

# Evaluation of Hepatoprotective Activity of Ethanolic Extract of *Crotalaria Pallida* in Paracetamol Induced Toxicity

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## Abstract:

The present study was designed to evaluate the possible protective effect of ethanolic extract of *Crotalaria pallida* Linn (EECP) against paracetamol induced hepatotoxicity in animals. The detailed preliminary phytochemical investigations rationalized its use as a drug of therapeutic importance. The ethanolic extract of the plant has phytoconstituents like flavonoids, terpenoids, sterols, alkaloids, saponins and tannins. The hepatoprotective effect was assessed using a battery of biochemical and histopathological tests. SGOT, SGPT, ALP, LDH, ACP were some of the biochemical tests done. In vivo tests for antioxidants (SOD, CAT, GSH, LPO) were conducted on albino mice and wistar rats. Ethanolic extract has hepatoprotective effects against liver toxicity induced by paracetamol as proven by macroscopical, microscopical, and biochemical analyses. The effects of EECP are comparable to that of Silymarin, the standard hepatoprotective drug. Accordingly, EECP could be used as an effective herbal product for the prevention of chemical-induced hepatic damage. Our results demonstrated that the progression of paracetamol-induced liver cirrhosis could be prevented or reduced using the ethanol extract of *Crotalaria pallida*. The plant extract exerted its hepatoprotective effect by preventing the harmful cascade of events induced. In conclusion, we can say that *Crotalaria pallida* has the ability to protect the liver from the damaging effects of paracetamol in toxic doses and stimulation of endogenous anti-oxidant defense system.

**Key words:** Hepatoprotective Activity, Ethanolic Extract, *Crotalaria Pallida*, Paracetamol

## INTRODUCTION

The liver is one of the most important organs of the body. It performs a fundamental role in the regulation of diverse physiological processes, and its activity is related to different vital functions, such as metabolism, secretion, and storage. Its capacity to detoxify endogenous (waste metabolites) and/or exogenous (toxic compounds) substances of organisms, as well as for synthesize useful agents, has been analyzed since the 1970s by many researchers[1-4]. The liver is also involved in the biochemical processes of growing, providing nutrients, supplying energy, and reproducing. In addition, it aids in the metabolism of carbohydrates and fats, in the secretion of bile, and in the storage of vitamins[5]. Because of all of these functions, hepatic diseases continue to among the principal threats to public health, and they are a problem worldwide[4,6]. Hepatic disease is a term that indicates damage to the cells, tissues, structure, or liver function, and this damage can be induced by biological factors (bacteria, virus, and parasites) and autoimmune diseases (immune hepatitis, primary biliary cirrhosis), as well as by the action of different chemicals, such as some drugs [high doses of paracetamol (PCM) and antitubercular drugs], toxic compounds [carbon tetrachloride (CCl<sub>4</sub>), thioacetamide, dimethylnitrosamine (DMN), D-galactosamine/lipopolysaccharide (GalN/LPS)], and unquestionably, excessive consumption of alcohol[7-9]. Despite enormous advances in modern medicine, there are no completely effective drugs that stimulate hepatic function, offer complete protection to the organ, or aid in regenerating hepatic cells[10]. Additionally, some drugs can induce adverse or side effects. Thus, it is necessary to identify alternative pharmaceuticals for the treatment of hepatic diseases, with the aim of these agents being more effective and

less toxic. The use of some plants and the consumption of different fruits have played fundamental roles in human health care. Approximately 80% of the world's population has employed traditional medicine for health care, which is based predominantly on plant materials[4,9]. Diverse scientific investigations of medicinal plants and the ingestion of fruits have indicated that the properties that are responsible for their beneficial effects could be attributed to the presence of chemical compounds or substances that are biologically active and that are non-essential nutrients for life, called phytochemicals[11]. *Crotalaria pallida* is a terrestrial herbaceous plant, annual, erect, up to 2 m high. Taproot white or brown. Stem fluted, solid, glabrous. Stipules present. Leaves trifoliate, alternate spiral, stalked; elliptic or obovate leaflets, more than 2 cm long / wide, hairy upper surface, margin entire, apex obtuse or rounded, pointed base, pinnately veined. Flowers hermaphrodites, grouped in terminal raceme, stalked, with 5 yellow petals. The fruit is a rounded pod.

The aim of the study evaluation of hepatoprotective activity of ethanolic extract of *Crotalaria Pallida* in paracetamol induced toxicity

## MATERIALS AND METHODS

### COLLECTION AND AUTHENTICATION OF PLANT MATERIALS

Fresh leaves of *Crotalaria pallida* L. were collected locally from Chittoor District, AP, India and authenticated by Prof. Madhav Shetty, Dept. of botany, Taxonomist, SV University, Tirupati. A voucher was kept in the Department of Pharmacognosy for reference. Leaves were separated from adulterants, shade dried and powdered coarsely. Paracetamol drug (Sun Pharmaceuticals Ltd) purchased from local medical store. All reagents procured were analytical grade.

### EXTRACTION OF PLANT MATERIALS

The air dried powdered material (100 g) was taken in 1000 ml Soxhlet apparatus (figure 12) and extracted with petroleum ether for 7 days to remove fatty material. At the end of 7<sup>th</sup> day the marc was taken out and it was dried and again subjected to extraction with absolute ethanol until the colour disappeared. Then the extract was concentrated by distillation. The final solution was evaporated to remove excess of remaining ethanol. Finally the colour consistency of ethanolic extract was noted.

### PHYTOCHEMICAL EVALUATION

#### QUALITATIVE ANALYSIS

Preliminary phytochemical analysis

The major contributions of phytochemical studies of plant physiology are in determining the chemical structure and characterization of chemical compounds. In identifying a plant constituent, isolation of the constituent and it is necessary first to determine the class of compound and then to identify a particular substance within that class. The class of compounds are usually clear from its response to color tests, solubility,  $R_f$  properties and UV spectral characteristics. However, equally informative data on a plant substance will be obtained from its special characteristics.

Preliminary screening is done for analysis of secondary metabolites. The phytochemical screening of the leaf extract was carried out.

### INDUCTION OF HEPATOTOXICITY, FREQUENCY AND DURATION OF TREATMENT

#### Pretreatment Group

All pretreated groups of albino mice except **control** groups receive a daily dose of Paracetamol (3g/ Kg of body weight), Silymarin (100mg/kg) and Ethanolic Extract of *Crotalaria pallida* Linn (**EECP**) (**250 and 500mg/kg**) for 14 days.

## EXPERIMENTAL DESIGN FOR HEPATOPROTECTIVE ACTIVITY

**Group 1:** Receives (Distilled water) as control for 14 days.

**Group 2:** Receives a daily dose of Paracetamol (3g/ Kg of body weight, p.o) for 14 days (p.o)

**Group 3:** Receives a daily dose of Paracetamol (3g/ Kg of body weight) and after one hour a daily dosage of Standard Silymarin (100mg/kg) of body weight for 14 days (p.o)

**Group4:** Receives a daily dose of Paracetamol (3g/ Kg of body weight) and one hour a daily dosage of EECP 250mg / Kg of body weight for 14 days (p.o)

**Group 5:** Receives a daily dose of Paracetamol (3g/ Kg of body weight) and one hour a daily dosage of EECP 500mg / Kg of body weight for 14 days (p.o)

### Sample collection

At the end of the 14<sup>th</sup> day treatment, samples of blood were withdrawn from the orbital sinus of mice from each group, under light ether anesthesia after fasting for 16 hours. The collected blood samples also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, ALP and BILIRUBIN. After separation of serum for biochemical estimation, the mice were sacrificed, liver of mice were isolated and washed with normal saline and stored for 12 h for in vivo antioxidant studies.

### Measurement of Body weight

The body weight of the animals was monitored daily by weighing on an electrical balance with accuracy to  $\pm 0.1$  g. All measurements were made every day between 8.30 and 9.15 h, immediately before administration of the distilled water in the case of control and the drugs paracetamol

### Preparation of tissue homogenate

The separated liver was homogenized with motor driven Teflon coated homogenizer with 0.1 M Tris-HCl buffer (pH 7.4) to get 10% homogenate. The homogenate was centrifuged at 10000 rpm for 10 min at 5°C. The supernatant was collected and used Antioxidant enzymes viz. Superoxide dismutase (SOD), Catalase (CAT), Glutathioneperoxidase (GPX), Reduced glutathione (GSH) and Lipid peroxidation (LPO) were determined in all the liver tissues of all the tested mice.

