Evaluation Of Hepatoprotective Activity On The Leaves Of *CORDIA MACLEODIL*

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ABSTRACT
Liver, the key organ of metabolism and excretion, is constantly endowed with the task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. The aim of the present study was to evaluate hepatoprotective activity of aqueous and ethanolic extracts of Cordia macleodil in wistar rats. It was found that Cordia macleodil aqueous and ethanolic extracts possess significant hepatoprotective activity
Keywords: *Hepatoprotective activity, Cordia macleodil*

INTRODUCTION
Plants have been used to treat diseases such as diabetes, jaundices, cardiovascular diseases, heavy metal poisoning, congestion of abdominal and pelvic cavities and scarlet fever.[1] It is estimated that out of 250,000 to 500, 000 species of plants only 1 to 2% of the terrestrial plants have been reasonably well investigated. Although today the synthetic drugs are larger in their number than the natural ones but still many synthetic drugs have their origin in the natural source and have been derived from plants and animals.[2]
Liver, the key organ of metabolism and excretion, is constantly endowed with the task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents.[3] Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequence. As such liver is highly affected primarily by toxic agents such as CCl_4, paracetamol, D-galactosamine, alcohol, rifampicin and thioacetamide through different mechanisms.[4]
The plant *Cordia macleodil* is a plant is a medium-sized, evergreen tree belonging to family, Boraginaceae.[5] *Cordia macleodii* is found in wet and dried up deciduous forests of India such as Chhattisgarh, Madhya Pradesh, Odisha, Chotanagpur and Maharashtra. This plant is utilised for its medicinal properties. In numerous communities the bark is utilised for the treatment of jaundice. The plant extract shows wound healing, hepato-protective and aphrodisiac properties. The leaves of the plant are also used for radical scavenging activity. [6, 7]
The present study has been undertaken to investigate the hepatoprotective activity of aqueous and ethanolic extracts of bark of *Cordia macleodii* against CCl_4 and ethanol induced hepatic damage in rats.

MATERIALS AND METHODS
Collection and authentication of plant materials
The barks of *Cordia macleodil* were collected in the month of July 2016 from local areas of Indore (M.P.) and were identified and authenticated by Dr. S.N. Dwivedi, Prof. and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, M.P. what's more, kept in our Laboratory, Voucher example No. J/BOT/H-140.

Preparation of powders

The barks of *Cordia macleodil* were dried under shade and afterward powdered with a mechanical processor. The powders were gone through sieve No. 40 and put away in an impermeable compartment for further employments.

Extraction procedure

The preparation of crude extract of bark of *Cordia macleodil* was done in Department of Pharmacy, The dried powder of barks of *Cordia macleodil* were separated with Ethanol (95%) in a soxhlet apparatus. Aqueous extract was set up by cold maceration process by utilizing separate amount of powder. The solvents were evacuated by refining under diminished weight and the subsequent semisolid mass was vacuum dried utilizing rotary flash evaporator.[8]

Acute oral toxicity studies

Acute oral toxicity was performed by using OECD guidelines – 423 (Organisation of Economic Co-Operation Development) – Fixed Dose Procedure. The purpose of this study is to allow selection of the appropriate starting dose for the main study. Acute oral toxicity of *Cordia macleodil* was performed in Wistar Albino Rats. The rats were kept for 4 hr of fasting prior to the experiment and body weight of the rats should be noted. Usually rats weighting 100-120 gm were used for acute toxicity studies. The dose was given to every rat orally according to body weight. The test for acute toxicity was performed at 5, 50, 300, and 2000mg/kg oral dose of aqueous and ethanolic extract of *Cordia macleodil* bark. Food was given for 1-2 hours after the administration of drug. During the first 4 hr. after the drug administration, animals were continuously observed for gross behavioral changes & then observation is continued for 24 hr & 72 hr in regular intervals for 14 days. The parameter such as hyperactivity, grooming, convulsions, sedation, hypothermia, change in fur colour, mortality, moribund stage or death were observed.[9]

Table No. 1 : Assessment of Acute toxicity studies.

