Assessment of ethanol and *Eclipta alba* L. extract on haematological parameters on liver of *Rattus rattus*

**Sumira Aziz and Mohd Ashraf Ganaie**

**Abstract**

The liver is an organ of prime importance and plays a significant role not only in metabolism and detoxification of xenogenous toxins and therapeutic agents, but also in the bio-regulation of blood coagulation and immuno-modulation. The investigation of the preliminary phyto chemical qualitative examination of ethanol and aqueous extracts of *Eclipta alba* L. shows presence of different haematological parameters. Haematological parameters namely RBCs, HB, WBC, PCV, and differential counts were monitored in this study because of their diagnostic significance and role in providing information concerning haematological changes caused by acetaminophen (paracetamol) induced toxicity. The effect of *Eclipta alba* on haematological parameters in control and acetaminophen induced hepatotoxic rats. The PVC was significantly reduced (P<0.05) only in hepatotoxic group treated with *Eclipta alba* extract while neutrophils were also reduced in hepatotoxic control. A single oral dose of the acetaminophen @ 750mg/kg body weight significantly (P<0.01), reduced Hb and increased MCV and MCH of the acetaminophen group as compared to control group. Simultaneous treatment with *Eclipta alba* extracts significantly increased the levels of Hb and decreased the levels of MCV and MCH. Agents capable of enhancing survival in the Acetaminophen inducing haemopoietic syndrome have typically been associated with accelerated haemopoietic regeneration.

**Keywords**: Liver, *Eclipta alba* L, Acetaminophen, haematological parameter and *Rattus rattus*

**Introduction**

The liver is an important organ in the metabolic homeostasis of the body. However, due to its metabolic features and localisation, it is very vulnerable to toxic effects of xenobiotic, alcohol, chemotherapeutic agents, virus and protozoan infections (Patel et al., 1988). Depending upon the severity of the hepatic cell injury, viral acute hepatitis can lead to chronic hepatitis, which if left untreated can result in cirrhosis or malignant lesions. Antioxidants have also been proposed as therapeutic agents to counteract liver diseases, since reactive species are known to play a crucial role in liver diseases induction and progression. Additionally, because plant compounds are xenobiotics, they can induce toxicity to the liver, which highlight the importance of performing studies with liver cells. Moreover, possible enzyme and protein induction conferred by these products could provide an opportunity to mechanisms of interaction with other important drugs.

**Chemical constituents of *Eclipta alba* L.**

Entire plant (India) is reported to contain an alkaloid nicotine (Pal et al., 1943) [20] but other reports (Gopal krishnan et al., 1992; Debelsmas et al., 1973; Al-Sharma et al., 1979; Aynehchi et al., 1985; Abu-Mustafa et al., 1977; Sinha et al., 1985) [11, 7, 4, 5, 2, 29] have shown its absence in the different parts of the plant. It is also reported to contain Alkanes-10hentriacontan-1-ol (Sikoria et al., 1982) [22] (RT-India), heptacosan-14-ol (Sikoria et al., 1982) [23] (RT-India) and heptacosane-n (Ali et al., 1997) [24] (Ar-India); Alkyn-tetradecane-4,6-diene-8-10-12 triyne (RT),heptadeca-1-ene-3-5-7-9-11-pentayne (RT), heptadeca-cis-1-7-diene-3, 5-9-11-tetrayne-8-methyl sulfonate (RT), heptadeca-trans-1-7-diene-3,5-9-11- tetrayne-5-methyl sulfonate, (RT) and cardiac glycosides (Debelmas et al., 1973) [25] (Ent-Nepal). Coumarins-Wedelolactone (Wagner et al., 1986; Govindachari et al., 1956; Mors et al., 1991; Fransa et al, 1995; Wagner et al., 1987; Melo et al, 1994; Mors et al, 1989; Sarg et al, 1981) [34, 12, 17, 10, 17, 15, 35, 25] and demethylwedelolactone (Wagner et al., 1986) [34]. Flavonoids- cynaroside (Sarg et al., 1981) [25] (Ar-Egypt), apigenin (Wagner et al., 1986) [34] (Ar-India) and unspecified (Aynehchiet al., 1985) [25] type (Ar-Iran) Lipids-heptacosan-5-one-1-ol-myristate.
Hepato-toxicity and its adverse effects

