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Research article

Hepatoprotective activity of ethanolic extract of *Euphorbia royleana linn* in carbon tetrachloride treated guinea pig

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Abstract

Background: The present study has been designed to explore and provide experimental evidences for the hepatoprotective activity of the stem of *Euphorbia royleana Linn*. **Material and Methods:** The herbs of *Euphorbia royleana Linn* (stem) were powdered, extracted by using soxhlet apparatus. Powdered stem were defatted using petroleum ether (60%-80%), chloroform (99%) and hydro-alcoholic (70% alcohol and 30% water) solvent for 72 hours each time. Animals in one group received standard drug Liv-52 (100 mg/kg, p.o.). Other two groups were treated with low and high dose of ethanolic extract & chloroform extract (200 and 400 mg/kg., p.o. respectively) for 10 days once daily. On the 11 day, carbon tetrachloride (2 ml/kg in 50% v/v olive oil, s.c.) was given to all groups for induction of hepatotoxicity. After that various biochemical parameters were measured like as; SGOT, SGPT, ALP, serum total protein, total bilirubin and Direct bilirubin. **Result:** The standard drug Liv-52 for 10 days before CCl₄ induction is administered to the animals and all the parameters were accessed after 11 days. Serum total protein level also elevates toward normal after treatment with standard drug. Ethanolic extract (EER) at dose of 400mg/Kg orally for 10 days shows most significant reduction in elevated level of SGOT, SGPT, ALP, total bilirubin and direct bilirubin level and EER also maintain the decreased levels of protein in the body as compared to the positive control group and standard treated group. Significant differences were observed change of body by ethanolic and methanol extract treated hepatotoxicity Guinea pig, when compared with the positive and normal animals. Concurrent histopathological studies of the liver these animals showed comparable regeneration by extract which were earlier encored by CCl₄. **Conclusion:** The present study showed that the ethanolic extract of *Euphorbia royleana Linn* has able to maintain the abnormal function of the liver when hepatotoxicity.. So it is may be concluded that *Euphorbia royleana Linn* is one of the herbal remedies which can be used as a liver ailment.

Keywords: Euphorbia royleana, Carban tetrachloride, Marker enzyme, Ethanolic extract.

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1. Introduction

The liver is the largest internal organ in the body (the skin being the largest organ overall. Which maintains the body's internal milieu and it is the most important organ, which plays a pivotal role in regulating the internal chemical environment and it plays various physiological processes in the body [1]. Liver dysfunction or hepatotoxicity is a major health related problem in society that challenges healthcare professional or physicians but also the pharmaceutical

industry and drug regulatory agencies of the world [2]. The most common complication includes: Infections such as hepatitis A, B, C and E, alcohol induced liver damage, fatty liver, cirrhosis, cancer, and drug induced liver damage (Acetaminophen and various anti-cancer drugs). Many diseases of the liver go hand in hand with jaundice which is caused by increased levels of bilirubin in the body. Excess bilirubin results in the breakup of the hemoglobin of aged RBC. Normally the liver removes bilirubin from the blood and excretes it through bile. There are also many pediatric liver diseases including biliary atresia, an alpha-1, antitrypsin

deficiency, alagille syndrome, progressive familial intrahepatic cholestasis, and Langerhans cell histiocytosis.

Euphorbia royleana Linn is a kind of medicinal plant which is mostly used in the Indian medicinal system as anti-inflammatory, antipyretic, analgesic, antimicrobial & anti-oxidant etc. For the treatment of hepatotoxicity, various allopathic medicines are available in a market but prolonged use of these agents produces adverse drug reactions such as weight gain, weakness, abdominal pain, black stool, dry mouth, insomnia, anxiety, bloating, peptic ulcer, etc. Management of hepatotoxicity with these agents avoids of any side effects is still a challenge to the medical profession. This has lead to an increased demand for natural product for hepatoprotective activity having fewer side effects. So, *Euphorbia royleana* Linn has been selected for the study. Myrsinane isolated from *Euphorbia* species as whole plant chloroform extract, showed significant analgesic activity when administered to mice at a dose of 5-20 mg/kg i.p. This activity is comparable to that of 100 mg/kg of aspirin or ibuprofen [3].

The aim of the present study was to explore and provide experimental evidences for the hepatoprotective activity of the stem of *Euphorbia royleana* Linn. In experiment Guinea pigs model of liver injury induced by carbon tetrachloride.

2. Material and method

Collection and authentication

The herb of *Euphorbia royleana* Linn was collected from Mathura (India) in the month of November 2014. The authentication of the herb was done by the experts of the National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. The voucher specimen was preserved in the department for further research in the laboratory.

Preparation of extract

The stem of *Euphorbia royleana* (Linn) was collected and washed with water to remove dust. Then they were shade dried for a few days. After proper drying they were powdered with the help of mortar & pestle to coarse powder and passed through sieve no. 12 to get uniform size of powder. The finely grinded crude drug was placed in a porous bag or "thimble" made up of strong filter paper, which was placed in the chamber of the Soxhlet apparatus. Powdered stem was defatted using petroleum ether (60%-80%) by heating solvent in the flask and its vapors were condensed in condenser. The condensed extract drops into the thimble containing the crude drug and when the level of liquid in the chamber (thimble) rises to the top of the siphon tube, the liquid contents of soxhlet chamber siphons into the round bottom flask. This process was continued and was carried out until not even single a drop of solvent

from the siphon tube leave residue when evaporated. After complete defatting of powdered stem, the marc was then extracted with chloroform (99%). Again the same procedure was followed as above until the formation of marc. Again marc was extracted with hydro-alcoholic (70% alcohol and 30% water) solvent for 72 hrs each time in the same way. The solvent was removed under reduced pressure. Crude extract was stored in airtight container in refrigerator for further studies [4].

