Antioxidant and Hepatoprotective Activity of 

*Tridax Procumbens* Linn, against Paracetamol

induced Hepatotoxicity in Male Albino Rats

Shardul S. Wagh

Department of Biochemistry, R. T. M. Nagpur University, L.I.T. Premises
Amravati Road Nagpur 440010, India
shardulwagh@gmail.com

Gangadhar B. Shinde

Department of Biochemistry, R.T.M. Nagpur University, L.I.T. premises
Amravati Road Nagpur 440010, India

Abstract

Hepatoprotective activity of *Tridax procumbens* L. a medicinal herb commonly used in folklore system for wound healing and also against jaundice, was evaluated against paracetamol (acetaminophen) induced hepatic damage in male albino rats. Paracetamol (2gm/kg body weight) induced hepatic damage was well manifested by significant increase in the activities of Alanine aminotransferase, Aspartate aminotransferase, Alkaline phosphatase in serum and enhanced lipid peroxidation. On the other hand, the activities of Superoxide dismutase and Catalase in liver tissue were lowered. Consequent to paracetamol induced hepatic injury, the Serum Bilirubin level was increased. Paracetamol toxicity, also resulted in, significant reduction in total serum protein and the hepatic glutathione and glycogen contents. The oral administration of varying doses of ethanolic extract of *Tridax procumbens* L. (100, 200, 300 and 400mg/kg body weight) for the period of 7 days reversed these altered parameters to normal levels indicating the antioxidative and hepatoprotective efficacy of *Tridax procumbens* L. against paracetamol induced liver injury.
Keywords: - *Tridax procumbens*, hepatoprotection, paracetamol, antioxidant, ethanolic extract.

1. Introduction

Hyper-physiological burden of free radicals causes imbalance in homeostatic phenomena between oxidants and antioxidants in the body. This imbalance leads to oxidative stress that is being suggested as the root cause of aging and various diseases like diabetes, cancer, and liver damage (in severe conditions like cirrhosis and hepatitis) (James et al., 2003).

Liver is the main organ which regulates many important metabolic functions. Hepatic injury is directly associated with these altered metabolic functions (Mitra et al., 1998). In past, several studies have been carried out to examine the effect of plants used traditionally by herbalists to support normal liver function and treat diseases of liver. So far various experimental evidences have confirmed the efficacy of plants such as *Silybum marrium* (milk thistle), *Curtuma longa* (turmeric) (Luper, 1999), *Nymphaea stellata* (Bhandarkar et al., 2004). In spite of significant advances in medicinal plant research and rapid strides in modern medicine, there continues to be a need for more precise, safe and effective treatment of liver disorders (Oliveria et al., 2005).

Paracetamol is a widely used over-the-counter drug for analgesic and antipyretic effects. Its use in overdose (suicidal or accidental) or with chronic alcohol abuse causes fulminant liver failure (Prescott, 2000; Gyamlani and Parikh, 2002). Paracetamol induced hepatic failure is the second leading cause of liver transplantation (Lee et al., 2004).

*Tridax procumbens* Linn. (asteraceae), a herb found throughout India is employed as indigenous medicine for a variety of ailments including jaundice (Saraf et al., 1991). It is commonly used in Indian traditional medicine as anticoagulant, antifungal and in dysentery (Ali et al., 2001). However very few reports are available regarding the hepatoprotective activity of *Tridax procumbens* Linn. (Saraf et al., 1991, Ravikumar et al., 2005). Keeping this fact in view the present study was undertaken to investigate the antioxidant and hepatoprotective activity of *Tridax procumbens* Linn., against paracetamol induced hepatic damage in albino rats.

