

# Evaluation of hepatoprotective activity of *Elaeocarpus ganitrus* leaf extract against CCl<sub>4</sub> induced liver damage

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## ABSTRACT

The extract of *Elaeocarpus ganitrus* was screened for its hepatoprotective activity in carbon tetrachloride induced liver damage in Wister albino rats. The extracts at dose of 250, 750 mg/kg were administered orally once daily. The substantially elevated serum enzymatic levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), total bilirubin, SOD and catalase were restored towards normalization significantly by the extracts. Silymarin was used as standard reference and exhibited significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity in rats. The results of this study strongly indicate that *Elaeocarpus ganitrus* have potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats.

**Keywords:** Serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, catalase, serum alkaline phosphatase.

## INTRODUCTION

Liver has a prominent role in the regulation of physiological processes. It is involved in varieties of vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. Hence liver diseases are among the most serious health ailments. They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon-tetrachloride, paracetamol, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections and autoimmune disorder. So it has become very much necessary to protect the liver from all these agents (Solomon Raju B *et.al*, 2011).

*Elaeocarpus ganitrus* is the king of herbal medicines which belongs to family Elaeocarpaceae. It is commonly known as Rudraksha and is popularly used in Ayurveda, Siddha and Unani system of medicine. It is a large evergreen tree which is found in Himalayan region and also in area of gangetic plain (Prabha Rashmi and Kaur Amrinder, 2014).

Biological studies that are undertaken show that *Elaeocarpus ganitrus* fruits contain glycosides, steroids, alkaloids and flavonoids. Apart from this, it has been found that the exocarp of the fruit supplies a nutritious reward, because it is particularly rich in carbohydrates (0.58 g per fruit) and proteins (0.12g per fruit), but lacking lipids. The leaves of *Elaeocarpus ganitrus* contain quercetin, Gallic and ellagic acids. Constituents such as elaeocarpiline, isoelaecarpiline, epielaecarpiline have also been reported. A minor non aromatic alkaloid, rudrakine was also found in the leaves of *Elaeocarpus ganitrus* Roxb which show the presence of Alkaloids, Glycosides, flavonoids, tannins and carbohydrates.

Traditionally the fruit of *Elaeocarpus ganitrus* used to treat various ailments. The flesh or pulp of drupe is green and fresh state which is sour in taste that stimulates appetite and is given in epilepsy, diseases of the head and mental illness. The fruit stone (seed kernel) is sweet, cooling and emollient. Externally the stone (fruit or drupe) is rubbed with water (like sandalwood) and then it is applied to small-pox eruptions. Similarly, it is applied on organs having burning sensation and in other conditions i.e. eruptions, measles and fevers (Balbir singh *et.al*, 2013). *Elaeocarpus ganitrus* is studied for various activities that includes antimicrobial, antidepressant, antihypertensive, immunomodulatory, antidiabetic, antioxidant, ameliorative. The present study was undertaken to find out the possible actions of *Elaeocarpus ganitrus* leaf extract for its hepatoprotective activity.

## MATERIALS AND METHODS

**Plant Collection:** Leaves of *Elaeocarpus ganitrus* was collected from G. mamidada, East Godavari district, Andhra Pradesh, INDIA, during the months of march and april - 2014. The collected leaves were separated from the stems and shade dried for about 40-50 days and then powdered using a mixer and then the powder is stored in an air tight container.

The collected sample of EGA was authenticated by Dr. K Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi.

**Extraction:** The prepared powder of EGA of about 85g was extracted using 850 ml of ethanol by subjecting to cold maceration. This is done by taking the powder into different conical flasks and then by adding the required volume of ethanol to it and placing it in the orbital shaker for about week days to remain the drug in contact with the solvent. Then after 7 days the conical flasks are removed from the orbital shaker and are subjected to decantation using separating funnel. Finally the extract is collected by evaporating the solvent using water bath where the temperature is maintained for about 30-40°C. The resulted extract weight is of 19.5g and it is sticky in nature (Sathish Kumar T *et.al*, 2008).

**Animals:** Male albino wistar rats weighing about 200-250g were selected for the study. They were purchased from Mahaveer enterprises, Hyderabad. The animals were habituated for about 10 days under experimental lab conditions. They were kept in polypropylene cages and are maintained at 27°C. They were fed with normal rat feed and water.