Physicochemical parameters

Determination of Loss on Drying

Loss on drying is a way of determination of moisture content in the crude drug. This method determines both water and volatile matter in crude drug. Loss on drying is the loss of mass expressed as percent w/w. Five grams of stem powder was accurately weighed and taken in a silica crucible. It was dried in an air oven at 106°C for one hour. Then cooled in desiccators and weighed out until a constant weight was achieved.

Determination of Solvent Extractive Values

An extractive value was used to identify the amount of active constituents present in a given amount of medicinal plant material when extracted with solvents. Determination of alcohol soluble extractive value: Air dried stem powder (5g) weighed accurately and transferred in a flat bottom stoppered flask then 100 mL of alcohol (90%) was added in to flask. It was macerated for 24 hr, frequently shaking during the first 6 hr and allowed to stand for 18 hr. After 24 hr, it was rapidly filtered. Filtrate (25 mL) was transferred in a flat bottom shallow dish and subjected to drying in an oven at 105°C till constant weight. Percentage w/w of alcohol soluble extractive value was calculated with reference to the air-dried drug.

Determination of water soluble extractive value: Air dried stem powder (5 g) weighed accurately and transferred in stoppered flat bottom flask then 100 mL distilled water was added. It was macerated for 24 hr, frequently shaking during the first 6 hr and allowed to stand for 18 hr. After 24 hr it was rapidly filtered. Filtrate (25 mL) was transferred in a flat bottom shallow dish and subjected to drying in an oven at 105°C till constant weight. Percentage w/w of water soluble extractive value was calculated with reference to the air-dried drug.

Experimental animals

Guinea pigs of either sex (n=48), 6 animals in each group with body weight of 400g -500g were used in this study. Animals were fed with food and water ad libitum, they were housed in large cages with a 12hour light/dark cycle and temperature controlled environment (25±5°C). The experimental protocol was

approved by Institutional Animal Ethical Committee (GLAIPR/CPCSEA/IAEC/2015/P.Col/R9) for use of laboratory animals.

Phytochemical analysis

The different extracts were obtained from the stems of *Euphorbia royleana* Linn by successive solvent extraction method these were subjected to various qualitative tests to detect presence or absence of common phytoconstituents like alkaloids, glycosides, carbohydrates, flavonoids, carotenoids, vitamins, minerals and phenolic compounds according to the standard methods described in practical pharmacognosy (Table:-1).

Table 1. Phytochemical Investigations

S.N	Name of Test	Chloroform extract	Ethanol extract
1	Alkaloids: a. Dragendroff's test b. Mayer's test c. Hager's test d. Wagner's test	Positive	Positive
2	Carbohydrate: a. Molisch's test b. Fehling's test c. Benedict's test	Negative	Negative
3	Protein and amino acids: a. Ninhydrin test b. xanthoproteic test c. Millon's test	Negative	Negative
4.	Saponins	Negative	Negative
5.	Flavonoids: a. Shinoda test b. zinc hydrochloride test c. Alkaline reagent test	Positive	Positive
6	Tannin and phenolic compound a. Gelatin test b. Ferric chloride test c. Alkaline reagent test d. Mitchell's test	Positive	Positive
7.	Steroids: a. Liebermann-Burchard test b. salkowski's test	Negative	Positive
8	Glycosides: a. Raymond's test b. Legal's test c. Bromine water test	Negative	Negative
9	Gums and Mucilage	Positive	Negative

Experimental design

Carbon tetra chloride (CCL₄) induced hepatotoxicity

Guinea pigs of either sex weighing between 400–500g were divided into seven groups of six animals each (n = 6).

Group I served as normal control or untreated control and received 1% CMC (10 mL/kg, p.o.) once daily for 10 days.

Group II carbon tetrachloride treated rats received 2 ml/kg subcutaneously diluted with olive oil on the 11th day.

Group III received standard drug Liv-52 (100 mg/kg, p.o.) for 10 days once daily and then received CCl₄ on the 11th day.

Groups IV were treated with low and high dose of Ethenolic extract of *Euphorbia royleana* (200 mg/kg., p.o.) for 10 days once daily. On the 11 day, carbon tetrachloride (2 mL/kg in 50% v/v olive oil, s.c.) was given to all groups except the rats in group I.

Groups V were treated with low and high dose of Ethenolic extract of *Euphorbia royleana* (400 mg/kg., p.o.) for 10 days once daily. On the 11 day, carbon tetrachloride (2 mL/kg in 50% v/v olive oil, s.c.) was given to all groups except the rats in group I.

Groups VI were treated with low and high dose of Chloroform extract of *Euphorbia royleana* (200 mg/kg., p.o.) for 10 days once daily. On the 11 day, carbon tetrachloride (2 mL/kg in 50% v/v olive oil, s.c.) was given to all groups except the rats in group I.

Groups VII were treated with low and high dose of Chloroform extract of *Euphorbia royleana* (400 mg/kg., p.o.) for 10 days once daily. On the 11 day, carbon tetrachloride (2 mL/kg in 50% v/v olive oil, s.c.) was given to all groups except the rats in group I.

Description of biochemical parameters

Estimation of SGOT (UV-kinetic method)

SGOT catalyzes the transfer of an amino group from L-aspartate to 2- oxoglutarate forming oxaloacetate and L-glutamate. The rate of this reaction was measured by an indicator reaction coupled with malate- dehydrogenase (MDH) in which the oxaloacetate formed was converted to malate in the presence of reduced nicotinamide adenine dinucleotide (NADH). The oxidation of NADH in this reaction was measured as a decrease in absorbance of NADH at 340nm which was proportional to SGOT activity [5].