2. Materials and methods

2.1 Plant material

*Tridax procumbens* Linn (Asteraceae) is pubescent herb with prostrate ascending stems and erect peduncles with terminal heads. Leaves are opposite, broadly lanceolate, coarsely toothed, erect head about 1 cm long. Flowers are white ligules about 4-5 mm long, disc-florets yellow. It is commonly called as
Tikki in local language or Coat buttons. The plant samples were collected at flowering stage from local region during September – November. The plant was authenticated at the Post Graduate Department of Botany, R.T.M. Nagpur University, Nagpur, India and voucher specimen (No.9114 dated 15/05/07) was preserved.

2.1.1 Preparation of plant extract
An extract of shade dried *Tridax procumbens* plant was prepared in 95% ethanol using soxhlet apparatus. Dried extract was re-suspended in known volume of Dimethyl sulphoxide (DMSO) and administered orally at various dose levels to treated groups only.

2.2 Animals
Male albino rats of Wistar strain weighing about 150 – 200g were housed individually in polypropylene cages and fed on standard pellet diet (Hindustan liver). Water was given *ad libitum*. The animals were maintained under standard laboratory conditions (temperature 24-28ºC, relative humidity 60-70% and 1:1 dark and light cycle). Ethical clearance for the handling of experimental animals was obtained from Institutional Animal Ethics Committee (Acc. No. 414/01/ab/CPCSEA/dt. 4th June 2001).

2.3 Experimental Design
Animals were divided into six groups each of six animals viz: Group – I, Normal control; Group – II, Experimental control and four treated (paracetamol + plant extract suspension) groups. Group – I (Normal control) received a single oral dose of DMSO only; Group – II (Experimental control) received a single toxic dose of paracetamol in DMSO (2g/kg body weight, orally) and four treated groups viz. Group – III, IV, V and VI each received a single toxic dose of paracetamol as per Group – II along with *Tridax procumbens* plant extract suspension in DMSO at a dose of 100, 200, 300 and 400mg/kg body weight *p. o.* (post oesophagus) respectively. Treatment with plant extract was started after 24 hrs of paracetamol administration. Total duration of treatment was 7 days.

Rats were sacrificed by cervical dislocation. Blood was collected and serum was separated. Liver was dissected out, washed with ice cold Phosphate Buffer Saline (PBS) (0.1 M, pH 7.4) and 10% tissue homogenate used for different biochemical analyses.

2.4 Biochemical analyses
Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) (Reitman and Frankel, 1957), Alkaline phosphatase (ALP) (Kind and King, 1954) in serum; Lipid peroxidation (LP) in terms of thiobarbituric acid reacting substances (TBARS) ( Stocks and Dormandy, 1971), Superoxide dismutase (SOD) (Roos et al., 1959), Catalase (CAT) (Aebi, 1983), in liver homogenate were assayed. Total serum protein (TSP) (Lowry et al., 1951) and bilirubin (Jendrassik and Grof, 1938) in serum and Glutathione (GSH) (Beutlar et al., 1963), Glycogen (GLY) (Hassid and Abrahm, 1957), in liver homogenate were estimated.
2.5 Observations

Table 1 Effect of *Tridax procumbens* extract on the activities of serum AST, ALT, ALP, Bilirubin and TSP levels.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AST (Units/ml)</th>
<th>ALT (Units/ml)</th>
<th>ALP (KA Units)</th>
<th>Bilirubin (mg%) (total)</th>
<th>TSP (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>72.58 ± 1.5</td>
<td>30.31 ± 0.69</td>
<td>388.66 ± 19.83</td>
<td>0.24 ± 0.01</td>
<td>192 ± 2.08</td>
</tr>
<tr>
<td>Group II</td>
<td>90.7 ± 2.8***</td>
<td>127.37 ± 3.21**</td>
<td>1253.07 ± 46.16***</td>
<td>4.58 ± 0.35***</td>
<td>67.2 ± 2.29***</td>
</tr>
<tr>
<td>Group III</td>
<td>84.44 ± 1.6***</td>
<td>54.96 ± 2.43**ns</td>
<td>495.43 ± 23.85***</td>
<td>1.36 ± 0.01***</td>
<td>140.8 ± 3.22***</td>
</tr>
<tr>
<td>Group IV</td>
<td>83.81 ± 1.75*</td>
<td>36.76 ± 1.34*</td>
<td>469.45 ± 19.24***</td>
<td>0.62 ± 0.17**ns</td>
<td>156.8 ± 3.67**</td>
</tr>
<tr>
<td>Group V</td>
<td>77.43 ± 4.33**</td>
<td>36.01 ± 2.6**ns</td>
<td>398.81 ± 17.21***</td>
<td>0.41 ± 0.01***</td>
<td>169.6 ± 4.86***</td>
</tr>
<tr>
<td>Group VI</td>
<td>74.61 ± 1.41**ns</td>
<td>35.33 ± 1.01*</td>
<td>395.62 ± 15.88***</td>
<td>0.30 ± 0.004***</td>
<td>195.2 ± 3.50***</td>
</tr>
</tbody>
</table>