Sr. No.	No. of Animals	Dose mg/kg	Results
1	3	5	No death
2	3	50	No death
3	3	300	No death
4	3	2000	1 death

The ethanolic and aqueous extracts of plant of *Cordia macleodil* were screened for acute toxicity study by OECD No. 423 Guideline for determination of LD50. The results showed that or both the extracts the LD_{50} was found to be 2500 mg/kg. Therefore their ED_{50} is 250 mg/kg.

Drugs and Chemicals:
All the chemicals were purchased from local market of Indore (M. P.).

Animals:
Male Wistar rats weighing 100-120 gms were used for this study. The animals were kept in polypropylene cages and maintained at 25 ± 5ºC and 60 ± 5% humidity under 12 h light/dark cycle. The animals were allowed free access standard pellet diet and water. The animal experiment was performed according to the guidelines laid by Institutional Animal Ethical Committee (IAEC).

Hepatoprotective activity
Experimental animal
Albino rats (100-120 gms) used in the present studies were procured from listed suppliers of Indore (M. P.), India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were acclimatized for a week before use.
A) CCl_4 Induced model
The rats were divided into 5 groups of 3 animals in each.
Group I : Received vehicle Gum acacia (5mg/kg.p.o) for 7 days, and served as normal control.
Group II : Received vehicle Gum acacia (5 mg/kg p.o) for 7 days once daily. Carbon tetrachloride 1ml/kg in 50%v/v olive oil on 7thday.
Group III : Received standard drug Silymarin (25 mg/kg) for 7 days once daily , CCl4 1ml/kg in 50%v/v olive oil on 7thday.
Group IV : Received aqueous extract of bark of *Cordia macleodil* (250mg/kg) for 7 days once daily; CCl_4 1ml/kg in 50%v/v olive oil on 7thday.
Group V : Received ethanolic extract of bark of *Cordia macleodil* (250mg/kg) for 7 days once daily; CCl_4 1ml/kg in 50%v/v olive oil on 7thday.
On the 7th day food and water were withdrawn after giving the last doses of aqueous and ethanolic extracts. After 36 hours the blood samples were collected and allowed to clot and serum was separated by centrifuge at 2500 rpm for 15 min and analyzed for various biochemical parameters.
Assessment of Liver Function
The liver was removed and weighed. Biochemical parameters i.e., Serum glutamic Pyruvate transaminase (SGPT), serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline phosphatase (ALP) were analyzed according to the reported methods.
Histopathological Studies
Liver slices fixed for 12 hrs in Bouin's solution were processed for paraffin embedding following standard micro technique. 5µ section of the livers stained with alum haematoxylin and eosin, were observed microscopically for histopathological changes i.e., normal liver, damaged and recovered liver were studied and compared.
B) Ethanol induced model
The rats were divided into 5 groups of 3 animals in each.
Group I : Received vehicle gum acacia (5mg/kg.p.o) for 5 days, and served as normal control.

Group II : Received vehicle gum acacia (5 mg/kg p.o) for 5 days once daily 50% Ethanol 5ml/kg on 5th day, and served as disease control.
Group III : Received Silymarin (25 mg/kg) for 5 days once daily, 50% Ethanol 5ml/kg on 5th day.
Group IV : Received aqueous extract of bark of *Cordia macleodil* (250mg/kg) for 5 days once daily and 50% Ethanol 5ml/kg on 5th day.
Group V : Received ethanolic extract of bark of *Cordia macleodil* (250mg/kg) for 5 days once daily and 50% Ethanol 5ml/kg on 5th day.
On the 5th day food and water were withdrawn after giving the last dose of aqueous and ethanolic extracts. After 24 hours the blood samples were collected and allowed to clot and serum was separated by centrifuge at 2500 rpm for 15 min and analyzed for various biochemical parameters.

Assessment of Liver Function
The liver was removed and weighed. Biochemical parameters i.e., Serum glutamic Pyruvate transaminase (SGPT), serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline phosphatase (ALP) were analyzed according to the reported methods.