**Permission from Ethic Committee:** The permission from the animal ethic committee was obtained through our institution in the month of July - 2014 for the use of animals and were maintained as per standard guidelines of the CPCSEA.

**Experimental design:** Animals were divided into 5 groups each consisting of 6 animals. The animals were fasted for 24 h prior to carbon tetrachloride treatment.

**Group I:** Control animals receive normal saline (2ml/kg p.o) throughout the experiment. These are treated as negative control.

**Group II:** Animals receive CCl<sub>4</sub> of about (1ml/kg) by subcutaneous route. These are treated as positive control.

**Group III:** As reference standard group received silymarin 25 mg/kg, p.o and simultaneously administered CCl<sub>4</sub>

**Group IV:** Received EGA of 250mg/kg body weight (bw) per oral and simultaneously administered CCl<sub>4</sub>

**Group V:** Received EGA of 750 mg/kg bw p.o and simultaneously with CCl<sub>4</sub>

Animals were treated as shown above for a period of 10 days. At the end of every 72 hrs i.e. on 4<sup>th</sup>, 7<sup>th</sup> and 10<sup>th</sup> day CCl<sub>4</sub> (30% in liquid paraffin 1 ml/kg, s.c) was administered to all groups other than group I. Group III received standard drug silymarin (25 mg/kg p.o) once a day and CCl<sub>4</sub> as mentioned above whereas group IV and V were treated with test extract dose of (250 and 750mg/kg, p.o) respectively. During this period of treatment, the rats were maintained under normal diet and water (Kalyani B *et.al*, 2011).

Carbon tetrachloride is prepared by diluting with olive oil in the ratio of 1:1. The standard solution of silymarin was prepared by dissolving the sample of silymarin in distilled water (Prakash T *et.al*, 2008). The ethanolic extract of *Elaeocarpus ganitrus* is prepared by dissolving it in the sterile distilled water (Sathish Kumar T *et.al*, 2008).

The biochemical (SGPT, SGOT, ALP, total and direct bilirubin, total protein), antioxidant parameters (reduced glutathione, super oxide dismutase, catalase, lipid peroxidation) were determined after 24 hrs of the last dose of CCl<sub>4</sub> i.e. on 11<sup>th</sup> day by collecting the blood through tail vein under mild ether anesthesia using disposable syringe and needle. Blood was allowed to clot at room temperature for 30min and then subjected to centrifugation (3000 rpm for 15min) and finally serum is collected. Then the animals were sacrificed and the liver was dissected out and subjected for morphological study such as wet liver weight and wet liver volume. The volume of wet liver was measured by displacement method and further the livers were placed in 10% formalin solution for histopathological study.

**Statistical Analysis:** All the values are presented as Mean ± Standard Error of Mean (SEM). Statistical significance was calculated by one way ANOVA followed by post hoc Dunnett's test multiple comparison test  $p < 0.05$  was considered to be statistically significant.

## RESULTS AND DISCUSSION

The evaluation of the hepatoprotective activity of ethanolic extract of *Elaeocarpus ganitrus* started with the extraction and phytochemical screening.

**Acute toxicity studies:** The data of LD<sub>50</sub> is collected from the review where it was already proven that the extract of EGA was nontoxic upto the dose of 5.0g/kg body weight. This was studied in mice by administering different doses of EGA. In the present study the data of acute toxicity studies for the ethanolic leaf extract of *Elaeocarpus ganitrus* is followed from the review mentioned above.

### Hepatoprotective activity:

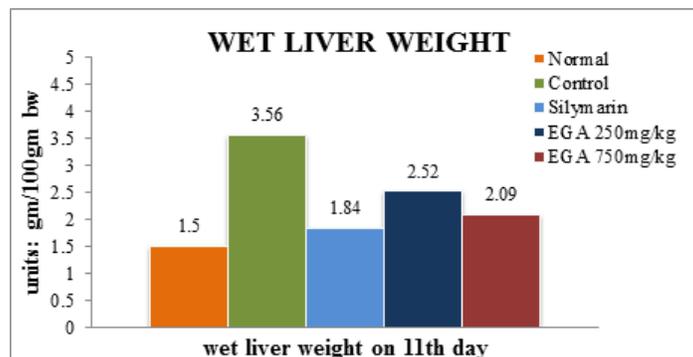
#### Physical parameters:

**a) Wet liver weight and volume:** Carbon tetra chloride (CCl<sub>4</sub>) treated rats resulted in the enlargement of liver which was an evident by increase in the wet liver weight and volume. The groups treated with silymarin and EGA showed significant restoration of wet liver weight and volume.

