STUDY OF ANTIOXIDANT ACTIVITY OF EUPHORBIA HIRTA LINN WHOLE PLANT IN MICE.

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ABSTRACT

The main objective is to investigate antioxidant activity of extract of Euphorbia hirta whole plant in mice both in-vitro and in-vivo methods. The in-vitro antioxidant activity was carried out to evaluate reducing power, Superoxide anion scavenging activity, hydroxyl radical scavenging activity, nitric oxide radical scavenging activity. The degree of protection was determined by measuring levels of biochemical markers like SGOT, SGPT, ALP, Bilirubin, the histopathological studies were also carried out. Significant increase in GSH level and scavenging activity and decreased lipid peroxidation. The results were comparable with the standard. In vitro antioxidant studies were carried out. Administration of alcoholic extract of Euphorbia hirta whole plant at 100 µg dose has demonstrated dose dependent increase in reducing power, which is comparable to that of std. sodium metabisulphate at 25 µg. Similar results are obtained in case of superoxide anion, hydroxyl radical scavenging activity, Nitric oxide radical scavenging activity. Alcoholic extract of Euphorbia hirta whole plant has significant invitro lipid peroxidation and scavenging activity among other polar extracts.

Keywords: Euphorbia hirta whole plant; marker enzymes; antioxidant; superoxide anion; hydroxyl radical scavenging.

INTRODUCTION

Liver diseases are largest health problem world wide. Liver disorders are mainly caused by toxic chemicals, excessive consumption of alcohol, infections, and autoimmune disorders. Excessive production of reactive oxygen species (ROS) plays a important role in pathogenesis and progression of various disease involving different organs such as liver.
Hepatotoxicity due to drugs appears to be the most common contributing factor. Among the many diseases that affect the liver, the most common is viral hepatitis (inflammation of liver caused by viral infection). Hepatitis can be caused by drugs, viruses, bacteria, mushrooms, parasites like amoebas, and giardiasis.

About 20,000 deaths are found every year due to liver disorders. Liver is also under the constant threat of oxidants and some of free radicals especially \( \text{H}_2\text{O}_2 \). Lipid peroxidation has been demonstrated as one of the important features after exposure to hepatotoxic substances and also is a measure of extent of hepatic damage. Experimentally liver diseases have been shown to be produced by the administration of \( \text{CCL}_4 \), thioacetamide, paracetamol. \( \text{CCL}_4 \) and paracetamol are being converted into reactive toxic metabolites by hepatic microsomal cytochrome P-450 in turn cause hepatotoxicity. Therefore in present study \( \text{CCL}_4 \) and paracetamol induced acute models have been used to assess hepatoprotective activity.

Inspite of phenomenal growth and advent for various human ailments, the medicine for treating hepatic disorders is not been available in allopathic system of medicine. However, there are several herbs/herbal products and herbal formulations available for treating hepatic disorders. Many herbs and their role in hepatic protection is established. However, there are many more such herbs/herbal products and are yet to be evaluated for their hepato protective property. In addition there is a increased interest in the herbs and herbal products regarding their medicinal properties.

Upon literature review it was found that there is no traditional or scientific reports available on the claimed antioxidant activity of the plant. In addition, the pharmacological profile of the plant is incomplete. There is one report that aerial parts of the plant are known to possess flavonoids. There were reports that some flavonoids known to possess antioxidant properties. Keeping these aspects in view, present study was undertaken to study and to correlate antioxidant property of whole plant of Euphorbia hirta L.

**MATERIALS AND METHODS**

**Collection of plant material and extraction**

*Euphorbia hirta* whole plant will be collected from local area, shade dried and coarse powdered. The powder obtained was subjected to successive soxhlet extraction with solvents with the increasing order of polarity i.e. pet. ether(60-80\(^{\circ}\)), chloroform(59.5-61.5\(^{\circ}\)), alcohol(64.5-65.5\(^{\circ}\)) and water.
In addition the shade dried powder was extracted directly with alcohol, which was used for biological investigations and preliminary phytochemical screening, acute toxic effect (LD₅₀), and evaluation of anti oxidant effect. The extracts were concentrated under reduced pressure and stored in a desiccator until further use and the percentage of corresponding extracts were calculated.

