IJNLS
INTERNATIONAL JOURNAL OF NATURE AND LIFE SCIENCES
Research article

International Journal of Nature and Life Sciences (IJNLS)
https://www.dergipark.gov.tr/ijnls
e-ISSN: 2602-2397
Vol. 4(1), June 2020, pp. 26-39

Morinda reticulata Gamble Tubers: A Potent Hepatoprotective Agent Against CCl4 and Paracetamol Induced Hepatotoxicity in Wistar Albino Rats

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Received: *17 February 2020,* ***Revised:*** *12 May 2020,* ***Published Online:*** *01 June 2020*

Abstract

Liver toxicityis the common issue in social life and it may induce by various chemicals and drugs. The hepatoprotective study was aimed to explore the extracts of *Morinda reticulata* Gamble for hepatoprotective activity against CCl4 and paracetamol induced hepatotoxicity on Wistar albino rats. Treatment group rats (4 groups, n=6 in each) were received either 200 mg/kg and 400 mg/kg of ethanol or aqueous extract of *M. reticulata* (p.o) and one group (n=6) was received 25 mg/kg of silymarin (p.o) twice daily for 7 days. CCl4 (1.5 ml/kg body weight, p.o) or paracetamol (2 g/kg) intoxication was done after the last dose of treatment and standard drug administration. Hepatoprotective potential was determined on the basis of measurement of hepatic serum marker enzymes such as Alanine Transaminase (AST), Aspartate transaminase (AST), Alkaline Phosphatase (ALP), Total bilirubin and liver weight. Further, liver tissue was subjected to histopathological studies. HPTLC studies were done to determine the presence of phytochemical constituents. Pretreatment with silymarin, ethanol and aqueous tuber extract of *M. reticulata* significantly ($p < 0.001$) reduced the CCl4 or paracetamol induced elevated AST, ALT, ALP, Total bilirubin level in serum and also reduced liver weight and total protein levels. HPTLC study of ethanol extract showed six distinct peaks for chemical constituents. The present study indicated that ethanol and aqueous tuber extract of *M. reticulata* has potential hepatoprotective effect against chemicals and it can be used as a clinical remedy for hepatotoxicity disorders.

Key words: Hepatoprotective, HPTLC fingerprint, *Morinda reticulata* Gamble, paracetamol, sylimarin

1. Introduction

The liver is an important metabolizing organ which effectively affects all physiological process of our body. It protects our body from various injurious substances and toxic metabolic by products which have been absorbed from intestinal tract (Ashoush et al., 2013). It is the most important site of metabolism and accountable for detoxifying any foreign material and other xenobiotics by converting and excreting waste and toxin (Howida and Abou, 2016). Xenobiotics are often reported to cause potential hepatic damage. Generally, phase I reaction is the first step in drug metabolism process where hepatic cytochrome p450 systems generate bioactive intermediate which may interact with various cellular organelles (e.g. mitochondria) leading to hepatocyte dysfunction and cellular damage. This bioactive intermediates are then inactivated through glucurono-, glutathione- or sulfa-conjugation in subsequent phase II reactions. In order to limit hepatotoxicity, the generation rate for phase I products should not exceed the liver's capacity to inactivate them. Depletion or deficiency of the compounds responsible for the phase II conjugation reactions may result in accumulation of toxic metabolites. Such is the case in patients who abuse alcohol and ingest acetaminophen. Drug induced liver injury is due to the contribution of several mechanisms among which mitochondrial dysfunction is the major cause by which the mitochondrial respiratory chain may be inhibited, diminishing ATP production and resulting in increased ROS levels. ROS generation, ATP depletion, and the aforementioned mitochondrial insults may combine to induce intracellular damage. Ultimately, hepatocytes commit to apoptosis, but this process requires energy (ATP), which may not be available due to mitochondrial dysfunction and depleted ATP stores. In this instance, hepatocyte death occurs through the necrotic pathway, which may enhance hepatic inflammation followed by hepatic diseases (David and Hamilton, 2010). In hepatic or liver disease, toxifying agents affect the cells or tissues or structure of hepatic cells thereby functions of the liver was disturbed (Uorakkottil et al., 2016). Long years back, plants are used as a part of medicine for the treatment of various disease. Herbal drugs and their formulations are commonly used than allopathic medicine due to their reasonable cost, better cultural suitability, improved compatibility, with the human body and less side effects (Saumendu et al., 2012).

Morinda reticulata Gamble is a highly endangered medicinal plant from India (Southern Western Ghats) and mostly available from the region of Kerala, India (Raju and Joshila 2017). It is an arborescent climber, growing to a height of 20 m. It is used for treatment of jaundice, stomach ailment and for blood purification (Thangaraj et al., 2014; Ijinu et al., 2011). Leucorrhoea and back pain can be treated by using *M. reticulata* (Ijinu et al., 2011). Extracts of various parts of *Morinda* species are used as laxatives in traditional medicines of India due to the abundance of anthraquinone derivatives (Raju and Joshila, 2017). Vast literature survey fails to report the hepatoprotective effect in animal experiment model because of its traditional use in jaundice. Hence, the present investigation, ethanol and aqueous tuber extract of *M. reticulata* was evaluated for the hepatoprotective activity CCl_4 and paracetamol induced hepatotoxicity using on Wistar albino rats.