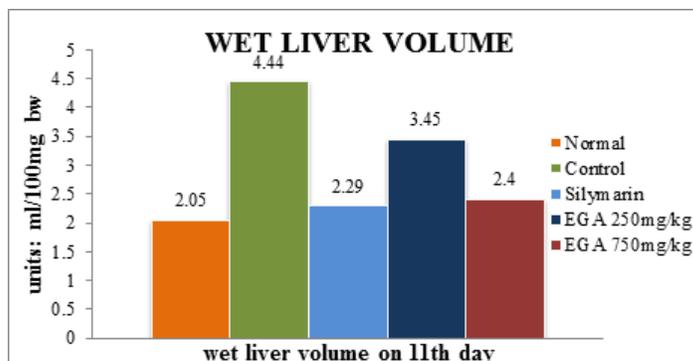
**Table 1: Effect of ethanolic extract of EGA on wet liver weight and volume**

Treated group	Wet liver weight (gm/100gm)	Wet liver volume (ml/100gm)
Normal Saline (2ml/kg)	1.50±0.40	2.05±0.40
Control (1ml/kg)	3.56±0.34 <sup>b</sup>	4.44±0.60 <sup>b</sup>
Standard (Silymarin) 25mg/kg	1.84±0.9**	2.29±0.40**
EGA (250mg/kg)	2.52±0.25*	3.45±0.10*
EGA (750 mg/kg)	2.09±0.10**	2.40±0.05**

Values are expressed as mean ± SEM and the data was analysed using one way ANOVA followed by Dunnett multiple comparison test, n=6. Here \* represents significant at p<0.05, \*\* represents very significant at p<0.01, \*\*\*p<0.001 represents highly significant as compared to control group and <sup>a</sup>p<0.001, <sup>b</sup>p<0.01, <sup>c</sup>p<0.05 as compared to the normal.



**Figure.1.Effect of ethanolic extract of EGA on wet liver weight**



**Figure.2.Effect of ethanolic extract of EGA on wet liver volume**

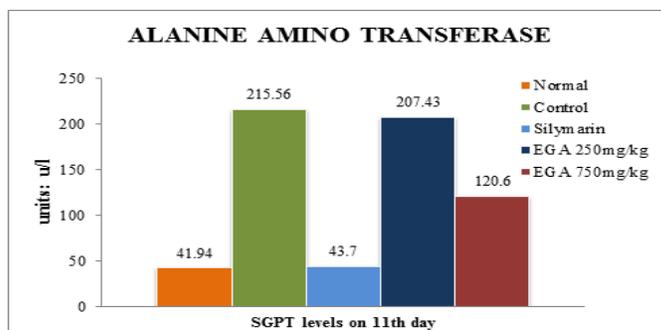
### Biochemical parameters

a) **Serum enzymes:** Rats treated with  $\text{CCl}_4$  developed significant hepatic damage which is observed by elevated serum levels of enzymes like SGPT, SGOT and ALP when compared to the normal group. Silymarin and EGA extract showed protection against  $\text{CCl}_4$  induced liver damage.

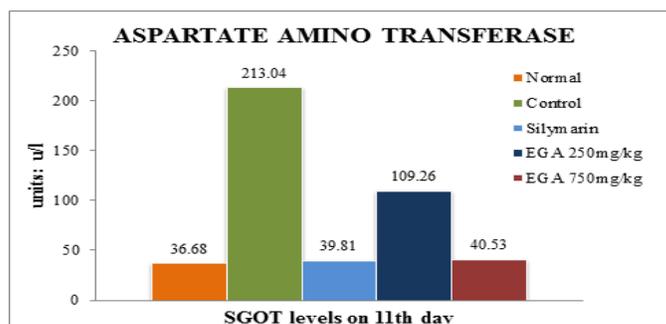
**Table.2.Effect of ethanolic extract of EGA on SGPT, SGOT, ALP**