Animals
Albino mice weighing 20-25g of either sex were used in this study. They procured from National Institute of Mental Health and Neuro Sciences, Bangalore. The animals acclimatize for one week underlaboratory conditions. They were housed in polypropylene cages and maintained at 27°C ± 2°C under 12 hours dark / light. They were fed with standard rat feed (Gold Mohur Lipton India Ltd.) and water ad libitum was provided. The litter in the cages was renewed thrice a week to ensure maximum comfort for animals and ethical clearance for handling the animals was obtained from Institutional Animals Ethical Committee prior to beginning of the project work.

I. Preliminary phytochemical screening
The preliminary phytochemical screening was carried out on petroleum ether, Chloroform, alcoholic extracts of Euphorbia hirta L whole plant for qualitative identification of type of phytoconstituents present.

A. Invitro antioxidant activity
The following in-vitro models were carried out to evaluate antioxidant activity.
1. Reducing power
2. Superoxide anion scavenging activity
3. Hydroxyl radical scavenging activity
4. Nitric oxide radical scavenging activity

Reducing power
The reducing power of alcoholic extract of Euphorbia hirta Linn whole plant was determined according to the method of Oyaizu (Oyaizu, 1986)

Procedure
Different doses of alcoholic extract of Euphorbia hirta Linn were mixed in 1 ml of distilled water so as to get 10µg, 25µg and 50µg concentration. This was mixed with phosphate buffer
(2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 ml) of trichloroacetic (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increase in reducing power.

The % inhibition of reducing power upon addition of varying doses of extracts was calculated by using the formula

\[
\text{% inhibition of reducing power} = \frac{\text{Control OD} - \text{Test OD} \times 100}{\text{Control OD}}
\]

**Superoxide anion scavenging activity**

Measurement of superoxide anion scavenging activity of Euphorbia hirta Linn whole plant was done based on the method described by Nishimiki (Nishimiki et al., 1972) and slightly modified.

About 1 ml of nitrobluetetrazolium (NBT) solution (156 µm NBT in 100 mM phosphate buffer, pH 7.4), 1 ml NADH solution (468 µm in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of sample solution of alcoholic extract of Euphorbia hirta linn whole plant in water was mixed. The reaction was started by adding 100 µl of Phenazine methosulphate (PMS) solution (60 µM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 minutes, and the absorbance at 560 nm was measured against blank.

Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. % inhibition of OD was calculated by using the formula

\[
\text{% inhibition of reducing power} = \frac{\text{Control OD} - \text{Test OD} \times 100}{\text{Control OD}}
\]

**Hydroxyl radical scavenging activity:**

In biochemical systems, superoxide radical and H₂O₂ react together to form the hydroxy radical, OH⁻, which can attract and destroy almost all known biochemical. Phenylhydrazine when added to erythrocytes ghost cause peroxidation of endogenous lipids and alteration of membrane fluidity. This peroxidation damage to Erythrocytes is probably initiated by active oxygen species like O₂⁻, OH⁻ and H₂O₂ which are generated in solution from auto-oxidation of phenyl hydrazine. This forms the basis of this experiment.
Procedure

Hydroxyl radical generation by phenylhydrazine has been measured by the 2-deoxyribose degradation, assay of Hathwell and Gutteridge in 50mM phosphate buffer (pH 7.4) containing 1 mM deoxyribose, 0.2 mM phenylhydrazine hydrochloride and other additions as necessary in a total volume of 1.6ml. Incubation was terminated after 1 hour or 4 hour and 1 ml each of 2.8% TCA and 1 %( w/v) thiobarbituric acid were added to the reaction mixture and heated for 10 minutes in a boiling water bath.

