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Hepatoprotective Effects of Ethanolic Extract of *Ipomoea Batatas* Leaves on Carbon Tetrachloride Induced Hepatotoxicity in Albino Wistar Rats

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ABSTRACT

In the present study, hepatoprotective effect of an ethanolic extract of *Ipomoea batatas* leaves, on liver injury induced by carbon tetrachloride (CCl4) was investigated. Wistar albino rats (n=6) were administered 300, 600 and 900 mg/kg body weight extract orally for 10 consecutive days. Marker enzymes SGOT, SGPT, serum protein and Bilirubin (total, direct and indirect) were estimated in serum whereas total protein (TP), Lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase were estimated in liver tissue as markers for oxidative stress. Histopathological assessment was also done on liver tissue. Ethanolic extract of *Ipomoea batatas* leaves administration for 10 days prevented the CCl4 induced hepatic injury and oxidative stress. Hepatoprotective effect of *I. batatas* in CCl4 induced liver poisoning was evident by decrease in elevated levels of SGOT, SGPT and bilirubin. Treatment with *I. batatas* increased the reduced levels of TP and SOD and decreased the elevated level of MDA concentration and catalase activity in CCl4 induced oxidative stress in the liver. The ability of ethanolic extract of *Ipomoea batatas* leaves to protect the liver toxicity in rats was further confirmed by histological findings in the liver tissue. In conclusion, it was observed that ethanolic extract of *Ipomoea batatas* leaves have a dose dependent potential to protect the liver against CCl4induced hepatic injury through its potent antioxidant activity in rats.

Keywords: Carbon tetrachloride, Vitamin-C, *Ipomoea batatas*, Serum enzymes & antioxidant enzymes.

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INTRODUCTION

Liver is an important organ which actively involved in regulation of metabolic function, detoxification, and secretory functions in the body. Hepatic injury is associated with distortion of these metabolic functions. Liver injury can be caused by drugs, chemicals, environmental pollutants, carcinogens, and various toxicant which results in dysfunction or distortion of these metabolic functions. Thus, liver diseases remain as one of the serious health problems. Despite tremendous progress in the conventional medical system, there is no effective hepatoprotective drugs are available which cure available which offers protection to the liver from the damage and help in the regeneration of hepatic cells[1, 2]. Several newly developed drugs are used for chronic liver disorder but they often have side effects. Therefore, demand for an essential research about suitable herbal drugs as an alternative or complement to conventional therapy has presently increased[3, 4]. Carbon tetrachloride is widely used for experimental inducer of liver damage. The principle causes of carbon tetrachloride induced hepatic damage are lipid peroxidation via decreased activities of antioxidant enzymes and generation of free radicals[5]. The antioxidant activity or the inhibition of the generation of free radicals is important factor in providing protection against hepatic damage[6]. Keeping this fact in view, the present study was undertaken to investigate the hepatoprotective activity of leaves against carbon tetrachloride induced hepatic damage in albino rats.

Ipomoea batatas leaves are an excellent source of flavanoids with antioxidative polyphenolic compounds like, caffeic, caffeoylquinic acid, di & tri-caffeoylquinic acid anthocyanins, as well as beta-carotene and are superior in regard to other vegetables. Excess consumption of these leaves does not lead to toxicity, since the polyphenols can be eliminated or deposited in the fat tissues. The plant phenols, because of their diversity and extensive distribution, are the most important group of natural antioxidants, and they contribute to the organoleptic and nutritional qualities of fruits and vegetables[7].

MATERIALS AND METHOD

Drugs and chemicals

All the drugs and chemicals used were of analytical grade. Carbon tetra chloride and Vitamin-C was purchased from S. D. Fine Chemicals Ltd, Mumbai were used in this study.

Plant material and preparation of extracts

The fresh leaves of *Ipomoea batatas* was collected from the fields near Kolar, district in the month of October and the plant material was identified & authenticated by a qualified botanist at

National Ayurveda Dietetics Research Institute (Govt. Central pharmacy), Jayanagar Bangalore. The ethanolic leaf extract of *Ipomoea batatas* was collected from an authentic supplier, M/S Green chem Pvt. Ltd, Bangalore as a gift sample.

Preliminary Phytochemical Screening

Ethanolic extract of *I. batatas* were subjected to preliminary phytochemical screening for the detection of various plants constituents like carbohydrates, Saponins, Flavanoids, Glycosides, Proteins, Alkaloids and Phenols[8, 9].

Animals

Inbred Wister albino rats weighing between 150-200 g were housed in a group of 5 to 6. All rats were feed with pelleted diet (Pranav Agro Industries Ltd, Sangli, India) and *water ad libitum*. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Department of Animal Welfare and Government of India.

Acute toxicity studies

The acute oral toxicity (AOT) studies were carried out by using albino Wistar rats weighing between 150-200g of either sex as per OECD 425 guidelines by employing the Up and Down method prior to evaluation of cardio protective activity.

Evaluation of Hepatoprotective effects of Ethanolic leaf extract of *Ipomoea batatas* on Carbon tetrachloride induced hepatotoxicity[10, 11]

The animals were randomly divided into 6 groups consists of 6 animals each. Wistar rats weighing (180-200g) either sex were selected under healthy conditions for experimental purpose.