Treated group	SGPT	SGOT	ALP
Normal Saline (2ml/kg)	41.94±1.25	36.68±0.60	105.80±0.87
CONTROL (1ml/kg)	215.56±2.45 <sup>b</sup>	213.04±2.69 <sup>b</sup>	317.43±2.89 <sup>b</sup>
Standard (Silymarin) 25mg/kg	43.70±1.08 <sup>**</sup>	39.81±1.03 <sup>**</sup>	108.40±0.42 <sup>**</sup>
EGA (250mg/kg)	207.43±0.81 <sup>*</sup>	109.26±0.7 <sup>**</sup>	308.77±0.69 <sup>*</sup>
EGA (750 mg/kg)	120.60±0.77 <sup>**</sup>	40.53±1.02 <sup>**</sup>	113.10±0.40 <sup>**</sup>

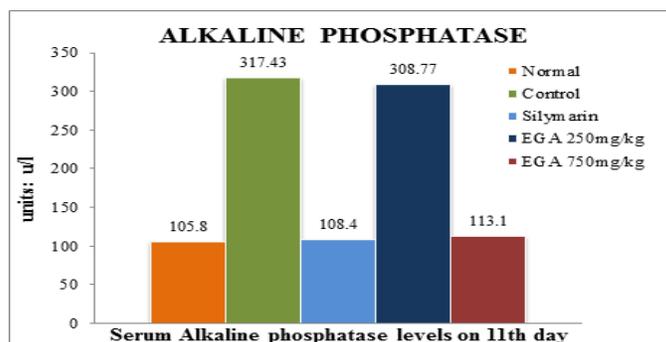
Values are expressed as mean  $\pm$  SEM and the data was analysed using one way ANOVA followed by dunnett multiple comparison test, n=6. Here \* represents significant at  $p < 0.05$ , \*\* represents very significant at  $p < 0.01$ , \*\*\* $p < 0.001$  represents highly significant as compared to control group and <sup>a</sup> $p < 0.001$ , <sup>b</sup> $p < 0.01$ , <sup>c</sup> $p < 0.05$  as compared to the normal.



**Figure.3.Effect of ethanolic extract of EGA on SGPT**



**Figure.4.Effect of ethanolic extract of EGA on SGOT**



**Figure.5.Effect of ethanolic extract of EGA on ALP**

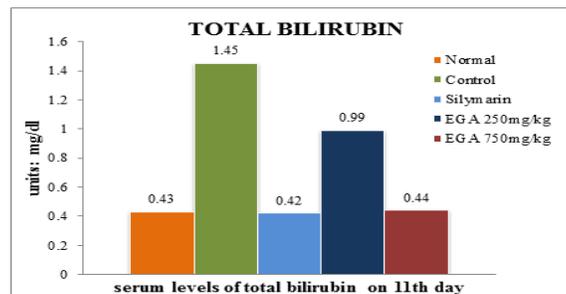
b) **Total bilirubin and total protein:** CCl<sub>4</sub> treated rats showed elevated levels of total and direct bilirubin which indicates hepatotoxicity. Silymarin and EGA extract showed protection against CCl<sub>4</sub> induced liver damage by reducing the levels of total bilirubin.

CCl<sub>4</sub> treated rats showed levels of total protein which indicates hepatotoxicity. Silymarin and EGA extract showed protection against CCl<sub>4</sub> induced liver damage by increasing the levels of total protein.

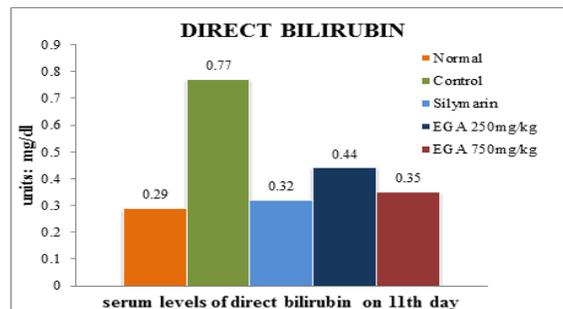
**Table.3.Effect of ethanolic extract of EGA on total, direct bilirubin and total protein**