The tubes were cooled and absorbance taken at 532 nm. Decrease in absorbance indicating the increase in the hydroxyl free radical scavenging activity. The % reduction in the OD is calculated.

Nitric oxide radical scavenging activity

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals\(^{11}\). This forms the basis of this experiment.

Procedure

The Nitric oxide radical scavenging method of alcoholic extract of Euphorbia hirta whole plant was determined according to the method of garret. Nitric oxide (NO) radical were generated from sodium nitroprusside solution at physiological pH Sodium nitroprusside (1ml of 10mM) were mixed with 1ml of alcoholic extract of Euphorbia hirta whole plant of different concentration like10µg, 25µg, 50µg, 100µg, in phosphate buffer (pH 7.4). The mixture was incubated at 25° C for 150 min. To 1 ml of the incubated solution, 1ml of Griess’s reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added.

Absorbance was read at 546 nm. % inhibition of OD is calculated by using the formula

\[
\% \ \text{inhibition of reducing power} = \frac{\text{Control OD} - \text{Test} \times 100}{\text{Control OD}}
\]

Invivo antioxidant activity

An attempt is made to assess the influence of pre-treatment with alcoholic extract of Euphorbia hirta whole plant on the lipid peroxidation in CCl\(_4\) and paracetamol induced...
hepatotoxicity in rats.

1. In vivo CCl₄ - induced lipid peroxidation.

**In vivo carbon tetra chloride induced lipid peroxidation**

Lipid peroxidation, is accepted to be on of the principal cause of CCL₄ induced liver injury, and is mediated by the production of free radical derivates of CCL₄.

**Procedure**

In the dose response experiment, animals were randomly assigned into 5 groups of 6 individuals.

Group-1 Animals ( -ve control )were administered with 1ml distill water p.o for 5 days.

Group-2 Animals (+ve control)were administered with 1ml distill water p.o.,for 5 days.

Group-3 Animals were administered with Silymarin 100mg/kg for 5days.

Group-4 Animals were administered with alcoholic extract 200 mg/kg for 5 days.

Group-5 Animals were administered with alcoholic extract 400 mg/kg for 5 days.

Group-1 receive liquid paraffin (1ml/kg) s.c., on 2ⁿᵈ and 3ʳᵈ day.

Group 2,3,4 and 5 received CCl₄:liquid paraffin (1:1) at a dose of 2ml/kg s.c., on 2ⁿᵈ and 3ʳᵈ day, after 30 min of vehile ,100mg/kg silymarin, 200 mg/kg alcoholic extract and 400mg/kg alcoholic extract of *Euphorbia hirta* whole plant. Animals were sacrificed on 5ᵗʰ day under mild ether anaesthesia . Hepatic tissues were collected and assessed.

A pilot study with different extracts revealed that alcoholic extract showed better response due to presence of flavonoids and its polar nature made us to select alcoholic extract.

Stock solution of TCA-TBA-HCL reagent: 15% w/v trichloroacetic acid; 0.375% w/v thiobarbituric acid; 0.25N hydrochloric acid. This solution may be mildly heated to assist in the dissolution of the thiobarbituric acid.

Combine 1.0ml of biological sample (0.1-2.0 mg of membrane protein or 0.1-0.2 µmol of lipid phosphate) with 2.0ml of TCA-TBA-HCL and mix thoroughly. The solution is heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. the absorbance of the sample is determined at 535 nm against a blank that contains all the reagents minus the lipid. The malondialdehyde concentration of the sample can be calculated by using an extinction coefficient of 1.56×10⁵ M⁻¹cm⁻¹.¹²
Determination of acute toxicity (LD50)

Method

The acute toxicity for petroleum ether, chloroform and alcoholic extracts of *Euphorbia hirta* whole plant were determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose OECD Guideline No. 420 method of CPCSEA was adopted for toxicity studies.

