HEPATOPROTECTIVE EFFECT OF *WEDELIA CALENDULACEAE* AGAINST THIOACETAMIDE INDUCED LIVER DAMAGE IN RATS

Pallab Kanti Haldar*, Malaya Gupta, Upal Kanti Mazumder, Chandi Charan Kandar, Laxmanan Manikandan

Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, India

**Summary**

The methanol extract of *Wedelia calendulacea* was tested for its hepatoprotective activity against thioacetamide-induced hepatotoxicity in Wister albino rats. The oral administration of the extract (100, 200 and 400mg/kg B.W.) significantly reduced thioacetamide induced hepatotoxicity in rats, as judged from the estimation of various biochemical parameters viz, Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (ALP), Lipid Peroxidation (LP), Glutathione (GSH), Total Protein (TP) and Bilirubin. These results were comparable with standard drug Silymarin (20mg/kg B.W., p.o.).

**Key words:** *Wedelia calendulacea*, hepatotoxicity, thioacetamide, silymarin.

*Corresponding Author*
**Address for Correspondence:**
Dr. Pallab Kanti Haldar,
Department of Pharmaceutical technology,
Jadavpur University, Kolkata, West Bengal, India-700 032.
Phone: +91-9433321047
E-mail: Pallab_Haldar@rediffmail.com
Introduction

Lipid peroxidation is a natural phenomenon involved peroxidative loss of unsaturated lipids thus bringing about lipid degradation and membrane disordering\(^1\). Peroxidised lipid has been considered to play a significant role in pathogenesis of several diseases and may be taken as the molecular mechanism of the cell injury under pathological condition\(^2\). A number of medicinal plants and their formulation are widely used for liver disease in ethnomedical practice as well as in traditional system of medicine in India\(^3\). *Wedelia calendulacea* (Compositae) is commonly known as Bhimraj found all over India and in traditional system of medicine it is used for the treatment of swelling, headache, hepatic disorder and diarrhoea\(^4\). The pharmacological investigation focuses on evaluation of the efficacy of the methanol extract of *Wedelia calendulacea* for its protection against thioacetamide induced liver damage.

Methods

The leaves of *Wedelia calendulacea* obtained from Raidighi, South 24 Parganas and authenticated by Botanical Survey of India, Shibpur, Howrah. A voucher specimen is kept in our laboratory for future references. Fresh leaves of *Wedelia calendulacea* (Compositae) were extracted with methanol by using Soxhlet apparatus (yield of methanol extract 4.8\%). The solvent was removed by evaporation the residue was kept in vaccume descicator for experimental purpose.

Adult male Wister albino rats weighing 150–180 g were purchased from Indian Institute of Chemical Biology, Kolkata. The animals were kept under uniform laboratory condition (Temperature 25-28\(^\circ\)C and 12 hours light/dark cycle) and fed with standard pellet diet (Hindusthan Lever, Mumbai) and water *ad libitum*.

Rats were divided into six groups each consisting of five animals (n= 5). The group I served as saline control (5ml/kg, i.p). The animals' in-group II received thiocetamide sodium subcutaneously (100mg/kg b.w.)\(^5\). Group III, IV and V were received methanol extract of *Wedelia calendulacea* (100, 200, 400 mg/kg b.w. p.o) for 21 days after 24 hours of the administration of thioacetamide (100 mg/kg s. c). Group IV was received standard drug silymarin 20 mg /kg p.o\(^6\) for 21 days after 24 hours of administration of thioacetamide (100mg/kg s. c).
Hours after the last dose and 18 hours of fasting condition the all rats were anaesthetized in ether chambers for estimation of various biochemical parameters. The blood was collected by cardiac puncture for the estimation of Serum glutamate oxaloacetate transaminase (SGOT) \(^7\), Serum glutamate pyruvate transaminase (SGPT) \(^8\), Serum alkaline phosphatase (ALP) \(^8\), Lipid peroxidation (LP) \(^9\), Glutathione (GSH) \(^10\), Total protein (TP) \(^11\) and bilirubin \(^12\). After sacrifice of all rats, the liver was isolated for estimation of lipid peroxidation and glutathione.

**Results**

The effect *Wedelia calendulaceae* on SGOT, SGPT, serum alkaline phosphatase, total bilirubin, protein and glutathione levels in rats with thioacetamide induced liver damage is summarized in Table 1 and 2. There was a significant increase in SGOT, SGPT, serum ALP and bilirubin levels and decrease in protein label in thioacetamide treated rats when compared with that of normal animals. The methanol extract of *Wedelia calendulaceae* significantly restored the altered biochemical parameters when it was compared with that of standard drug silymarin (20mg/kg b.w. orally).