Group 1- Normal control received distilled water (vehicle) for 10 days and on every 72 hours it received liquid paraffin 1ml/kg i.e. on 3rd, 6th, 9th day.

Group 2- Toxic control received CCl_4 1ml/kg i.p with liquid paraffin v/v in a ratio of 1:1 at every 72 hours, i.e. on 3rd, 6th, 9th day.

Group 3- Standard control received 50mg/kg of vitamin-C for ten days and received CCl_4 1ml/kg i.p with liquid paraffin v/v in a ratio of 1:1 at every 72 hours, i.e. on 3rd, 6th, 9th day in the experimental period.

Group 4- Treatment group received 300 mg/ kg of ethanolic extract of *Ipomoea batatas* orally for 10 days and received CCl4 1ml/kg i.p (1:1 dilution with liquid paraffin v/v) on every 72 hours, i.e. on 3rd, 6th, 9th day in the experimental period.

Group 5- Treatment group received 600 mg/ kg of ethanolic extract of *Ipomoea batatas* orally for 10 days and received CCl4 1ml/kg i.p (1:1 dilution with liquid paraffin v/v) on every 72

hours, i.e. on 3rd, 6th, 9th day in the experimental period.

Group 6- Treatment group received 900 mg/ kg of ethanolic extract of *Ipomoea batatas* orally for 10 days and received CCl_4 1ml/kg i.p (1:1 dilution with liquid paraffin v/v) on every 72 h, i.e. on 3^{rd}, 6^{th}, 9^{th} day in the experimental period.

After 24hours (11^{th} day) rats were anaesthetized and blood samples were collected by retro orbital method, and the serum was separated and used for the estimation of serum biomarkers i.e. SGOT, SGPT, Total & Direct Bilirubin and Serum proteins[12,13]. Following, the animal was sacrificed by cervical dislocation and the Liver specimen was collected and used for histopathology and for tissue biochemical estimations tissue proteins, lipid peroxidation [14], SOD and catalase[15, 16].

Histopathological evaluation

Livers from rats of different groups were fixed in 10% neutral formalin solution, dehydrated in graded alcohol and embedded in paraffin. Fine sections obtained were mounted on glass slides and counter-stained with haematoxylin–eosin (H&E) dye for light microscopic observation. [17]

Statistical analysis

Values were expressed as mean ± SEM from 6 animals. Statistical differences were evaluated using a One-way analysis of variance (ANOVA) followed by Dunnet's t-test. Results were considered to be statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The phytochemical screening of the ethanolic extract of *Ipomoea batatas* revealed the presence of carbohydrates, Saponins, flavanoids, glycosides, alkaloids, phenols, proteins & amino acids.

Table 1: Phytochemical Screening of ethanolic extracts of the leaf of *Ipomoea batatas*

S. No.	Phytoconstituents	Ethanolic extracts of whole plant of *Clitoria ternatea*	Aqueous extracts of whole plant of *Clitoria ternatea*
1	Carbohydrates	+	+
2	Saponins	+	+
3	Flavanoids	+	+
4	Glycosides	+	+
5	Proteins & amino acids	+	+
6	Alkaloids	+	+
7	Phenols	+	+

(+) sign indicates presence of phytoconstituents in the extract and (-) sign indicates absence of phytoconstituents in the extract.

Table 2: Effect of leaves extract of *Ipomoea batatas* on SGPT, SGOT, Total & Direct Bilirubin.

Drug treatment	SGPT (IU/L)	SGOT (IU/L)	Bilirubin total (mg/dL)	Bilirubin Direct (mg/dL)
Normal control Purified water	93.13±3.74	151.1±6.87	0.66±0.01	0.23±0.02
CCL_4(1ml/kg on 3, 6, 9th day)	347.2±5.4$^{\#\#\#}$	200.5±6.51$^{\#\#\#}$	1.17±0.09$^{\#\#\#}$	0.35±0.05$^{\#\#}$
Vitamin –C (50 mg/kg/day) CCL_4	105.2±5.63***	172.2±6.95***	0.70±0.02***	0.28±0.02 ^{NS}
EEIB (300 mg/ kg/day) CCL_4	305.3±36.39**	196.4±9.08 ^{NS}	1.20±0.07 ^{NS}	0.40±0.04 ^{NS}
EEIB (600 mg/ kg/day) CCL_4	205.4±16.78***	174.3±8.93***	0.76±0.05***	0.27±0.08 ^{NS}
EEIB (900 mg/ kg/day) CCL_4	117.9±10.12***	157.5±5.86***	0.72±0.03***	0.27±0.05**

EEIB: Ethanolic extract of *Ipomoea batatas*

Each value is expressed as Mean ± SD for groups of 6 animals in each group.

*P<0.05, **P<0.01, ***P<0.001 will be considered as significant when compared to toxic group. $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, will be considered as significant when compared to the normal group. One way ANOVA followed by Dunnett's multiple comparison tests.

