TMT) was prepared after defatting the drug with petroleum ether. Ethanol (70%) is a well-documented solvent for the majority of polar constituents of a plant. The extracts were dried under reduced pressure using a Rota-flash evaporator and heating. All extracts were screened for phytoconstituents using simple chemical tests (Kokate, 1996; Khandelawal, 2005).

Preliminary phytochemical investigations indicated the presence of sterols in the petroleum ether extract, saponins, cardiac glycosides, triterpenoids and bitters in the ethanol extract and carbohydrates and constituents of the ethanol extracts in an aqueous extract. The phytoconstituents present in the ethanol (70%) extract were the same as the ethanol and aqueous extracts.

Animals

Albino rats (150-200 g) and mice (18-25 g) of both sexes were obtained from Sri Venkateshwar Enterprise, Bangalore. The animals were kept in polypropylene cages in groups of six to eight, with a 12 h of light and dark cycle in the institution’s animal house. The animals were fed with standard rodent diet and provided with water ad libitum. After one week of acclimatization, the animals were used for experiments. Approval from the Institutional animal ethical committee for use of animals was obtained as per the Indian CPCSEA guidelines prior to the experiment. The research followed international ethical standards for the care and use of laboratory animals.

Toxicity studies

Acute toxicity of the TMT was determined using albino mice according to OECD guideline 420 (fi-
The LD$_{50}$ of the TMT was found to be 200 mg/kg. Therefore 1/10$^{th}$ (20 mg/kg) and 1/5$^{th}$ (40 mg/kg) doses were selected for further studies.

**Paracetamol-induced hepatotoxicity**

The paracetamol-induced hepatotoxicity and protective effect of the extract was studied according to Chattopadhyaya (2003). Healthy albino rats were divided into five groups of six animals each. Animals group I and II received saline (1 ml/kg) for 7 days. Group III received 100 mg/kg silymarin (standard drug) orally for seven days. Animals in group IV and V received 20 and 40 mg/kg, respectively, of TMT orally. But on the fifth day, 30 min after the administration of the saline, silymarin and test extract to the respective groups, the animals in group II to V were orally administered paracetamol 2 g/kg. Blood samples (1 ml) were collected from the retro-orbital plexus for evaluating the serum biochemical parameters. After 48 h of paracetamol administration, the rats were killed under mild ether anesthesia. The liver was extracted, the blood blotted off, washed with saline, stored in 10% formalin and taken for histopathological studies to evaluate the details of the hepatic architecture in each group microscopically.

**Biochemical studies**

The collected blood sample (1 ml) was centrifuged (2000 rpm for 10 min) to obtain serum and subjected to various biochemical studies like SGPT (Teitz, 1976), SGOT (Teitz, 1976), ALP (Teitz, 1983), bilirubin (total and direct) (Michelson, 1986), serum cholesterol (Young, 1973) and serum triglyceride (Buccolo, 1973) determinations.

**In vivo lipid peroxidation**

The degree of lipid peroxidation was measured according to Buege (1978) by monitoring thiobarbituric reactive substance formation. One ml of a biological sample (0.1-2.0 mg of membrane protein or 0.1-2.0 μmol of lipid phosphate) was combined with 20 ml of TCA-TBA-HCL reagent and mixed thoroughly. The solution was heated for 15 min and cooled. The precipitate formed was removed by centrifugation at 1000 rpm for 10 min and absorbance of sample was read at 535 nm (Systronics UV-Visible spectrophotometer 118) against a reagent blank.

**Determination of in vivo tissue GSH**

GSH estimation was performed using a modified Ellman procedure (Aykac et al., 1985). The liver tissue was homogenized in ice-cold trichloroacetic acid (1 g tissue in 10 ml 10% TCA) in an Ultratrax tissue homogenizer (Omi TH tissue homogenizer, OC/TH 220). The mixture was centrifuged at 3000 rpm for 10 min. The supernatant (0.5 ml) was added to 2 ml of (0.3 M) disodium hydrogen phosphate solution. Later, 0.2 ml of dithiobisnitrobenzoate (0.4 mg/ml in 1% sodium acetate) was added and absorbance was read at 412 nm.