2. Materials and Methods

2.1. Chemicals

Paracetamol procured from Farmson analgesics, Gujarath, India. Silybon-140 was procured from Micro labs Limited, Himachal Pradesh Biochemical estimation kits were purchased from Aggappe, Kerala, India. Analytical grade reagents and chemicals were used in this experiment.

2.2. Plant materials

The tubers of *Morinda reticulata* Gamble was collected in the month of December 2014 from vicinity of kazhakoottam, Thiruvananthapuram, Kerala, India. The plant material was identified and authenticated by Prof. Ramanibhai, Head of the Department, Department of Botany, Christian College, Kattakkada, Thiruvananthapuram. The herbarium specimen of the same is deposited at the Department of Pharmacognosy Sree Krishna College of Pharmacy and Research, Parassala, Thiruvananthapuram, Kerala (H.S.P. No: SKCPRC/COGNOSY/HS2014/003).

2.3. Preparation of the extract

Cleaned and dried tubers of *M. reticulata* was coarse powdered and successively extracted in a soxhlet by using petroleum ether, chloroform, ethanol and water. The extracts were concentrated under reduced pressure and temperature. Ethanol extract of *M. reticulata* (EEMR) and aqueous extract of *M. reticulata* (AEMR) were used to evaluate hepatoprotective potential in animal model.

2.4. Phytochemical analysis

The extracts were subjected to the analysis of various phytoconstituents by preliminary phytochemical studies (Trease and Evans, 1989).

2.5. Experimental animal

Wistar rats (180-230 g) of both sexes were selected and were acclimatized under standard laboratory environmental conditions for one week before the experiment where they fed with standard rodent feed and water *ad libitum*. Use of animals as per the experiment was approved by the IAEC (Institutional Animal Ethics Committee) of Sree krishna college of Pharmacy and Research Centre (CPCSEA registration number: 1551/PO/Re/S/11/CPCSEA the approved protocol number is Joshila J U/M. Pharm / KUHS/2014/a&b).

2.6. Acute toxicity study

As per OECD-423 guidelines acute oral toxicity in rats was carried out. The animals were subjected for toxicity study using ethanol and aqueous extract at a dose of 2000 mg/kg orally in a group of three female rats and observed at regular intervals of 1, 2, 4, 8, 12 and 24 hours.

2.7. Carbon tetrachloride induced hepatotoxicity

Total forty two rats (180-230 g) of both sexes were selected and divided into seven groups of 6 rats in each and were treated as Molehin et al., 2017 method.

Group I received vehicle (1 ml) orally for seven days.

Group II received vehicle (1 ml, orally) twice daily for seven days and CCl_4 (1.5 ml/kg) on seventh day (1:1 of CCl_4 in olive oil).

Group III received Silymarin (25 mg/kg, p.o.) twice daily for seven days and CCl_4 (1.5 ml/kg, orally) on seventh day (1:1 of CCl_4 in olive oil).

Group IV received ethanol extract of *M. reticulata* (EEMR 200 mg/ kg, orally) twice daily for seven days CCl_4 (1.5ml/kg, orally) on seventh day (1:1 of CCl_4 in olive oil).

Group V received ethanol extract of *M. reticulata* (EEMR 400 mg/ kg, orally) twice daily for seven days and CCl_4 (1.5ml/kg, orally) on seventh day (1:1 of CCl_4 in olive oil).

Group VI received aqueous extract of *M. reticulata* (AEMR 200 mg/kg, orally) twice daily for seven days CCl_4 (1.5ml/kg, orally) on seventh day (1:1 of CCl_4 in olive oil).

Group VII received aqueous extract of *M. reticulata* (AEMR 400 mg/ kg, orally) twice daily for seven days CCl_4 (1.5ml/kg, orally) on seventh day (1:1 of CCl_4 in olive oil).

On 8^{th} day, 18 h after the last dose of CCl_4, overnight fast and under light ether anaesthesia blood was withdrawn from the retro orbital plexus later were sacrificed for isolation of liver by administration of excess of anaesthetic agent.

2.8. Paracetamol induced hepatotoxicity

Wistar albino rats (180-230 g) of both sexes were used. Animals were divided into seven groups of six animals in each; Silymarin (25 mg/kg) is used as the standard drug. Treatment regimen was followed as that of Deepak et al., 2007.

Group I received 1 ml vehicle administered orally for seven days.

Group II received 1 ml vehicle (orally) twice daily for seven days and paracetamol (2 g/kg, p.o) on seventh day.