Estimation of SGPT (UV-kinetic method)

SGPT catalyzes the transfer of an amino group from L-alanine to 2-ketoglutarate with the formation of pyruvate and L-glutamate. The pyruvate so formed is permitted to react with NADH to produce lactate. The rate of this reaction is measured by an indicator reaction coupled with LDH in the presence of NADPH (nicotinamide adenine dinucleotide). The oxidation of NADH in this reaction was measured as a decrease in the absorbance of NADH at 340nm, which was proportional to SGPT activity.

unconjugated bilirubin is solubilised using a surfactant and then it reacts similar to direct bilirubin.

Reagents and chemicals

SGOT estimation kit, total protein estimation kit, the ALP estimation kit, SGPT estimation kit & Bilirubin estimation kit were purchased from Span diagnostics ltd., Surat, India. CCl₄ for inducing hepatotoxicity in guinea pigs was purchased from CDH, Lucknow, India. Liv-for treatment of hepatotoxicity was purchased from Himalaya herbal healthcare.

Table 2. Effect of *Euphorbia royleana* Linn Extract on SGOT, SGPT, ALP, Total Protein, Total Bilirubin, Direct Bilirubin.

Group	SGOT	SGPT	ALP	TP	TB	DB
Normal	38.84 ± 3.35	30.37 ± 2.46	91.13 ± 5.90	8.09 ± 0.28	0.83 ± 0.11	0.71 ± 0.11
Positive Control (CCl ₄)	137.71 ± 2.59	183.52 ± 2.87	205.12 ± 4.96	3.43 ± 0.14	1.80 ± 0.29	1.61 ± 0.14
Liv-52 Treated (100mg/Kg)	44.55 ± 2.21	34.31 ± 2.58	99.17 ± 2.83	6.27 ± 0.15	0.95 ± 0.12	0.83 ± 0.11
EER (200mg/Kg)	83.82 ± 3.09	66.065 ± 3.03	140.37 ± 3.34	5.52 ± 0.12	1.30 ± 0.12	1.34 ± 0.11
EER (400mg/Kg)	62.97 ± 3.35	50.5 ± 1.80	127.36 ± 3.60	6.05 ± 0.12	1.19 ± 0.11	1.27 ± 0.13
CER (200mg/Kg)	108.71 ± 2.54	152.04 ± 2.30	192.80 ± 3.07	4.93 ± 0.11	1.77 ± 0.1	1.53 ± 0.11
CER (400mg/Kg)	93.30 ± 2.72	112.98 ± 3.76	162.99 ± 4.20	5.02 ± 0.10	1.86 ± 0.11	1.48 ± 0.15

SGOT= Serum Glutamate Oxaloacetate Transaminase, SGPT= Serum Glutamate Pyruvate Transaminase, ALP= Alkaline Phosphatase, TP= Total Protein, TB= Total Bilirubin, DB= Direct Bilirubin. EER= Ethanolic extract of *Euphorbia royleana* stem, CER= Chloroform extract of *Euphorbia royleana* stem. All values are expressed as mean ± S.D., n=6 animals in each group.

Estimation of ALP

ALP as an alkaline pH 10.3 catalyses the hydrolysis colourless p-nitrophenyl phosphate to form p-nitrophenol and phosphate. The rate measured as an increase in absorbance, which is proportional to the ALP activity in the sample.

Estimation of serum total protein

The peptide bonds of proteins react with cupric ions in alkaline solution to form a coloured chelates. The absorbance was measured at 578nm. The Biuret reagent contains sodium-potassium tartrate which helps in maintaining solubility of this complex at alkaline pH. The absorbance of the final colour was proportional to the concentration of total protein in the sample [6].

Estimation of serum bilirubin

Bilirubin reacts with diazotized sulphanilic acid in acidic medium to form pink coloured azobilirubin with absorbance directly proportional to bilirubin concentration. Direct bilirubin, being water soluble directly reacts in acidic medium. However, indirect or

Statistical analysis

All values were expressed as mean ± standard deviation (S.D.). Statistical analysis was performed by using graphpad and sigmastat software. Data obtained from various groups were significantly analyzed using one-way ANOVA, P<0.05 was considered to be statistically significant.

3. Result

Evaluation of hepatoprotective Activity in CCl₄ induced liver damage in guinea pigs

Oral administrations of hydro alcoholic extract of plant *Euphorbia royleana* Linn. has shown variations in various biochemical parameters in ccl₄ induced liver damage of the animal (Table:-2). They are as follows:

Effect on SGOT

CCl₄ administration caused significant (p<0.05) elevation of SGOT by inducing Liver damage in control animals as compared to normal animals. While,

standard drug Liv-52 treatment reduced the increase in SGOT concentration in animals treated with CCl₄ in standard group as compared to positive control group. There was dose-dependent reduction in serum SGOT levels. Ethanolic extract of drug (200 mg/Kg & 400 mg/Kg., p.o.) significantly ($p < 0.05$) reduces the elevated SGOT level in animals as compared to positive control group. While chloroform extract of drug (200 mg/Kg & 400 mg/Kg., p.o.) showed less significant ($p < 0.05$) decrease in SGOT level as compared to ethanolic extract treated group (Fig. 1).

Effect on SGPT

Treatment with CCl₄ caused significant ($p < 0.05$) elevation in SGPT levels in animals as compared to normal animals. Standard drug Liv-52 treatment reduced significantly ($p < 0.05$) SGPT concentration as compared to positive control group. Dose-dependent reduction in serum SGOT levels was observed. Ethanolic extract of drug (200 mg/Kg & 400 mg/Kg., p.o.) significantly ($p < 0.05$) reduced the elevated SGPT level in animals as compared to positive control group. While chloroform extract of drug (200 mg/Kg & 400 mg/Kg., p.o.) showed less significant ($p < 0.05$) reduction in elevated SGPT level as compared to ethanolic extract treated group (Fig. 2).