Histopathological Studies
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Hepatoprotective studies (CCl_4 Induced Model)
Liver play key role in regulation of physiological processes. it is involved in several functions such as metabolism, secretion and storage. Further more detoxification of a variety of drugs and xenobiotics occurs in liver. The bile secreted by the liver has, among other things, an important role in digestion .liver diseases are the most serious ailments. The results of biochemical parameter reveal that the elevation of enzyme level in CCl_4 treated group are almost restored to the normal level in the extract treated group.

CCl_4 a hepatotoxin gets converted into CCl_3O^- radical in liver by action of enzymes and these attacks the unsaturated fatty acids of membranes in presence of oxygen to give lipid peroxide consequently .The functional integrity of hepatic mitochondria is altered, leading to liver damage, thereby levels of marker enzymes SGPT, SGOT, ALP, bile, proteins are found to be elevated in cytoplasm and in blood as these are released into blood.

Effect on SGPT
Aqueous and ethanolic extracts of bark of *Cordia macleodil* showed significant hepatoprotective activity as they reduced SGPT to 78.22± 4.82 and 76.20 ±2.28 as compared to the hepatotoxic control 112.4 ±4.20. and hence the extract bark of *Cordia macleodil* showed significant hepatoprotective activity. The results of treatment with extract of bark of *Cordia macleodil* are tabulated in Table No.2.

SGPT is a cytosolic enzyme primarily present in the liver. The level of SGPT in serum increase due to leakage of this cellular enzyme into plasma by CCl_4 induced hepatic injury. Serum level of SGPT can increase due to damage of the tissue producing acute hepatic necrosis. Since the extract of bark of *Cordia macleodil* significantly reduced the level of SGPT, this suggests that the extracts possess significant hepatoprotective activity.

Effect on SGOT

Aqueous and ethanolic extracts of bark of *Cordia macleodil* showed significant hepatoprotective activity as they reduced SGOT to 202.24± 8.64 and 200.22± 4.20 as compared to the hepatotoxic control 298.00 ±4.60 and hence the extract of bark of *Cordia macleodil* showed significant hepatoprotective activity. The results of treatment with extracts of bark of *Cordia macleodil* are tabulated in Table No.2.

SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney. Liver toxicity elevated the SGOT level in serum due to the damage to the tissue producing acute necrosis such as several viral hepatitis and acute cholestasis. Since the extracts of bark of *Cordia macleodil* significantly reduced the level of SGOT, this suggests that the extracts possess significant hepatoprotective activity.

Effect on ALP

Aqueous and ethanolic extracts of bark of *Cordia macleodil* showed significant hepatoprotective activity as they reduced ALP to 208.00± 6.48 and 206.20± 4.88 as compared to the hepatotoxic control 318.46± 10.82 and hence the extract of bark of *Cordia macleodil* showed significant hepatoprotective activity. The results of treatment with extract of bark of *Cordia macleodil* are tabulated in Table No.2.

In case of toxic liver, alkaline phosphate levels are very high, which may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchyma or duct cells. Since the extracts of bark of *Cordia macleodil* significantly reduced the level of ALP and this suggests that the extracts possess significant hepatoprotective activity.

Effect on liver weight

The liver weight of animals treated with both the aqueous and ethanolic extracts of the bark of *Cordia macleodil* were compared with that of the standard drug Silymarin (25 mg/kg) treated ones.

The aqueous and ethanolic extracts of the bark of *Cordia macleodil* exhibited significant decrease in the weight of liver as that of the standard drug Silymarin and thus suggest that the extracts of *Cordia macleodil* possess significant hepatoprotective activity.

RESULTS AND DISCUSSION

Table No. 2. Effect of aqueous and ethanolic extracts of bark of *Cordia macleodil* on CCl_4 induced hepatotoxicity in rats.

Treatment	Total bilirubin (mg%)	Direct bilirubin (mg%)	SGOT(µ/min/l)	SGPT(µ/min/l)	ALP(µ/min/l)
Normal	0.38 ± 0.06	0.20 ± 0.08	168.04 ± 2.80	58.6 ± 2.26	186.0 ± 8.4
Induced	9.82 ± 2.82	6.28 ± 3.36	298.00 ± 4.60	112.4 ± 4.20	318.46 ± 10.82
Standard	0.54 ±0.02**	0.42 ±2.86**	186.48 ±8.52**	68.42 ± 8.46**	196.00 ±8.24**
Aqueous extract (250mg/kg)	0.68 ± 0.24*	0.58 ± 2.20*	198.00 ± 8.24*	84.28 ± 6.26*	212.48 ± 8.64*
Ethanolic extract (250mg/kg)	0.66 ± 0.46*	0.56 ± 0.28*	196.48 ±6.82**	86.46 ± 2.26*	210.42 ± 6.42*

Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of * P<0.01, ** P<0.001, when compared with respective control.