**Statistical analysis**

Results were expressed as the means ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by the Tukey-Kramer multiple comparisons test. A p value less than 0.05 was considered as statistically significant. The percentage changes in lipid peroxidation and tissue GSH were calculated using the formula,

\[
\text{% change} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100
\]

**RESULTS**

**Effect of TMT on serum enzymes, bilirubin, cholesterol and triglycerides**

Levels of SGPT, SGOT, total and direct bilirubin and ALP were increased in the paracetamol-treated group. Treatment with a 40 mg/kg dose of TMT decreased the levels of these biomarker enzymes to 75-80% of the initial value. There was no significant rise in total cholesterol and triglyceride levels in the paracetamol-treated group. These levels were...
brought to near normal levels by the extract at a 40 mg/kg dose. The changes in biochemical markers are shown in Table 1. Results obtained with 40 mg/kg of TMT were significant in comparison to a 100 mg/kg dose of the standard drug silymarin, but the reduction in marker levels was only 50-60% compared to the negative control group.

**In vivo lipid peroxidation**

Paracetamol administration caused lipid peroxidation which was prevented with the TMT treatment. The standard drug silymarin at 100 mg/kg showed 60.95% inhibition, whereas the 20 and 40 mg/kg doses of TMT showed 49.06% and 55.74% inhibition, respectively. However, in comparison to the negative control group the inhibition was nearly 80% at the 40 mg/kg dose. The results are shown in Table 2.

**In vivo GSH**

There was a marked depletion in the GSH level in the paracetamol treated groups. Silymarin at 100 mg/kg restored tissue GSH by 76.98%. Treatment with the ethanol (70%) extract caused a dose-dependent restoration in GSH levels. However, at both doses the increase in GSH level was less compared to the standard drug (Table 3).

**DISCUSSION**

Biochemical markers such as SGPT, SGOT, ALP, bilirubin, total cholesterol and triglycerides are used to assess the functioning of the liver. In the present study there was a marked increase in the levels of these markers with paracetamol treatment and the administration of the test extract (TMT) restored...
ETHANOL EXTRACT OF MOMORDICA TUBEROsa TUBERS

Table 2. Effect of 70% ethanol extract of tubers of M. tuberosa on in vivo lipid peroxidation in paracetamol-induced hepatotoxicity.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Absorbance</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>0.257 ± 0.004</td>
<td>----</td>
</tr>
<tr>
<td>(1 ml vehicle)</td>
<td></td>
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<tr>
<td>Positive control</td>
<td>0.689 ± 0.008</td>
<td>----</td>
</tr>
<tr>
<td>paracetamol (2 g/kg po.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracetamol + Standard (Silymarin) (2 g/kg po. +100 mg/kg, po)</td>
<td>0.269 ± 0.003***</td>
<td>60.95</td>
</tr>
<tr>
<td>Paracetamol + ethanol (70%) extract (2 g/kg po. +20 mg/kg po.)</td>
<td>0.351±0.0006***</td>
<td>49.06</td>
</tr>
<tr>
<td>Paracetamol + ethanol (70%) extract (2 g/kg po. + 40 mg/kg po)</td>
<td>0.305±0.006***</td>
<td>55.74</td>
</tr>
</tbody>
</table>

(Values are mean ± SEM of six rats/treatment. Significance***p<0.001, compared to paracetamol treatment.

these to near normal levels. The effect, however, was less compared to standard silymarin (100 mg/kg).

The elevation of the SGOT level is significantly higher compared to SGPT in the paracetamol model. This is attributed to the presence of SGOT in the nephrons and paracetamol-induced nephrotoxicity. Hence, the leakage of SGOT from nephron to serum results in its increased level. The SGPT level, specific only to the liver, represents a better parameter for assessing damage (Malaya et al., 2004). The extract reduced the elevated levels of direct and total bilirubin at a 40 mg/kg dose. There was no significant increase in the total cholesterol and triglyceride levels with the paracetamol treatment. However, the extract showed an effect again at the higher dose. Paracetamol may increase the synthesis of fatty acids and decrease the release of hepatic lipoproteins.