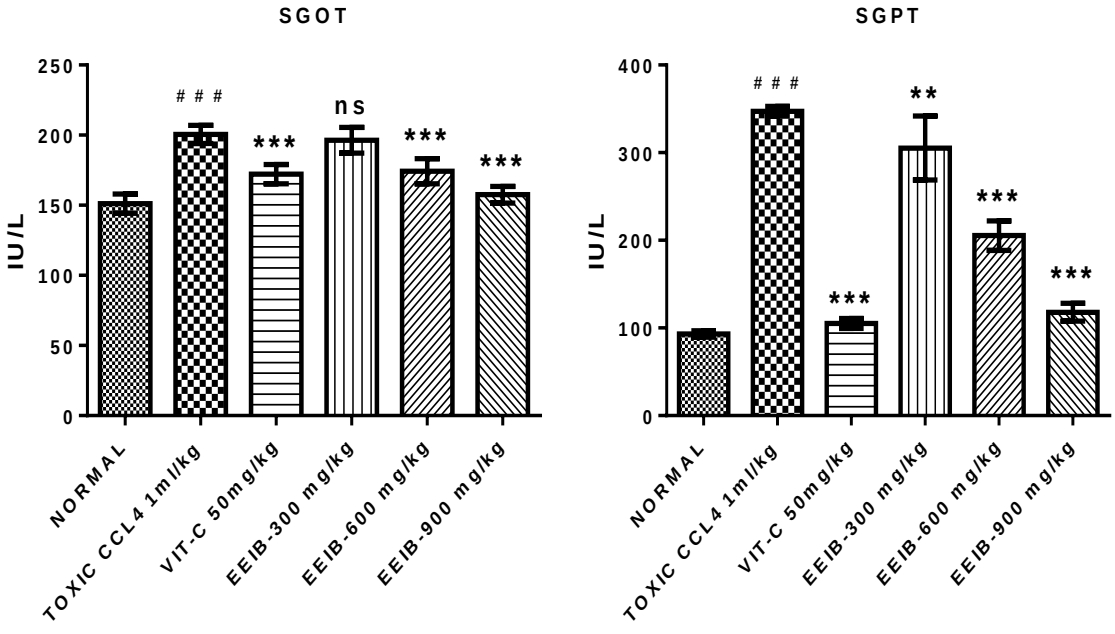


Figure 1: Effect of vehicle, CCl_4, Vit-C & extract on SGOT & SGPT.

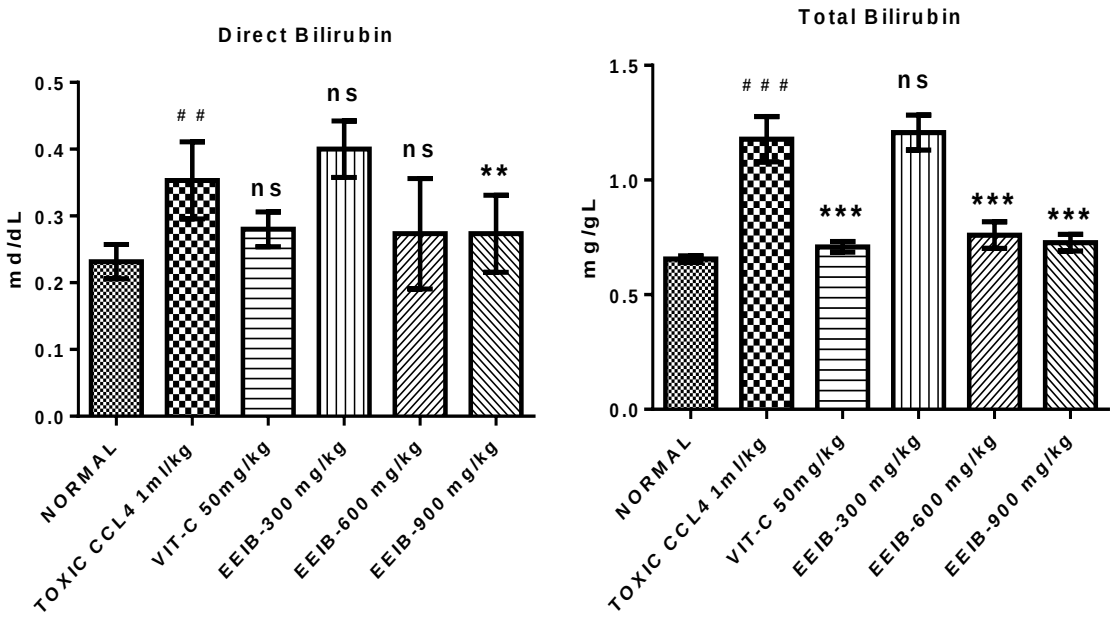


Figure 2: Effect of vehicle, CCl_4, Vit-C & extract on direct & indirect bilirubin.

SGOT: The rats treated with alone CCL_4 showed an elevated levels of SGOT (200.5±6.51) with mean ± SD & significance of P<0.001 when compared to normal (151.1±6.87). The SGOT level in the extract dose 300mg/kg was not significant as compared to toxic group The rats pre-treated with Vitamin C 50mg/kg & extracts at a dose of 600, 900mg/kg resorted the levels of enzyme (172.2±6.95) & (174.3±8.93), (157.5±5.86) respectively with significance of P<0.001when compared to CCL_4 treated group. Thus the EEIB showed a better action compared to vitamin C.