A pilot study with different extracts revealed that alcoholic extract showed better response due to presence of flavonoids and its polar nature made us to select alcoholic extract.

Statistical analysis

Results were expressed as mean ± SEM, (n=6). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by student’s ‘t’ test value less than <0.05 was considered to be statistically significant. *P<0.05, **<0.01 and***<0.001, when compared with control.

RESULTS

Preparation of extract and properties

Results: Successive soxhlet extract process has yielded 4.8% yellow waxy coloured petroleum ether extract, 2.6% of brownish green colored and sticky chloroform extract, 20.2% of dark brown colored alcoholic extract.

Table No. 1: Successive soxhlet extraction of *Euphorbia hirta* whole plant

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Solvent</th>
<th>Colour and consistency</th>
<th>Percentage Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pet.ether</td>
<td>Yellow and waxy</td>
<td>4.8%</td>
</tr>
<tr>
<td>2</td>
<td>chloroform</td>
<td>Brownish green and sticky</td>
<td>2.6%</td>
</tr>
<tr>
<td>3</td>
<td>Alcohol</td>
<td>dark brown</td>
<td>20.2%</td>
</tr>
</tbody>
</table>

Preliminary Phytochemical analysis of *Euphorbia hirta* whole plant:

Results: It is observed from the phytochemical study that steroids, glycosides, tannins and alkaloid sand flavonoids are present in pet.ether, chloroform, and alcohol extract. Phenolic compounds are only present in alcoholic extract.
Table No 2.: Preliminary phytochemical screening of *Euphorbia hirta* whole plant extract.

<table>
<thead>
<tr>
<th>Types of phytochemical constituents</th>
<th>Petroleum Ether extract</th>
<th>Chloroform Extract</th>
<th>Alcoholic Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Indicates presence  
- Indicates absence.

**Acute toxicity (LD<sub>50</sub>) studies**

An attempt was made to identify LD<sub>50</sub> of whole plant of *Euphorbia hirta* since no mortality was observed at 2000mg/kg. It was thought that 2000mg/kg was the cut off dose. Therefore 1/10<sup>th</sup> and 1/5<sup>th</sup> dose (i.e 200mg/kg and 400mg/kg) were selected for all further in vivo studies.

**Effect of the extract of *Euphorbia hirta* on nitric oxide radical scavenging activity**

**Results**: The alcoholic extracts has demonstrated dose dependent increase in the reduction of NO radical scavenging activity. Whereas 25µg sodium metabisulphate (std.) has reduced the NO radical scavenging activity. However the test extracts even at 100µg has shown lesser inhibition than standard, which has produced almost equivalent inhibition at 100µg dose.

**Reducing property of extract of *Euphorbia hirta***

**Results**: The alcoholic extracts has demonstrated dose dependent increase in the reducing property. Whereas 25µg sodium metabisulphate (std.) has reducing property. The test extract at 100µg dose has higher reducing property when compared to 25µg sodium metabisulphate.
Superoxide anion scavenging activity extract of *Euphorbia hirta*

**Results:** The alcoholic extracts has demonstrated dose dependent increase in the superoxide anion scavenging activity. The extract at 50µm has more reducing property than at 25µm of sodium metabisulphate.

Hydroxyl radical scavenging activity extract of *Euphorbia hirta*

**Results:** The alcoholic extracts have demonstrated dose dependent increase in the hydroxyl radical scavenging activity. The extract at 100µm has shown lesser inhibition than at 25µm of sodium metabisulphate.

