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A STUDY OF PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *EUPHORBIA HIRTA LINN WHOLE* PLANT IN RATS & MICE

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ABSTRACT

Objective: To investigate hepatoprotective of extract of *Euphorbia hirtawhole* plant in rats and mice. **Methods:** The hepatoprotective activity of the alcoholic extract of *Euphorbia hirta*whole plant was tested against carbon tetrachloride and paracetamol induced hepatotoxicity in albino rats. The degree of protection was determined by measuring levels of biochemical markers like SGOT, SGPT, ALP, Bilirubin, the histopathological studies were also carried out.Silymarin was used as the standard drug for comparison. In vitro antioxidant studies were also carried out. **Results:** Administration of alcoholic extract of *Euphorbia hirta*whole plant (200 and 400 mg/kg) markedly reduced carbon tetrachloride and paracetamol induced elevation of SGOT, SGPT, ALP, Bilirubin and cholesterol whereas HDL levels were elevated. Significant increase in GSH level and scavenging activity and decreased lipid peroxidation. The results were comparable with the standard. A comparative histopathological study of liver exhibited all most normal architecture as compared to control group. **Conclusion:** Alcoholic extract of *Euphorbia hirta* whole plant possess significant hepatoprotective activity, showed excellent resortstion of depleted levels of GSH. 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; paracetamol; hepatoprotective; antioxidant ; Carbon tetrachloride.

INTRODUCTION

The liver is the largest glandular organ in the body, and has more functions than any other human organ. A person's entire blood supply passes through the liver several times a day. Liver produces and secretes bile, it also produces prothrombin and fibrinogen, both blood clotting factor, and heparin, a mucopolysacchride sulfuric acid ester that helps keep blood from clotting within the circulatory system[1]. It plays a vital role in regulating various physiological processes. It is also involved in several vital functions such as metabolism secretion and storage. It has a great capacity to detoxicate toxic substances and synthesize useful principles. In addition it aids metabolism of carbohydrates, proteins, fat, detoxification, secretion of bile and storage of vitamins. The role played by this organ is removal of substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign compounds ,culminating in liver dysfunction[2].

Liver is the key organ in regulating homeostasis in the body. Liver diseases are largest health problem worldwide. Liver disorders are mainly caused by toxic chemicals, excessive consumption of alcohol, infections, autoimmune disorders. Excessive production of reactive oxygen species(ROS) plays an important role in pathogenesis and progression of various disease involving different organs such as liver[4].Hepatotoxicity due to drugs appear to be the most common contributing factor.The liver is expected not only to perform physiological functions but also to protect against the hazardous of harmful drugs and chemicals[3].

About 20,000 deaths found every year due to liver disorders[1]. Liver is also under the constant threat of oxidants and some of free radicals especially H_2O_2. Lipid peroxidation has been demonstrated as one of the important feature after exposure to hepatotoxic substances and also is a measure of extent of hepatic damage[5]. Experimentally liver diseases have .been shown to be produced by the administration of ccl_4,thioacetamide,paracetamol[6].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 ccl_4 and paracetamol induced acute models have been used to assess hepatoprotective activity[7].

Upon literature review it was found that there is no traditional or scientific reports available on the claimed hepatoprotective property 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[8].There were reports that some flavonoids known to possess antioxidant and hepato protective properties[9]. Keeping these aspects in view, present study was undertaken to study

and to correlate antioxidant and hepatoprotective 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.epet.ether(60-80^0), chloroform(59.5-61.5^0), alcohol (64.5-65.5^0) and water[10].

In addition the shade dried powder was extracted directly with alcohol, which was used for biological investigations and preliminary phytochemical screening, acute toxic effect (LD_{50}) ,invitro antioxidant studies and evaluation of hepatoprotectiveeffect.vThe extracts were concentrated under reduced pressure and stored in a desiccator until further use and the percentage of corresponding extracts were calculated.

Animals

Albino Rats (wistar strain) weighing 120-150g and albino mice weighing 20-25gof either sex were used in this study. They procured from National Institute of MentalHealth 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 (GoldMohur 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.[11,12.]

II. Antioxidant activity

A. *Invitro* antioxidant activity

The following in-vitro models were carried out to evaluate antioxidant activity.

- Reducing power
- Superoxide anion scavenging activity

- Hydroxyl radical scavenging activity
- Nitric oxide radical scavenging activity

1. Reducing power

The reducing power of alcoholic extract of Euphorbia hirta Linn whole plant was determined according to the method of **Oyaizu (Oyaizu, 1986)**[13]

Procedure

Different doses of alcoholic extract of Euphorbia hirta Linn were mixed in 1 ml ofdistilled water so as to get 10µ g, 25µg and 50µg concentration. This was mixed withphosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). Themixture 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 700nm. 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

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

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.[76]

About 1 ml of nitrobluetetrazolium (NBT) solution (156µ m NBT in 100 mMphosphate buffer, pH 7.4), 1 ml NADH solution (468µ m in 100 mm phosphate buffer, pH7.4) and 0.1 ml of sample solution of alcoholic extract of Euphorbia hirtalinn whole plant in water was mixed. The reaction was started by adding 100µl of Phenazinemethosulphate PMS) solution (60µ 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 superoxideanion scavenging activity.% inhibition of OD was calculated by using the formula mentioned earlier in the estimation of reducing power.

Hydroxyl radical scavenging activity

In biochemical systems , superoxide radical and H_2O_2 react together to form the hydroxy radical, OH *, which can attract and destroy almost all known biochemical mediators[14]. 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 O_2^*, OH^* and H_2O_2 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 the2-deoxyribose degradation, assay of**Hathwell and Gutteridge**[78] in 50mM phosphatebuffer (pH 7.4) containing 1 mMdeoxyribose, 0.2 mMphenylhydrazine hydrochlorideand other additions as necessary in a total volume of 1.6ml, incubation was terminatedafter 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 % reductionin the OD is calculated.