Table no. 3. Effect of aqueous and ethanolic extracts of bark of *Cordia macleodil* on liver weight variation of CCl_4 induced hepatotoxicity in rats.

Treatment	Liver weight in gm/100g
Normal	5.28 ± 0.86
Induced (CCl_4)	7.49 ± 0.02
Standard (Silymarin25mg/kg)	5.88 ± 2.24**
Aqueous extract (250mg/kg)	6.12 ± 0.26*
Ethanolic extract (250mg/kg)	6.10 ± 0.44*

Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of * P<0.01, ** P<0.001, when compared with respective control

Hepatoprotective studies (Ethanol Induced Model)

The result of biochemical parameter revealed that the elevation of enzyme level in ethanol treated group, are almost restored to the normal level in the extract treated group.

Effect on SGPT

Aqueous and ethanolic extracts of bark of *Cordia macleodil* showed significant hepatoprotective activity as they reduced SGPT to 78.26± 6.24 and 76.66 ±4.22 as compared to the hepatotoxic control 128±2.28 and hence the extracts of bark of *Cordia macleodil* showed significant hepatoprotective activity. The results of treatment with extracts of *Cordia macleodil* are tabulated in Table No.4.

SGPT is a cytosolic enzyme primarily present in the liver. The level of SGPT in serum increase due to leakage of this cellular enzyme into plasma by ethanol induced hepatic injury. Serum level of SGPT can increase due to damage of the tissue producing acute hepatic necrosis. Since the extracts of bark of *Cordia macleodil* significantly reduced the level of SGPT, this suggests that the extracts possess significant hepatoprotective activity.

Effect on SGOT

Aqueous and ethanolic extracts of bark of *Cordia macleodil* showed significant hepatoprotective activity as they reduced SGOT to 186.8 ± 8.44 and 188.6± 6.64 as compared to the hepatotoxic control 320.6± 10.26 and hence the extracts showed significant hepatoprotective activity. The results of treatment with extracts of bark of *Cordia macleodil* are tabulated in Table No.4.

SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney. Liver toxicity elevated the SGOT level in serum due to the damage to the tissue producing acute necrosis such as several viral hepatitis and acute cholestasis. Since the extracts of *Cordia macleodil* significantly reduced the level of SGOT, this suggests that the extracts possess significant hepatoprotective activity.

Effect on ALP

Aqueous and ethanolic extracts of bark of *Cordia macleodil* showed significant hepatoprotective activity as they reduced ALP to 206.26± 9.42 and 210.84± 6.44 as compared to the hepatotoxic control 340.22± 14.6.The results of treatment with extracts of bark of *Cordia macleodil* are tabulated in Table No. 4. and hence the extracts of bark of *Cordia macleodil* showed significant hepatoprotective activity.

In case of toxic liver, alkaline phosphate levels are very high, which may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchyma or duct cells. Since the extracts of bark of *Cordia macleodil* significantly reduced the level of ALP, this suggests that the extract possesses significant hepatoprotective activity.

Effect on liver weight

The liver weight of animals treated with both the aqueous and ethanolic extracts of the bark of *Cordia macleodil* were compared with that of the standard drug Silymarin (25mg/kg) treated ones.

The aqueous and ethanolic extracts of the bark of *Cordia macleodil* exhibited significant decrease in the weight of liver and thus suggest that the extracts of *Cordia macleodil* possess significant hepatoprotective activity.