Hepato-toxicity is a word derived from hepatic toxicity and refers to damage that is caused to the liver due to chemical driven damage. Chemicals that cause damage to the liver are referred to as hepatotoxins and include carbon tetrachloride, alcohol, dantrolene sodium, valproic acid, and isonicotinic acid hydrazide. This sort of damage can be a result of side effects due to certain types of medicines but may also be a result of certain natural chemicals and chemicals employed in industry and laboratories. The most common form of liver poisoning observed in western countries due to medication is from that caused by paracetamol poisoning known as acetaminophen. Sometimes certain medicines are not poisonous or toxic in their compounds but do become toxic when broken down by the liver. Liver plays a vital role in the metabolism and elimination of various exogenous and endogenous compounds. As a result of its continuous involvement, it is susceptible to toxic injuries caused by certain agents and any damage to hepatic cells disturbs body metabolism. In recent times lots of interest has been generated in order to monitor the liver protective effects of various principles of crude drugs that have been alleged to be natural substances. This method was shown to be quite protective activity.

Preparation of Extract (Harborne, 1988)
The whole plant was dried under shade and then powered with a mechanical grinder to obtain a coarse powder, which was then subjected to successive extraction in a maceration apparatus using petroleum ether (60-80°C), chloroform and methanol. Solvent elimination under reduced pressure afforded the chloroform extract (2 % v/w yield) and methanol extract (17 % v/w yield) respectively. The resulting chloroform and methanol extracts were then used for hepato protective and in vivo antioxidant studies.

Experimental animals
Wistar albino rats (150-200g) and Swiss albino mice (20-25g), of either sex roughly the same age (8-10 weeks), obtained from the Experimental Animal Care Centre, Division of Pharmacology, IIIM(CSIR), Jammu were used. The animals were housed under constant temperature (22 ± 2°C), humidity (55%) and light/dark conditions (12/12 h). They were provided with Purina chow and free access to drinking water ad libitum.

Hepato-protective activity Determination
As part of our investigation on clarification of liver-protective principles of crude drugs that have been alleged to be remedies for hepatitis, a method was used to study the hepato-protective activity using acetaminophen induced cytotoxicity in order to monitor the liver protective effects of various natural substances. This method was shown to be quite successful in protecting against various chemicals that cause liver damage.

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Common name</th>
<th>Family</th>
<th>Parts used</th>
<th>Month of collection</th>
<th>Season of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eclipta alba</td>
<td>False Daisy or Bhringraj</td>
<td>Asteraceae</td>
<td>Leaves</td>
<td>April-May.</td>
<td>Summer</td>
</tr>
</tbody>
</table>

The agent should protect against such damage, especially of one which facilitates regeneration by proliferation of parenchymal cells after damage and arrest growth of fibrous tissue. There is no remedy for liver diseases which are so prevalent in the population. The treatment is mainly symptomatic. (Rege et al, 1984) [23] Scientists and some industrialists deliberated on various prospective plant remedies for ailments of liver disorder management. In the decade 70s, the world scientific community concentrated on a herbal plant Vincarosoo. Then in 80s the attention was focused on Panax ginseng. Now, the news of multifarious activities of the Neem tree indicates that it may become centre for research in 90s. Indian Council of Medical Research, New Delhi, in its reviewed research on traditional medicine, had adopted liver diseases as one among six thrust areas and for multidisciplinary study. Screening of active constituents from Kutki (Picrorhiza-kurroa). Bhoomy-amalaki (Phyllanthusniruri) have shown marked protection against jaundice. Hepatitis continues to be a major health problem in urban areas in India, and several studies in viral hepatitis were under investigation by the ICMR. For example, extracts of milk thistle (Silibum-marianum) fruits under investigation for the treatment of alcoholic hepatitis. According to Indian Society of Gastroenterology, Mulethi (Glycyrrhizaglabra) prevents multiplication of viruses inside liver cells. The disorder of liver may be acute or chronic hepatitis (inflammatory liver diseases), hepatitis (non-inflammatory liver diseases) and liver cirrhosis (fibrosis of the liver). Liver enzymes act as an index of sub-clinical hepatic damage. Serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic pyruvic transaminase (SGOT), Serum lactate dehydrogenase (LDH) and Serum alkaline phosphatase are reported as an index of hepatic injury and cholestasis (Doreswamy et al, 1995) [3].
suitable for primary screening of hepatoprotective activity of extracts, fraction and constituents of plant origin, because
a. Many samples can be screened at one time at a relatively low cost
b. the amount of sample required are small
c. variation of result is very little
d. the reproducibility of results are good.

