Hepatoprotective Effect of Leaves of *Morinda tinctoria* Roxb. Against Non-alcoholic Fatty Liver Disease in Rats

Anil K. Yadav[1,2*] and Anoop Singh[1]

[1]*Department of Pharmacy, Bhagwant University, Sikar Road, Ajmer, 305004, Rajasthan, India.*
[2]*Institute of Pharmacy, Pranveer Singh Institute of Technology (PSIT), Kanpur – 209305,*
Uttar Pradesh, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aim: To study the hepatoprotective and antioxidant activity of 50% aqueous-alcoholic leaves extract of *Morinda tinctoria* (*Rubiaceae*) against non-alcoholic fatty liver disease.
Study Design: All experiments involving animals complies with the ethical standards of animal handling and approved by institutional animal ethical and welfare committee of the Institute of Pharmacy, PSIT (1273/AC/09/ CPCSEA) and plant were collected from Ranan Nagar, Madurai, Tamil Nadu, India and was authenticated by Dr. Navin K. Ambasht, Head and Associate Professor, Botany Department, Christ Church College, Kanpur, Uttar Pradesh, India.
Place and Duration of Study: The study was carried out at Institute of Pharmacy, PSIT, Kanpur, Uttar Pradesh, India, during 2018-21.
Methodology: The hepatoprotective potential of *Morinda tinctoria* leaves extract (*MTLE*) 150 and 300 mg/kg body weight was studied on Methionine and Choline deficient diet, High Fat Diet, Cholesterol and Cholate diet, and Streptozotocin + HFD induced non-alcoholic fatty liver disease. At the end of the treatment blood sample was collected from direct cardiac puncture and analysed for various parameter like alanine aminotransferase, aspartate transaminase, low density

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Corresponding author: E-mail: anu7364@gmail.com;

lipoprotein, high density lipoprotein, triglycerides, total cholesterol, free fatty acid and malondialdehyde.

Results: The phytochemical investigation of extract showed presence of alkaloids, flavonoids, amino acid, saponin, tannins, phenols, carbohydrate and for the first time the present study showed that *Morinda tinctoria* leaves extract reduced level of alanine aminotransferase, aspartate transaminase, triglycerides, total cholesterol, low density lipoprotein, free fatty acid, malondialdehyde and enhance the level of Superoxide dismutase, High Density Lipoprotein and it also returned hepatic damage towards normal which further supports hepatoprotective and antioxidant activity of *M. tinctoria.* leaves extracts.

Conclusions: *M. tinctoria* leaves extract showed maximum curation in the dose 300 mg/kg body weight against non-alcoholic fatty liver disease.

1. INTRODUCTION

Mannanunai is a species of Morinda tinctoria (Rubiaceae), sometimes known as Indian mulberry. It is found in South India's unoccupied agricultural and uncultivated lands [1]. Arthritis, diarrhoea, viral infection, astringent, stomach ulcer, liver disorders, and diabetes are among the conditions for which the plant is utilized. The plant in leaves is reported to as cytoprotective [2], antimicrobial and anti-inflammatory [3], anticonvulsant [4], macro-vertebra colonization [5], antimicrobial [6], in vitro antioxidant activity [7] and removal of ammonia from polluted waters [8]. The fruits are wound healing [9], antihyperglycemic and antidiabetic [10], antiinflammatory [11]. Chronic liver disease (CLD) is a major cause of mortality, morbidity, and health care resource utilization worldwide [12]. This increase was mostly observed in low and low-middle-income countries of Asia and Africa [13]. Non-alcoholic fatty liver disease is a liver disease related with obesity, insulin resistance, type 2 diabetes mellitus (T2DM), hypertension, hyperlipidaemia, and metabolic syndrome. The subtype of non-alcoholic fatty liver disease that is histologically categorised as non-alcoholic steatohepatitis (NASH) has a potentially progressive course leading to liver fibrosis, cirrhosis, hepatocellular carcinoma (HCC) and liver transplantation. All of these complications of NASH can pose significant health, economic, and Patient-experience burdens to the patients, their families and the society [14]. Recent studies have demonstrated that NASH is one of the most important causes of liver transplantation in the USA, and it will become the leading cause for the request of liver donors in the next decades [15]. Obesity elevated the risk of non-alcoholic fatty liver disease [16-25]. World Health Organization (WHO) defined the overweight as a body mass index (BMI) is higher than or equal to 25 and obesity is defined as a BMI higher than or equal to 30. The body mass index has been very useful population-level measure to define overweight and obesity, because the measurement applies to both sexes and adults of all ages. Nevertheless, due to diverse populations in Asia, the World Health Organization has classified the different BMI levels by risk. The patient who's BMIs in between 18.5 to 23 kg/m^2 are considered to have increasing but acceptable degree of risk for obesity-related conditions; patients who's BMIs in between 23 to 27.5 kg/m^2 have an increased risk for obesity-related conditions, and the patients who's BMIs is 27.5 kg/m^2 or higher have a high risk for obesity-related conditions [16]. Specified the importance of visceral obesity as a risk factor for a number of obstacles of metabolic syndrome, analysis of waist circumference may be the more accurate. However, the advantages and disadvantage of BMI vs. waist circumference analysis continue to be debated. In this context, it may be best to assess the risk and progression of non-alcoholic fatty liver disease both based on BMI and waist circumference [26-27]. In this study we performed phytochemical screening, hepatoprotective and antioxidant activity of *Morinda tinctoria* against non-alcoholic fatty liver disease.