Table no. 4. Effect of aqueous and ethanolic extracts of bark of *Cordia macleodil* on ethanol induced hepatotoxicity in rats

Treatment	Total bilirubin (mg%)	Direct bilirubin (mg%)	SGOT(µ/min/l)	SGPT(µ/min/l)	ALP(µ/min/l)
Normal	0.38 ± 0.06	0.20 ± 0.08	168.04 ± 2.8	58.6 ± 2.26	186.0 ± 8.4
Induced(ethanol)	6.28 ± 2.26	5.86 ± 3.33	320.6 ± 10.26	128.0 ± 2.28	340.22 ± 14.6
Standard (Silymarin 25mg/kg)	0.44 ± 4.20**	0.38 ± 2.84**	176.0 ± 8.92**	74.22 ± 4.86**	192.48 ± 10.26**
Aqueous extract (250mg/kg)	0.62 ± 4.86*	0.52 ± 3.86*	198.6 ± 9.86*	82.24±5.63*	212.64± 8.94*
Ethanolic extract (250mg/kg)	0.64 ± 4.42*	0.56 ±3.92*	196 .4± 6.26*	86.42±2.84*	218.22±8.42*

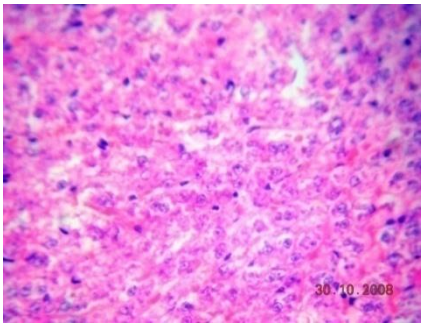
Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of * P<0.01, ** P<0.001, when compared with respective control.

Table No. 5. Effect of aqueous and ethanolic extracts of bark of *Cordia macleodil* on liver weight variation of ethanol induced hepatotoxicity in rats

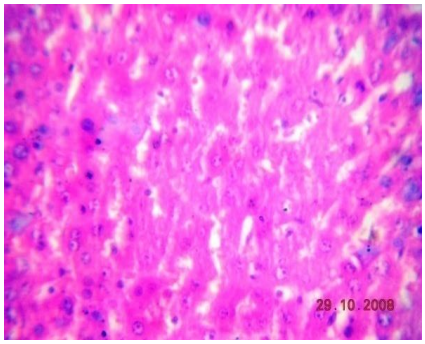
Treatment	Liver weight in gm/100g
Normal	5.28 ± 0.86
Induced (ethanol)	8.62 ± 0.22
Standard (Silymarin 25mg/kg)	5.86 ± 4.28**
Aqueous extract (250mg/kg)	6.48 ± 6.42*
Ethanolic extract (250mg/kg)	6.56 ± 0.48*

Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of * P<0.01, ** P<0.001, when compared with respective control.

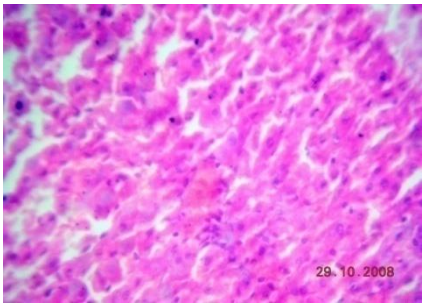
Figure No. 1. Histopathological Diagram of liver of rats in CCl_4 Induced hepatotoxicity



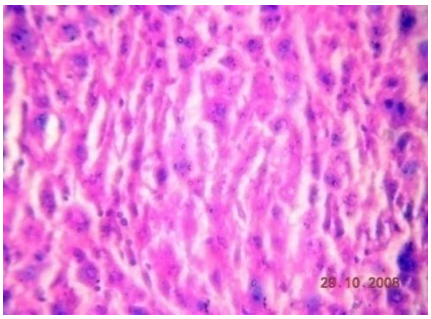
Normal: The architecture is normal. The central veins, sinusoids and portal triads appear normal.The hepatocytes show show moderate cytoplasm and round to oval nuclei. There is no periportal inflammation.



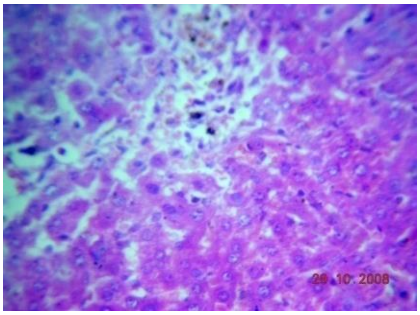
CCl_4 induced: The architecture is partly effaced. The central veins, sinusoids and portal triads appear congested. The hepatocytes show feathery degeneration and show moderate cytoplasm and round to oval nuclei. There is periportal inflammation.