Group III received Silymarin (25 mg/kg) orally twice daily for seven days and paracetamol (2 g/kg, orally) on seventh day.

Group IV received ethanol extract of *M. reticulata (*EEMR 200 mg/kg) orally twice daily for seven days and paracetamol (2 g/kg, orally) on seventh day.

Group V received ethanol extract of *M. reticulata* (EEMR 400 mg/kg) orally for seven days and paracetamol (2 g/kg, orally) on seventh day.

Group VI received aqueous extract of *M. reticulata* (AEMR 200 mg/kg) twice daily for seven days and paracetamol (2 g/kg, orally) on seventh day.

Group received aqueous extract of *M. reticulata (*AEMR 400 mg/kg) orally twice daily for seven days and paracetamol (2 g/kg, orally) on seventh day.

After the last dose of paracetamol, animals were subjected to overnight fast and under light ether anaesthesia blood was withdrawn from the retro orbital plexus for biochemical studies later were sacrificed for isolation of liver by administration of excess of anaesthetic agent.

2.9. Evaluation of hepatoprotective parameters

Liver weight of animal estimated and was expressed in grams. Biochemical estimation of serum marker enzymes was estimated by using standard analytical kits from Aggappe, Kerala, India. ALT, AST, ALP (IU/L), total bilirubin (mg/dL) were estimated on an auto analyser.

2.10. HPTLC study

5µL of *M. reticulata* ethanol extracts were spotted (total 8 spots) on TLC plate. Sharp band (6 mm width) of samples were spotted on 10cm x 10cm size pre coated silica gel aluminum plate $60F_{254}$ by using spray technique with a help of Camag 100 µL sample syringe and Camag Linomat V automatic sample applicator. The spotted bands should be 10 mm above from the bottom edge of the plate and each spots with a distance of 11.4 mm.

Prior to chromatography procedure, TLC plates were washed with ethanol and activated at 60 °C for 5 min. Respective mobile phases(chloroform: methanol (9:1)) were added in TLC twin trough developing chamber. TLC developing chambers were closed with lids and to allowed for saturation of solvent vapour. The sample loaded plates were kept in their particular mobile phase up to 70 mm. The developing chamber assembles were kept aside for saturation and development of chromatogram for 30 min at room temperature. After 30 min, developed plates were allowed to dry by hot air. The plates were photo-documented at UV 254 and 366 nm using photo documentation chamber. Finally, the plates were placed on scanner stage and were scanned at 200-400 nm. The images of band were obtained from photo documentation chamber under UV light at 254 and 366 nm.

2.11. Histopathological study

Livers were isolated immediately after sacrificing the animal and washed with ice cold normal saline, trimmed and placed in 10% formaldehyde. The organs were sectioned and stained with hematoxylin and eosin (H&E). The structure were examined under light microscope10X and 40X by a pathologist blinded to the groups under study.

2.12. Statistical analysis

The values which were presented in the study as mean ± standard error of mean (SEM). Statistical significance was calculated by using one-way ANOVA followed by Students-Newman-Keuls comparison test where $p < 0.001$ was considered as statistically significant.

3. Results

Phytochemical screening revealed the presence of following constituents in each extract, petroleum ether extract showed the presence of phenolic compound, benzene extract shows the presence of carbohydrate, anthraquinone, amino acid and protein. Carbohydrate, anthraquinone and saponine were presnt in chloroform extract. Ethanol extract showed the presence of carbohydrate, anthraquinone, tannins, flavanoid, saponine and phenolic compound. Carbohydrate, anthraquinone, tannins, flavanoid and phenolic compound were present in aqueous extract. No mortality was found in acute toxicity study with 2000 mg/kg of extracts per body weight of animals.

3.1. CCl_4 induced hepatotoxicity

3.1.1. Liver weight

Rats were pretreated (p.o) with both doses of EEMR (400, 200 mg/kg) and AEMR (400, 200 mg/kg) for seven consecutive days and CCl_4 induction on seventh day causes changes in liver weight of animals in different group and were summarized in Table 1. Liver weight was significantly ($p < 0.001$) increased in only CCl_4 (1.5 ml/kg) administered animals, whereas rats pretreated with higher dose EEMR (5.9 ± 0.01) and standard Silymarin (5.8 ± 0.01) does not cause any notable change in liver weight.

Table 1. Effect of EEMR and AEMR on biochemical parameters on CCl_4 induced hepatotoxicity.