## RESULTS

### PHYTOCHEMICAL STUDIES

#### *Extractive value and percentage yield of EECP*

The nature of the extract obtained following cold maceration with 90% v/v of ethanol and percentage yield of leaves of EECP is shown in Table 2.

**Table 1. Percentage yield and nature of EECP**

Plant / Extract	Nature of the extract	Extraction Yield (% w/w)
Ethanol extract of whole plant <i>Crotalaria pallida</i> L.	Dark green semisolid	8.34 %

### *Preliminary phytochemical screening of plant extract*

The ethanolic extract of *Crotalaria pallida* L whole plant was analyzed for the presence of flavonoids, amino acids, tannins, steroids, glycosides and reducing sugars, etc., according to standard methods of Harborne et al., (2005); Kasture et al., (2003) and Gurudeep et al., (2003).

**Table 2. Preliminary phytochemical analysis of *Crotalaria pallida* Linn**

Phytoconstituents	Ethanol Extract
Reducing sugars	+
Glycosides	-
Alkaloids	+
Steroids	+
Flavonoids	+
Proteins	-
Amino Acids	+
Tannins	+
Fixed oils & fats	+
Gum & mucilage	-
Saponins	+

+ Present; - Absent

### *Phytochemical studies*

Ethanol extract of whole plant of *Crotalaria pallida* was found to be 8.34% w/w. The qualitative analysis of the EECF reveals that the presence of flavonoids, reducing sugars, alkaloids, steroids, tannins, saponins, triterpenoids, & amino acids.

Hepatotoxicity is a common side effect of various drugs and xenobiotics. Paracetamol is a NSAID which is harmless in normal therapeutic doses and causes liver toxicity in high doses in humans.

### **BODY WEIGHT**

**Table 3. Effect of ETHANOLIC EXTRACT OF *Crotalaria pallida* L. on Body Weight(Physical Parameter) Analysis on Paracetamol Induced Hepatotoxicity:**

GROUP	INITIAL BODYWEIGHT	FINAL BODY WEIGHT
CONTROL	23.67±0.236	37.333±1.429

ONLY PARA	33.83±0.543	16.166±5.179*
PARA + STD	28.00±0.837	16.166±7.231
PARA + L.D	29.50±0.671	16.000±7.197
PARA + H.D	31.83±0.910	22.333±7.214

Values are expressed as the mean  $\pm$  S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ns- no significant \*P < 0.001, \*\*P < 0.01, \*\*\*P < 0.05 calculate by comparing treated group with control group.

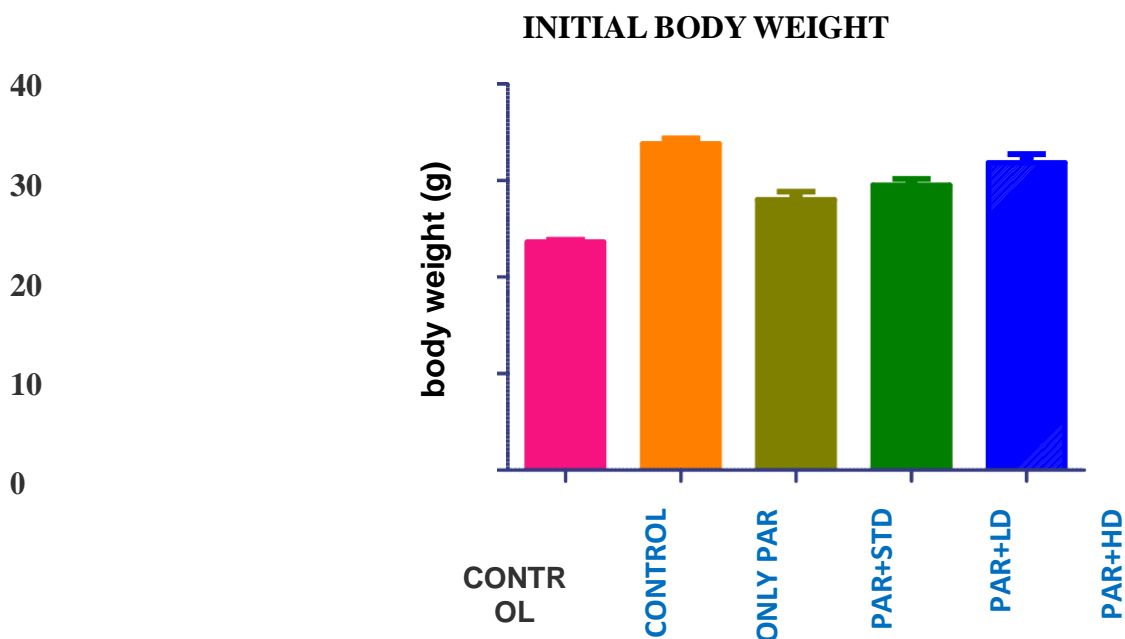
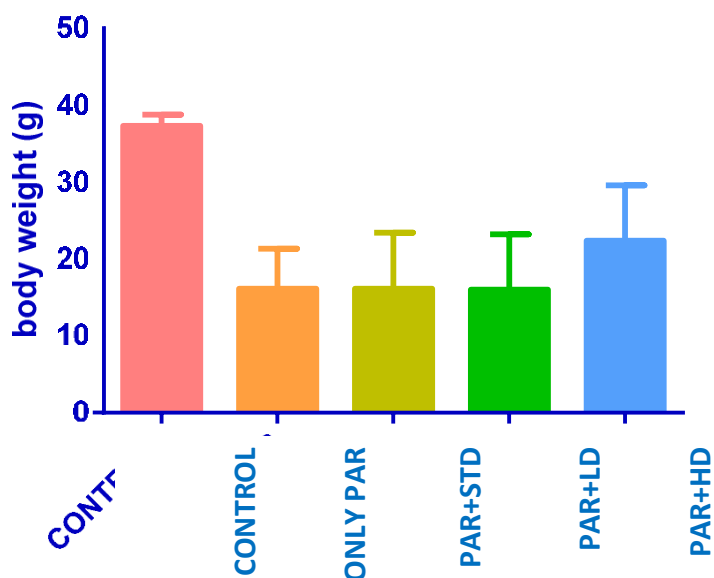


Figure 1: Effect of EECp on Body weight of albino mice

### FINAL BODY WEIGHT



Liver damage is caused when paracetamol is administered to albino mice. This is manifested in a lower

body weight. The body weight of normal mice showed significantly increased in mice following paracetamol treatment ( $16.166 \pm 5.179$ ). In EECF treated mice at the doses of 250 and 500mg/kg treated mice, the final body weights became ( $16.000 \pm 7.197$ ) and ( $22.333 \pm 7.214$ ) respectively. However, administration of paracetamol with standard silymarin and the low dose and high dose EECF significantly reduced the relative body weight.

## LIVER WEIGHT

### Effect of ETHANOLIC EXTRACT OF *Crotalaria pallida* L. on Liver Weight in Paracetamol Induced Hepatotoxicity:

Table 4.

GROUP	LIVER WEIGHT
CONTROL	$0.721 \pm 0.236$
ONLY PARA	$1.522 \pm 0.524$
PARA + STD	$1.033 \pm 0.463$
PARA + L.D	$0.857 \pm 0.385^{**}$
PARA + H.D	$1.188 \pm 0.398^{*}$

Values are expressed as the mean  $\pm$  S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnet's ns- no significant  $^{*}P < 0.001$ ,  $^{**}P < 0.01$ ,  $^{***}P < 0.05$  calculate by comparing treated group with CONTROL group.

