The Pharma Innovation Journal 2017; 6(11): 671-675

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(11): 671-675

www.thepharmajournal.com
Received: 01-09-2017
Accepted: 02-10-2017

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

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Abstract
The liver is an organ of prime importance and plays a significant role not only in metabolism and detoxification of exogenous 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 haemotological 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, which can induce several steps of liver damages–from inflammatory to fibrotic processes. Impairment of the liver generally occurs from excessive exposure to 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 krishanan *et al*., 1992,: Debelmas *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-hentriacontan-1-ol (Sikroria *et al*., 1982) [27] (Rt-India), heptacosan-14-ol (Sikroria *et al*., 1982) [27] (Rt-India) and hepatacosane-n (Ali *et al*., 1997) [3] (Ar-India); Alkyne-tetradeca-4-6-diene-8-10- 12 triyne (Rt),trideca-1-ene-3-5-7-9-11-pentayne (Rt), trideca-cis-1-7-diene-3- 5-9-11-tetrayne-8-methyl sulfonate (Rt), trideca-trans-1-7-diene-3-5-9-11- tetrayne-5-methyl sulfonate, (Rt) and cardiac glycosides (Debelmas*et al*., 1973) [8] (Ent-Nepal). Coumarins-Wedelolactone (Wagner *et al*., 1986: Govindachari *et al*, 1956,: Mors *et al*., 1991: Franca *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 demethylwadelolactone (Wagner *et al*., 1986) [34]. Flavonoids- cynaroside (Sarg *et al*., 1981) [25] (Ar-Egypt), apigenin (Wagner *et al*., 1986) [34] (Ar-India) and unspecified (Aynehchi*et al*., 1985) [5] type (Ar-Iran) Lipids-heptacosan-5-one-1-ol-myristate

(Ali *et al*., 1997) [3] (Ar-India) andpentadeca-1ol-palmitate-11-hydroxy (Ali *et al*., 1997) [3] (Ar-India) are also reported to be present. Roots and aerial parts are reported to contain various types of number of (more than 26 number) Polyacetylene Sulphurcompounds like bithienyl derivatives, dithiophene derivatives. Sihgh *et al*., 1985,: Singh *et al*., 1992) [29, 28], terthienyl derivatives, thiophene derivatives (Bohlmann *et al*., 1990,: Triterpenes-Bamyrin (Sarg *et al*., 1981) [25] (Ar-Egypt), Enchinocystic acid (Zhang *et al*., 1996) [36] (Ent-China), Eclalbasaponins (Yahara *et al*., 1994, Yahara *et al*., 1997) triterpene acid glycoside (Sarg *et al*., 1981) [25], Ecliptasaponin A (Zhang *et al*., 1996) [36], Ecliptasaponin B (Zhang *et al*., 1996) [36] and Ecliptasaponin D (Zhang *et al*., 1997) [37]; oleanolic acid (Zhang *et al*., 1996) [36]; Steroids-B-sitosterol (Mors *et al*., 1989) [16] (Ar-Brazil) and stigmasterol (Mors. *et al*., 1991) [17], Ar-Brazil (Melo *et al*, 1994) [14], Rt-India (Sikroria *et al*., 1982) [27]; haemolytic saponins (Debelmas *et al*., 1973,: Aynehchi *et al*., 1985,: Abu-Mustafa *et al*., 1977,: Sinha *et al*., 1985) [8, 5, 2, 29], Steroidalalkaloids-Ecliptalbine (Abdel- Kader *et al*., 1998) (Lf-Suriname), Verazine andits derivatives (Abdel- Kader *et al*., 1998) (Lf-Suriname) are reported to be present in *E. prostrata.*

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 to find out a natural remedy for hepatic disorders caused by toxins like alcohol and hepatitis virus (Patel *et.al*, 1998) [22]. 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 Vincarosea. 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 revived 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 (Phyllan-thusniruri) 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 (Silybum-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), hepatosis (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 lactic dehydrogenase (LDH) and Serum alkaline phosphatase are reported as an index of hepatic injury and cholestasis (Doreswamy *et al*, 1995) [9].

Material and methods

Collection and Identification of Plant material

The plants of *Eclipta alba L.* were collected in April directly from the Nehru Nagar surroundings of Bhopal Madhya Pradesh, India and authenticated with the help of botanist at college and voucher samples were preserved for reference in the herbarium. The collected leaves were dehydrated in hot air dryer at 40 ± 5ºC (one batch) and were stored for further experiments. The plant part used for study are given in (Table 1).

Table 1: Showing Plant material and its part used.