SGPT: The extract dose with 600 & 900mg showed better activity than vitamin C. The rats treated with alone CCL_4 showed an elevated levels of SGPT (347.2±5.44) with mean ± SD & significance of P<0.001 when compared to normal (93.13±3.74). The rats pre-treated with Vitamin C 50mg/kg & extract at a dose of 600, 900mg/kg resorted the levels of enzyme (105.2±5.63) & (205.4±16.78), (117.9±10.12) respectively with significance of P<0.001, but extract 300mg/kg showed significance (305.3±36.39) with P<0.01, when compared to CCL_4 treated group.

Total, Indirect & Direct Bilirubin: CCL_4 elevated significantly the serum Total Bilirubin & In-Direct Bilirubin (1.17±0.09), (0.82±0.09) with significance P<0.001 & Direct bilirubin (0.35±0.05) with significance P<0.01 when compared to normal (0.66±0.01), (0.42.0.02) & (0.23±0.02) respectively. Significant reduction in the Total Bilirubin & In-Direct Bilirubin was observed at a dose of 600 & 900mg/kg & vitamin-C (0.76±0.05, 0.72±0.03), (0.48±0.07, 0.45±0.04) & (0.70±0.02, 0.42±0.02) with significance P<0.001, but dose 300 mg/kg showed no significance

when compared to toxic group. Vitamin- C, 300 & 600 mg/kg showed no significance for direct bilirubin and 900 mg/kg showed significant with (0.27±0.05) P<0.01.

The rats treated with CCL_4 1ml/kg developed a significant hepatic damage observed by elevated concentrations of SGOT, SGPT, and Total & Direct bilirubin as compared to normal rats.

Table 3: Effect leaves extract of *Ipomoea batatas* on & In-Direct Bilirubin, Serum & tissue protein & SOD.

Drug treatment	Indirect Bilirubin(mg/dL)	Serum Protein (g/dL)	Tissue Protein (g/dL)	SOD Units/ mg Protein
Normal control Purified water	0.42±0.02	12.06±0.17	10.14±0.42	14.13±1.24
CCL_4(1ml/kg on 3, 6, 9th day)	0.82±0.09###	4.22±0.23###	3.13±0.28###	4.08±0.84###
Vitamin –C (50 mg/kg/day)CCL_4	0.42±0.02***	8.93±0.45***	9.14±0.32***	13.04±0.92***
EEIB (300 mg/ kg/day) CCL_4	0.80±0.04 NS	4.71±0.49 NS	3.26±0.37 NS	3.53±0.90 NS
EEIB (600 mg/ kg/day) CCL_4	0.48±0.07***	6.69±0.40***	5.89±0.24***	7.50±0.68***
EEIB (900 mg/ kg/day) CCL_4	0.45±0.04***	8.73±0.51***	8.34±0.45 ***	11.18±0.50***

Table 4: Effect leaves extract of *Ipomoea batatas* LPO & catalase

Drug treatment	LPO nmoles/MDA/min/mg of protein	Catalase micromole of H_2O_2 Consumed/min
Normal control Purified water	18.94±0.72	191.8±9.62
CCL_4(1ml/kg on 3, 6, 9th day)	63.37±1.39###	103.5±8.01###
Vitamin –C (50 mg/kg/day)CCL_4	23.23±1.56***	181.5±11.76***
EEIB (300 mg/ kg/day) CCL_4	61.09±1.81*	106.2±5.91NS
EEIB (600 mg/ kg/day) CCL_4	52.72±1.38***	122.2±7.38**
EEIB (900 mg/ kg/day) CCL_4	42.25±1.41***	165.5±8.44***

Each value is expressed as Mean ± SD for groups of 6 animals in each group.

*P<0.05, **P<0.01, ***P<0.001 will be considered as significant when compared to toxic group. #P<0.05, ##P<0.01, ###P<0.001, will be considered as significant when compared to the normal group. One way ANOVA followed by Dunnett's multiple comparison tests.