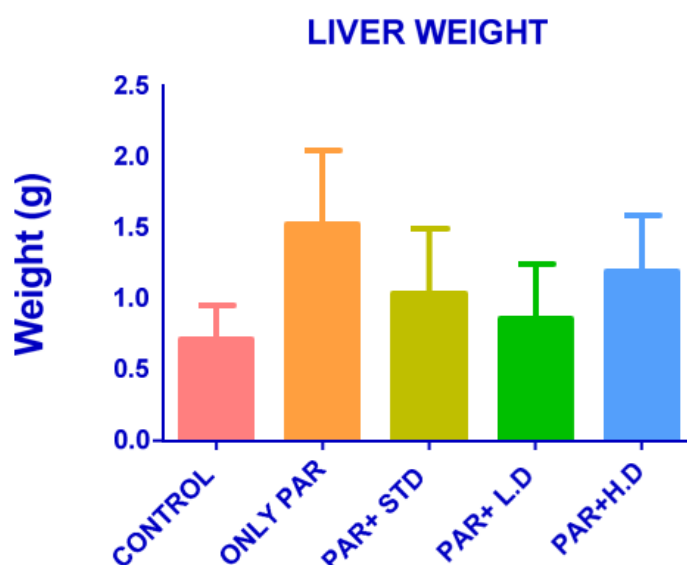


Figure 2: Effect of EECF on the liver weight of albino mice

The relative liver weight of the Acetaminophen treated groups shows an increase in relative liver weight when compared to the control group. A further significant increase in relative liver weight is shown in the Silymarin + Acetaminophen treated group ( $1.033 \pm 0.463$ ) when compared to the control group. The EECF treated groups clearly shows a decrease in weight for low dose 250mg/kg ( $0.857 \pm 0.385$ ) and a medium

increase in relative liver weight at 500mg/kg ( $1.188 \pm 0.398$ ) which is the higher dose (HD) administered.

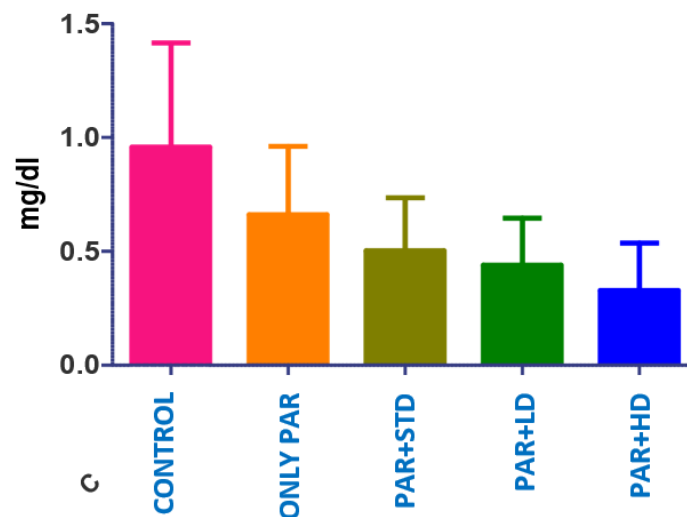
**EFFECT OF ETHANOLIC EXTRACT OF *Bauhinia tomentosa* Linn on TOTAL BILIRUBIN, DIRECT BILIRUBIN**

**Table 5.**

GROUP	TOTAL BILIRUBIN	DIRECT BILIRUBIN
CONTROL	$0.958 \pm 0.456$	$0.897 \pm 0.237$
ONLY PARA	$0.660 \pm 0.299$	$0.970 \pm 0.012^{ns}$
PARA + STD	$0.503 \pm 0.232$	$0.347 \pm 0.064^{ns}$
PARA + L.D	$0.440 \pm 0.207$	$0.240 \pm 0.021^*$
PARA + H.D	$0.328 \pm 0.206$	$0.243 \pm 0.086^*$

Values are expressed as the mean  $\pm$  S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ns- no significant \* $P < 0.001$ , \*\* $P < 0.01$ , \*\*\* $P < 0.05$  calculate by comparing treated group with control group.

Bilirubin is an orange-yellow pigment, a waste product primarily produced by the normal breakdown of heme. Heme is a component of hemoglobin, which is found in red blood cells (RBCs). Bilirubin is ultimately processed by the liver to allow its elimination from the body. Any condition that accelerates the breakdown of RBCs or affects the processing and elimination of bilirubin may cause an elevated blood level.



**Figure 3: Effect of EECP on serum bilirubin**

The above table shows that the levels of bilirubin is decreased in the group 2 (only paracetamol) ( $0.660 \pm 0.299$ ) and shows gradual decrease on treatment with standard drug silimarin, low dose EECP and high dose EECP.

This test shows that there is reduced breakdown of total bilirubin pointing to hepato protective activity.

ACTIVITY OF DIRECT BILIRUBIN

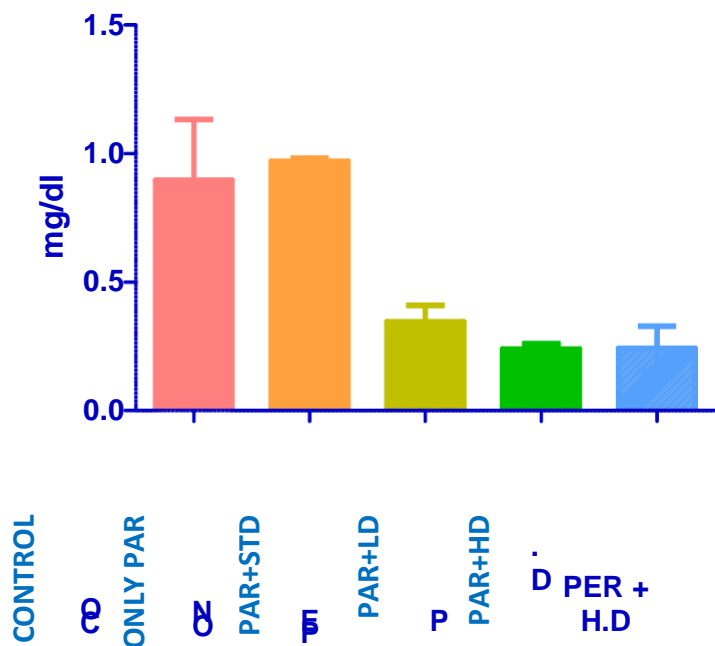


Figure 4: Effect of EECP on direct bilirubin

Bilirubin that is bound to a certain protein is called unconjugated, or indirect, bilirubin. Conjugated, or direct, bilirubin travels freely through the bloodstream to the liver. Most of this bilirubin passes into the small intestine. The levels of direct bilirubin show significant reduction on treatment with standard drug silymarin and low dose EECP( $0.240\pm0.021$ ) and high dose EECP( $0.243\pm0.086$ ).

ACTIVITY OF TOTAL PROTEIN

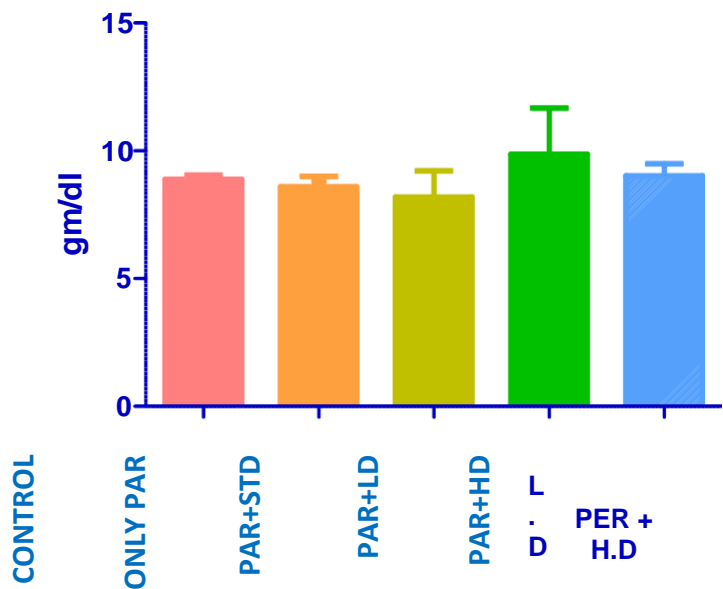


Figure 5: Effect of EECP on total serum protein

# EFFECT OF ETHANOLIC EXTRACT OF *Crotalaria pallida* ON SERUM PROTEIN IN PARACETAMOL INDUCED HEPATOTOXICITY

Table 7.

GROUP	TOTAL PROTEIN
CONTROL	8.90±0.153
ONLY PARA	8.60±0.404
PARA + STD	8.20±1.02
PARA + L.D	9.87±1.81
PARA +H.D	9.03±0.463

Values are expressed as the mean  $\pm$  S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ns- no significant \*P < 0.001, \*\*P < 0.01, \*\*\*P < 0.05 calculate by comparing treated group with CONTROL group.