Effect on ALP

The liver damage was induced by CCl₄ treatment which resulted in significant ($p < 0.05$) elevation of serum enzyme alkaline phosphatase as compared to normal animals. Animals administered with standard drug (Liv-52) showed a significant ($p < 0.05$) decrease in ALP concentration as compared to positive control group. Treatment with ethanolic extract of drug (200 mg/Kg & 400 mg/Kg., p.o.) significantly ($p < 0.05$) reduced the elevated ALP level in animals as compared to positive control group. While chloroform extract (200 mg/Kg & 400 mg/Kg., p.o.) showed less significant ($p < 0.05$) reduction in elevated SGPT level as compared to ethanolic extract treated group (Fig. 3).

Effect on total protein

In positive control group animals treated with CCl₄. There was significant ($p < 0.05$) decrease in serum total protein concentration. This decrease was found due to liver damage when compared to normal animals. Administration of Liv-52 caused significant ($p < 0.05$) increase in total protein as compared to positive control group. Animals treated with ethanolic extract of drug (200 mg/Kg & 400 mg/Kg., p.o.) shows significant ($p < 0.05$) elevation in reduced total protein concentration level in CCl₄ treated animals. While chloroform extract of drug (200 mg/Kg & 400 mg/Kg., p.o.) showed less significant ($p < 0.05$) elevation in total protein level as compared to ethanolic extract treated group (Fig. 4).

Effect on total bilirubin

The total bilirubin concentration was found to be significantly ($p < 0.05$) increased in animals with liver damage by CCl₄ (i.e. positive control group). In standard group, Liv-52 administration significantly ($p < 0.05$) reduced total bilirubin concentration. Treatment with ethanolic extract of drug (200 mg/Kg & 400 mg/Kg., p.o.) significantly ($p < 0.05$) reduced the elevated total bilirubin level in animals as compared to positive control group. While chloroform extract (200 mg/Kg & 400 mg/Kg., p.o.) showed less significant ($p < 0.05$) reduction in elevated total bilirubin level as compared to ethanolic extract treated group (Fig. 5).

Effect on direct bilirubin

CCl₄ significantly ($p < 0.05$) elevates the direct bilirubin concentration in positive control animals as compared to normal animals. Treatment with standard drug Liv-52 significantly ($p < 0.05$) reduces the elevated direct bilirubin level in standard group. Treatment with ethanolic extract of drug (200 mg/Kg & 400 mg/Kg., p.o.) significantly ($p < 0.05$) reduces the elevated direct bilirubin level in animals as compared to positive control group. While chloroform extract of drug (200 mg/Kg & 400 mg/Kg., p.o.) shows less significant ($p < 0.05$) reduction in elevated direct bilirubin level as compared to ethanolic extract treated group (Fig. 6).

Effect of *Euphorbia royleana* Linn on histopathology observation

Examination of liver tissue of hepatotoxicity with CCl₄ indicated that the hepatic lobules appeared more (or) less like control (Fig. 7).

4. Discussion

Liver is a most important organ of vertebrates and some animals. It is the second largest organ of the body after skin. It plays a major role in detoxification of various metabolites, protein synthesis and the production of biochemicals necessary for digestion. Drug induced liver disease is the most common cause of acute liver failure and is a great challenge to physicians [7].

The present study was performed to assess the hepatoprotective activity of stem of the plant *Euphorbia royleana* in guinea pigs, against carbon tetrachloride (CCl₄) as a hepatotoxic agent to prove its claim in folklore practice against liver disorders. The changes associated with CCl₄ induced liver damage seemed to be similar to as acute viral hepatitis [8]. CCl₄ is a widely used experimental hepatotoxicant and it is biotransformed by cytochrome P-450 enzyme in liver it produces trichloromethyl free radical, (CCl₃) This free radical a covalently binds to cell membranes and organelles to elicit lipid peroxidation and disturbs Ca²⁺ hemostasis which finally results in cell death [9].

Hepatotoxic doses of CCl₄ deplete the normal levels of hepatic glutathione protects hepatocytes by combining with the reactive metabolite of CCl₄ thus preventing its covalent binding to liver proteins [10]. In living systems, liver is deemed to be highly sensitive organ toward toxic agents. It was noted that different enzyme activities such as SGOT, SGPT, ALP, total bilirubin, direct bilirubin and total protein are indicators of

clinical and experimental liver damage [11]. In the present investigation, it was observed that the animals treated with CCl₄ resulted in hepatic damage as showed by the abnormal levels of serum markers. The changes in marker levels reflect changes in hepatic structural integrity [12].