Treated group	TOTAL BIL.	DIRECT BIL.	TOT. PROTEIN
Normal saline (2ml/kg)	0.42±0.04	0.29±0.03	9.30±0.26
Control (1ml/kg)	1.45±0.08	0.77±0.03	3.72±0.20
Standard (Silymarin) 25mg/kg	0.42±0.04**	0.32±0.04**	8.31±0.24**
EGA (250mg/kg)	0.99±0.05*	0.44±0.04**	5.36±0.32*
EGA (750 mg/kg)	0.44±0.05**	0.35±0.03**	8.09±0.33**

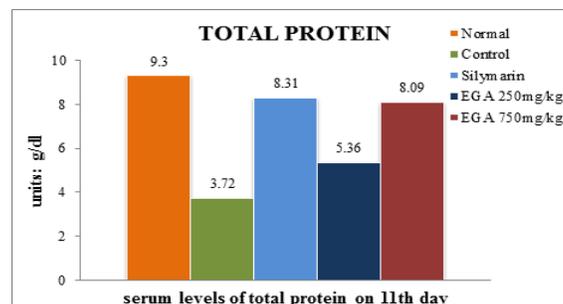
Values are expressed as mean ± SEM and the data was analysed using one way ANOVA followed by dunnett multiple comparison test, n=6. Here \* represents significant at p<0.05, \*\* represents very significant at p<0.01, \*\*\*p<0.001 represents highly significant as compared to control group and <sup>a</sup>p<0.001, <sup>b</sup>p<0.01, <sup>c</sup>p<0.05 as compared to the normal.



**Figure.6.Effect of ethanolic extract of EGA on Total bilirubin**



**Figure.7.Effect of ethanolic extract of EGA on Direct bilirubin**



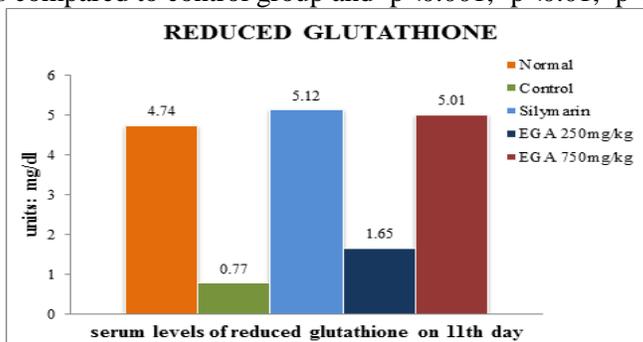
**Figure.8.Effect of ethanolic extract of EGA on Total protein**

**Antioxidant parameters:** CCl<sub>4</sub> treated rats showed decreased levels of reduced glutathione, super oxide dismutase, lipid peroxidation and catalase when compared to the normal which indicates liver damage. Silymarin and EGA extract showed protection against CCl<sub>4</sub> induced liver damage by increasing the levels of these enzymes.

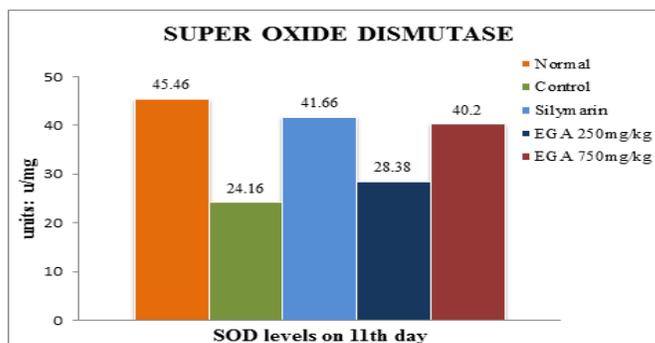
**Table.4.Effect of ethanolic extract of EGA on GSH, SOD, LPO, CAT**

Treated group	GSH	SOD	LPO	CAT
Normal Saline (2ml/kg)	4.74±0.05	45.46±0.66	0.88±0.27	72.09±0.81
Control (1ml/kg)	0.77±0.06	24.16±0.84	7.11±0.59	31.37±0.84
Standard (Silymarin) 25mg/kg	5.12±0.07**	41.66±0.37**	1.40±0.37**	68.79±0.74**
EGA (250mg/kg)	1.65±0.01*	28.38±0.7*	6.12±0.65*	35.89±0.91*
EGA (750 mg/kg)	5.01±0.02**	40.20±0.94**	2.61±0.58**	71.61±0.82**