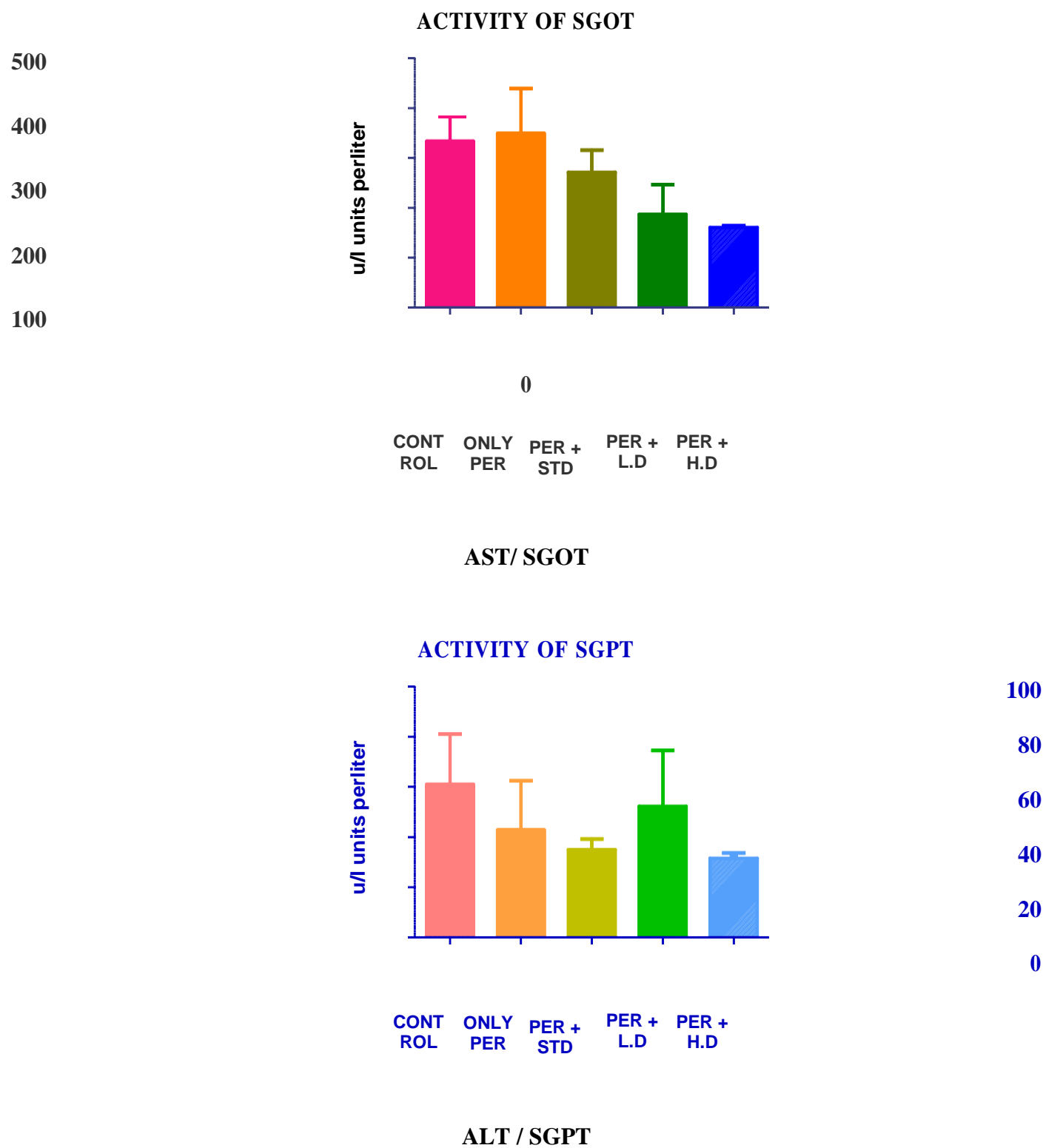
The level of serum protein which includes albumin and globulin are largely unchanged after treatment with paracetamol. The group 3 which received paracetamol and standard drug silymarin showed a slight decrease (8.20±1.02) and the group 4 (paracetamol + low dose) shows a slight elevation in the level of total proteins (9.87±1.81).

## EFFECT OF ETHANOLIC EXTRACT OF *Crotalaria pallida* L. ON SGOT, SGPT, ALP

Table 8:

GROUP	SGOT	SGPT	ALP
CONTROL	334.5±45.87	61.17±19.92	299.37±54.57
ONLY PARA	349.7±89.96	42.93±19.61	336.3±27.89
PARA + STD	271.3±44.92*	34.93±4.313**	356.3±35.41
PARA + L.D	186.9±60.03**	52.30±22.27	325.9±29.27*
PARA +H.D	160.4±3.467**	31.67±2.00**	255.8±79.96**

Values are expressed as the mean  $\pm$  S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ns- no significant \*P < 0.001, \*\*P < 0.01, \*\*\*P < 0.05 calculate by comparing treated group with CONTROL group.



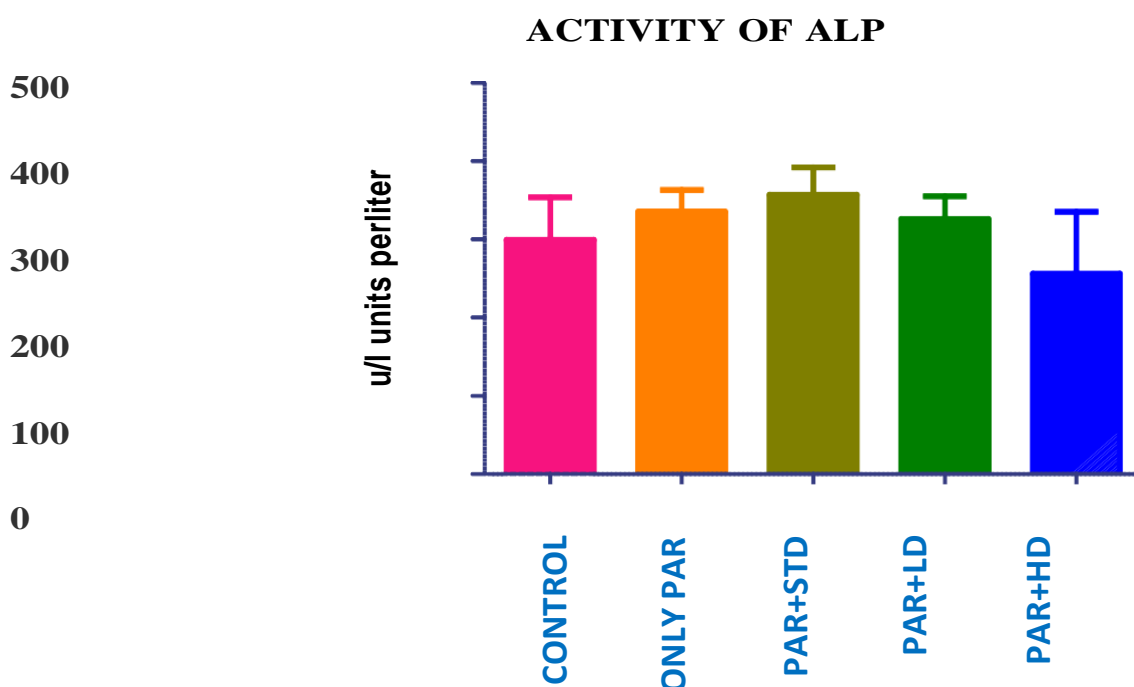
**Figure 6: Effect of EECP on SGOT and SGPT**

AST – Aspartate amino transferase  
SGOT - Serum Glutamate Oxaloacetate Transaminase  
ALT – Alanineamino transferase  
SGPT - Serum Glutathione peroxidase

The level of AST in the paracetamol only group on AST/SGOT shows an increase pointing to possible hepatotoxicity ( $349.7 \pm 89.96$ ). The AST levels are lowered than the control in the groups 3(paracetamol with std) with an even more significant decrease in 250mg/kg EECP( $186.9 \pm 60.03$ ) and 500mg/kg EECP groups( $160.4 \pm 3.467$ ). This graph shows significant hepatoprotective activity of ethanolic extract of *Crotalaria pallida*

The level of serum ALT is lower than the control groups of mice in the study. Paracetamol with silymarin standard( $34.93 \pm 4.313$ ) and Paracetamol with High dose EECP( $31.67 \pm 2.00$ ) show comparable levels of hepatoprotectivity.