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total bilirubin (mg/dL)	Liver weight (g)
Normal	61.8±0.08	34.4±0.01	89.4±0.51	0.42±0.01	5.7±0.07
CCl_4 (1.5 ml/kg)	161.6±0.13	149±1.03	228.9±0.5	0.96±0.01	8.64±0.05
Silymarin (25 mg/kg) + CCl_4 (1.5 ml/kg)	68.2±0.13	42.6±0.07	96.9±0.92	0.44±0.03	5.8±0.05
EEMR (200 mg/kg) + CCl_4 (1.5 ml/kg)	78.6±1.49[a]	59.6±1.73[a]	104.7±1.23[a]	0.53±0.01[a]	6.6±0.01[a]
EEMR (400 mg/kg) +CCl_4 (1.5 ml/kg)	65.4±0.15[a]	39.8±2.37[a]	92.6±0.62[a]	0.41±0.01[a]	5.9±0.01[a]
AEMR (200 mg/kg) + CCl_4 (1.5 ml/kg)	101.6±0.86[a]	79.7±0.14[a]	122.7±2.42[a]	0.63±0.01[a]	7.16±0.09[a]
AEMR (400 mg/kg) + CCl_4 (1.5ml/kg)	70.4±0.83[a]	52.3±0.02[a]	98.6±0.73[a]	0.49±0.01[a]	6.44±0.06[a]

Data expressed as Mean ± SEM for five observations $p < 0.001$ compared to respective CCl_4 induced group. The data analysed by one way ANOVA followed by students - newman - keuls: a-$p < 0.001$. EEMR: Ethanol extract of *M. reticulata*; AEMR: Aqueous extract of *M. reticulata.*

3.1.2. Serum biomarkers

The effect of CCl_4 on the levels of liver marker enzymes in serum such as AST, ALT, ALP and total bilirubin were summarized in Table 1. The serum levels of above biochemical parameters were increased significantly ($p < 0.001$) in only CCl_4 treated rats than normal rat. Pretreatment with both the doses of EEMR and AEMR extracts showed excellent protection over lowering of the above raised serum markers near as that of normal. Highly significant protection was detected with EEMR 400 mg/kg dose administered group.

3.2. Paracetamol induced hepatotoxicity

3.2.1. Liver weight

Weight of liver in rats were significantly ($p < 0.001$) increased after oral administration of paracetamol whereas, rats which were continuously pretreated with both the doses of EEMR (400, 200 mg/kg) and AEMR

(400, 200 mg/kg) for seven days, significantly ($p < 0.001$) reduced the liver weight to normal level, the results obtained were summarized in Table 2. 400 mg/kg of EEMR (4.95 ± 0.03) showed better result comparable to standard drug, silymarin (5.13 ± 0.03). Higher dose of AEMR also shows highly significant ($p < 0.001$) effect on reduction of liver weight.

Table 2. Effect of EEMR and AEMR on serum biochemical parameters on paracetamol induced hepatotoxicity.

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total bilirubin (mg/dL)	Liver weight (g)
Normal	52.1±0.78	29.4±2.68	92.7±2.78	0.38±0.01	4.8±0.02
Paracetamol (2 g/kg)	190.2±2.17	161.4±1.09	240.6±1.78	0.79±0.01	7.9±0.01
Silymarin (25 mg/kg) + Paracetamol (2 g/kg)	56.2±0.83	34.6±2.31	93.6±0.93	0.34±0.01	5.13±0.03
EEMR (200 mg/kg) + Paracetamol (2 g/kg)	71.4±2.16[a]	48.4±1.73[a]	114.4±2.23[a]	0.51±0.01[a]	5.59±0.01[a]
EEMR (400 mg/kg) + Paracetamol (2 g/kg)	58.8±1.73[a]	37.4±0.72[a]	92.6±0.62[a]	0.42±0.01[a]	4.95±0.03[a]
AEMR (200 mg/kg) + Paracetamol (2 g/kg)	82.7±1.78[a]	61.7±2.17[a]	141.8±0.91[a]	0.68±0.01[b]	6.56±0.03[b]
AEMR (400 mg/kg) + Paracetamol (2 g/kg)	65.6±2.83[a]	47.3±2.83[a]	110.6±1.23[a]	0.48±0.01[a]	5.39±0.01[a]

Data expressed as mean ± SEM for five observations $p < 0.001$ compared to respective CCl_4 induced group. The data analysed by one way ANOVA followed by students - newman - keuls: a-$p < 0.001$, b-$p < 0.01$ EEMR: Ethanol extract of *M. reticulata*. AEMR: Aqueous extract of *M. reticulata.*

3.2.2. Serum bio markers

The impact of continuous seven days treatment with both the doses of EEMR (400, 200 mg/kg) and AEMR (400, 200 mg/kg) on serum liver enzymes were summarized in Table 2. AST, ALT, ALP, Total bilirubin level is increased significantly in paracetamol in toxicated control group. Pretreatment with EEMR as well as AEMR maintains enzyme level to normal even after intoxication with paracetamol. Higher dose of EEMR was found with highly significant effect on reduction and maintenance of these enzymes level.