**Discussion**

Liver cirrhosis is characterized by the nodular transformation of the hepatic parenchymal and the appearance of widespread fibrosis, with an abnormal reconstruction of the lobular architecture \(^13\). Liver fibrogenesis represents the uniform response of the liver to toxic, infectious or metabolic agents and is characterized by an excessive accumulation of extracellular matrix component \(^14\). Construction of hepatic sinusoids in fibrosis impairs hepatic function, increases portal vein pressure and reduces the exchange of macromolecules between the sinusoidal blood and hepatocytes \(^15\). The end-stage of fibrogenesis is cirrhosis, which in humans, results in deteriorated organ function and life-threatening secondary sequelae \(^15\).
**Table 1.** Effect of methanol extract of *Wedelia calendulacea* on SGPT, SGOT, ALP, total protein and bilirubin in thioacetamide induced hepatic damage in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Total Protein (mg/dl)</th>
<th>Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline Control (5ml/kg, 0.9%w/v)</td>
<td>79.01±0.13</td>
<td>65.16±0.13</td>
<td>31.98±0.74</td>
<td>7.49±0.21</td>
<td>1.09±0.01</td>
</tr>
<tr>
<td>Thioacetamide (100mg/kg)</td>
<td>177.61±0.19</td>
<td>156.51±0.38</td>
<td>91.12±0.93</td>
<td>3.59±0.14</td>
<td>2.95±0.07</td>
</tr>
<tr>
<td>Thioacetamide (100mg/kg)+MEWC (100mg/kg)</td>
<td>159.33±0.59</td>
<td>138.71±0.91</td>
<td>73.31±0.61</td>
<td>4.51±0.11*</td>
<td>2.17±0.02*</td>
</tr>
<tr>
<td>Thioacetamide (100mg/kg)+MEWC (200mg/kg)</td>
<td>135.12±0.84*</td>
<td>113.43±0.75*</td>
<td>54.33±0.26*</td>
<td>4.96±0.13*</td>
<td>1.96±0.01*</td>
</tr>
<tr>
<td>Thioacetamide (100mg/kg)+MEWC (400mg/kg)</td>
<td>102.32±0.14*</td>
<td>85.13±0.27*</td>
<td>41.19±0.39*</td>
<td>5.27±0.21*</td>
<td>1.56±0.03*</td>
</tr>
<tr>
<td>Thioacetamide (100mg/kg)+Silymarin (25mg/kg)</td>
<td>85.69±0.29*</td>
<td>74.33±0.23*</td>
<td>35.11±0.32*</td>
<td>6.29±0.17*</td>
<td>1.31±0.02*</td>
</tr>
</tbody>
</table>

IU/L: International Unit per Liter
Each value represents the mean ±S.E.M. for 5 rats in each group and the test of significance (p) of the results were evaluated by students ‘t’ test. *p<0.05 in comparison with thioacetamide group.
Table 2. Effect of methanol extract of *Wedelia calendulacea* on Thiobarbituric Acid Reactive Substance (TBARS) and glutathione levels in Thioacetamide induced hepatic damage in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TBARS (moles/g of wet tissue)</th>
<th>Glutathion (IU/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline Control (5ml/kg, 0.9%w/v)</td>
<td>0.80±0.01</td>
<td>12.93±0.23</td>
</tr>
<tr>
<td>Thioacetamide (100mg/kg)</td>
<td>1.79±0.03</td>
<td>11.01±0.18</td>
</tr>
<tr>
<td>Thioacetamide (100mg/kg)+MEWC (100mg/kg)</td>
<td>1.48±0.05</td>
<td>12.73±0.34</td>
</tr>
<tr>
<td>Thioacetamide (100mg/kg)+MEWC (200mg/kg)</td>
<td>1.13±0.02*</td>
<td>14.91±0.41*</td>
</tr>
<tr>
<td>Thioacetamide (100mg/kg)+MEWC (400mg/kg)</td>
<td>0.93±0.01*</td>
<td>16.15±0.38*</td>
</tr>
<tr>
<td>Thioacetamide (100mg/kg)+Silymarin (25mg/kg)</td>
<td>0.78±0.02*</td>
<td>19.01±0.27*</td>
</tr>
</tbody>
</table>

TBARS: Thiobarbituric Acid Reactive Substance
Each value represents the mean ±S.E.M. for 5 rats in each group and the test of significance (*p*) of the results were evaluated by students ‘t’ test. *p<0.05 in comparison with thioacetamide group.
Reactive oxygen species (ROS), proinflammatory cytokines, glutathione depletion and nitric oxide have all been implicated in the pathogenesis of hepatic failure. Thioacetamide is known to be a hepatotoxin via generation of free radicals, resulting in ROS-mediated acute hepatitis and induces apoptosis of hepatocytes in the liver.

SGOT, SGPT, ALP and bilirubin levels were increased and decreased total protein content (Table-1) in the animals treated with thioacetamide. The methanol extract significantly restored the altered biochemical parameters. The glutathione level was significantly decreased and the lipid peroxidation level was an increased in (Table-2) thioacetamide treated animal. Methanol extract of Wedelia calendulaceae decreased the lipid peroxidation level and increased the glutathione content in thioacetamide induced hepatic damage animals.

Thioacetamide is fungicide, which was recognized as potent hepatotoxic, and carcinogens in rats. It was found that chronic administration of thioacetamide produced cirrhosis in rats. It is well known that thioacetamide is converted into a highly toxic metabolite N-acetyl-p-benzoquinone imine (NAPBI) via cytochrome p-450 pathway. This highly toxic metabolic is normally conjugated with glutathione and excreted in the urine a conjugates]. Thioactamide is a potent inductors of cytochrome p450 and produces a highly reactive NAPBQI which combines with sulphahydryl groups of proteins and cause rapid depletion to intracellular GSH, thereby developing acute hepatic necrosis. The breakdown of Glutathione dependent antioxidant defensive system increases the intracellular flux of oxygen free radical thereby creating an oxidative stress and initiates apoptosis. It has been well established that elevated levels of SGOT, SGPT are indicative of cellular leakage and loss of thioacetamide induces fatty liver and cell necrosis.

The methanol extract of Wedelia calendulaceae significantly restored the elevated serum enzyme level in thioacetamide fed rats when compared to the untreated animals. The methanol extract of Wedelia calendulaceae also decreased the lipid peroxidation level and increased the glutathione content in thioacetamide fed rats. Thus from the present investigation it may inferred that the methanol extract of Wedelia calendulaceae possess potent protective activity against thioacetamide induced hepatic damage in rats.
Acknowledgement

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Reference