Table No:3 Anti-oxidant activity of Euphoria Hirta Linn whole plant extract

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Standard</th>
<th>10µg</th>
<th>25µg</th>
<th>50µg</th>
<th>100µg</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NITRIC OXIDE</td>
<td>0.441±0.004\textsuperscript{a}</td>
<td>0.076±0.001\textsuperscript{b}</td>
<td>0.617±0.001\textsuperscript{c}</td>
<td>0.416±0.001\textsuperscript{d}</td>
<td>0.383±0.001\textsuperscript{e}</td>
<td>0.0116±0.001\textsuperscript{f}</td>
<td>6568.94875</td>
</tr>
<tr>
<td>RADICAL scavenging</td>
<td>Euphoria Hirta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANTI-OXIDANT ACTIVITY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REDUCING POWER (OR)</td>
<td>0.305±0.001\textsuperscript{a}</td>
<td>0.535±0.01\textsuperscript{b}</td>
<td>0.319±0.001\textsuperscript{c}</td>
<td>0.425±0.001\textsuperscript{d}</td>
<td>0.526±0.001\textsuperscript{e}</td>
<td>0.625±0.001\textsuperscript{f}</td>
<td>39288.0008</td>
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<tr>
<td>REDUCING ACTIVITY</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Euphoria Hirta</td>
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</tr>
<tr>
<td>SUPEROXIDE ANION</td>
<td>0.665±0.001\textsuperscript{a}</td>
<td>0.364±0.01\textsuperscript{b}</td>
<td>0.575±0.001\textsuperscript{c}</td>
<td>0.546±0.001\textsuperscript{d}</td>
<td>0.445±0.001\textsuperscript{e}</td>
<td>0.379±0.001\textsuperscript{b}</td>
<td>35797.4179</td>
</tr>
<tr>
<td>RADICAL scavenging</td>
<td>Euphoria Hirta</td>
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<td></td>
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<tr>
<td>ACTIVITY</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>HYDROXYL RADICAL</td>
<td>0.443±0.001\textsuperscript{a}</td>
<td>0.075±0.01\textsuperscript{b}</td>
<td>0.633±0.001\textsuperscript{c}</td>
<td>0.436±0.001\textsuperscript{d}</td>
<td>0.395±0.001\textsuperscript{e}</td>
<td>0.0104±0.001\textsuperscript{f}</td>
<td>31568.1697</td>
</tr>
<tr>
<td>scavenging activity</td>
<td>2hr</td>
<td></td>
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<tr>
<td>Euphoria Hirta</td>
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<tr>
<td>HYDROXYL RADICAL</td>
<td>0.441±0.001\textsuperscript{a}</td>
<td>0.076±0.01\textsuperscript{b}</td>
<td>0.612±0.001\textsuperscript{c}</td>
<td>0.415±0.001\textsuperscript{d}</td>
<td>0.385±0.001\textsuperscript{e}</td>
<td>0.095±0.001\textsuperscript{f}</td>
<td>11294.0659</td>
</tr>
<tr>
<td>scavenging activity</td>
<td>4 hr</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Euphoria Hirta</td>
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<td></td>
</tr>
</tbody>
</table>

ANOVA with Dunnett’s Multiple comparisons, $\alpha = 0.05$, means with different superscripts in same row differ significantly.
Effect of alcoholic extract of *Euphorbia hirta* whole plant on in vivo lipid peroxidation in CCL₄ induced hepatotoxicity.

There is a dose dependent inhibition of in vivo lipid peroxidation by alcoholic extract. 400mg/kg alcoholic extract has greater inhibition than 100mg/kg silymarin.

Table No:4 Effect of Euphoria Hirta Linn whole plant extract on antioxidant parameters in CCl₄ induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group -I (neg control)</th>
<th>Group -II (+ve control)</th>
<th>Group - III Silymarin 100 mg/kg</th>
<th>Group -IV Euphoria Hirta 200 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/mg protein)</td>
<td>1.22±0.02a</td>
<td>1.32±0.04b</td>
<td>1.27±0.02c</td>
<td>1.29±0.04d</td>
</tr>
<tr>
<td>SOD (activity/mg protein)</td>
<td>1.49±0.01a</td>
<td>1.57±0.01b</td>
<td>1.51±0.01c</td>
<td>1.52±0.01d</td>
</tr>
<tr>
<td>CAT (activity/mg protein)</td>
<td>1.44±0.02a</td>
<td>1.37±0.01b</td>
<td>1.38±0.01c</td>
<td>1.39±0.01d</td>
</tr>
<tr>
<td>GSH (µ mol/mg protein)</td>
<td>1.934±0.001a</td>
<td>1.437±0.001b</td>
<td>1.844±0.001c</td>
<td>1.677±0.001d</td>
</tr>
</tbody>
</table>