Nitric oxide radical scavenging activity[15]

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. 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

diaminedihydrochloride) was added. Absorbance was read at 546 nm.

% inhibition of OD is calculated by using the formula mentioned earlier.

B. *Invivo* antioxidant activity

An attempt is made to assess the influence of pre-treatment with alcoholic extract of *Euphorbia hirta*whole plant on the levels of Glutathione in-vivo in CCl_4 and paracetamol induced hepatotoxicity and on the lipid peroxidation in CCl_4 and paracetamol induced hepatotoxicity in rats.

1. Glutathione (GSH) estimation in $CCL_4$and paracetamol induced hepatotoxicity in rats.
2. In vivo CCl_4 - induced lipid peroxidation.

1.Glutathione estimation

Glutathione is present in all type of living cells. Tissues such as mammalian liver normally contain high levels of reduced Glutathione. It has been suggested that GSH protects thiol groups in protein from oxidation, functions as an intracellular redox buffer and serves as a reservoir of cysteine[16].

The role of GSH in determining the extent of liver damage has been demonstrated in experiments where the hepatic concentration of GSH is altered by toxin treatments Depletion of GSH contents has been reported to potentiate hepatic necrosis and covalent bonding of toxic metabolites to cellular macromolecules[17].

1.1 GSH estimation in CCL_4induced hepatotoxicity in rats

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

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

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

Group-3 Animals were administered withSilymarin 100mg/kg BW for 5days.

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

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

Group-1 receive liquid paraffin (1ml/kg) s.c., on 2nd and 3rd day .Group 2,3,4 and 5 received CCl_4:liquid paraffin (1:1) at a dose of 2ml/kg s.c., on 2nd and 3rd day, after 30 min of vehicle, 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.

Tissue samples were homogenized in ice cold tricholoroacetic acid (1gm tissue plus 10 ml 10% TCA) in a ultra turraxhomozenizer, after centrifugation at 3000rpm for 10 min, 0.5 ml supernatant was added to 2 ml of 0.3 M disodium hydrogen phosphate solution. A 0.2 ml solution of dithiobisnitrobenzoate(0.4 mg/ml in 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing.% increase in OD is directly proportional to the increase in the levels of Glutathione . Hence % increase in OD is calculated.

GSH estimation in paracetamol induced hepatotoxicity

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

Group-1 Animals (-ve control)were administered with 1ml distilled water p.o.,for 7 days.

Group-2 Animals (+ve control)were administered with 1ml distilled water p.o., for 7 days.

Group-3 Animals were administered withSilymarin 100mg/kg BW for 7 days.

Group-4 Animals were administered with alcoholic extract 200 mg/kg BW for 7 days.

Group-5 Animals were administered with alcoholic extract 400 mg/kg BW for 7 days.

On 5thday, 30 min after the administration of normal saline, 100mg/kg silymarin, 200 mg/kg alcoholic extract and 400mg/kg alcoholic extract of *Euphorbia hirta* whole plant to group 2, 3, 4 & 5 respectively, paracetamol 2g/kg was given orally. After 48 hrs of paracetamol feeding rats were sacrificed under mild ether anesthesia.Hepatic tissues were collected and assessed.

Tissue samples were homogenized in ice cold tricholoroacetic acid (1gm tissue plus 10 ml 10% TCA) in a ultra turraxhomozenizer,after centrifugation at 3000rpm for 10 min, 0.5 ml supernatant was added to 2 ml of 0.3 M disodium hydrogen phosphate solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml in 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing.% increase in OD is directly proportional to the increase in the levels of Glutathione.

2. *In-vivo* carbon tetra chloride induced lipid peroxidation[19]

Lipid peroxidation, is accepted to be on of the principal cause of CCL_4 induced liver injury, and is mediated by the production of free radical derivates of CCL_4[20].

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 distilled water p.o.for 5 days.

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

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

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

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

Group-1 receive liquid paraffin (1ml/kg) s.c., on 2nd and 3rd day .Group 2,3,4 and 5 received CCl_4: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 selectalcoholic extract.

III. Determination of acute toxicity(LD_{50})

Method

According to OECD Guidelines No. 420 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[21].

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.

Evaluation of Hepatoprotective Activity in CCl_4 induced hepatotoxicity

The method of Suja SR. et al was followed[22]

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

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

Group-2 Animals (+ve control)were administered with 1ml distilled water p.o.for5days.
Group-3 Animals were administered withSilymarin 100mg/kg BW for 5days
Group-4 Animals were administered with alcoholic extract 200 mg/kg BW for 5days.
Group-5 Animals were administered with alcoholic extract 400 mg/kg BW for 5 days.

Group-1 receive liquid paraffin (1ml/kg) s.c., on 2nd and 3rd day .Group 2,3,4 and 5 received CCl_4:liquid paraffin (1:1) at a dose of 2ml/kg s.c., on 2nd and 3rd day,After 30 min of vehicle, 100mg/kg silymarin, 200 mg/kg alcoholic extract and 400mg/kg alcoholic extract of *Euphorbia hirta* whole plant. Animals were sacrificed under mild ether anesthesia.

Blood samples were collected for evaluating the serum biochemical parameters and liver was dissected out , blotted off blood ,washed with saline and stored in 10% formalin and preceded for histopathology to evaluate the details of hepatic architecture in each group microscopically.