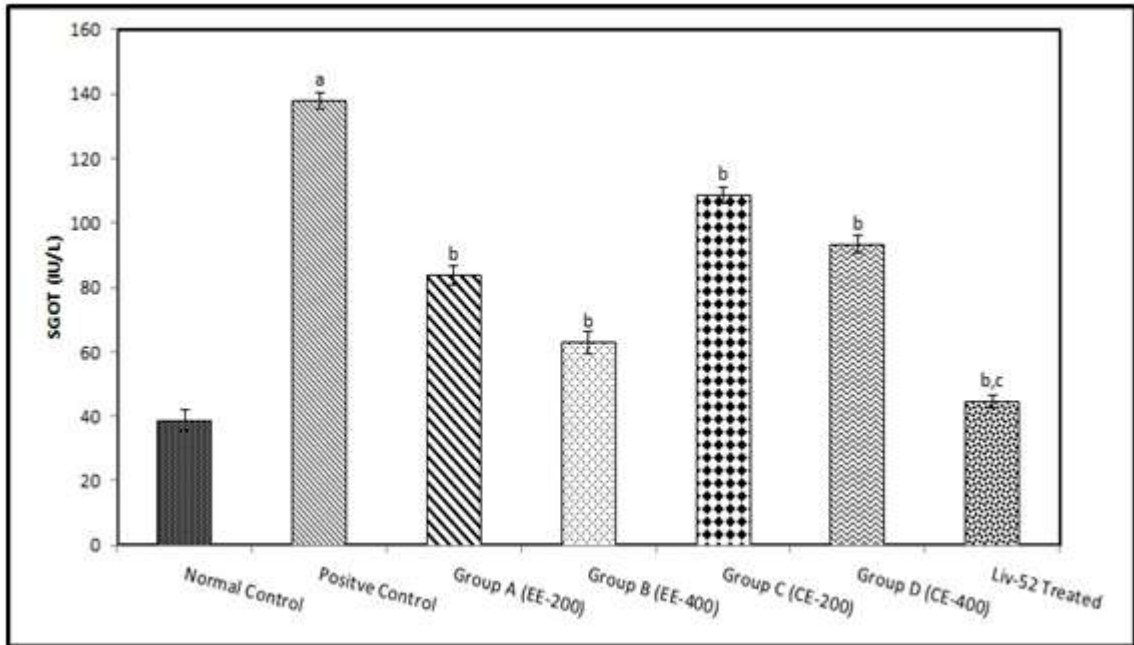


Figure 1 Effect of *Euphorbia royleana* Linn extract on SGOT. Values are expressed as mean \pm S.D., a= p <0.05 vs. normal control; b= p <0.05 vs. positive control; c= p <0.05 vs. Group B (EE-400).

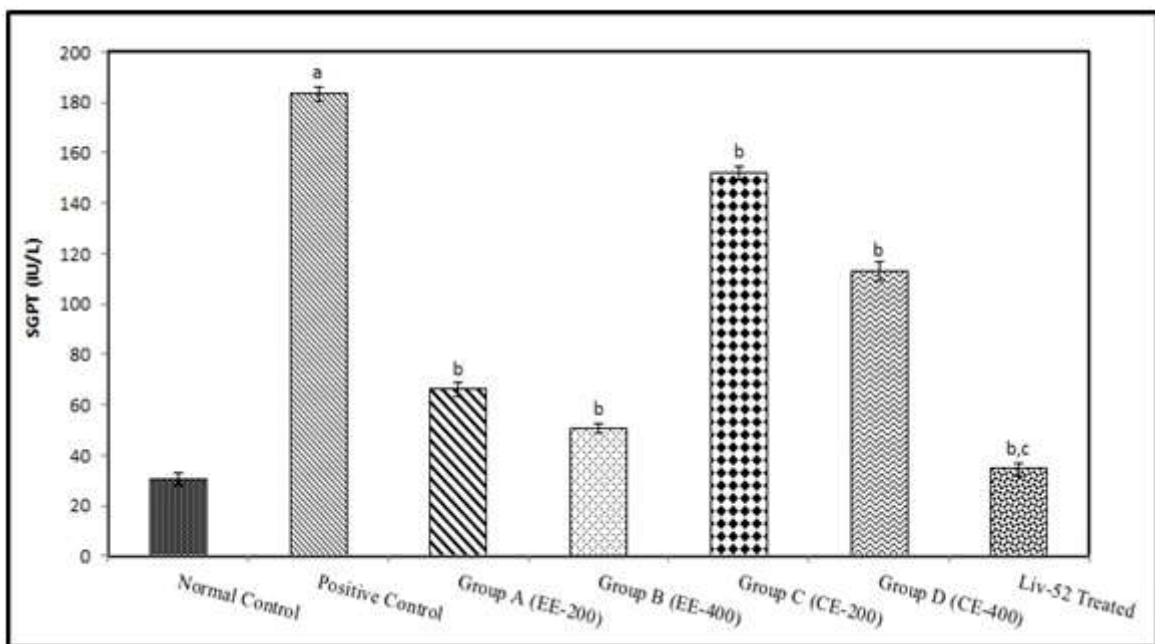


Figure 2 Effect of *Euphorbia royleana* Linn extract on SGPT. Values are expressed as mean \pm S.D., a= p <0.05 vs. normal control; b= p <0.05 vs. positive control; c= p <0.05 vs. Group B (EE-400).