Haematological analysis
Blood samples were collected via venous puncture into sterile sample tubes containing the anticoagulant, EDTA. Blood haemoglobin concentration (HB), Red blood cell (RBC) count, White blood cell (WBC) count, Haematocrit (HCT), Mean haemoglobin concentration (MHC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC) as well as Platelet (PLT) count were analysed using an automated analyser, Cell Dyna, model 331430, Abbott laboratories, IL USA.

Results
The Present study was focused at evaluating the hepatoprotective effect of chloroform and methanol extract of *Eclipta alba* L. by acetaminophen (paracetamol) induced liver damage in rats. The chloroform and methanol extracts were studied for their hepatoprotective effects on acetaminophen (750mg/kg) induced acute liver damage on Wister albino rats.

Extraction
The *Eclipta alba*, leaves powder was subjected to maceration. The percentage yield, colour, consistency and solubility in water were noted.

Determination of Hepato-protective activity of *Eclipta alba* L. Leaves extract
Haematological parameters
Haematological parameters namely RBCs, HB, WBC, PCV, and differential counts were monitored in this study because of their diagnostic significance and role in providing information concerning haematological changes caused by acetaminophen (paracetamol) induced toxicity. The effect of *Eclipta alba* on haematological parameters in control and acetaminophen induced hepatotoxic rats are given in (table 4). The PVC was significantly reduced (P<0.05) only in hepatotoxic group treated with *Eclipta alba* extract while neutrophils were also reduced in hepatotoxic control. A single oral dose of the acetaminophen @ 750mg/kg body weight significantly (p<0.01), reduced Hb and increased MCV and MCH of the acetaminophen group as compared to control group (table 4). Simultaneous treatment with *Eclipta alba* extracts significantly increased the levels of Hb and decreased the levels of MCV and MCH. Agents capable of enhancing survival in the Acetaminophen inducing haemopoietic syndrome have typically been associated with accelerated haemopoietic regeneration. The decline in haematological constituents may be attributed to a direct damage by acetaminophen. In the present study, acetaminophen (paracetamol) causes depletion in the RBC count or erythrocytes (Table 4). *Eclipta alba* L. treated groups (III-VI) showed recovery of normal erythrocytes levels. There was also noticeable depletion in haemoglobin concentration in hepatotoxic rats. The decrease in the Haemoglobin content may be due to decrease in RBCs number or leakage of the RBC membrane to plasma after toxicity. In the present study *Eclipta alba* (L.) treated rats maintained higher level of haemoglobin in dose dependent manner as compared to normal, indicating *Eclipta alba* L. may be having a protective action on the haemoglobin content. The hematocrit (Ht or HCT) or packed cell volume (PCV) is the proportion of blood volume that is occupied by red blood cells. A depression in the haematocrit value can be due to bone marrow and blood erythropoietic cells and thus maintaining the normal percentage of HCT or PCV.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Hepatotoxic control group</th>
<th>Methanol extract 250mg+ Acetaminophen 750mg/kg</th>
<th>Methanol extract 500mg+ Acetaminophen 750mg/kg</th>
<th>Chloroform Extract 250mg+ Acetaminophen 750mg/kg</th>
<th>Chloroform extract 500mg+ acetaminophen 750mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10^6/mm³)</td>
<td>8.67±0.072</td>
<td>6.40±0.092</td>
<td>7.47±0.0821</td>
<td>8.52±0.079</td>
<td>8.11±0.096</td>
<td>8.76±0.103</td>
</tr>
<tr>
<td>White blood cells(10^3/mm³)</td>
<td>6500±288.68</td>
<td>6100±556.78</td>
<td>6500±540.06</td>
<td>5000±204.12</td>
<td>5000±524.40</td>
<td>5250±629.15</td>
</tr>
<tr>
<td>PVC</td>
<td>39.33±0.67</td>
<td>35.20±1.93</td>
<td>37.25±3.04</td>
<td>33.75±0.25</td>
<td>32.00±1.79</td>
<td>30.25±1.31</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>18.2±1.76</td>
<td>20.00±0.50a</td>
<td>20.40±0.58</td>
<td>20.00±0.82</td>
<td>21.00±1.078</td>
<td>20.20±0.48</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>81.67±1.76</td>
<td>78.20±0.58a</td>
<td>79.50±0.96</td>
<td>79.00±0.86</td>
<td>79.60±1.078</td>
<td>78.00±0.41a</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>8.10±0.29</td>
<td>6.07±0.18</td>
<td>6.93±0.26</td>
<td>7.10±0.70</td>
<td>7.30±0.26</td>
<td>7.00±0.28</td>
</tr>
<tr>
<td>MCV(μm³)</td>
<td>72.9±7.97</td>
<td>133.4±23</td>
<td>105.7±15.6</td>
<td>88.0±10.2</td>
<td>80.4±17.4</td>
<td>79.4±7.97</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>20.9±2.21</td>
<td>31.1±3.7</td>
<td>26.7±4.04</td>
<td>22.7±1.75</td>
<td>21.6±3.62</td>
<td>19.6±1.72</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>29.1±1.88</td>
<td>24.1±1.74</td>
<td>25.9±2.31</td>
<td>26.7±1.98</td>
<td>28.2±1.77</td>
<td>26.9±1.87</td>
</tr>
</tbody>
</table>