Paracetamol directly inhibits cellular proliferation and induces oxidative stress, resulting in increased lipid peroxidation, depleted ATP levels and altered Ca++ homeostasis - conditions considered to be fatal to the cell (Remirez et al., 1995; Lee et al.,1999). The liver is the main organ involved in the metabolism of biological toxins and medicinal agents. Such metabolism is always associated with a disturbance of hepatocyte biochemistry and the generation of reactive oxygen species (Fernandez, 2005). Many types of liver damage, ranging from subclinical icteric hepatitis to necroinflammatory hepatitis, cirrhosis and carcinoma, have been shown to be associated with redox imbalance and oxidative stress (Vrba et al., 2002). Therefore, a potential novel approach, namely developing antioxidant drugs to treat and protect liver injury and liver disease, has been proposed (Bansal et al., 2005). This strategy is aimed at devising and incorporating antioxidants into the therapeutics for the control of viral infections or protecting from alcohol or other toxin damage. It is suggested that antioxidants are capable of reducing hepatic inflammation and fibrosis, thus slowing or even preventing progression to cirrhosis.

The ethanol extract of M. tuberosa contained triterpenoids, saponins, cardiac glycosides, bitters and carbohydrates. Some saponins have been found to have antioxidative or reductive activity. A group of saponins produced in legumes, such as the group B soy saponins, contain an antioxidant moiety attached at C23 (Yoshiki et al.,
This unique sugar residue, 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), allows saponins to scavenge superoxides by forming hydroperoxide intermediates, thus preventing biomolecular damage by free radicals (Yoshiki et al., 1995).

Paracetamol was selected as a model toxin since it is known to be bioactivated by specific cytochrome P-450s to N-acetyl-p-benzoquinoneimine (NAPQI), a reactive metabolite which at high doses causes protein and non-protein thiol depletion, lipid peroxidation and cytotoxicity. A one-electron reduction metabolite of NAPQI mediates the cytotoxic effects of NAPQI. These semiquinone radicals, in turn, can bind directly to cellular macromolecules to produce toxicity. Alternatively, the radicals can be reoxidized back to their original quinones by donating one electron to molecular oxygen under aerobic conditions. This donation of one electron then generates a reduced oxygen radical species and hydroxyl radical. It is evident from the present results that the antioxidant property of TMT prevented the formation of an oxygen radical species and hydroxyl radical, thereby reducing tissue damage. This is confirmed further with TMT showing significant restoration of GSH and reduced lipid peroxidation. Therefore, the liver protective activity of TMT may be due to its antioxidant potential.

Our earlier work (Kumar et al., 2008) on the tubers of this plant established the antioxidant nature of an ethanol (70%) extract. Hence the liver protective activity of TMT may be attributed to its antioxidant principles. Tubers of *M. tuberosa* are reported to be abortifacient (Kirtikar and Basu, 1991) and its use as a liver protective is contraindicated in pregnancy. In conclusion, the present study demonstrates the liver protective activity of the tubers of *M. tuberosa*, which could be due to its antioxidant activity. Further investigation is under way to isolate, characterize and screen the active principles possessing antioxidant and liver protective property.

**Acknowledgments -** The authors wish to thank the managements and principals of the V.L.College of Pharmacy, Raichur and S.C.S. College of Pharmacy, Harapanahalli for their whole-hearted support and research facilities.

**REFERENCES**


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**Table 3.** Effect of 70% ethanol extract of tubers of *M. tuberosa* on *in vivo* GSH levels in paracetamol-induced hepatotoxicity.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Absorbance</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (1 ml vehicle)</td>
<td>0.932 ± 0.009</td>
<td>----</td>
</tr>
<tr>
<td>Positive control paracetamol(2 g/kg po.)</td>
<td>0.352 ± 0.014</td>
<td>----</td>
</tr>
<tr>
<td>Paracetamol + Standard (Silymarin)</td>
<td>0.623 ± 0.011***</td>
<td>76.98</td>
</tr>
<tr>
<td>(2 g/kg.po.+100 mg/kg po.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracetamol + ethanol (70%) extract</td>
<td>0.468±0.001***</td>
<td>32.95</td>
</tr>
<tr>
<td>(2 g/kg.po + 20 mg/kg po)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracetamol + ethanol (70%) extract</td>
<td>0.612±0.006***</td>
<td>73.86</td>
</tr>
<tr>
<td>(2 g/kg. po + 40 mg/kg po.)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Values are the mean ± SEM of six rats/treatment. Significance ***p<0.001, compared to paracetamol treatment).