3.3. HPTLC study

In order to know the presence of phytoconstituents in the administered crude extracts, HPTLC study was carried out to get fingerprint of the active constituents. The mobile phase selected for the HPTLC studies of ethanol extract was chloroform: methanol at the ratio of 9:1. By performing TLC with the mobile phase identified after trial and error method by trying different mobile phase. Detection of spot was executed under UV 254 nm and 366 nm and after application of Godin reagent spray (1 % vanillin in ethanol and 2 % H_2SO_4) performed at 105 °C. The result was depicted in Figure 1. The result of each peak with respective R_f was depicted in Table 3. The chromatogram developed with ethanol extract of *M. reticulata* showed six distinct peaks in which unknown phytocontstituent produced a peak at six positions was the highest (0.79) R_f value.

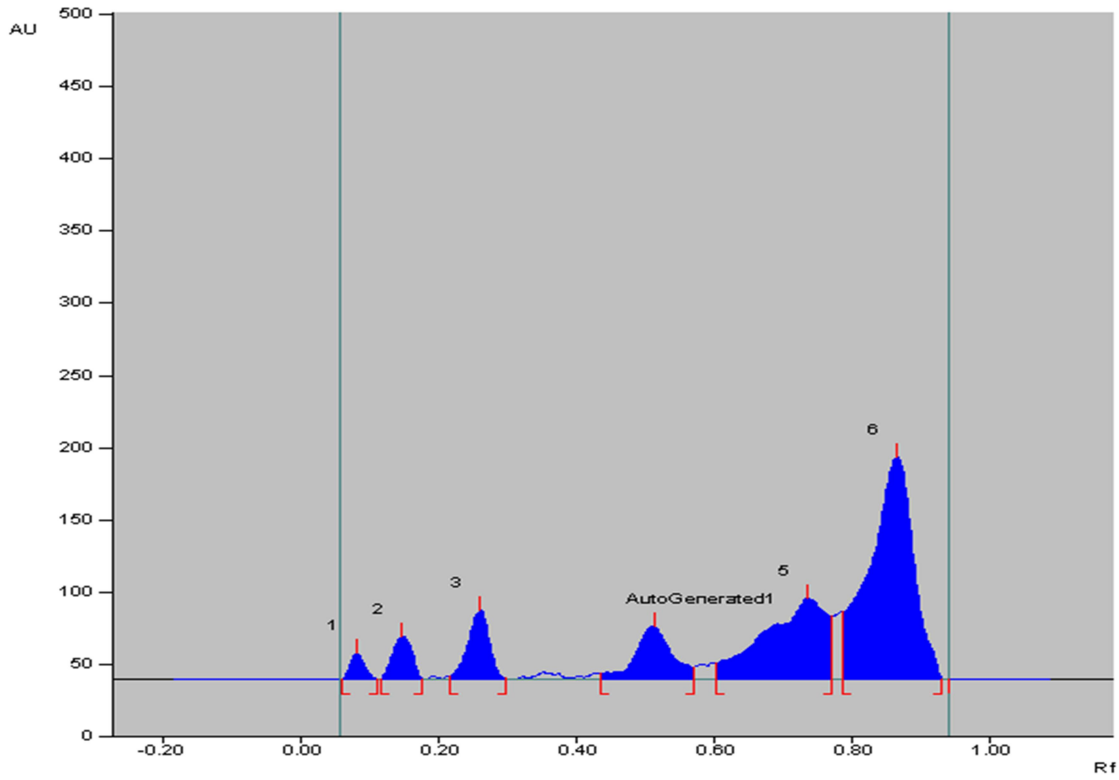


Figure 1. HPTLC chromatogram of ethanol extract of *M. reticulata* tuber showing different peaks of phytoconstituents.