Values are mean±SEM; *P<0.05 Values with different super scripts differ significantly different from group.

Fig. 1: Effect Euphoria Hirta Linn whole plant extract on antioxidant parameters in CCl₄ induced hepatotoxicity in rats.
MDA=nmol/mg protein; SOD=activity/mg protein; CAT(activity/mg protein); GSH=µmol/mg protein;

Table No:05 Effect of Euphoria Hirta Linn whole plant extract on antioxidant parameters in paracetamol-induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group -I (-ve control)</th>
<th>Group -II (+ve control)</th>
<th>Group -III Silymarin 100 mg/kg.</th>
<th>Group -IV Euphoria Hirta 200 mg/kg.</th>
<th>Group -V Euphoria Hirta 400 mg/kg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/mg protein)</td>
<td>0.32±0.02a</td>
<td>0.42±0.04a</td>
<td>0.16±0.02a</td>
<td>0.12±0.04a</td>
<td>0.17±0.02a</td>
</tr>
<tr>
<td>SOD (activity/mg protein)</td>
<td>0.39±0.01b</td>
<td>0.47±0.01b</td>
<td>0.44±0.01b</td>
<td>0.47±0.01b</td>
<td>0.41±0.01b</td>
</tr>
<tr>
<td>CAT (activity/mg protein)</td>
<td>0.34±0.02c</td>
<td>0.27±0.01c</td>
<td>0.35±0.01c</td>
<td>0.27±0.01c</td>
<td>0.28±0.01c</td>
</tr>
<tr>
<td>GSH (µmol/mg protein)</td>
<td>0.834±0.00d</td>
<td>0.216±0.001d</td>
<td>0.465±0.001d</td>
<td>0.554±0.001d</td>
<td>0.645±0.001d</td>
</tr>
</tbody>
</table>

Values are mean±SEM; *P<0.05 , Values with different superscripts differ significantly different from group-I

Fig 2: Effect of Euphoria Hirta Linn whole plant extract on antioxidant parameters in paracetamol induced hepatotoxicity in rats

MDA=nmol/mg protein; SOD=activity/mg protein; CAT(activity/mg protein); GSH=µmol/mg protein;
DISCUSSION

Hepatic system of an organism is involved in metabolic activities of it. In this process it is exposed to various challenges and hence, hepatic system is not only evolved to various challenges and hence, hepatic system is not only evolved to perform its function but also to protect itself from the various challenges like exposure to antibiotics/xenobiotics, chemicals, microbes, etc. Liver is such an organ that its physiological role and its self protective mechanism are well developed and orchestrated. In spite of such a balanced internal milieu, hepatic aberrations, damage and necrosis commonly occurring due to over exposure to hepato-toxic causes to such an extent that it over powers the protective mechanism.

Hepatic injury caused by various hepatotoxins disturbs the balance in the various metabolic activities. In addition hepatic necrosis resulting in the elevation of serum levels of various biochemical marker enzymes e.g. SGPT, SGOT, ALP, etc.

In spite of ultramodern advances in medical sciences, pharmaco-therapeutic treatment with synthetic drugs is not yet realized. However, there are several herbs and herbal formulation which are found to be claimed to be beneficial in treating hepatic disorders. In the present study one of the locally available herb Euphorbia hirtawhole plant were selected based on the basis of claims of native practitioner and available phytochemical profile of the plant.