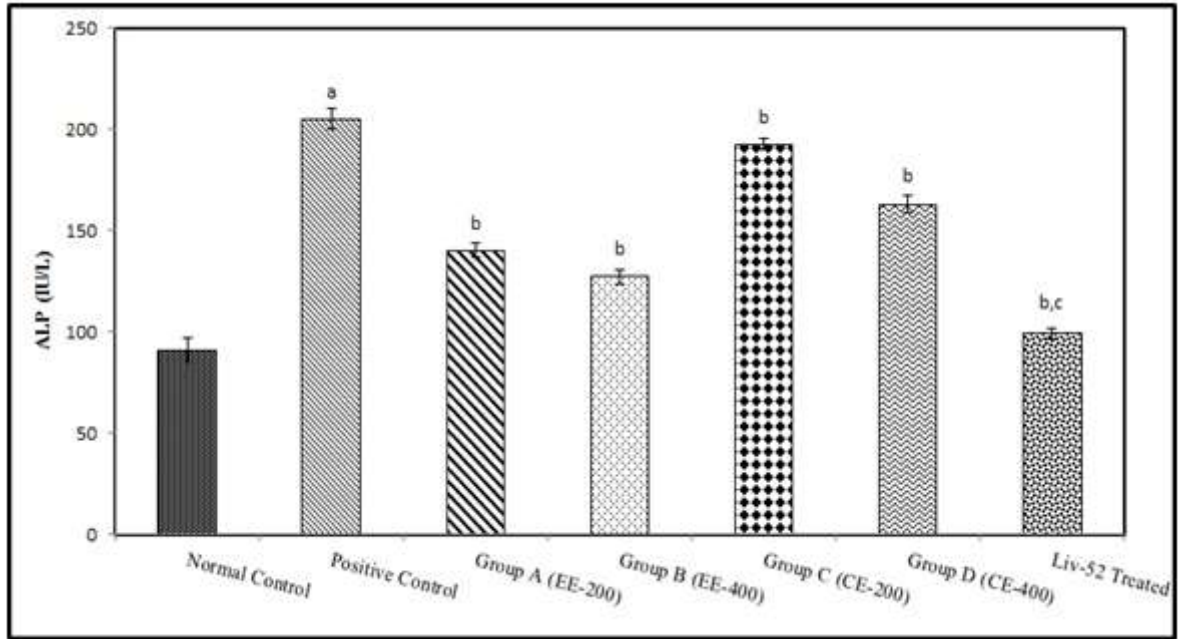


Figure 3 Effect of *Euphorbia royleana* Linn extract on ALP. Values are expressed as mean \pm S.D., a= p <0.05 vs. normal control; b= p <0.05 vs. positive control; c= p <0.05 vs. Group B (EE-400).

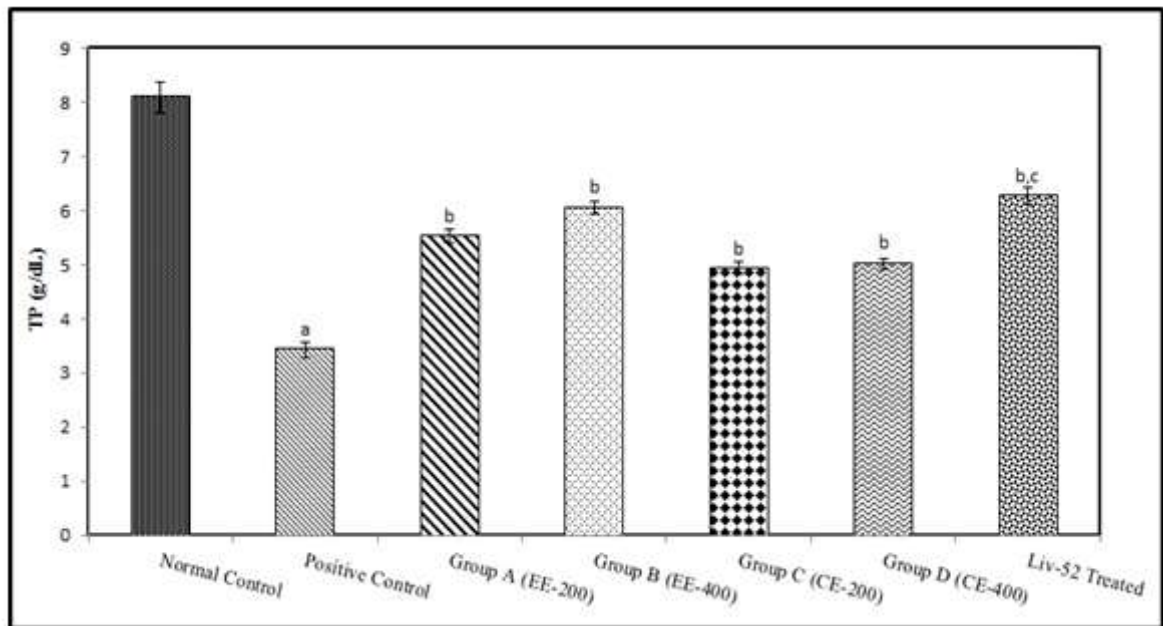


Figure 4 Effect of *Euphorbia royleana* Linn extract on Total protein. Values are expressed as mean \pm S.D., a= p <0.05 vs. normal control; b= p <0.05 vs. positive control; c= p <0.05 vs. Group B (EE-400).

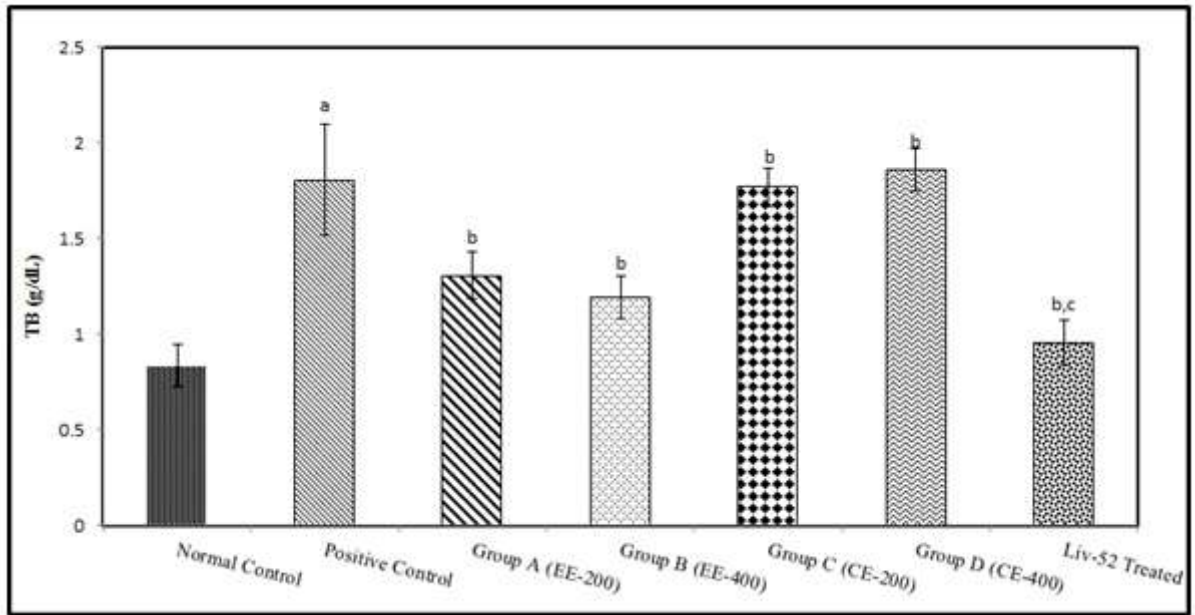


Figure 5 Effect of *Euphorbia royleana* Linn extract on Total bilirubin. Values are expressed as mean \pm S.D., a= p <0.05 vs. normal control; b= p <0.05 vs. positive control; c= p <0.05 vs. Group B (EE-400).