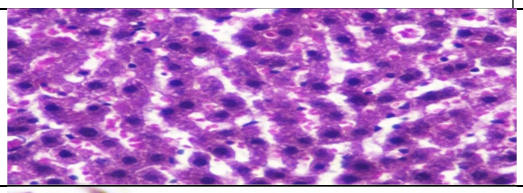
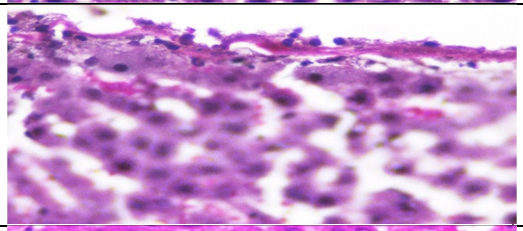
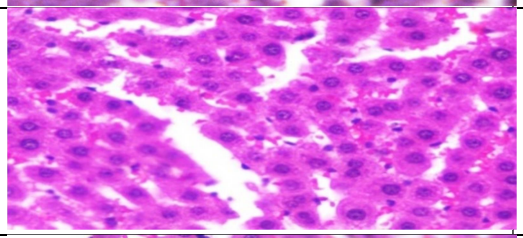
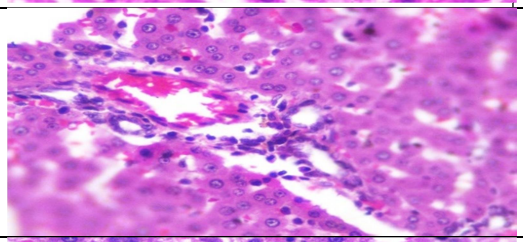
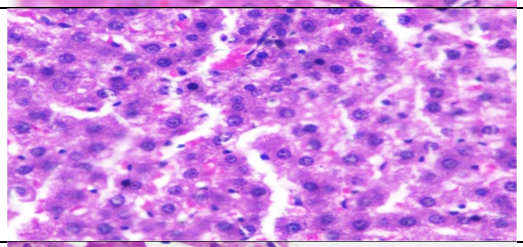
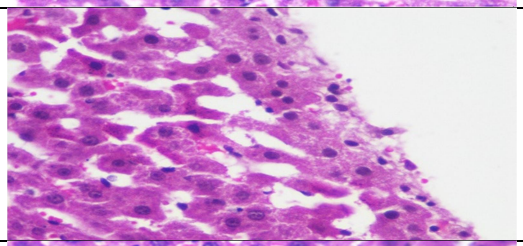
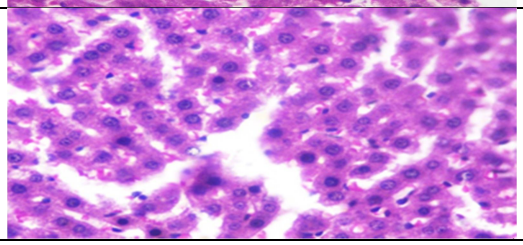
Table 3. HPTLC profile with respect to R_f value of ethanol extract of *M. reticulata.*

Peak	Start position	Start height	Max position	Max height	Max %	End position	End height	Area	Area %	Assigned substance
1	0.06 Rf	0.1 AU	0.08 Rf	18.2 AU	5.33	0.11 Rf	0.2 AU	240.0 AU	1.95	unknown
2	0.12 Rf	0.3 AU	0.15 Rf	29.7 AU	8.69	0.18 Rf	0.3 AU	524.7 AU	4.27	unknown
3	0.22 Rf	2.0 AU	0.26 Rf	48.0 AU	14.03	0.30 Rf	0.6 AU	908.6 AU	7.39	unknown
4	0.44 Rf	3.9 AU	0.51 Rf	36.6 AU	10.69	0.57 Rf	8.5 AU	1224.0 AU	9.95	auto generated
5	0.60 Rf	11.2 AU	0.74 Rf	55.7 AU	16.28	0.77 Rf	43.4 AU	3174.9 AU	25.8	unknown
6	0.79 Rf	46.5 AU	0.87 Rf	153.8 AU	44.98	0.93 Rf	0.2 AU	6227.4 AU	50.6	unknown

3.4. Histopathological results

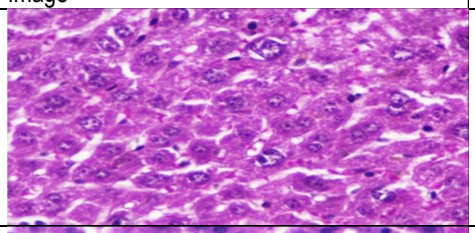
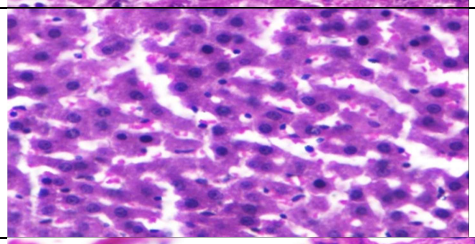
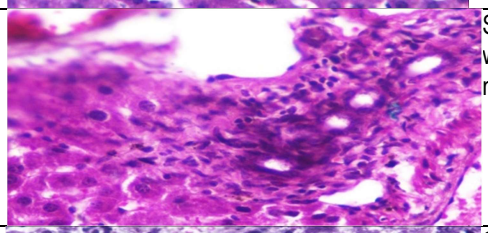
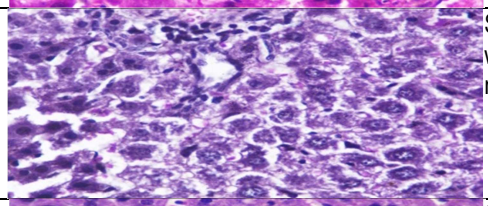
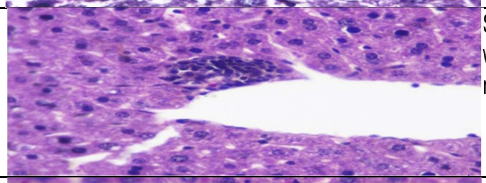
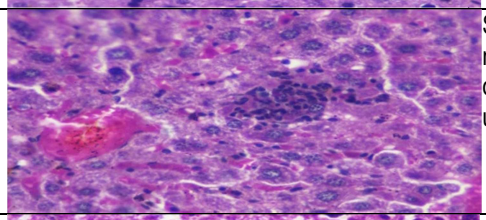
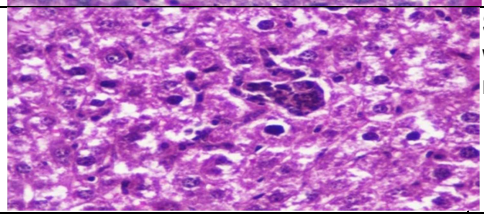
Pathological reports of the sectioned livers were viewed under microscope and the results were presented in Table 4 and 5.