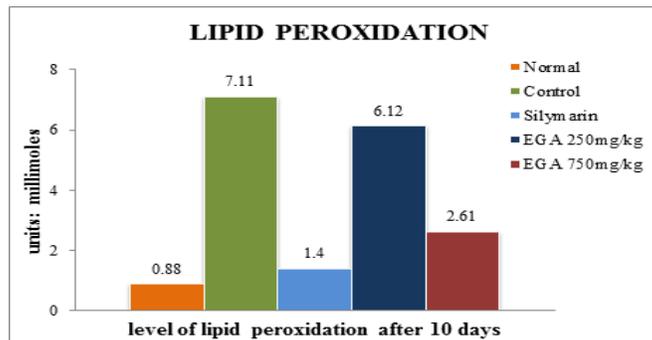
Values are expressed as mean ± SEM and the data was analysed using one way ANOVA followed by Dunnett multiple comparison test, n=6. Here \* represents significant at p<0.05, \*\* represents very significant at p<0.01, \*\*\*p<0.001 represents highly significant as compared to control group and <sup>a</sup>p<0.001, <sup>b</sup>p<0.01, <sup>c</sup>p<0.05 as compared to the normal.



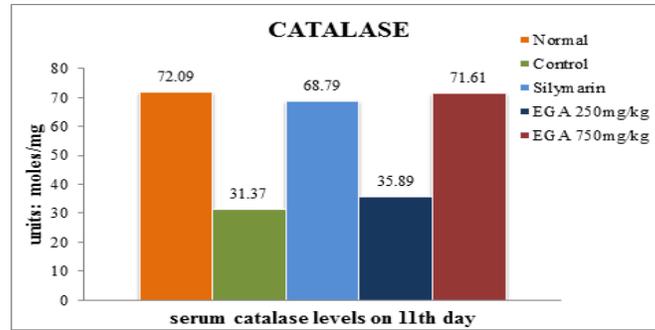
**Figure.9.Effect of ethanolic extract of EGA on Reduced glutathione**



**Figure.10.Effect of ethanolic extract of EGA on Super oxide dismutase**



**Figure.11.Effect of ethanolic extract of EGA on Lipid peroxidation**



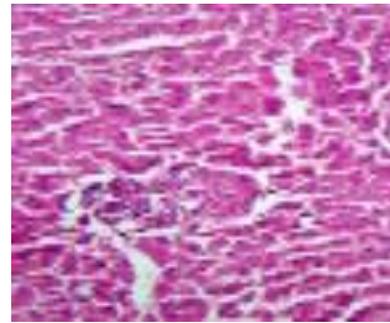
**Figure.12.Effect of ethanolic extract of EGA on Catalase**

### Histopathological studies:

- a. Normal control group:** The architecture of liver parenchyma appeared intact.
- b. Toxicant group:** The architecture of liver parenchyma appeared partly affected. There were seen degenerating hepatocytes in the middle of the normal hepatocytes.
- c. Silymarin:** The architecture of liver parenchyma appeared intact. Focal areas showed degenerating and regenerating hepatocytes.
- d. EGA extract (750mg/kg):** The architecture of liver parenchyma appeared partly intact. There were seen some regenerating hepatocytes in the middle of normal hepatocytes.



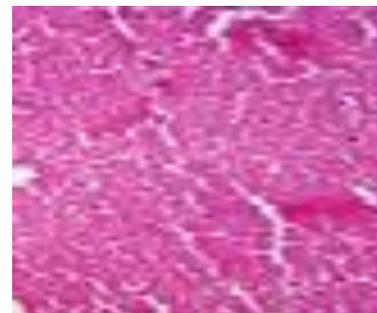
**Figure.13.Light micro graphs of tissue section from rat liver after 11 days showing the eosin and hematoxylin stained hepatocytes of normal group in magnification of 200X**



**Figure.14.Light micro graphs of tissue section from rat liver after 11 days showing the eosin and hematoxylin stained hepatocytes of toxicant group in magnification of 200X**



**Figure.15.Light micro graphs of tissue section from rat liver after 11 days showing the eosin and hematoxylin stained hepatocytes of standard (silymarin) group in magnification of 200X**



**Figure.16.Light micro graphs of tissue section from rat liver after 11 days showing the eosin and hematoxylin stained hepatocytes of extract (750mg/kg) group in magnification of 200X**

$\text{CCl}_4$  is a xenobiotic that produces hepatotoxicity in various experimental animals.  $\text{CCl}_4$  is metabolized by cytochrome  $\text{P}_{450}$  to form a reactive trichloromethyl radical ( $\text{CCl}_3$ ) and a trichloromethyl peroxy radical ( $\text{CCl}_3\text{O}_2$ ). Both radicals are capable of binding to DNA, lipids, proteins or carbohydrates, leading to lipid peroxidation, cell necrosis.