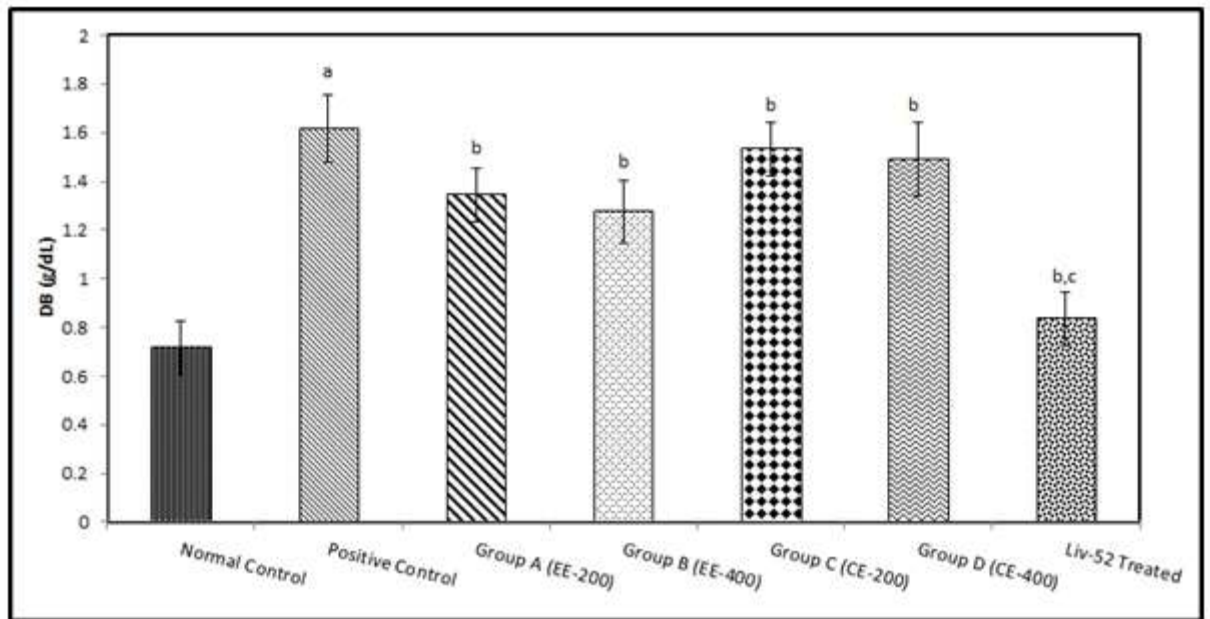
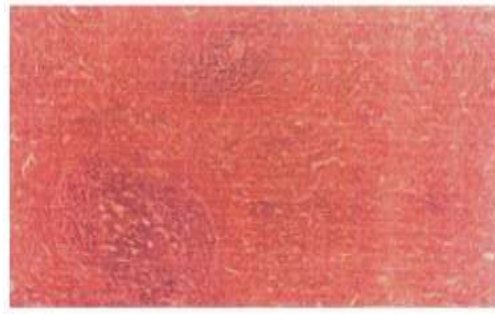
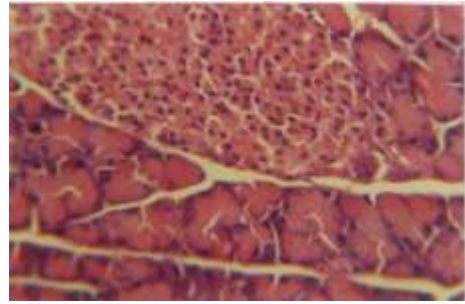


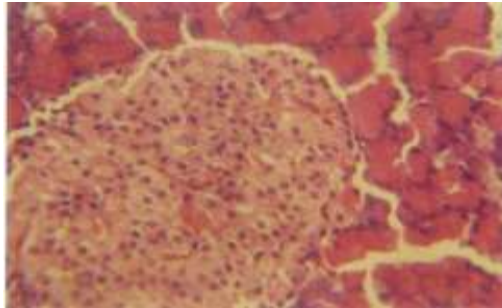
Figure 6 Effect of *Euphorbia royleana* Linn extract on Direct Bilirubin. Values are expressed as mean \pm S.D., a= p <0.05 vs. normal control; b= p <0.05 vs. positive control; c= p <0.05 vs. Group B (EE-400).