In severe tissue damage ALT activity is higher than AST and the ALT:AST ratio becomes  $\geq 1$  (normally  $< 1$ ). Some increase in the activities of ALT and AST are seen in extrahepatic cholestasis. In both cirrhosis and carcinoma activity of AST is found to be higher than the ALT. ALT is a more liver specific enzyme as increased ALT activity in serum is hardly seen in tissues other than liver cell damage.



**Figure 7: Effect of EECP on Alkaline phosphatase**

The mice that were administered only paracetamol showed a higher value of alkaline phosphatase than the control ( $336.3 \pm 27.89$ ). The paracetamol and std drug silymarin group showed an even higher increase in ALP ( $356.3 \pm 35.41$ )

This shows liver damage in these groups. Damaged liver cells release increased amounts of ALP into the blood. ALP is especially high in the edges of cells that join to form bile ducts. If one or more of them are obstructed, for example by a tumor, then blood levels of ALP will often be high.

The paracetamol and Low Dose EECP ( $325.9 \pm 29.27$ ) group showed a slight increase in ALP compared to groups 2 and 3. However, paracetamol with High dose EECP showed significant protection from liver damage by registering a lower level of ALP than the control groups itself ( $255.8 \pm 79.96$ ). This is a significant find in the evaluation of hepatoprotective activity of *Crotalaria pallida*

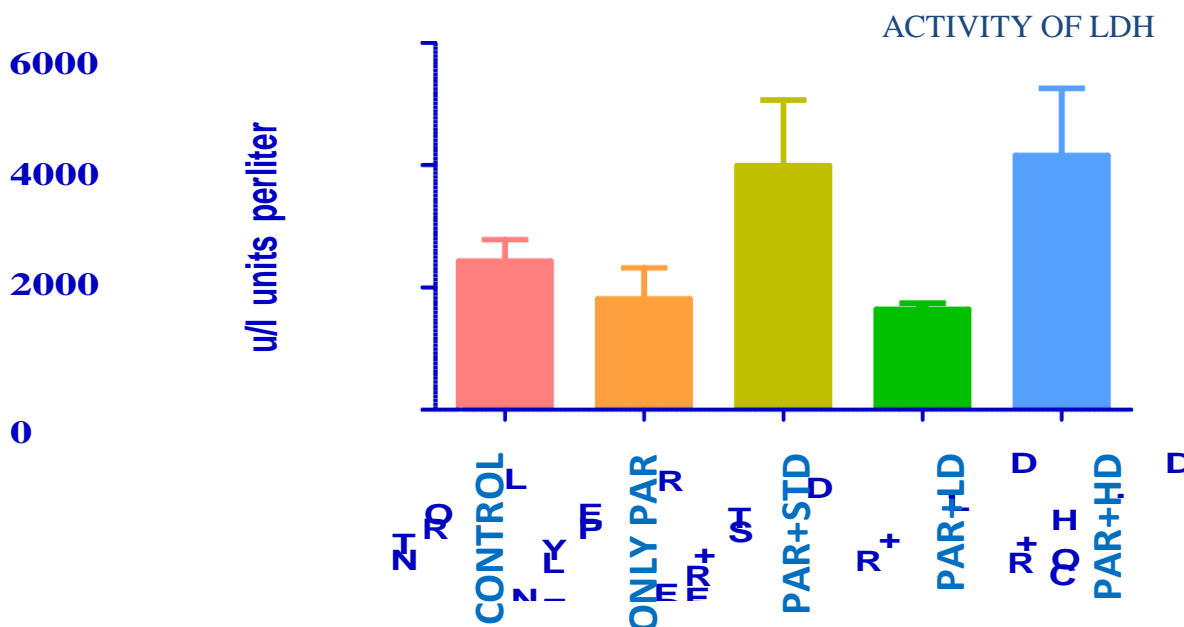
## ACTIVITY OF LDH

Table 9

GROUP	LDH
CONTROL	2426±325.6
ONLY PARA	1808±325.6
PARA + STD	3966±682.9*
PARA + L.D	1636±65.86**
PARA +H.D	4158±698.5*

Values are expressed as the mean  $\pm$  S.D; Statistical significance (p)calculated byone way ANOVA followed by dunnett's ns- no significant \*P< 0.001, \*\*P < 0.01, \*\*\*P < 0.05 calculate by comparing treated group with CONTROL group.

LDH is an enzyme found in all living tissue. Because it is released during tissuedamage, it is a marker of common injuries and disease.



**Figure 8: Effect of EECP on LDH** LDH: Lactate dehydrogenase

In our study, we see group 2 mice having a low level of LDH showing the pharmacological effect of paracetamol. Group 3 shows that paracetamol and silymarin combination causes marked elevation of LDH. Paracetamol and HD of EECP (4158±698.5) show marked elevation as well. Paracetamol and low dose EECP lowers Lactate dehydrogenase levels showing significant hepatoprotective property. (1636±65.86)

# EFFECT OF ETHANOLIC EXTRACT OF *Crotalaria pallida* L. ON HOMOGENISED LIVER TISSUE IN PARACETAMOL INDUCED HEPATOTOXICITY

Table 10:

GROUP	TOTAL PROTEIN
CONTROL	0.368±0.164
ONLY PARA	0.504±0.225
PARA + STD	0.248±0.111**
PARA + L.D	0.304±0.136*
PARA +H.D	0.291±0.131*

Values are expressed as the mean  $\pm$  S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ns- no significant \*P < 0.001, \*\*P < 0.01, \*\*\*P < 0.05 calculate by comparing treated group with CONTROL group.

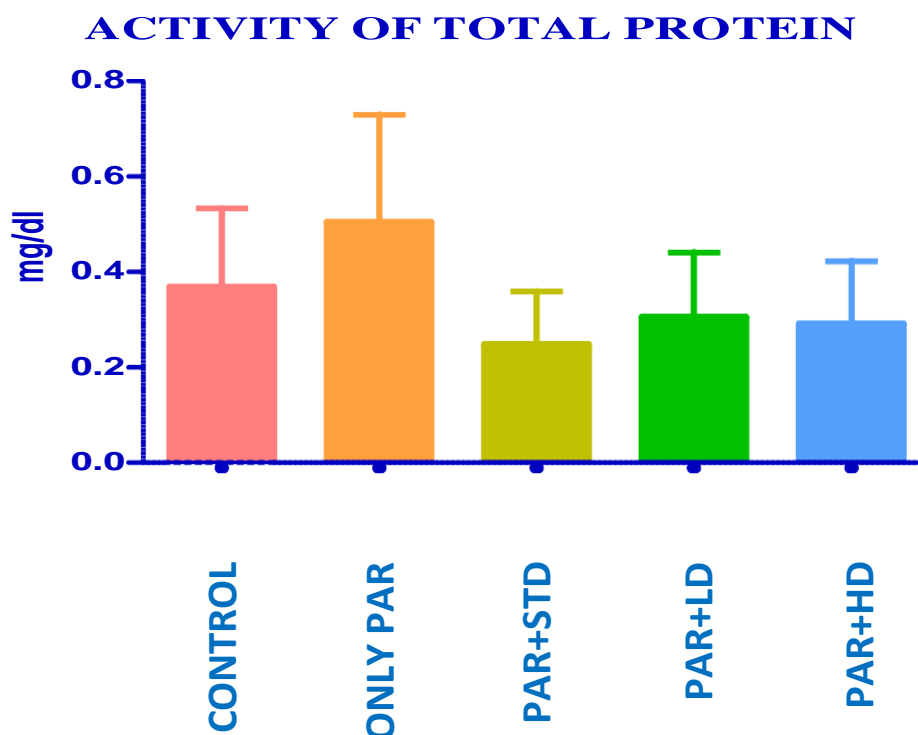


Figure 9: Effect of EECF on homogenized liver tissue

Paracetamol and silymarin group and both dosages of EECF (0.304±0.136), (0.291±0.131) lower the protein levels in homogenized liver of albino mice. The determination of cellular protein content is very advantageous over other markers due to early formation, greater stability and reliability and also their longer life-span.