Table 4. Histopathology reports of liver: CCl_4 induced hepatotoxicity.

Code	Group details	Image	Histopathology report
HTCNC	Normal control (liver)		Section showed that all the cellular functional units were within normal limits.
HTCDC	Disease control (liver)		Section showed the infiltrates with mononuclear, capsular, diffuse and mild alteration in cellular functional units.
HTCS	Silymarin (25 mg/kg) (liver)		Section showed that the cellular functional units were within normal limits.
HTCEC1	EEMR (200 mg/kg) (liver)		Section showed that infiltrates with mononuclear, capsular, diffuse and mild alteration in cellular functional units.
HTCEC2	EEMR (400 mg/kg) (liver)		Section showed that the cellular functional units were within normal limits.
HTCAC1	AEMR (200 mg/kg) (liver)		Section showed the infiltrates with mononuclear, capsular, diffuse and minimal damage to the cellular functional units.
HTCAC2	AEMR (400 mg/kg) (liver)		Section showed that the cellular functional units were within normal limits.

HTCNC: Hepatotoxicity CCl_4 normal control, HTCDC: Hepatotoxicity CCl_4 disease control, HTCS: Hepatotoxicity CCl_4 standard, HTCEC1: Hepatotoxicity CCl_4 ethanol concentration 1, HTCEC2- Hepatotoxicity CCl_4 ethanol concentration 2, HTCAC1: Hepatotoxicity CCl_4 aqueous concentration 1, HTCAC2: Hepatotoxicity CCl_4 aqueous concentration 2.

Table 5. Histopathology reports of liver paracetamol induced hepatotoxicity.

Code	Group Details	Image	Histopathology report
HTPNC	Normal control (liver)		Section showed that all the cellular functional units were within normal limits.
HTPDC	Disease control (liver)		Section showed that extra medullary with haematopoiesis, random and multifocal. Moderate damage to the cellular functional units.
HTPS	Silymarin (25 mg/kg) (liver)		Section showed that minimal damage with infiltrates, mononuclear and multifocal.
HTPEC1	EEMR (200 mg/kg) (liver)		Section showed that minimal damage with infiltrates, mononuclear and multifocal.
HTPEC2	EEMR (400 mg/kg) (liver)		Section showed that minimal damage with infiltrates, mononuclear and multifocal.
HTPAC1	AEMR (200 mg/kg) (liver)		Section showed that infiltrates with mononuclear, focal and minimal damage to the cellular functional units.
HTPAC2	AEMR (400 mg/kg) (liver)		Section showed that minimal damage with infiltrates, mononuclear and multifocal.

HTPNC: Hepatotoxicity paracetamol normal control, HTPDC: Hepatotoxicity paracetamol disease control, HTPS: Hepatotoxicity paracetamol standard, HTPEC1: Hepatotoxicity paracetamol ethanol concentration 1, HTPEC2: Hepatotoxicity paracetamol ethanol concentration 2, HTPAC1: Hepatotoxicity paracetamol aqueous concentration 1, HTPAC2: Hepatotoxicity paracetamol aqueous concentration 2.

4. Discussion

Preliminary phytochemical screening of extracts of *M. reticulata* Gamble showed the presence of carbohydrate, alkaloid, anthraquinone glycoside, flavanoid, saponine, amino acid and phenolic compound as biologically active phytoconstituent and may be responsible for their respective pharmacological properties.

The acute toxicity study report revealed 2000 mg/kg administration of the ethanol and aqueous tuber extract of *M. reticulata*in rats indicated that the plants none or low toxicity on animal.

Hepatotoxicity is due to overuse of refers to drugs or xenobiotics which causes liver dysfunction or liver damage (Navarro et al., 2006). The chemicals that cause liver injury are called hepatotoxins or hepatotoxicants. In the present study Carbon Tetrachloride (CCl_4) (Molehin et al., 2017; Akram et al., 2011) and paracetamol (Deepak et al., 2007; Garima et al., 2015) were used as hepatotoxicants to induce liver damage in rats.