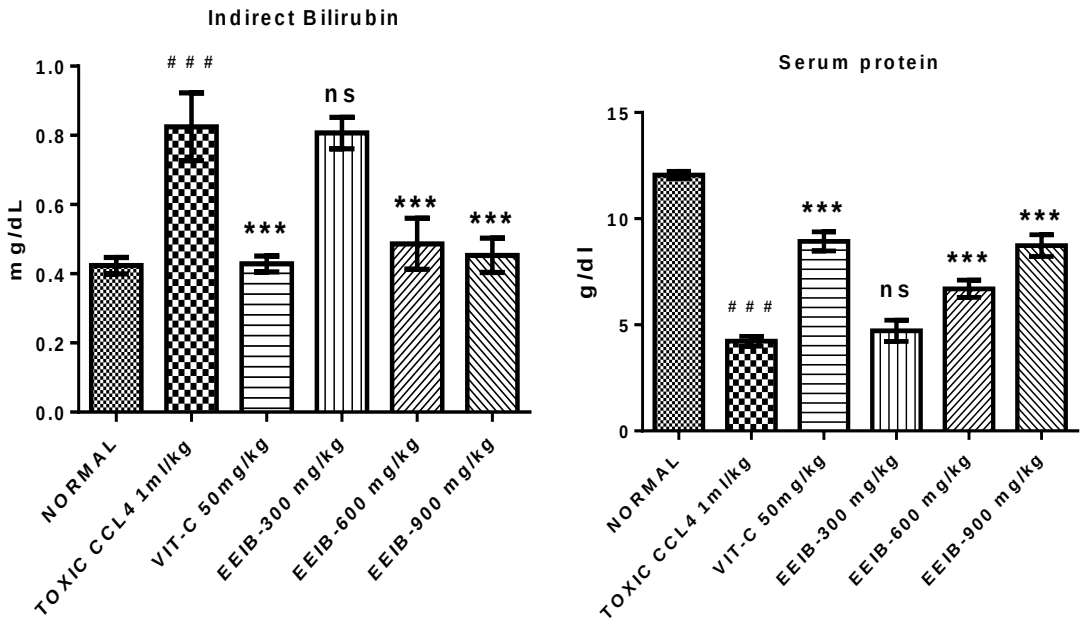


Figure 3: Effect of vehicle, CCl_4, Vit-C & extract on serum protein & indirect bilirubin.