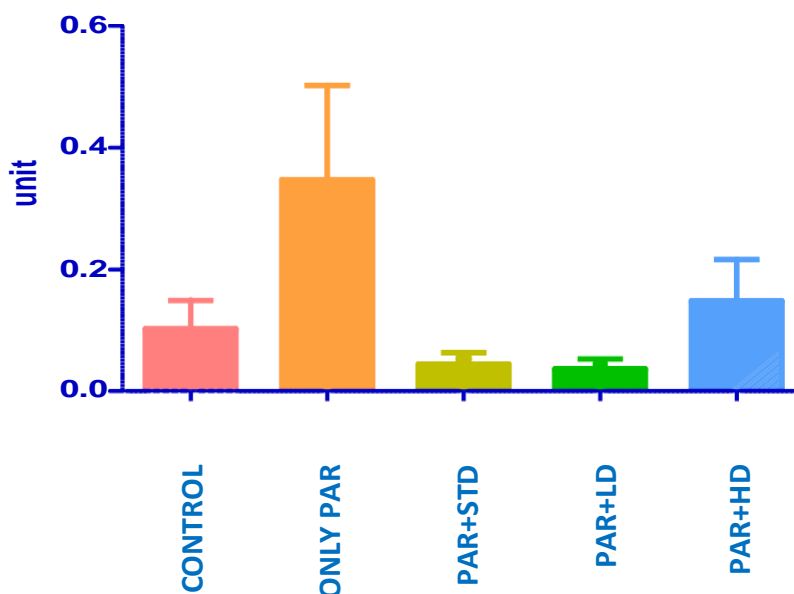
# EFFECT OF ETHANOLIC EXTRACT OF *Crotalaria pallida* L. ON SERUM BIOCHEMICAL MARKERS IN PARACETAMOL INDUCED HEPATOTOXICITY

Table 11

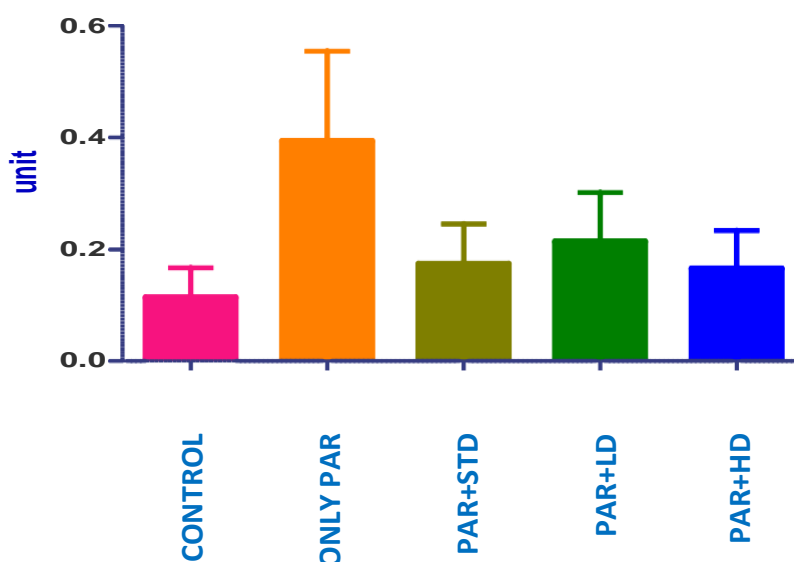
GROUP	SOD	CATALASE
CONTROL	0.107±0.047	0.114±0.053
ONLY PARA	0.346±0.155	0.393±0.160
PARA + STD	0.044±0.019**	0.174±0.071*
PARA + L.D	0.036±0.017**	0.214±0.088**
PARA +H.D	0.147±0.068*	0.166±0.068**

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant \*P< 0.001, \*\*P < 0.01, \*\*\*P < 0.05 calculate by comparing treated group with CONTROL group.

## ACTIVITY OF SOD



## ACTIVITY OF CAT



**Figure 10: Effect of EECP on SOD and CAT**

SOD: Superoxide dismutase CAT: Catalase

The study shows that silymarin ( $0.044 \pm 0.019$ ), 250mg/kg ( $0.036 \pm 0.017$ ) and 500 mg/kg ( $0.147 \pm 0.068$ ) extract lower the levels of Superoxide dismutase enzymes. SOD, a key enzyme in free radical protection, increases significantly in the liver tissue of group 2 that received only paracetamol ( $0.346 \pm 0.155$ ) suggesting that products of free radical reactions are involved in pathogenesis. A significant decrease offered by the both doses of EECP shows that the hepatoprotective activity of *Crotalaria pallida* is comparable to standard drug Silymarin

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). Both standard and test doses shows a decline in catalase levels ( $0.214 \pm 0.088$ ,  $0.166 \pm 0.068$ ).

# EFFECT OF ETHANOLIC EXTRACT OF *Crotalaria pallida* ON SERUM BIOCHEMICAL MARKERS IN PARACETAMOL INDUCED HEPATOTOXICITY

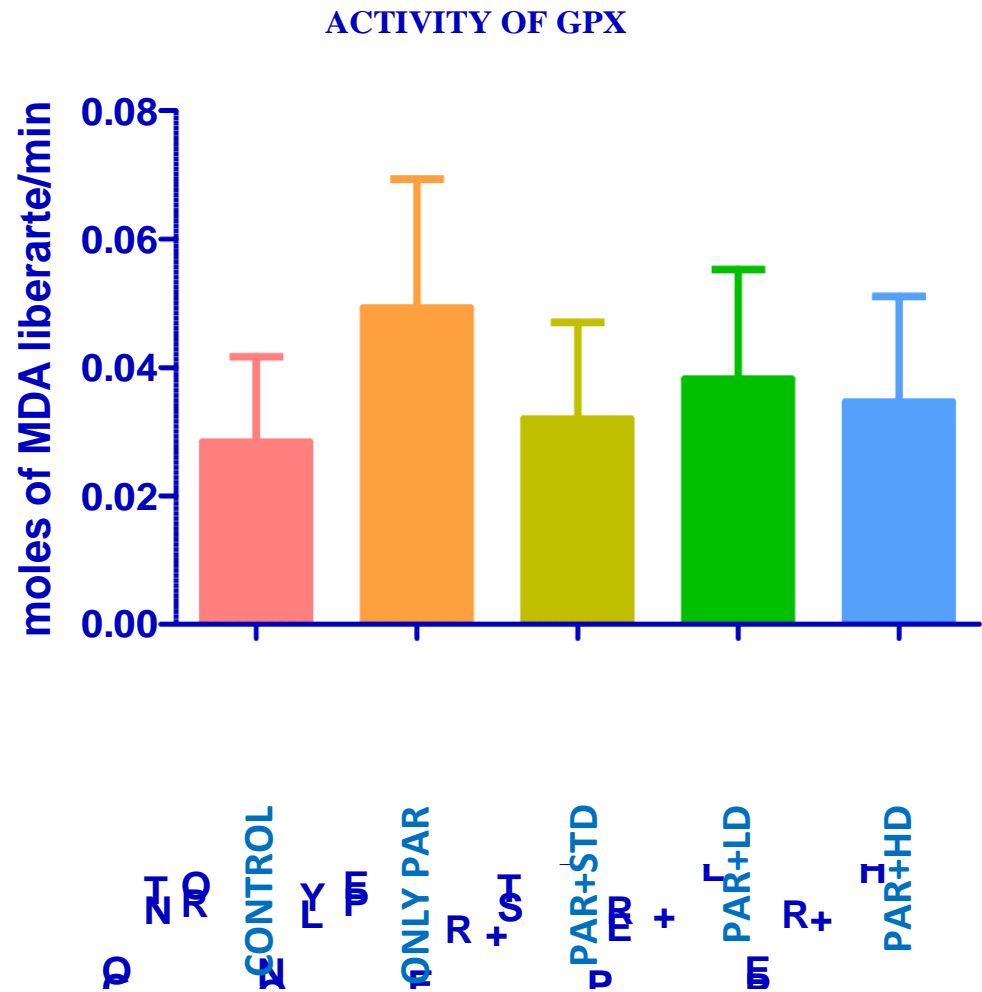
Table 12

GROUP	GPX
CONTROL	0.028±0.013
ONLY PARA	0.049±0.020
PARA + STD	0.032±0.015
PARA + L.D	0.038±0.017
PARA +H.D	0.035±0.016*

Values are expressed as the mean  $\pm$  S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's ns- no significant \*P< 0.001, \*\*P < 0.01, \*\*\*P < 0.05 calculate by comparing treated group with CONTROL group.