2. MATERIALS AND METHODS

2.1 Plant materials

The leaves of *Morinda tinctoria* (*Rubiaceae*) were collected from Ranan Nagar, Madurai, Tamil Nadu, India. The dried leaves materials were mechanically powdered, sheaved using 80 meshes and soaked in 50% aqueous-alcoholic solution for 72 hours at room temperature. Aqueous-alcohol extract was filtered and

concentrated under pressure in rotary evaporator at 50°C and dried to a constant weight in an oven at 35°C. The total yield of the extracts obtained after removing the solvents 32.2% (w/w) and was stored in an air tight container about 20°C until used.

2.2 Phytochemical Screening

The preliminary phytochemical screening revealed the presence of flavonoids, saponin, protein, amino acids, and carbohydrates in 50% aqueous-alcoholic extracts of *M. tinctoria*.

2.3 Animals

Healthy male and female Sprague Dawley rats of aged 5-8 weeks was purchased from local market and resides in polyethylene cage in a room of 23 ± 2°C with a 12 hr light and 12 hr dark cycle, were acclimatized for one week at normal diet in the institute animal house of PSIT, Institute of Pharmacy. In the all experiments involving animals complies with the ethical standards of animal handling and approved by institutional animal ethical and welfare committee of the Institute of Pharmacy, PSIT (1273/AC/09/CPCSEA/12thApr.2017).

2.4 Experimental Design

The animals were divided into five groups (n = 6), with Group I serving as control and receiving only the vehicle (1 mL/kg/day of 1% CMC; p.o.), Group II serving as negative control and receiving no treatment, Group III receiving standard at a dose of 100 mg/kg body weight, and Groups IV and V receiving *MTLE* at doses of 150 and 300 mg/kg body weight. These were given orally twice a day, at 10:00 and 16:00 hr.

2.4.1 Methionine and Choline deficient diet (MCD diet)

For the elicitation of non-alcoholic fatty liver disease in SD rat's fed methionine-choline deficient diet for 4-21 days. Methionine - choline deficient diet possess high sugar and moderate fat content (40% sucrose and 10% fat), but lacking in methionine and choline, which are required for hepatic β-oxidation and the creation of very low density lipoproteins. All the diet was γ- irradiated which make the diet safer by reducing the number of harmful bacteria and parasite. The diet was matched in all nutrients except L- methionine (2g/kg) and choline chloride (2g/kg) which was present in MCD formula. MCD diets provided 17% kacl as protein, 62%kacl as carbohydrate (70:30, sucrose: starch) and 19%

kcal as fat. All the animals had free access to diet and drinking water at the duration of study [28]. The rats were fasted for 4hr prior anesthetized and killed through cervical dislocation at the end of nourishing duration. Livers were collected and a portion of fresh tissue was fixed in 10% buffered formalin.

2.4.2 High Fat Diet (HFD)

The male and female Sprague Dawley rats were used for the elicitation of non-alcoholic fatty liver disease, rats were fed ad libitum a high fat diet, high carbohydrate diet (Western diet) with 42% kcal from fat and containing 01% cholesterol with high fructose-glucose solution (Sugar Water 23.1 g/l d-fructose + 18.9g/l d-glucose) for 16 weeks [28]. Control group fed standard chow diet with normal water. All the animals had free access to diet and drinking water at the duration of study. The rats were fasted for 4hr prior anesthetized and killed through cervical dislocation at the end of nourishing duration. Livers were collected and a portion of fresh tissue was fixed in 10% buffered formalin.