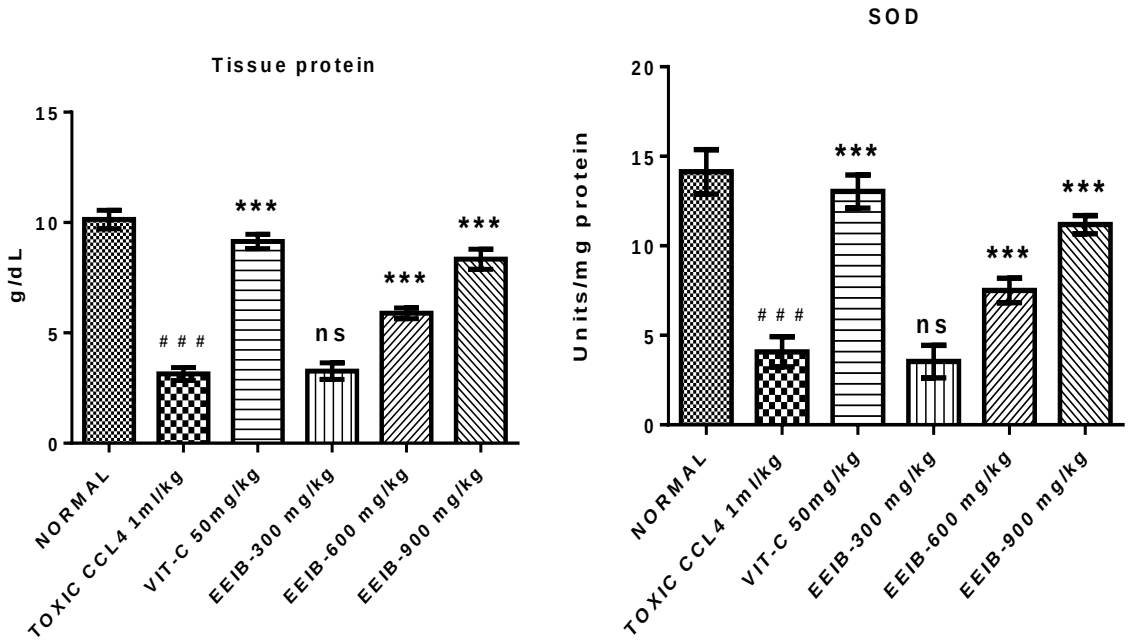


Figure 4: Effect of vehicle, CCl_4, Vit-C & extract on SOD & tissue protein

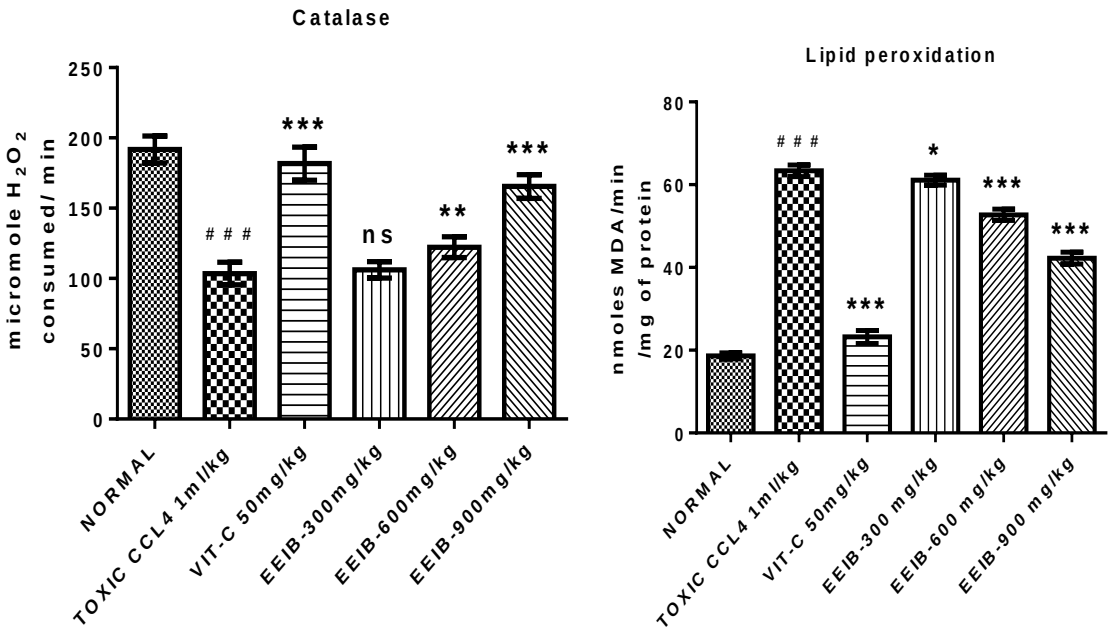


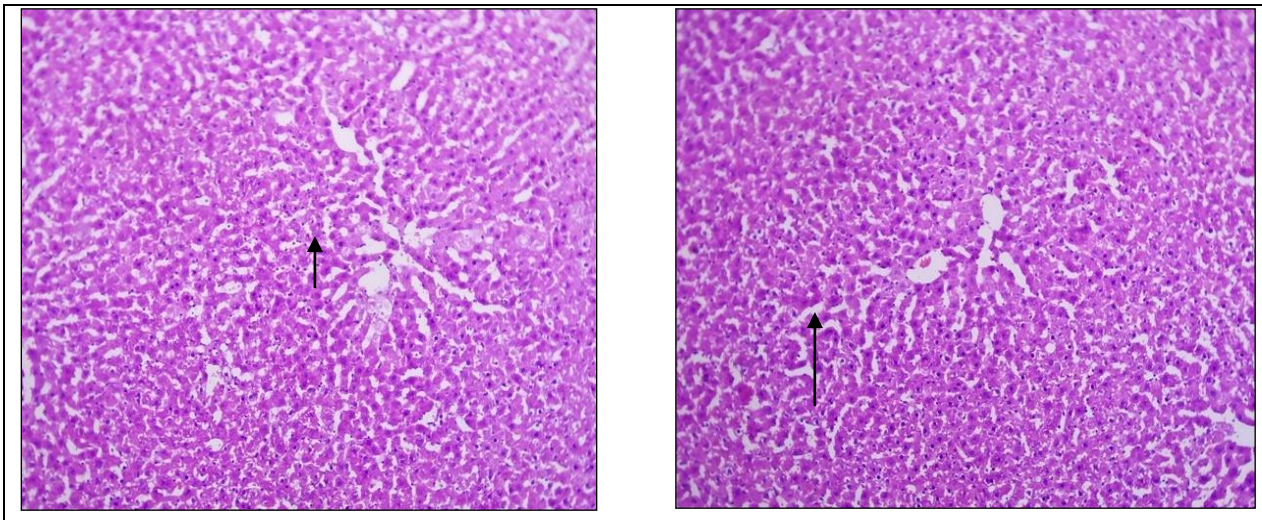
Figure 5: Effect of vehicle, CCl_4, Vit-C & extract on catalase, LPO

Tissue and serum proteins: CCL_4 induce significant decrease (P<0.001) in tissue & serum proteins (3.13±0.28 & 4.22±0.23) respectively, when compared to the normal group (10.14±0.42, 12.06±0.17). Rats pretreated with vitamin-C 50mg/kg & extract of 600, 900mg/kg (9.14±0.32, 8.93±0.45) & (5.89±0.24, 6.69±0.40 & 8.34±0.45, 8.73±0.51) resorted the protein levels nearer to vitamin C with a significance of P<0.001. Extract with 300mg/kg showed no significance in serum & tissue proteins when compared to CCL group.

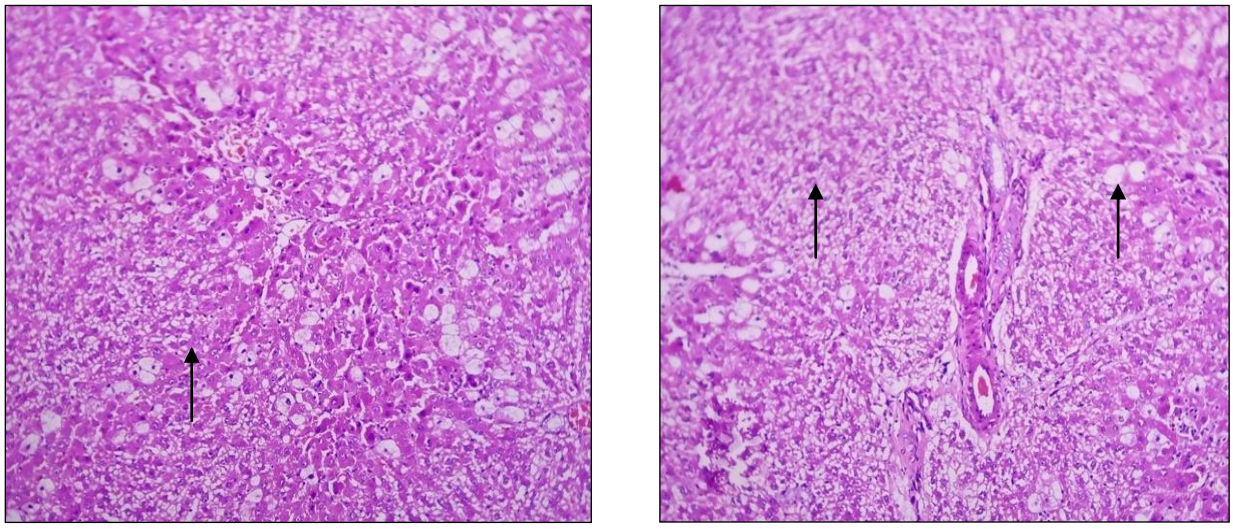
Antioxidant activity: SOD, Lipid peroxidation & catalase.