Standard (Silymarin 25mg/kg): The central veins show dilatation and congestion. The hepatocytes show feathery degeneration. The portal triads show mild peri-portal inflammation composed of lymphocytes.

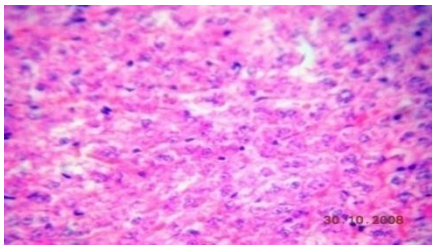


Aqueous extract (250mg/kg): The central veins appear normal. The hepatocytes show moderate cytoplasm and enlarged pleomorphic nuclei. The portal triads show mild peri-portal inflammation composed of lymphocytes.

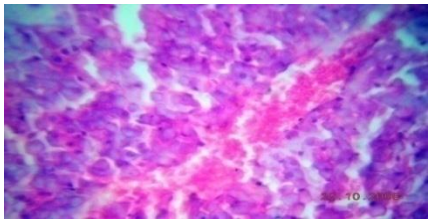


Ethanolic extract (250mg/kg): Section shows dilated and congested central veins. There is prominent periportal patchy necrosis and inflammation composed of mono-nuclear cells.

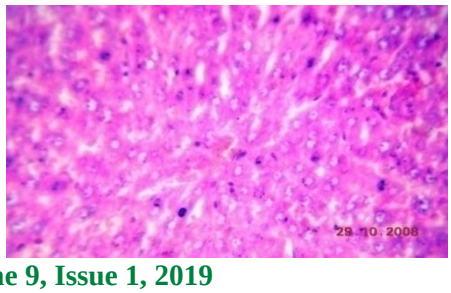
Figure No. 2. Histopathological Diagram of liver of rats in ethanol Induced hepatotoxicity



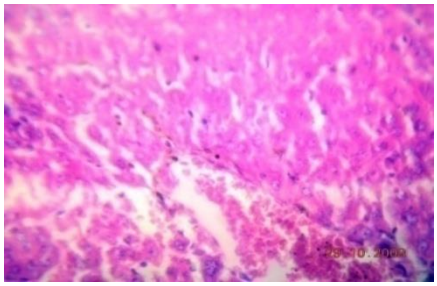
Normal: The architecture is normal. The central veins, sinusoids and portal triads appear normal. The hepatocytes show show moderate cytoplasm and round to oval nuclei. There is no periportal inflammation.



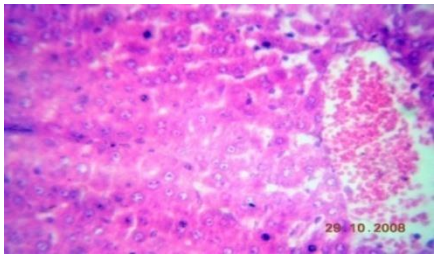
Ethanol induced: Section shows large dilated and congested central veins. The hepatocytes are normal. The portal triads appear normal.



Standard (Silymarin 25mg/kg): The architecture is normal. The hepatocytes shows moderate cytoplasm and round nuclei. The portal triads appear normal.



Aqueous extract (250mg/kg): The architecture is normal. The hepatocytes shows moderate cytoplasm and round nuclei. The portal triads appear normal.



Ethanolic extract (250mg/kg): The architecture is normal. The hepatocytes shows moderate cytoplasm and round nuclei. The portal triads show mild peri-ortal inflammation.

CONCLUSION

The present study has demonstrated that the aqueous and ethanolic extracts of *Cordia macleodii* have hepatoprotective effect against CCl_4 and ethanol induced hepatotoxicity in rats. Thus, this result suggests that aqueous and ethanolic extracts of barks of *Cordia macleodii* have capacity to regenerate and repair liver.

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