GPx – Glutathione peroxidase

The main biological role of GPx is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water.



**Figure 11: Effect of EECP on Glutathione peroxidase**

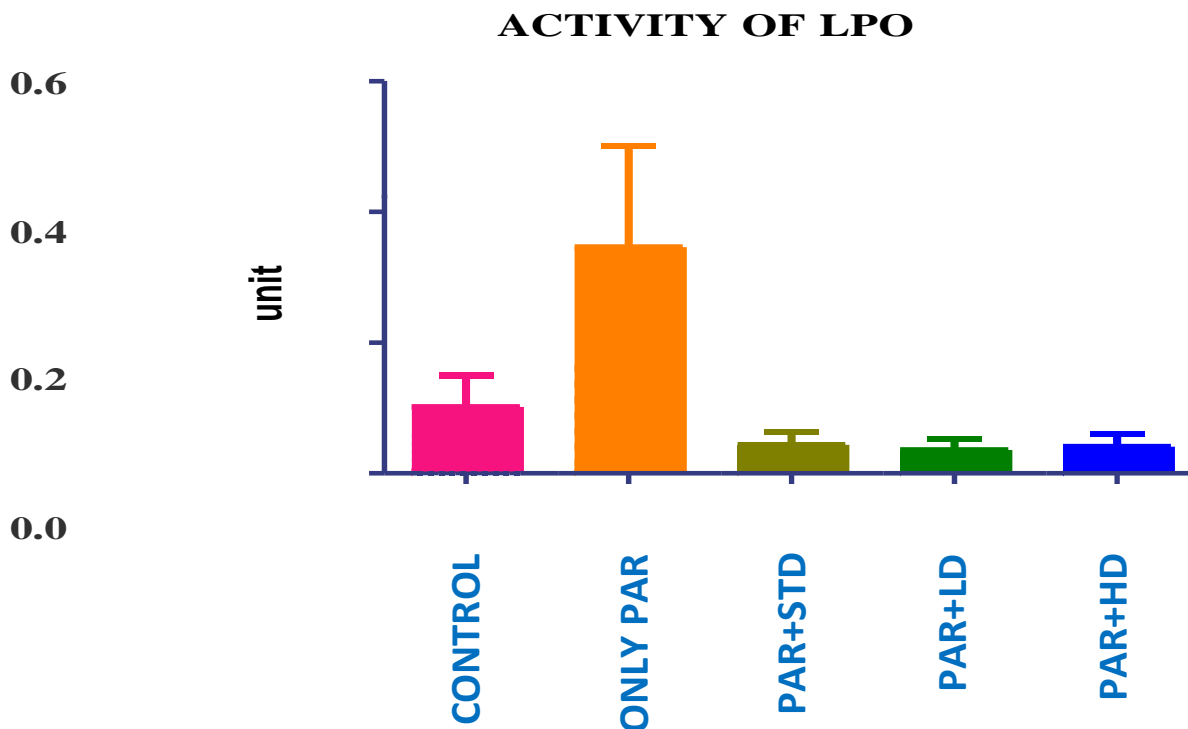
The mice given paracetamol show marked elevation of GPx levels ( $0.049 \pm 0.020$ ). The standard drug silymarin shows a marked reduction of GPx activity. The Low dose EECP ( $0.038 \pm 0.017$ ) and high dose EECP reduce the level of glutathione peroxidase but not as well as the standard drug ( $0.035 \pm 0.016$ ).

**EFFECT OF ETHANOLIC EXTRACT OF *Crotalaria pallida* L. on SERUM BIOCHEMICAL MARKERS IN PARACETAMOL INDUCED HEPATOTOXICITY**

**Table 13: LPO assay**

GROUP	LPO
CONTROL	0.102±0.047
ONLY PARA	0.346±0.155
PARA + STD	0.044±0.019
PARA + L.D	0.036±0.017**
PARA +H.D	0.040±0.019**

Values are expressed as the mean  $\pm$  S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ns- no significant \*P < 0.001, \*\*P < 0.01, \*\*\*P < 0.05 calculate by comparing treated group with CONTROL group.



**Figure 12: Effect of EECP on LPO**

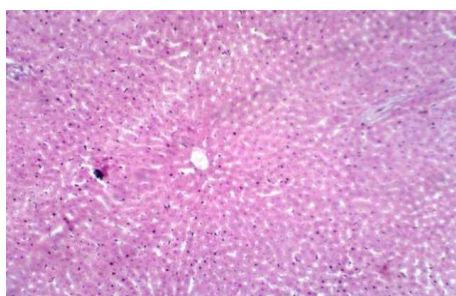
LPO: Lipid peroxidase

Lipid peroxidation is the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage ( $0.346 \pm 0.155$ ). Paracetamol causes marked elevation of Lipid peroxidase in albino mice. The control and the test groups drastically reduce the LPO levels showing that the EECP in both low and high doses ( $250\text{mg/kg}$  and  $500\text{mg/kg}$ )  $0.036 \pm 0.017$ ,  $0.040 \pm 0.019$  protect the liver as well as Silymarin in paracetamol induced hepatotoxicity.

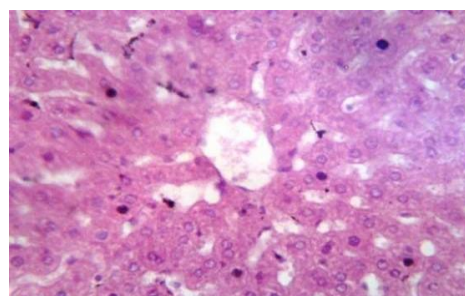
EECP shows promising anti-oxidant, free radical scavenging and anti-lipid peroxidase activities.

#### **EFFECT OF - ETHANOLIC EXTRACT OF *Crotalaria pallida* L. ON HISTOPATHOLOGICAL CHANGES LIVER AFTER 14 DAYS OF PARACETAMOL INDUCED HEPATOTOXICITY**

**Figure 13: GROUP-I CONTROL (VEHICLE)**



10 X



40X

**SPECIMEN** : Liver

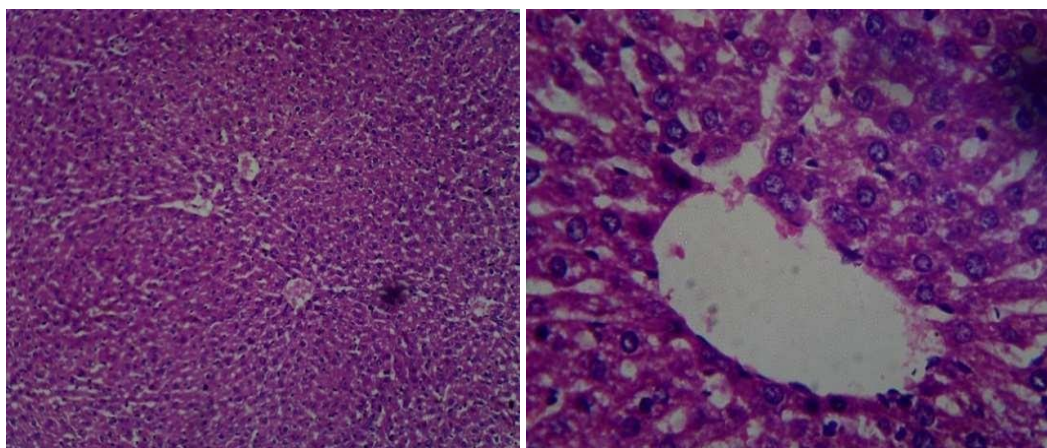
**Group – I** : Control

**MICROSCOPIC APPEARANCE:**

Section shows liver with normal lobular architecture. The portal tracts show normal morphology. The hepatocytes are normal. The sinusoids and central vein show normal. There is no inflammation / fibrosis / toxic change.