Rats treated with CCL_4 showed a significant increase in the levels of LPO (63.37±1.39), and decrease in the levels of catalase (103.5±8.01) & SOD (4.08±0.84) with a significance of P<0.001 when compared to the normal group. Rats pretreated with vitamin C & 900mg of extract, increased the levels of SOD (13.04±0.92), (11.18±0.50) catalase (181.5±11.76) (165.5±8.44) & decreased LPO (23.23±1.56) (42.25±1.41) levels with significance P<0.001 correspondingly. Extract with 600 mg showed the significance of P<0.001 for both LPO (52.72±1.38) & SOD (7.50±0.68) followed by P<0.01 significance for catalase (122.2±7.38). The low dose of extract showed no significance for SOD, catalase & LPO had a significance of P<0.05 (61.09±1.81).

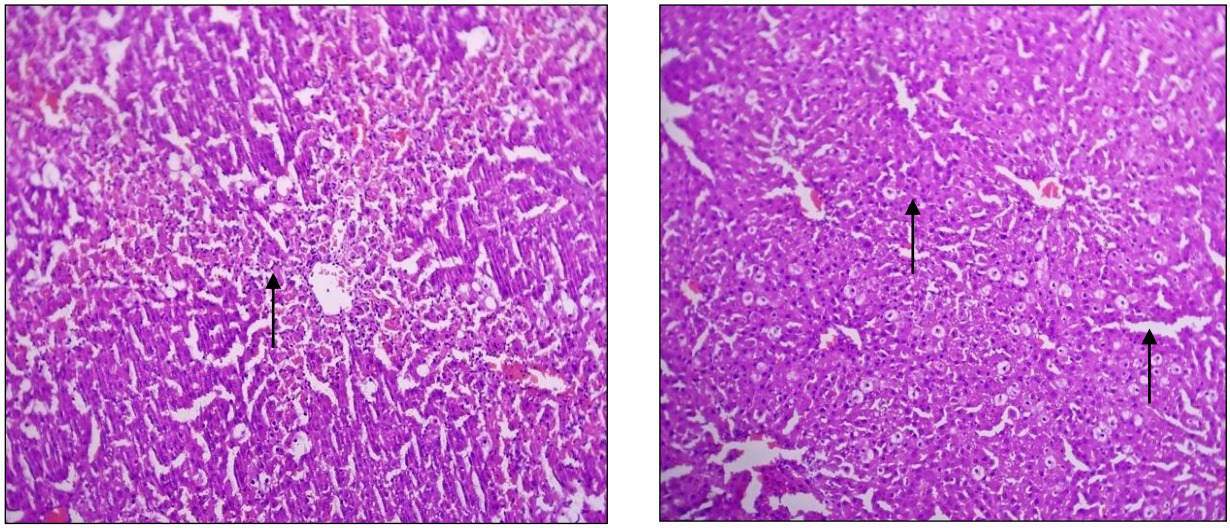
Histopathological studies of rat's liver.