* Represent the values that are significant (P<0.05) with hepatotoxic control group
Effect of Eclipta alba L. MEEA and CEEA extracts heametological parameters

Discussion
In the present study, we have been planning to elucidate hepato-protective potential of Eclipta alba against chemically induced hepato-carcenogenasis in Rattus rattus. Treatment of diseases associated with the liver is very vital, and must be done with importance and extensive care. Many herbal remedies for liver diseases are known but only a few of them have been pharmacologically assessed for their efficacy. It is very important to assess natural products for their efficacy in the treatments they are used for. It is especially very important to assess remedies for liver diseases due to the liver’s fragility and relation to other vital organs, and yet it’s numerous vital roles detrimental to the survival of a person.

In recent times, due to economic factors, people are in need of available, easily accessible and less costly medication, even with the slightest knowledge of efficacy, and minimum idea of toxicity. It is believed by most people that since herbal remedies are natural, they are non-toxic. Toxicity of natural remedies have however been reported. Even scientifically proven hepato protective plant was found to contain hepatotoxins as well (Bramanti et al, 1978; MacGregor et al, 1989; Oshima, 1995) [6, 13]. Thus, work on hepato protective herbal remedies remain a challenge (Schuppan et al, 1999) [26].

Paracetamol (acetaminophen) is a commonly and widely used analgesic and antipyretic agent. Hepato toxic doses of acetaminophen deplete the normal levels of hepatic glutathione, when NAPQI covalently binds to cysteine groups on proteins to form 3-(cystein-S-yl) acetaminophen adducts (Timenstein and Nelson, 1989) [23]. The glutathione protects hepatocytes by combining with the reactive metabolite of paracetamol thus preventing their covalent binding to liver proteins (Vermsulen et al, 1992) [33].

Haematological parameters namely PCV, WBC and differential count were monitored in this study because of their diagnostic significance and role in providing information concerning haematological changes caused by acetaminophen (paracetmol) induced toxicity.

In the present study increase in PCV (p<0.05) in heptotoxic rats treated with 500mg/kg b.wt Eclipta alba extract supports the increase in the levels of haemoglobin with treatment of Eclipta alba in the previous work by (Tabassum and Agarwal, 2004) [10]. This may be due to the presence of haematinic factors in Eclipta alba. The non-significant change in WBC level in the animals was not in line with the work of (Saeed et al, 1996) [24], where the levels of haemoglobin and WBC were significantly increased. This could be attributed to a rare case of haemo cytopenia often associated with acetaminophen (paracetmol) over dose. The increase in the levels of lymphocytes and neutrophils levels of the heptotoxic rats treated with Eclipta alba supports the study bone by (Trirumalai et al, 2011) [31] which stated that antioxidant phyto-chemicals that can be found in Eclipta alba are known to protect them.

References


21. Rege N, Dahanukar S, Karanidiker SM. Hepato protective effect of *Piper longum* against CCl4 induced liver damage, Indian Drugs, 1984; 31(2):569.