Since leaves of the plant possess flavonoids, these are known to possess anti-oxidant property, it was thought to screen the whole plant for hepatoprotective property by using various models of experimentally induced hepatitis and for antioxidant property.

In the present study various extracts of whole plant Euphorbia hirtawere prepared by using successive soxhlet procedure. They are subjected to preliminary phytochemical tests. It is observed that steroids, Carbohydrates, glycosides, tannins and phenolic are present in pet. ether, chloroform and ethanol extract. Flavonoids are present only in chloroform and ethanol extract.

Since there are reports that the flavonoids are containing antioxidant activity, it was planned to screen alcoholic extract for reducing power, superoxide anion, hydroxyl radical scavenging activity, Nitric oxide radical scavenging activity.
It is evident from our study that alcoholic extract of *Euphorbia hirta* whole plant at 100µg dose has demonstrated dose dependent increase in reducing power, which is comparable to that of std. sodium metabisulphate at 25µg.

Similar results are obtained in case of superoxide anion, hydroxyl radical scavenging activity, nitric oxide radical scavenging activity\(^\text{17}\).

Alcoholic extract which was selected was subjected to screening of acute toxicity by using CPCSEA guide line no.420 (fixed dose method). Since no death was observed at 2000mg/kg it was thought that 2000mg/kg was cut off dose and 1/10\(^\text{th}\) (200mg/kg) and 1/5\(^\text{th}\) (400mg/kg) of cut off dose were selected for further studies.

This clarity indicated that the alcoholic extract of *Euphorbia hirta* whole plant possess hepatoprotective activity.

The term ‘antioxidant’ refers to the activity of numerous vitamins, minerals and other phytochemicals to protect against the damage caused by reactive oxygen species (ROS). By their ability to react with the damage many structure in the body. It is well known that reactive oxygen species (ROS) are involved in many pathological disorders such as atherosclerosis and related cardiovascular diseases, diabetes, and cancer. Reactive oxygen species, generated in vitro mainly by neutrophils, macrophages, and xanthine-oxidase system appear to be responsible in these illnesses by inducing lipid peroxidation via a chain reaction process\(^\text{18}\).

Most living species have protective systems against oxidative stress and toxic effects of ROS. Several studies have demonstrated that the antioxidant properties of plant compounds could be correlated with oxidative stress defense. Thus, antioxidant compounds can be used to counteract oxidative damage by reacting with free radicals, chelating free catalytic metals, and also by acting as oxygen scavengers\(^\text{19}\).

However, further studies are needed to isolate the active principle and identify the lead molecule responsible for Anti oxidant activity.

However, our study has demonstrated that alcoholic extract of *Euphorbia hirta* whole plant possess antioxidant property.
CONCLUSION

The *Euphorbia hirta* whole plant contains alkaloids, steroids, glycosides, flavonoids, tannins, carbohydrates and proteins. Similarly all the above mentioned extracts demonstrated the dose dependent superoxide anion scavenging activity, reducing power, hydroxyl free radical scavenging activity, nitric oxide radical scavenging activity in in – vitro models. Histopathological observations have revealed that treatment with alcoholic extract of *Euphorbia hirta* whole plant has improved the hepatic anatomy i.e. hepatic generations are seen in both the models of hepatic injury.

REFERENCES

9) Kuppuswamy raju, govindaraju anbuganapathi, veluswamy gokulakrishan, balasubramanian rajkapoor, balasundaram jayakar and sellamuthu manian . Effect of
dried fruit of solanum nigrum linn against CCL\textsubscript{4} induced hepatic damage in rats. boil pharm bull.2003; 26(11): 1618-1619.

10) Sumithi P , Thinuralsundara T, Hepatoprotective activity of Aegle Marmelos in ccl4 induced toxicity An In-Vivo Study Journal of Phytology.3(9): 05-09.