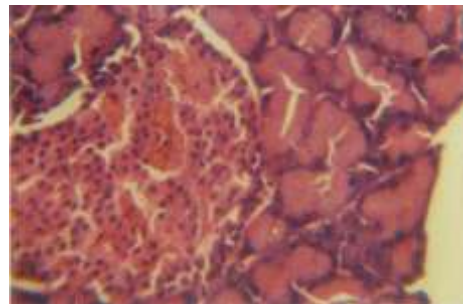
(a) Group 1 Normal control



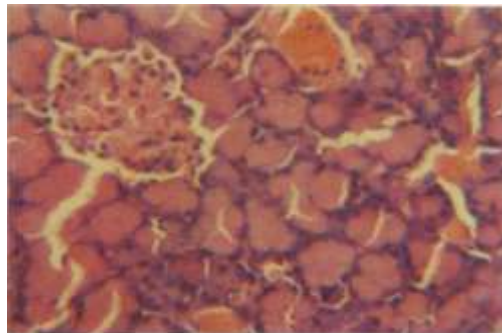
(b) Group 2 Positive control



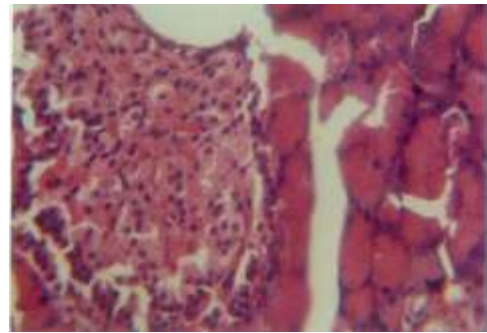
(c) Group 3 Standard drug Liv-52



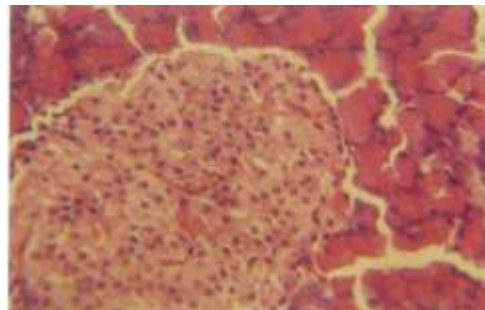
(d) Group 4 Ethenolic extract of *Euphorbia royleana* (200 mg/kg)



(e) Group 5 Ethenolic extract of *Euphorbia royleana* (400 mg/kg)



(f) Group 6 Chloroform extract of *Euphorbia royleana* (200 mg/kg)



(g) Group 7 Chloroform extract of *Euphorbia royleana* (200 mg/kg)

Figure:-7(a-g) Histopathology of ethanol and Chloroform extracts of leaves of *Euphorbia royleana* Linn in CCL₄ induced hepatotoxicity Guinea pigs.

There are two main ingredients of the medicine is first Capparis spinosa-The hepatoprotective activity of stem of the plant Capparis spinous has already been reported. Second Cichorium incubus- Animal studies showed it has a great antihepatotoxic potential [13-14]. Hepatoprotection provided by Liv-52 appears to rest on four properties. First its activity against lipid peroxidation as a result of free radical scavenging which increases the cellular content of glutathione. Second its ability to regulate membrane permeability and to increase stability in the presence of xenobiotic damage Third capacity to regulate nuclear expression by means of a steroid-like effect. Fourth inhibition of the transformation of stellate hepatocytes into myofibroblasts, which are in charge of the deposition of collagen fibres leading to cirrhosis.

In living systems, liver is considered to be highly sensitive organ toward toxic agents. It was found that the study of different enzyme activities such as SGOT, SGPT, ALP, total bilirubin, direct bilirubin and total protein has great value in the assessment of clinical and experimental liver damage. In the present investigation, it was observed that the animals treated with CCl₄ resulted in hepatic damage as showed by the abnormal levels of serum markers. The changes in marker levels reflect changes in hepatic structural integrity. Liver is a major storage organ which stores various serum enzymes like SGOT, SGPT which are involved in transamination reactions for various amino acids. In liver diseases like liver necrosis, cirrhosis and jaundice, liver cannot store these enzymes due to malfunctioning, they get leaked into blood. Hence elevated serum SGOT and SGPT can be observed in liver toxicity [15].

Alkaline phosphatase is an isoenzyme which is synthesized mainly in the liver and has a critical role in dephosphorylation of various biomolecules. in case of liver toxicity, Alkaline phosphatase levels are raised in the case of liver toxicity, which may be due to cellular leakage and loss of functional integrity of the cell membrane [16]. One of the most important functions of the liver is detoxification of bilirubin which is a breakdown product of heme. The bilirubin is taken up by liver parenchyma cells from the blood and conjugates with glucuronic acid in presence of enzyme glucuronyltransferase. Later conjugated product is excreted into bile. Hepatotoxicity impairs the livers normal functions and detoxification of bilirubin get impaired and its abnormal levels are observed [17]. The albumin and globulin are the core components of total protein in the plasma mainly synthesized by the liver [18]. The levels of total protein were reduced in CCl₄ induced hepatotoxicity which is attributed to the initial damage and is localized in the endoplasmic reticulum where there is the loss of P450 enzyme which leads to functional failure of liver Subsequently there is a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver [19]. Hence serum total protein is the critical biomarker of liver function.

In our study Treatment with standard drug Liv-52 (100mg/Kg; p.o.) and *Euphorbia royleana* Linn. of ethanolic and chloroform extract (200mg/kg & 400mg/kg; p.o.) was shows significant improvement in abnormal levels of SGOT. SGPT. ALP, total protein, total bilirubin and direct bilirubin level. Administration of 200 and 400 mg/kg body weight of ethanolic and chloroform extract *Euphorbia royleana* Linn. significantly decreased the blood glucose level in these Guinea pig suggesting that it has hepatoprotective properties. The decreased body weight in hepatotoxicity Guinea pig is due to excessive breakdown of tissue proteins [20]. Treatment with *Euphorbia royleana* Linn. improved body weight significantly in a dose dependent manner, indicating prevention of muscle wasting due to liver failure condition. In histopathology liver caused by hepatotoxicity to normal pattern.

Conclusion

Our study demonstrates that the activity of ethanolic extract of *Euphorbia royleana* Linn in carbon tetrachloride treated Guinea pig can be effective treatment against liver injury.

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Conflict of interest

There is no conflict of interest.

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