Evaluation of Hepatoprotective Activity in paracetamol induced hepatotoxicity

The method of RR Chattopadhyay was followed[23]

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

Group-1 Animals (-ve control)were administered with 1ml/kg distill water p.o., for 7 days.
Group-2 Animals (+ve control)were administered with 1ml/kg distill water p.o., for 7 days.
Group-3 Animals were administered withSilymarin 100mg/kg BW for 7 days
Group-4 Animals were administered with alcoholic extract 200 mg/kg BW for7 days.
Group-5 Animals were administered with alcoholic extract 400 mg/kg BW for 7 days.

On 5thday , 30 min after the administration of normal saline , 100mg/kg silymarin, 200 mg/kg alcoholic extract and 400mg/kg alcoholic extract of *Euphorbia hirta* whole plant to group 2, 3 , 4 & 5 respectively, paracetamol 2g/kg was given orally. After 48
hrs of paracetamol feeding rats were sacrificed under mild ether anesthesia. Blood samples were collected for evaluating the serum biochemical parameters and liver was dissected out , blotted off blood ,washed with saline and stored in 10% formalin and preceded for histopathology to evaluate the details of hepatic architecture in each group microscopically.

The biochemical parameters estimated includes

- Serum glutamate pyruvate transaminase (SGPT)

- Serum gluamate oxaloacetate transaminase (SGOT)
- Serum alkaline phosphatase (ALP)
- Serum bilirubin (total and direct)
- Serum cholesterol
- Serum HDL,

Histopathology[24]

Small pieces of liver tissues were collected in 10% formalin for proper fixation.These tissues were processed and embedded in paraffin wax. Section of 5-6 microns in thickness were cut and stained with hematoxylin and eosin. All the sections of the tissueswere examined under microscope for the analyzing the altered architecture of the liverand paracetamol challenge and improved liver architecture due to pretreatment with test extracts and standard drug.

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

Prepration 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 *Ephorbiahirta*whole plant

Sl.No.	Solvent	Colour and consistency	Percentage Yield
1	Pet.ether	Yellow and waxy	4.8%
2	Chloroform	Brownish green and sticky	2.6%
3	Alcohol	dark brown	20.2%

Preliminary Phytochemical analysis of Euphorbia hirta whole plant

Results: It is observed from the phytochemical study that steroids, glycosides, tannins and alkaloidsand flavonoids are present in pet.ether, chloroformand alcohol extract .Phenolic compounds are only present in alcoholic extract.

Table No.2 : Preliminary phytochemical screening of *Euphorbia hirta* whole plant extract.

Types of phytochemical constituents	Petroleum Ether extract	Chloroform Extract	Alcoholic Extract
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponin Glycosides	+	+	+
Tannins	_	+	+
Steroids	+	+	+
Proteins	_	+	+
phenolic compounds	_	_	+
Fats and oils	+	+	+

+ Indicates presence - Indicates absence.

Acute toxicity (LD_{50}) studies

An attempt was madeto identify LD_{50} 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 $1/10^{th}$and $1/5^{th}$ 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 theNO 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 *Euphorbiahirta*

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 has 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.

	Control	Standard	10µg	25µg	50µg	100µg	F Value
NITRIC OXIDE RADICAL SCAVANGING ACTIVITY **Euphoria Hirta**	0.441± 0.004[a]	0.076± 0.001[b]	0.617± 0.001[c]	0.416± 0.001[d]	0.383± 0.001[e]	0.0116± 0.001[f]	6568.94875
REDUCING POWER (OR) REDUCING ACTIVITY **Euphoria Hirta**	0.305± 0.001[a]	0.535± 0.001[b]	0.319± 0.001[c]	0.425± 0.001[d]	0.526± 0.001[e]	0.625± 0.001[f]	39288.0008
SUPEROXIDE ANION RADICAL SCAVENGING ACTIVITY **Euphoria Hirta**	0.665± 0.001[a]	0.364± 0.001[b]	0.575± 0.001[c]	0.546± 0.001[d]	0.445± 0.001[e]	0.379± 0.001[b]	35797.4179
HYDROXYL RADICAL SCAVENGING ACTIVITY 2hr	0.443± 0.001[a]	0.075± 0.001[b]	0.633± 0.001[c]	0.436± 0.001[d]	0.395± 0.001[e]	0.0104± 0.001[f]	31568.1697
HYDROXYL RADICAL SCAVENGING ACTIVITY 4 hr **Euphoria Hirta**	0.441± 0.001[a]	0.076± 0.001[b]	0.612± 0.001[c]	0.415± 0.001[d]	0.385± 0.001[e]	0.095± 0.001[f]	11294.0659

ANOVA with Dunnets Multiple comparisons, $\alpha = 0.05$, means with different superscripts in same row differ significantly.

Influence of alcoholic extract of whole plant on GSH levels of *Euphorbiahirta*in CCL_4 induced hepatotoxicity in rats

Results: There is marked depletion of GSH levels in CCL_4 treated groups. 100mg/kg silymarin has shown dose dependent increase in the levels of GSH. 400mg/kg alcoholic extract of the plant has increased the GSH levels which is almost equal to standard silymarin.

Effect of alcoholic extract of *Euphorbia hirta* whole plant on in vivo lipid Peroxidation in CCL_4 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/kgsilymarin.

Table No 4: Effect of Euphoria Hirta Linn whole plant extract on antioxidant parameters in CCL_4 induced hepatotoxicity in rats.