CCl_4 induced liver damage is the most common method (AlHarbi *e*t al., 2012) used for the screening of hepatoprotective drugs. Formation of fatty liver and hepatic cell necrosis are the extended symptom of hepatotoxicity in CCl_4 intoxication. In early stage of hepatotoxic cell damage, CCl_4 also inhibit protein synthesis in liver. Researcher reported that hepatic cellular cytochrome P_{450} enzyme convert the CCl_4 to trichloromethyl ($^{\cdot}CCl_3$) which is a free radical. This free radical can be source of hepatic injury causing element (Xiao-Wen et al., 2010). The immense generation of free radicals in the CCl_4 induced liver damage followed by an increased level of lipid peroxidation. CCl_4 induced liver toxicity leads to a tremendous depletion of glutathione, membrane damage, depression of protein synthesis and lose of enzyme activity (Gini and Muraleedhara, 2008). CCl_4 induced hepatocellular necrosis in rat represent an adequate experimental model of cirrhosis in man (Alhassan et al., 2009).

Acetaminophen or paracetamol is a commonly available as an over the counter (OTC) drug for the treatment of pain. The safest therapeutic dose of paracetamol is up to 4 g/day for adults. If it is exceed more than the daily dose leads to acute liver failure (Rotem et al., 2012). Damage of hepatic cell is revealed by an increased level of liver specific enzymes. After liver cell damage these enzymes are released from cytoplasm to portal circulation. In this study also found that significant increased level of liver enzymes such as AST, ALT, ALP and total bilirubin in blood and also observed that there was a change in liver weight in the CCl_4 as well as in paracetamol treated groups. This could be taken as an indicator of liver damage caused by CCl_4 and paracetamol.

The administration of CCl_4 (1.5ml/kg) or paracetamol (2 g/kg) to rats, either in the pretreatment or curative studies, did not cause the death of any of the animals neither did it affect any physical properties (body weight and general behaviour). There was a notable increase however livers weight after the induction with the above mentioned hepatoxicants. Generally, intoxicated liver is expected to enlarge and increase in weight (Rotem et al., 2012). This is due to the intrusion of fatty acids as well as glycerol into the liver cell upon after the damage of cell membranes. The increased liver weight was however not significantly affected by treatment with the extract.

Prior treatment with extracts in rats before inducing toxicity was aimed to conditioning the hepatocytes to accelerate regeneration of parenchymal cells, thereby protection against fragility and stabilizing cell membrane that will prevent the leakage of liver specific marker enzymes into the circulation (Thabrew and Joice, 1987). Monitoring of both AST and ALT are normally indicated in certain drug or chemical which are expected to cause liver damage due to its toxic or any harmful effects. In this study also found that significant increased level of liver enzymes such as AST, ALT, ALP and total bilirubin in only CCl_4 and paracetamol animal blood. This could be

taken as an indicator of liver damage caused by CCl_4 and paracetamol. Existence of these liver specific marker enzymes in blood is due to liver damage which is always connected with hepatonecrosis (Naik and Panda, 2008). Animals which were treated with both the doses of EEMR and AEMR showed that the levels of liver specific marker enzymes were near to normal, indicating that *M. reticulata* extracts protect the liver cell against liver damage. In this study higher dose of ethanol extract of *M. reticulata* was found more effective in controlling the AST, ALT level almost similar as that of normal control group animals.

Total serum bilirubin level was elevated in condition such as excessive haemolysis, liver diseases and obstruction of the biliary tract. Liberation of bilirubin occurs during the destruction of hemoglobin and are conjugated in liver to diglucoroxide and then excreted in bile. Bilirubin gets accumulate in plasma whenever presence of liver disorder or obstruction in biliary tract or increased rate of hemolysis (Naik and Panda, 2008). In this study also found that significant increased level of total bilirubin in blood in the CCl_4 as well as in paracetamol treated groups. Treatment with extracts of *M. reticulata* (both the doses of EEMR and AEMR) showed that the levels of total bilirubin reduced to normal when compare with only CCl_4 and paracetamol administered rats. This indicates that both the extracts of *M. reticulata* protect the liver cell against liver damage.

Plant with phytoconsistuent such as flavonoids, tannins, phenolic compounds has diverse biological activity. The present study, HPTLC study report concluded that it possess more than 6 phytoconsistuent with corresponding Rf values. These phytoconstituents might be played an important role on the protection of liver from the toxicity causing agents.

5. Conclusion

The present study results revealed that, hepatoprotective capacity of *M. reticulata* might be due to its phytoconstituents. Presences of this phytoconstituents were confirmed by HPTLC study. Therefore, *M. reticulata* can be useful as natural hepatoprotective and cure of liver diseases. It is recommended that phytoconstients present in *M. reticulata* can be identified individually and its more activities can be revealed in future studies.

Conflicts of Interests

Authors declare that there is no conflict of interests

Acknowledgments

The authors gratefully acknowledge Dr. Sr. Betty Carla, Director and Dr. Vinod B, Principal, St. Joseph's College of Pharmacy for providing support and facilities for this research work.

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