Table 2

Effect of *Tridax procumbens* extract on LP, GSH, SOD, CAT and Glycogen content of liver

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LP (η Moles of MDA formed/mg protein)</th>
<th>SOD (units of activity/mg protein)</th>
<th>CAT (µMoles of H₂O₂ decomposed/mg protein)</th>
<th>GSH (µg/mg protein)</th>
<th>Glycogen (mg/gm of wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>27.31 ± 0.60</td>
<td>16.52 ± 1.08</td>
<td>114.83 ± 2.10</td>
<td>4.38 ± 0.22</td>
<td>7.315 ± 0.98</td>
</tr>
<tr>
<td>Group II</td>
<td>731.25 ± 6.72***</td>
<td>10.96 ± 0.88***</td>
<td>50.43 ± 2.29***</td>
<td>2.17 ± 0.03***</td>
<td>4.89 ± 0.50***</td>
</tr>
<tr>
<td>Group III</td>
<td>236.58 ± 3.62**ns</td>
<td>11.83 ± 1.01*</td>
<td>61.19 ± 2.01***</td>
<td>2.31 ± 0.13**</td>
<td>4.90 ± 0.50**ns</td>
</tr>
<tr>
<td>Group IV</td>
<td>199.91 ± 2.93*</td>
<td>13.04 ± 1.12**</td>
<td>88.28 ± 2.06***</td>
<td>2.66 ± 0.09*</td>
<td>6.03 ± 1.02*</td>
</tr>
<tr>
<td>Group V</td>
<td>140.59 ± 1.68*</td>
<td>16.44 ± 1.00***</td>
<td>115.46 ± 5.74***</td>
<td>4.76 ± 0.10*</td>
<td>7.41 ± 1.26***</td>
</tr>
<tr>
<td>Group VI</td>
<td>91.29 ± 2.96*</td>
<td>17.61 ± 0.98***</td>
<td>119.34 ± 2.35***</td>
<td>5.15 ± 0.13**</td>
<td>8.03 ± 1.11*</td>
</tr>
</tbody>
</table>

All Values are mean ± S.D. (n = 6), Group II is compared with group I, Group III, IV, V and VI compared with Group II, * p<0.05; **p<0.01; ***p<0.001and ns = not significant.
Antioxidant and hepatoprotective activity

3. Results and discussion

Overdose of paracetamol causes a potentially fatal, hepatic centrilobular necrosis. The hepatotoxicity of paracetamol has been attributed to the formation of a toxic metabolite, \textit{N-acetyl-p-benzoquinoneimine} (NAPQI) by the action of cytochrome P4502E1 (Lee et al., 1996).

In the present investigation, paracetamol administration resulted in elevated activities of AST, ALT and ALP in serum (Group II) against their respective control values (Group I). Similarly, serum bilirubin level was also found to be increased significantly as a result of paracetamol toxicity (Group II). On the other hand, total serum protein level was lowered in response to paracetamol administration when compared with control (Table 1).