Parameter	Group -I(-ve control)	Group –II (+ve control)	Group – III Silymarin 100mg/kg.	Group –IV Euphoria Hirta 200 mg/kg	Group -V Euphoria Hirta 400 mg/kg
MDA (nmol/mg protein)	1.22±0.02a	1.32±0.04b	1.27±0.02c	1.29±0.04d	1.25±0.02e
SOD(activity/mg protein)	1.49±0.01a	1.57±0.01b	1.51±0.01c	1.52±0.01d	1.51±0.01e
CAT(activity/mg protein)	1.44±0.02a	1.37±0.01b	1.38±0.01c	1.39±0.01d	1.42±0.01e
GSH (µ mol/mg protein)	1.934±0.001a	1.437±0.001b	1.844±0.001c	1.677±0.001d	1.816±0.001e

MDA=nmol/mg protein; SOD=activity/mg protein; CAT(activity/mg protein); GSH=µ mol/mg protein;
Values are mean±SEM;*P<0.05 ,Values with different super scripts differ significantly different from group

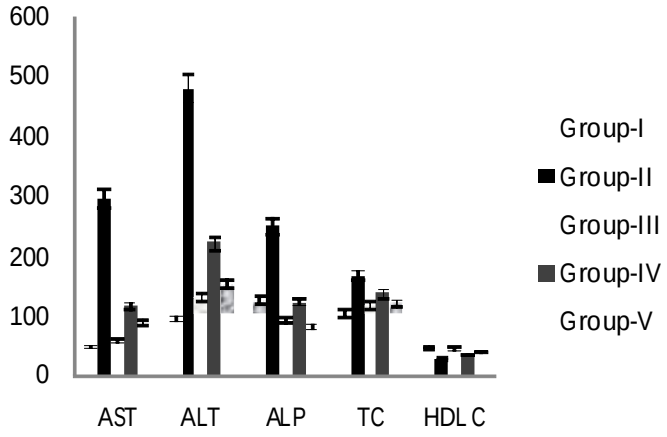


Fig. 5 : Effect Euphoria Hirta Linn whole plant extract on antioxidant parameters in CCl_4 induced hepatotoxicity in rats.

Influence of alcoholic extract of *Euphorbia hirta* whole plant on GSH levels in Paracetamol induced hepatotoxicity in rats

Results: There is marked depletion of GSH levels in paracetamol treated groups. 100mg/kg silymarin has shown dose dependent increase in the levels of GSH. 400mg/kg alcoholic extract of the plant has increased the GSH levels which is almost equal to standard silymarin.

Effect of whole plant on biochemical alcoholic extract of *Euphorbia hirta* markers in CCl_4 induced hepatotoxicity

Result:

There is a increase in SGPT levels observed in CCl_4 treated group(396.473U/L). The extract has shown a dose dependent effect. SGPT levels were restored to 99.09U/L by 400mg/kg alcoholic extract of the whole plant which is near to the effect of 100mg/kg silymarin i.e.67.77U/L.

SGOT levels has been increased significantly in CCl_4 treated group i.e., 581.167U/L . 400mg/kg alcoholic extract of the whole plant reduced the elevated levels of SGOT to 263.72U/L which is near to silymarin effect 231.71U/L.

Table No: 5: Effect of Euphoria hirta Linn whole plant extract on biochemical parameters in CCl_4 induced hepatotoxicity in rats.

Parameter	Group -I (-ve control).	Group -II (+ve control).	Group - III Silymarin 100 mg/kg.	Group -IV Euphoria Hirta 200 mg/kg	Group -V Euphoria Hirta 400 mg/kg
AST (U/ml)	58.553±0.0a	396.473±0.02b	67.775±0.0c	217.198±0.01d	99.091±0.0e
ALT (U/ml)	105.293±0.0a	581.167±1.08b	231.715±0.02c	321.877±0.05d	263.725±0.01e
ALP (U/ml)	226.14±0.08a	350.502±0.01b	92.240±0.00c	222.935±0.01d	91.846±0.00e
Total bilirubin (mg/dl)	1.992±0.00a	5.242±0.01b	2.237±0.00c	3.083±0.00d	2.543±0.00e
Directbilirubin (mg/dl)	1.185±0.00a	2.496±0.00b	1.376±0.00c	1.72±0.00d	1.546±0.00e
Cholesterol (mg/dl)	204.257±0.71a	267.653±0.01b	218.098±0.01c	237.14±0.01d	120.938±0.01e
HDL(mg/dl)	57.436±0.00a	37.605±0.00b	54.792±0.00c	43.853±0.00d	50.623±0.00e

Values are mean±SEM;*P<0.05 ,Values with different superscripts differ significantly different from group-I
AST = U/ml; ALT = U/ml; ALP = U/ml; LDH = U/ml; C=mg/dl HDL C=mg/dl

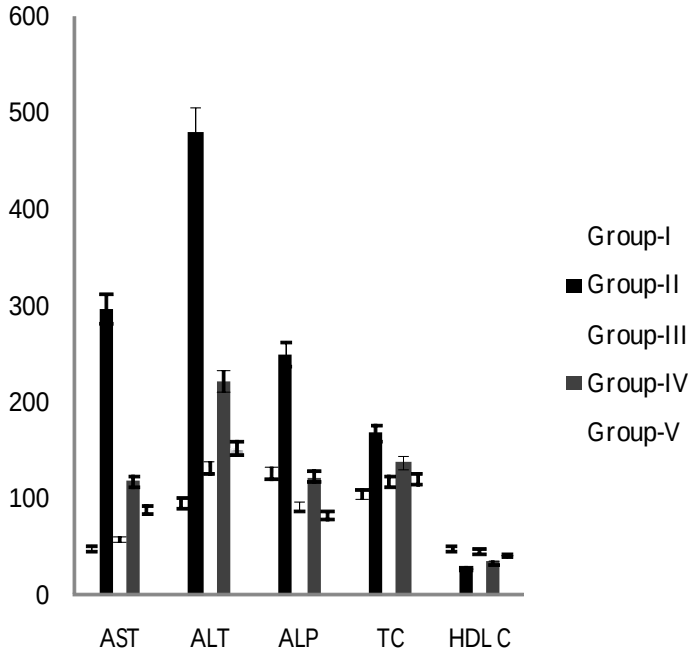


Fig.7: Effect of EuphoriaHirta Linn whole plant extract on biochemical parameters in CCl_4 induced hepatotoxicity in rats