**Group – II** : **ONLY PARACETAMOL**

**Figure 26: Paracetamol induced toxicity.**



10X

40 X

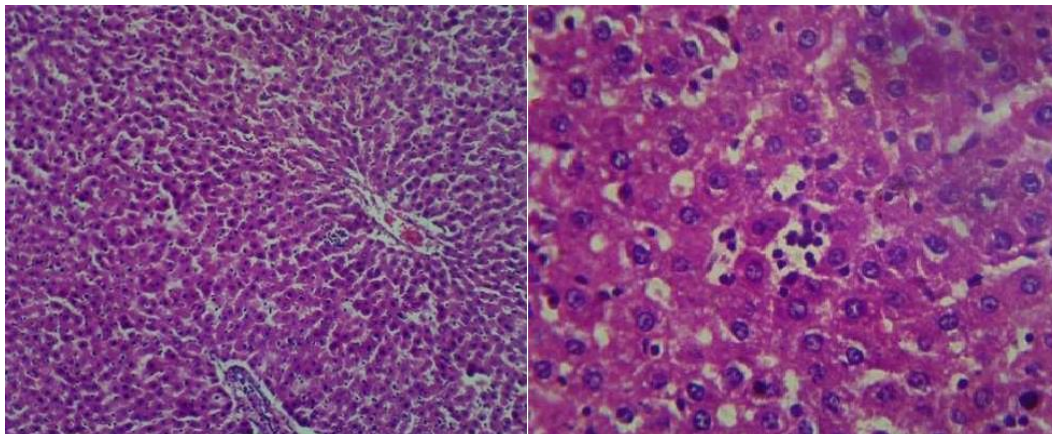
**SPECIMEN** : Liver

**Group – II** : **ONLY PARACETAMOL**

**MICROSCOPIC APPEARANCE:**

Liver section of paracetamol treated mice showing extensive areas of hepatocellular necrosis and inflammatory cell infiltration. Most of the centrilobular hepatocytes were swollen with marked cytoplasmic vacuolation and pyknotic nuclei with obliterated intervening hepatic sinusoids. The sinusoids show mild dilatation. Central veins are congested.

**Figure : 14 GROUP-III- PARACETAMOL + STD**



10X

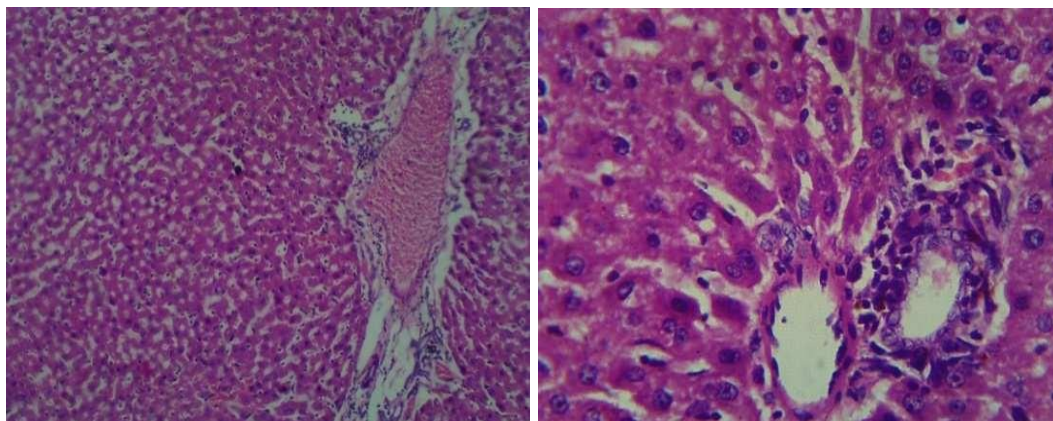
40X

**SPECIMEN : Liver**

**Group – III : PARACETAMOL + STD**

**MICROSCOPIC APPEARANCE:**

Section shows liver with normal lobular architecture. The portal tracts show mild inflammation. The hepatocytes are normal. The sinusoids are dilated. The central vein show congested. There is no fibrosis / toxic change.

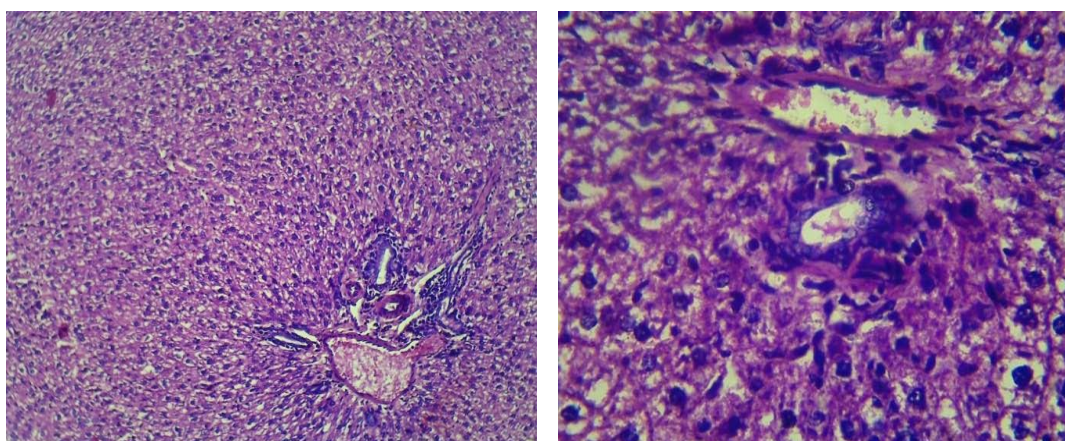
**GROUP- IV – PARACETAMOL + L.D****Figure 15: Paracetamol + 250mg/kg EECP**

10x

40x

**SPECIMEN : Liver****Group – IV : PARACETAMOL + L.D****MICROSCOPIC APPEARANCE:**

Section shows liver with normal lobular architecture. The portal tracts show mild inflammation. The hepatocytes are normal. The sinusoids are dilated. The central vein show congested. There is no fibrosis / toxic change.

**GROUP-V- PARACETAMOL + H.D****Figure 16: Paracetamol + 500mg/kg EECP**

10x

40x

**SPECIMEN : Liver**

### **Group – V: PARACETAMOL+ H.DMICROSCOPIC APPEARANCE:**

Section shows liver with normal lobular architecture. The portal tracts show normal. The hepatocytes are normal. The sinusoids and central veins are normal. There is no fibrosis / toxic change/ inflammation. Histopathological findings are shown for controls and exposed rats. Histopathological findings revealed that the administration of paracetamol resulted in necrosis of hepatocytes as well as deposition of fats in the tissues when compared with controls, but the severity was reduced in those groups of animals pretreated with 100 mg/kg of silymarin, 500 mg/kg and 250 mg/kg of the ethanolic extract of *Crotalaria pallida*

### **SUMMARY AND CONCLUSION**

The present study was designed to evaluate the possible protective effect of ethanolic extract of *Crotalaria pallida* Linn (EECP) against paracetamol induced hepatotoxicity in animals.

A literature survey revealed that more studies were needed for this plant to ascertain the hepatoprotective potential.

The detailed preliminary phytochemical investigations rationalized its use as a drug of therapeutic importance. The ethanolic extract of the plant has phytoconstituents like flavonoids, terpenoids, steroids, alkaloids, saponins and tannins.

The hepatoprotective effect was assessed using a battery of biochemical and histopathological tests. SGOT, SGPT, ALP, LDH, ACP were some of the biochemical tests done. In vivo tests for antioxidants (SOD, CAT, GSH, LPO) were conducted on albino mice and wistar rats.

In paracetamol induced hepatotoxicity, a lower dose and a high dose of extract were used and compared with the hepatoprotective activity of standard drug silymarin. Control group and an only drug group were also used.

The EECP showed marked hepatoprotective activity in lowered levels of body weight, positive effect on total bilirubin, total protein and on liver enzymes. Histological sections of liver showed that centrilobular necrosis, the pathognomonic feature of hepatotoxicity, which appeared in paracetamol-intoxicated mice, was strikingly reduced in EECP treated mice. Furthermore, the congestion and inflammatory cell infiltration evoked by paracetamol was considerably decreased by EECP indicating its possible antihepatotoxic action.

In conclusion, we can say that *Crotalaria pallida*. has the ability to protect the liver from the damaging effects of paracetamol toxic doses and stimulation of endogenous anti-oxidant defense system.

In the near future, a further study is warranted to isolate, characterize and screen the active components of *Crotalaria pallida* that have the hepatoprotective activity.

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