Plant Name	Common name	Family	Parts used	Month of collection	Season of collection
Eclipta alba	False Daisy or Bhringraj	Asteraceae	Leaves	April-May.	Summer

Preparation of Extract (Harbone, 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^{0}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

suitable for primary screening of hepato-protective 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 veinous 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 Dyne, 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 hepato-protective 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

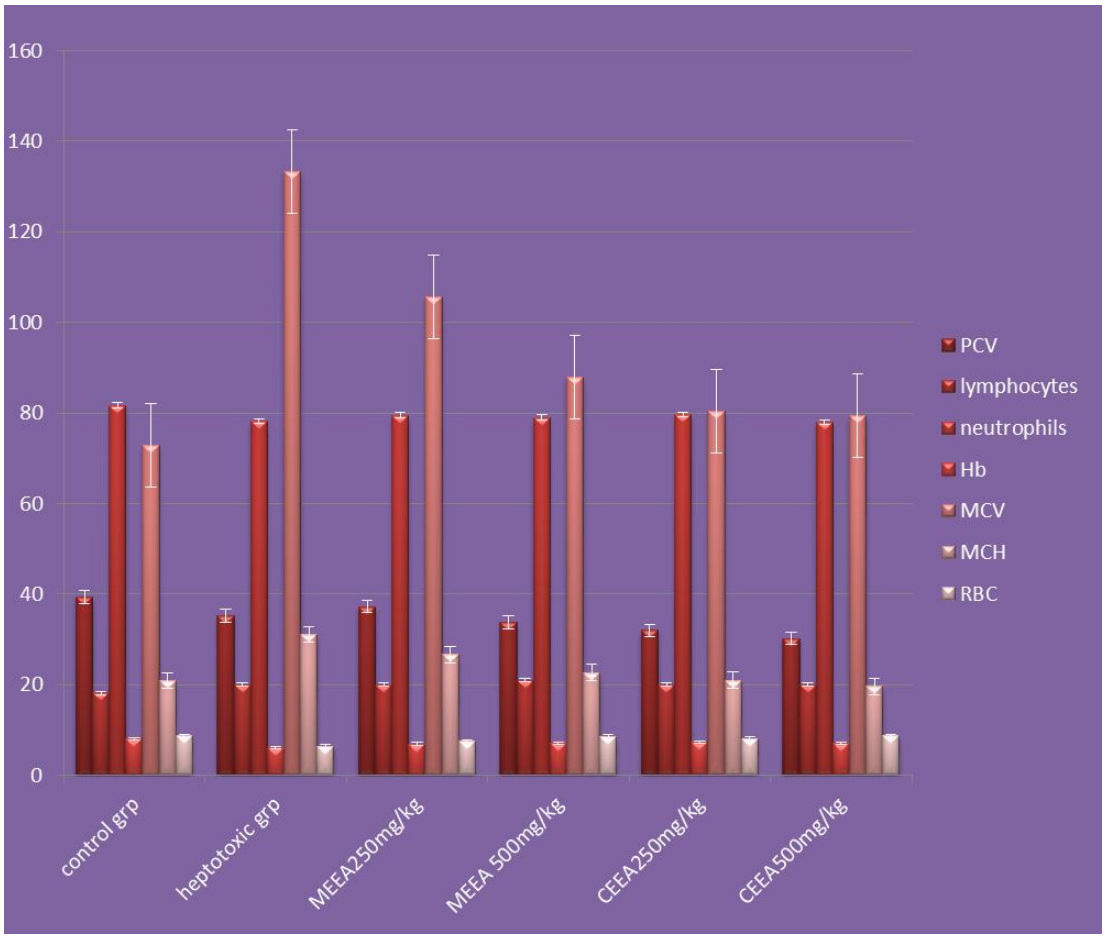
Haematological parameters

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 depletion in the synthesis of haemoglobin after hepatotoxicity. 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 attributed to total cell depletion in peripheral blood aided by disturbances in steady state mechanisms in blood forming organs as well as an increase in plasma volume after toxicity. In our study, *E. alba* treated (group III-VI) the PCV value increase and it indicates that *E. alba* may be providing protection to bone marrow and blood erythropoeitic cells and thus maintaining the normal percentage of HCT or PCV.

Table 4: Effect of *Eclipta alba* Methanol extract and Chloroform extract on Haematological parameters.

Parameters	Control group	Hepatotoxic control group	Methanol extract 250mg+ Acetaminophen 750mg/kg	Methanol extract 500mg+ acetaminophen 750mg/kg	Chloroform Extract 250mg+ Acetaminophen 750mg/kg	Chloroform extract 500mg+ acetaminophen 750mg/kg
RBC (10^6 /$mm^{3)}$	8.67±0.072	6.40a±0.092	7.47a±0.0821	8.52± 0.079	8.11a±0.069	8.76±0.103
White blood cells(10m/$m^{3)}$	6500±288.68	6100±556.78	6500±540.06	5000±204.12	5000±524.40	5250±629.15
PVC	39.33±0.67	35.20±1.93	37.25±3.04	33.75±0.25	32.00±1.79a	30.25±1.31a
Lymphocytes	18.23±1.76	20.00±0.50a	20.40±0.58	20.00±0.82	21.00±1.078	20.20±0.48
Neutrophils	81.67±1.76	78.20±0.58a	79.50±0.96	79.00±0.86	79.60±1.078	78.00±0.41a
Hb (g/dl)	8.10±0.29	6.07±0.18	6.93±0.26	7.10±0.70	7.30±0.26	7.00±0.28
MCV(μm3)	72.9±7.97	133.4±23	105.7±15.6	88.0±10.2	80.4±17.4	79.4 ±7.97
MCH (pg)	20.9±2.21	31.1±4.37	26.7±4.04	22.7±1.75	21.6±3.62	19.6±1.72
MCHC (%)	29.1±1.88	24.1±1.74	25.9±2.31	26.7±1.98	28.2±1.77	26.9±1.87

[a] 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) [32]. 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) [30]. 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.

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