In the case of total and direct bilirubin, a dose dependent effect of the extent of the extract observed. 400mg/kg alcoholic extract of the has reduced the elevated levels of total and direct bilirubin levels by CCl_4 is 5.242mg/dl and 2.496mg/dl to 2.543mg/dl and 1.546mg/dl respectively. The result of 400mg/kg alcoholic extract was found to be comparable with the results of 100mg/kg silymarin on the same marker enzymes. There is an increase in ALP levels observed in CCl_4 treated group (350.502U/L). The extract has shown dose dependent effect. ALP levels were restored to 91.846U/L. By 400mg/kg alcoholic extract of the whole plant which is near effect of 100mg/kg silymarin i.e,92.240U/L.

Effect of alcoholic extract of *Euphorbia hirta*whole plant on biochemical markers in paracetamol induced hepatotoxicity

Result: There is an increase in SGPT levels observed in paracetamol treated group(181.21U/L). The extract has shown a dose dependent effect. SGPT levels were restored to 76.86U/L by 400mg/kg alcoholic extract of the whole plant which is near to the effect of 100mg/kg silymarin i.e.64.57U/L.SGOT levels has been increased significantly in

paracetamol treated group i.e., 303.14U/L . 400mg/kg alcoholic extract of the whole plant reduced the elevated levels of SGOT to 035.66U/L which is near to silymarin effect 035.25U/L. In the case of total and direct bilirubin, a dose dependent effect of the extent of the extract observed. 400mg/kg alcoholic extract of has reduced the elevated levels of total and direct bilirubin levels by paracetamol2.41mg/dl and 0.68mg/dl to 0.07mg/dl and 0.308mg/dl respectively. The result of 400mg/kg extract was found to be comparible with the results of 100mg/kg silymarin on the same marker enzymes.

There is an increase in ALP levels observed in paracetamoltreated group (436.31U/L). The extract has shown dose dependent effect. ALP levels were restored to 181.66U/L by 400 mg/kg alcoholic extract of the whole plant which is near effect of 100mg/kg silymarin i.e,157.99U/L.

Table No 6: Effect of Euphoria Hirta Linn whole plant extract on biochemical parameters in paracetamol-induced hepatotoxicity in rats.

Parameter	Group-I	Group-II	Group- III	Group-IV	Group-V
	(-ve control).	(+ve control).	Silymarin 100 mg/kg.	Euphoria Hirta 200 mg/kg	Euphoria Hirta 400 mg/kg
AST (U/ml)	58.57±0.001a	181.212±0.014b	64.57±0.002c	061.25±0.011d	76.86±0.002e
ALT (U/ml)	031.72±0.001a	303.14±0.013b	035.253±0.015a	180.66±4.496c	035.66±16.63a
ALP (U/ml)	135.532±0.009a	436.31±0.02b	157.99±8.338c	286.41±0.018d	181.66±0.016e
Total bilirubin (mg/dl)	0.92±0.00a	2.41±0.001b	0.034±0.001a	0.44±0.001c	0.07±0.002a
Direct bilirubin (mg/dl)	0.25±0.00a	0.68±0.00b	0.255±0.00a	0.379±0.00c	0.308±0.00d
Cholesterol (mg/dl)	93.45±0.009a	147.83±0.012b	106.195±0.013c	116.34±0.012d	108.683±0.015c
HDL(mg/dl)	37.13±0.001a	18.45±0.011b	35.12±0.001c	23.40±0.001d	31.255±0.001e

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

AST = U/ml; ALT = U/ml; ALP = U/ml; LDH = U/ml; C=mg/dl HDL C=mg/dl

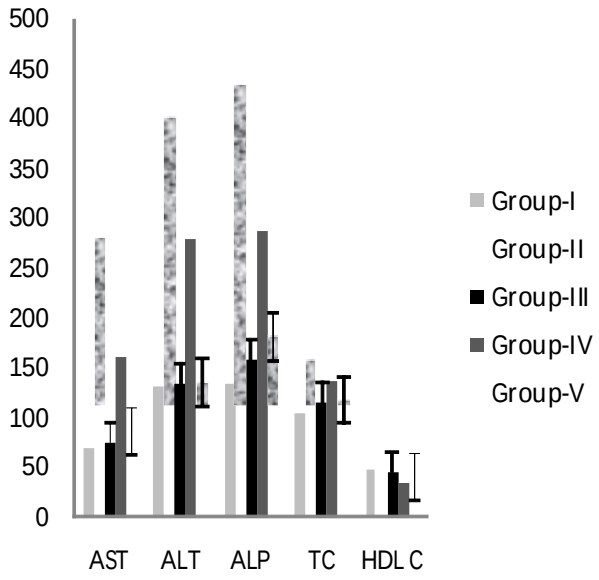


Fig.8: Effect of Euphoria hirta Linn whole plant extract on biochemical parameters inparacetamol induced hepatotoxicity in rats

Histopathological Studies in CCl_4 induced hepatotoxicity

Result:

Group A: In case of normal control (-ve control), hepatic globular structure, central Vein, portal tract and kuffer cells look normal.

Suggestive: **Normal liver** (Plate-9).