Abnormally higher activities of serum ALT, AST and ALP after paracetamol administration as observed in the present study is an indication of the development of hepatic injury, which is responsible for leakage of cellular enzymes into the blood. When liver plasma membrane gets damaged, a variety of enzymes normally located in the cytosol are released into the circulation (Mitra et al., 1998). Oral administration of various doses of \textit{Tridax procumbens} (TP) ethanolic extract to paracetamol intoxicated rats resulted in gradual normalization of the activities of AST, ALT and ALP. This evidently suggests the protective effect of the extract in improving the functional integrity of liver cells.

Serum bilirubin is considered as an index for the assessment of hepatic function and any abnormal increase indicates hepatobiliary disease and severe disturbance of hepatocellular architecture (Martin and Friedman, 1992). Paracetamol administration resulted in increased serum bilirubin level (Group II), (Table 1) thereby suggesting severe hepatic injury and confirming the hepatotoxic
nature of paracetamol. Treatment with TP ethanolic extract significantly decreased the elevated level of total bilirubin in serum towards normalcy indicating its hepatoprotective efficacy.

Hepatotoxin impairs the capacity of liver to synthesize albumin (Dubey et al., 1994). Decreased total serum protein level (Table 1) in paracetamol treated rats may be attributed to impaired protein synthesis by damaged liver tissue. Subsequent treatment of paracetamol intoxicated rats with TP ethanolic extract increased the total serum protein (TSP) level (Table 1). This further signifies the curative nature of extract against paracetamol toxicity.

Hepatic lipid peroxidation (LP), expressed as TBARS (thiobarbituric acid reacting substances), increased significantly in paracetamol toxicity. While, the activities of protective enzymes such as Superoxide dismutase (SOD) and catalase (CAT) and glutathione and glycogen content in liver tissue were lowered after paracetamol administration (Table 2). Enhanced LP and reduced activities of SOD and CAT is an indication of generation of free radical stress as a mark of hepatic damage due to paracetamol toxicity. Marked reductions in the activities of these free radical scavenging enzymes, SOD and CAT, associated with paracetamol toxicity were significantly reversed to normal on oral feeding of TP ethanolic extract in a dose dependent manner conferring the antilipid peroxidative ability to the extract.

Paracetamol gets metabolically activated to a reactive metabolite NAPQI by cytochrome P4502E1 (Lee et al., 1996). NAPQI, in turn, is detoxified by conjugating with glutathione (GSH). Thus, GSH constitute the first line of defence against paracetamol induced generation of free radicals (James, et al., 2003). In paracetamol toxicity, total hepatic GSH was found to be depleted (Table 2) due to the damage caused to hepatic cells. As a result, formation of NAPQI-glutathione conjugate is diminished. Administration of TP ethanolic extract effectively replenished the paracetamol induced depletion of hepatic GSH presumably due to diminished production of toxic metabolite, NAPQI through the inhibition of cytoP450 enzyme system.

Paracetamol induced damage of hepatocytes is also a reason behind decreased glycogen content of liver tissue (Table 2). Significant increase in hepatic glycogen level was observed after administration of the extract indicating improvement in hepatic status.

Histopathological examination of liver sections of the normal control group (Group I) showed normal cellular architecture with distinct hepatic cells (plate 1). However, distinct hepatic necrosis was noted after paracetamol administration (Group II) with destruction of hepatic cells (plate 2.). *Tridax procumbens* treatment (Group III, IV, V & VI) to such paracetamol intoxicated rats showed recovery of the hepatocytes from necrosis. This also suggests that the plant extract has a tremendous potential to reverse the changes induced by paracetamol toxicity back to normal.

The curative efficacy of TP ethanolic extract was dose dependent as evidenced by gradual reversal of the altered values of various biochemical markers back to normal following oral administration. This may, probably be through promotional activation of antioxidative enzymes and regeneration of
hepatocytes that restore the structural and functional integrity of liver. Thus, the present investigation confirms the hepatoprotective action of TP against paracetamol induced hepatotoxicity in rats.

References


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