When liver plasma membrane is damaged variety of enzymes found in the cytoplasm were released into the circulatory system which results in elevated levels of such enzymes in serum.  $\text{CCl}_4$  when injected into the animals there is a significant increase in all these enzymes. The estimation of these enzymes in the serum is an important biomarker to find the extent and type of liver damage.

The total protein levels decreased due to the hepatotoxin intoxication. In the present study,  $\text{CCl}_4$  intoxication reduced the serum total protein levels. The pretreatment of EGA restored the total protein levels as mentioned in table no: 3. The rise in protein level suggests the stabilization of endoplasmic reticulum leading to protein synthesis.

The liver marker enzymes (AST, ALT and ALP) are cytoplasmic in nature; upon liver injury these enzymes enter into the circulatory system due to altered permeability of membrane. In this study, significant increase in AST and ALT levels in the serum is observed after administration of  $\text{CCl}_4$ . ALP level also increased after  $\text{CCl}_4$  administration. The increased levels of these enzymes significantly decreased by pretreatment with EGA extract as according to the data mentioned in table no: 2. Reduction in the levels of AST, ALT and ALP towards the normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by  $\text{CCl}_4$ .

Thus, administration of  $\text{CCl}_4$  elevates the serum levels of SGOT, SGPT, ALP and bilirubin (Total and direct) significantly due to its enzymatic activation of  $\text{CCl}_3$  free radical, which in turn alters the structure and function of liver cells.

Many studies have demonstrated that the hepatoprotective effect of plant extracts may be related to its antioxidant capacity to scavenge reactive oxygen species.  $\text{CCl}_4$  intoxication reduced the total protein level in liver homogenate, which restored significantly with the pretreatment of EGA.

Liver cells possess antioxidant defence system consisting of antioxidants such as GSH and antioxidant enzymes such as catalase and SOD to protect own cells against oxidative stress, which causes destruction of cell components and cell death. In the present study,  $\text{CCl}_4$  intoxication reduced the level of GSH, which was significantly restored in EGA treated (higher dose) rats.

Increase in the level of lipid peroxidation in liver reflects hepatocellular damage. In the present study,  $\text{CCl}_4$  induced liver damage is associated with the increased levels of lipid peroxidation which is significantly reduced in case of EGA treated (higher dose) rats.

Trichloromethyl peroxy radical, the metabolic product of  $\text{CCl}_4$  binds covalently to the macromolecules and causes peroxidative degradation of cellular membrane leading to the necrosis of hepatocytes. In the present study, the hepatic antioxidant enzymatic activity of catalase and SOD significantly decreased in  $\text{CCl}_4$  intoxicated rats as compared with normal rats. The decreased enzymatic activity would result in cell injury. The catalase and SOD levels are elevated by administration of EGA to  $\text{CCl}_4$  intoxicated rats suggesting that it has the ability to restore the enzyme activity towards normalization in  $\text{CCl}_4$  damaged liver.

In  $\text{CCl}_4$  treated animals, there is severe damage to the hepatic cells and also disturbance in the architecture of the liver. In the present study, the animals treated with the EGA extract exhibited reduced hepatic damage and reduced disturbance in the architecture of the liver indicating that it has the hepatoprotective activity.

Finally, depending upon the improvement of physical, biochemical, antioxidant parameters and histopathological studies, it is said that the ethanolic leaf extract of *Elaeocarpus ganitrus* has hepatoprotective activity and thus supports the traditional application of the same under the modern science.

## CONCLUSION

In view of the findings of the study it can be concluded that ethanolic leaf extract of *Elaeocarpus ganitrus* possesses significant hepatoprotective effect against  $\text{CCl}_4$ -induced hepatotoxicity.

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