Group B: In the case of carbon tetrachloride treated group(+ve control), hepatic globular architecture was normal, hepatic cells has shown various degree of fatty degeneration like ballooning of hepatocytes, fatty cyst, infiltration of lymphocytes and proliferation of kuffer cells. Liver sinusoids were Congested .

Suggestive: **Fatty liver** (Plate- 10).

Group C: In the case of 100 mg/kg silymarin treated group the hepatic globular architecture was normal. There were occasional fatty cells and few cells have shown hyaline and cytoplasm. There were occasional areas of lymphocytic infiltration and kuffer cell proliferation.

Suggestive: **Regenerative liver or hepatic tissue** (Plate-11).

Group D: In case of 200 mg/kg alcoholic extract of *Euphorbia hirta Linn*treated group the hepatic globular architecture was normal. A few areas shown lymphocytic infiltration. Majority of hepatocytes are normal.

Suggestive: **Light regeneration of hepatocytes** (Plate- 12).

Group E: In the case of 400mg/kg alcoholic extract of *Euphorbia hirta Linn* treated group the hepatic globular architecture was maintained. A few areas of kuffer cells proliferation and sinus appear to be normal.

Suggestive: **Regeneration of hepatocytes** (Plate-13).

Histopathological Studies in Paracetamol induced hepatotoxicity

Result

Group A: In case of normal control (-ve control), hepatic globular structure, central Vein, portal tract and kuffer cells look normal.

Suggestive: **Normal liver** (Plate- 14).

Group B: In the case of paracetamol treated group(+ve control), hepatic globular architecture was normal, hepatic cells has shown various degree of fatty degeneration like ballooning of hepatocytes, fatty cyst, infiltration of lymphocytes and proliferation of kuffer cells. Liver sinusoids were Congested .Centri-lobular necrosis was observed.

Suggestive: **Fatty liver** (Plate-15).

Group C: In the case of 100 mg/kg silymarin treated group the hepatic globular architecture was normal. There were occasional fatty cells and few cells have shown hyaline and cytoplasm. There were occasional areas of lymphocytic infiltration and kuffer cell proliferation.

Suggestive: **Regenerative liver or hepatic tissue** (Plate-16).

Group D: In case of 200 mg/kg alcoholic extract of *Euphorbia hirta Linn.* Treated group the hepatic globular architecture was normal. A few areas shown lymphocytic infiltration. Majority of hepatocytes are normal.

Suggestive: **Light regeneration of hepatocytes** (Plate-17)

Group E: In the case of 400 mg/kg alcoholic extract of *Euphorbia hirta Linn* Treated group the hepatic globular architecture was maintained.

A few areas of kuffer cells proliferation and sinus appear to be normal.

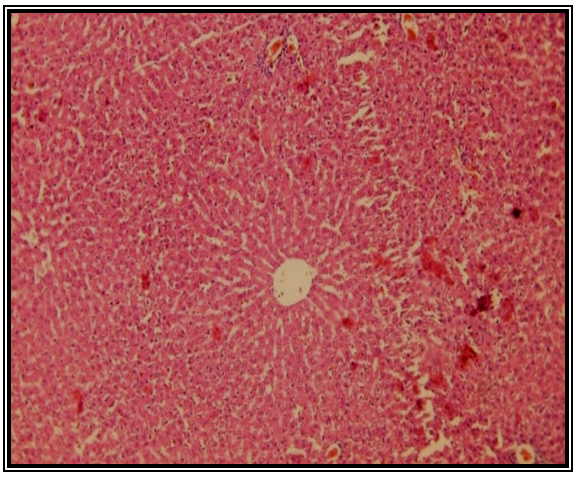


Plate- 9: Liver architecture of normal control

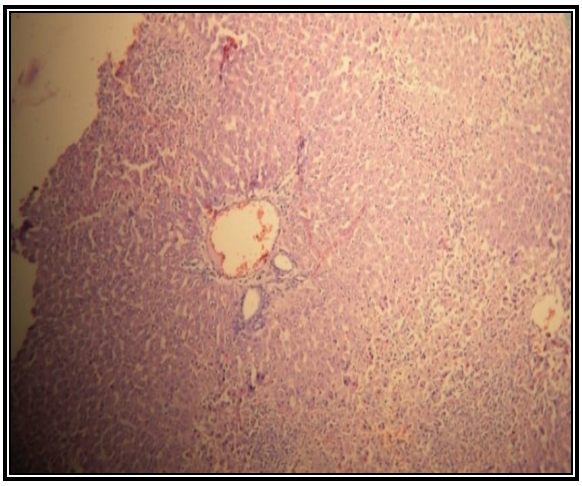


Plate-10 : Liver architecture of CCL_4 treatment

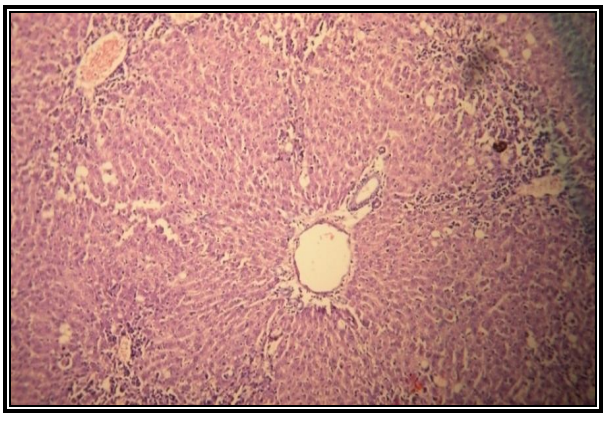
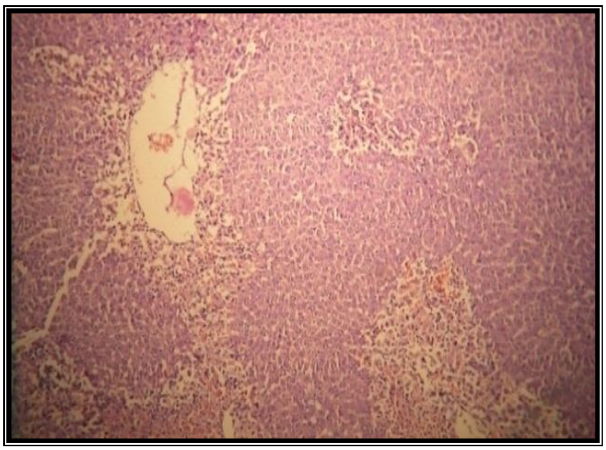