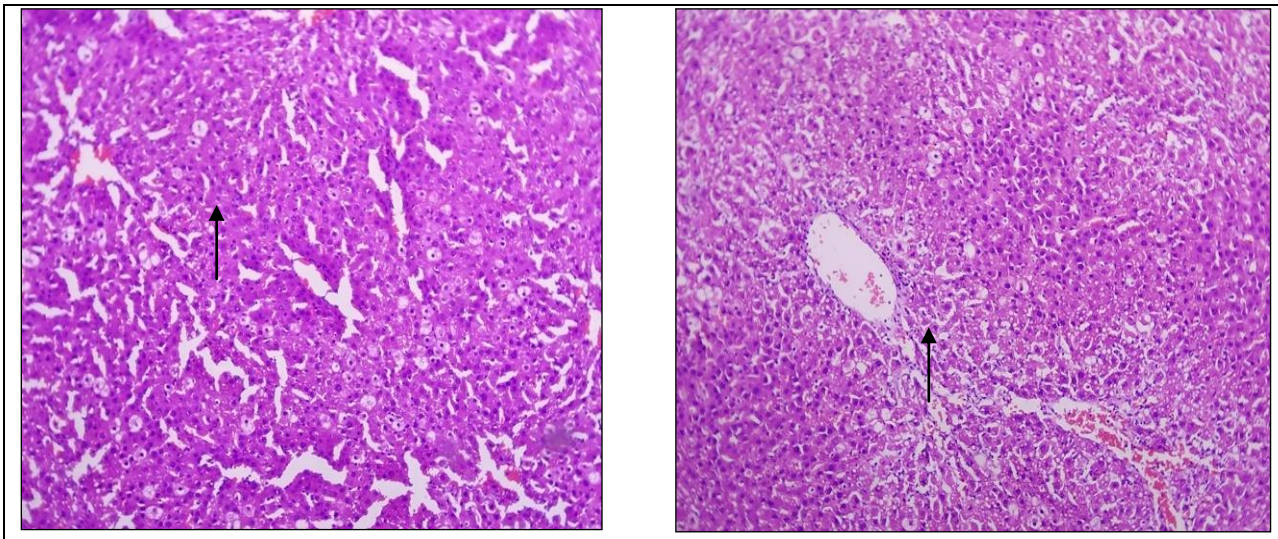
G1-Normal control



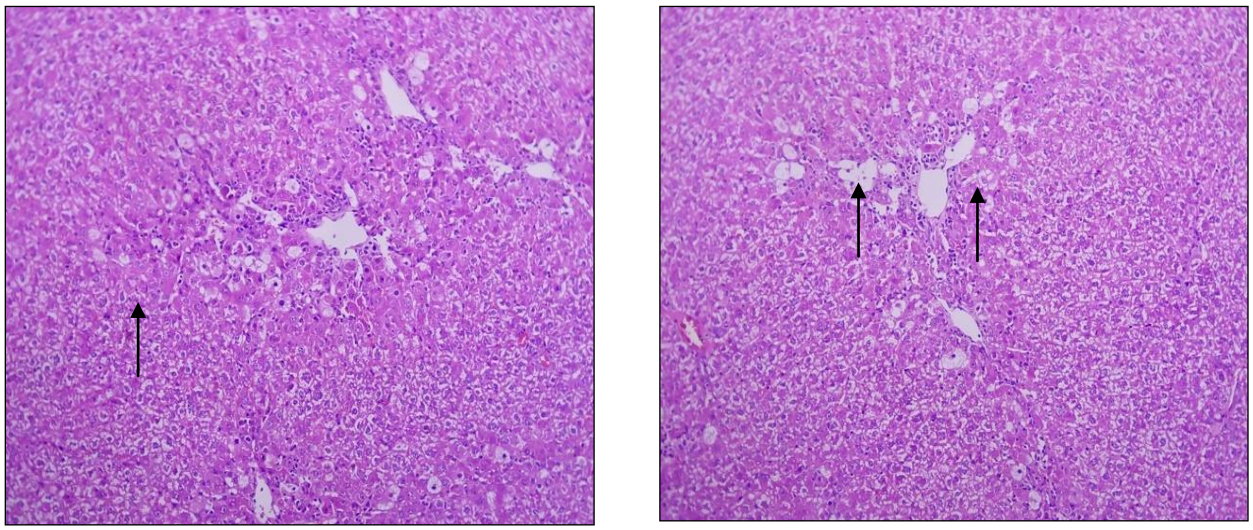
G2-Toxic control (Carbon tetrachloride 1ml/kg)



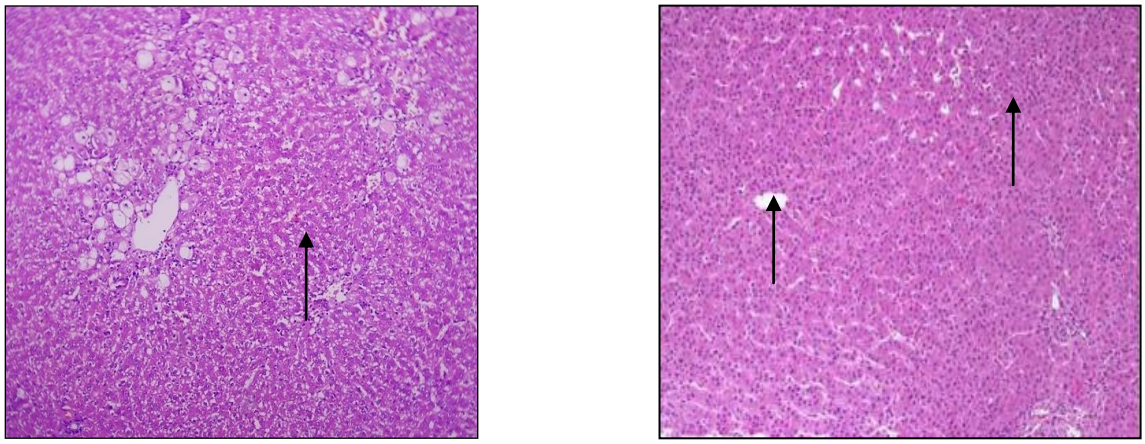
G2-Standard(Vitamin –C 50mg/kg)



G3- EEIB - 300mg/kg



G3- EEIB - 600mg/kg



G3- EEIB - 900mg/kg

Figure 6: Histopathological studies of rat's liver.

CONCLUSION

Thus the ethanolic leaf extract of *Ipomoea batatas* have shown a significant antioxidant property. The free radical scavenging property may be the possible mechanism by the extract, protected the liver with comparison to the standard drug vitamin C against carbon tetrachloride induced hepatic injury in rats.

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