Plate- 11 : Liver architecture of CCL_4 +100mg/kg Silymarin



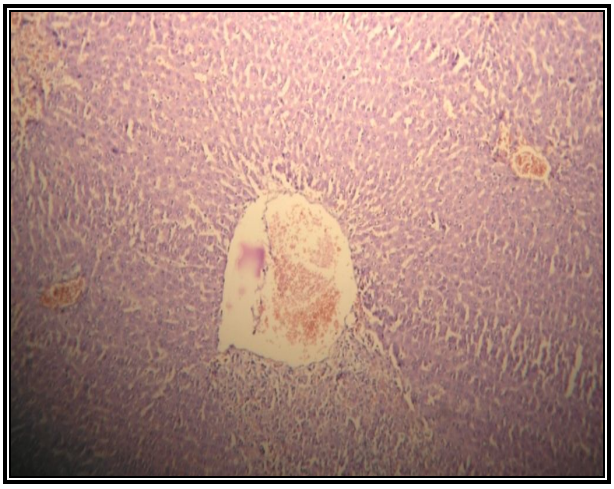


Plate- 13 : Liver architecture of CCL_4 +400mg/kg alcoholic extract of Euphorbia hirta whole plant.

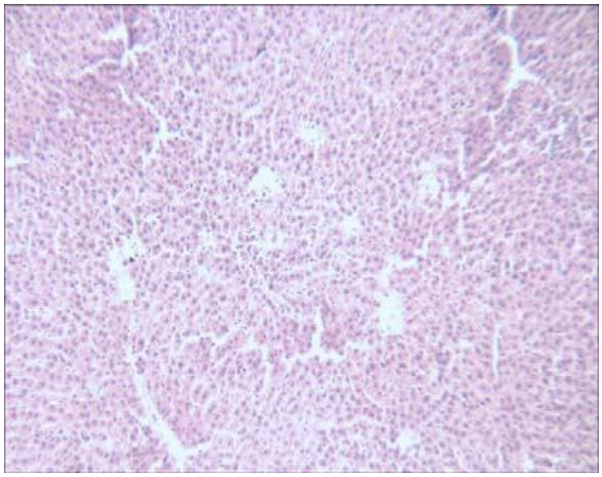


Plate- 14: Liver architecture of normal control

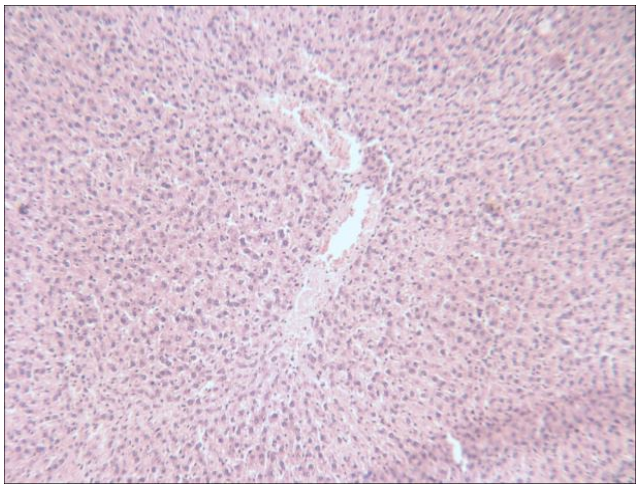


Plate- 15: liver architecture of Paracetamol (PCM) treatment

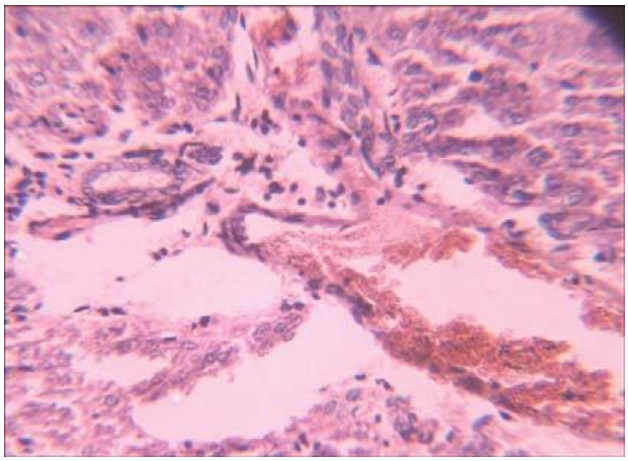


Plate-16 : Liver architecture of PCM+100mg/kg Silymarin treatment

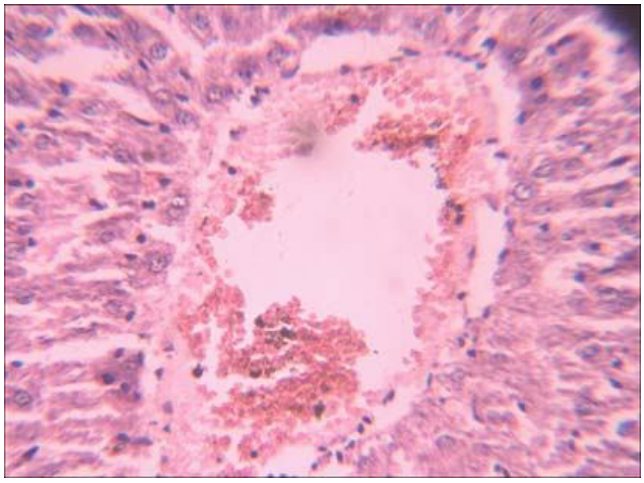


Plate- 17: Liver architecture of PCM+200mg/kg alcoholic extract of Euphorbia hirta whole plant.

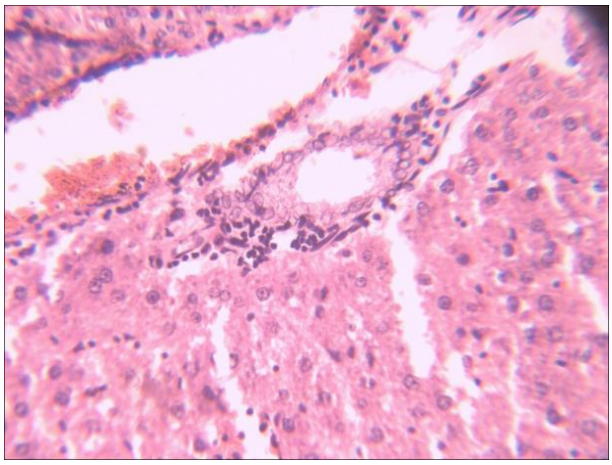


Plate- 18 : Liver architecture of PCM+400mg/kg alcoholic extract of Euphorbia hirta whole plant.

DISCUSSION

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.

Inspite 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 hirta*whole 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 hirta* were prepared by using successive soxhlet procedure. They are subjected to preliminary phytochemical 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.

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 that2000mg/kg was cut off dose and $1/10^{th}$ (200mg/kg) and $1/5^{th}$(400mg/kg) of cut off dose were selected for further studies.

Hepatic injury induced by CCL_4 is the best characterized system of the xenobiotics induced hepatotoxicity and paracetamol induced hepatotoxicity is another commonly used model for screening hepatoprotectivity of drugs[86,95] In the present study these two models were adopted for screening the hepatoprotectivity of whole plant of*Euphorbia hirta.*

It is evident from the results that pretreatment alcoholic extract of *Euphorbia hirta*whole plant has significantly reduced the elevated levels of SGPT, SGOT, ALT and bilirubin (total and direct). Similarly there was a reduction inlipid peroxidation and increase in GSH levels in the treated groups when compared with +vecontrol group. Elevated levels of cholesterol and decreased levels of HDL are brought back to near normal levels.

This clarity indicated that the alcoholic extract of *Euphorbia hirta*whole plant possess hepatoprotective activity. The photographic plate of histopathological studies further supported this and there is a dose dependent enhancement in the regeneration of hepatocytes.

The present study indicate that alcoholic extract of *Euphorbia hirta*whole plant probably protects the liver by scavenging the highly reactive Trichloromethyl and Trichloromethylperoxy radical, increasing the GSH levels and decrease the lipid peroxidation. However , further studies are needed to confirm and to out the possibility of involvement of cytochrome P450 enzyme inhibition.

The result of +ve control of paracetamol (2gm/kg p.o) are in conformity of reported results i.e elevated levels of marker enzymes (SGPT, SGOT, ALP) and depletion of GSH levels, decrease in HDL levels and increase in cholesterol and bilirubin levels. Histopathological

observation also demonstrated fatty degeneration , proliferation of kuffer cells , centrolobular necrosis , congestion of liver sinusoids, which are indicative of fatty liver . In this model elevation of levels of SGOT was more pronounced when compared to SGPT. Treatment with alcoholic extract of *Euphorbia hirta*whole plant has brought backthealterd biochemical markers like SGPT,SGOT, ALP,cholesterol and bilirubin levels,GSH levels near normal levels.

Even Histopathological studies have shown similar type improvement in the anatomy of liver.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

There was an elevation of liver tissue GSH levels with treatment of alcoholic extract of *Euphorbia hirta*whole plant possess Reducing power, Superoxide anion scavenging activity, Hydroxyl radical scavenging activity, Nitric oxide radical scavenging activity. These results are indicating that hepatoprotective activity of alcoholic extract of *Euphorbia hirta*whole plant against paracetamol may be due to scavenging of NAPQI free radical and other free radicals and increasing the levels of GSH.

However, higher dose i.e 400mg/kg has demonstrated hepatoprotective activity comparable to that of dose of silymarin 100mg/kg. this may be due to the fact that silymarin is an isolated component, whereas extract may contain lesser quantity of activity principle.However, our study has demonstrated that alcoholic extract of *Euphorbia hirta*whole plant possess hepatoprotective property against CCL_4 and paracetamol induced hepatotoxicity in rats. In addition they also possess antioxidant property.

CONCLUSIONS

- 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.
- Alcoholic extract of *Euphorbia hirta*whole plant increased the hepatic tissue content of GSH.
- Treatment with alcoholic extract of *Euphorbia hirta*whole plant has reduced the elevated serum levels of SGPT, SGOT,ALP, cholesterol, bilirubin(both direct and free) in both

CCL_4 and paracetamol induced hepatotoxicity in rats and reduced HDL levels are increased.

- 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.
- Our study finally justifies the claim of the native practitioner.
- This locally available herb can be used to treat the hepatic disorders.

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