2.4.3 Cholesterol and Cholate Diet (CCD)

Cholesterol in the diet is an important risk factor for non-alcoholic steatohepatitis because it makes the liver sensitive to tumor necrosis factor- and Fas-induced steatohepatitis [29]. The presence of cholic acid stimulates cholesterol and fat absorption while inhibiting cholesterol conversion to bile acids, limiting cholesterol elimination and raising cholesterol levels, particularly low-density lipoprotein cholesterol [30]. Over the course of 6–24 weeks, cholesterol and Cholate diet comprising 1.25 percent cholesterol and 0.5 percent Cholate are used. All the animals had free access to diet and drinking water at the duration of study. The rats were fasted for 4hr prior anesthetized and killed through cervical dislocation at the end of nourishing duration. Livers were collected and a portion of fresh tissue was fixed in 10% buffered formalin.

2.4.4 Streptozotocin + High fat diet

Low dose of streptozotocin (STZ) intraperitoneal administration in new born Sprague Dawley rat's diabetes is caused by a chemical inflammation and destruction of the pancreatic islets [31]. This model combines with high fat diet can establish a model of non-alcoholic fatty liver disease. In this model new born two days after birth Sprague-Dawley rats were give *streptozotocin* (200μg) and then surviving Sprague Dawley rats were

started high fat diet at four weeks old. These rats developed simple steatosis at six weeks, non-alcoholic steatohepatitis with inflammatory foci and ballooning at eight weeks, progressive pericellular fibrosis at twelve weeks and multiple HCC at twenty weeks of age [32]. The level of transaminase and fasting glycemia are elevated at six weeks of age. This model recapitulates several important histological feature of human non-alcoholic fatty liver disease and is also important in terms of oxidative stress. However, rather than a systemic inflammatory insulin resistant milieu, streptozotocin recreating beta cell activity is distinct from the human state [33]. However in similar model where rats was given streptozotocin followed by high fat diet, a investigator failed to demonstrate concordance between rats and humans with respects to differentially expressed gene [34].

2.5 Evaluation of Serum Biochemical Variables

The serum was separated by centrifugation at 3000 rpm for 10 minutes at 4°C from collected blood sample and stored at -22°C for further biochemical analysis. The alanine amino-transferase, aspartate transaminase, triglycerides, total cholesterol, low density lipoproteins and high density lipoproteins was analyzed by using a commercial kit with their instruction and using the multifunctional analyzer (AU600, Olympus). At 505 nm the absorbance of alanine amino-transferase and aspartate transaminase were read and the enzyme activity were calculated as U/L. The level of triglycerides and total cholesterol was determined as mmol/L using absorbance measurements at 510 nm.

2.5.1 Evaluation of Malondialdehyde formation in lipid peroxidation

The liver homogenate (10% w/v) was made by homogenising liver tissue in 150 mmol/L tris-HCL buffered saline (pH 7.2) using a polytron homogenizer. A spectrophotometer was used to quantify the level of malondialdehyde in the liver tissue at 532 nm (U-2001 Hitachi Ltd). The results are expressed as nmol/mg protein in liver tissue.

2.5.2 Evaluation of liver LDL/HDL and FFA activity

For the evaluation of liver tissue low density lipoprotein, high density lipoprotein and free fatty acid had use the commercial kit and follow the instructions of protocols gave by the manufacturer. The absorbance of low density lipoprotein, high density lipoprotein and free fatty acid reaction were read at 546nm and data was showed as mmol/L.

3. RESULTS AND DISCUSSION

3.1 Acute Toxicity Test

Up to 2000 mg/kg body weight, the 50% aqueous alcoholic leaves extract of *Morinda tinctoria* (*MTLE*) showed no signs or symptoms of toxicity, hence it was deemed safe and effective.

3.2 Effect of *Morinda tinctoria* on Body Weight and Liver Coefficient

After fed with Methionine and Choline deficient diet, High Fat Diet, Cholesterol and Cholate diet and Streptozotocin+HFD, the body mass of Sprague Dawley rats in the model groups were notably raised as comparison to that control group of Sprague Dawley-rats ($P < 0.01$, Fig. 1A). Meantime after *MTLE* treatment for six (6) weeks the raised in the body mass for Sprague Dawley-rats in the 150 and 300mg/kg b.w., the rats of *Morinda tinctoria* leaves extract treated groups was lower than in the control and model groups. ($P < 0.01$, Fig. 1A), which indicates Obesity may be prevented by using *Morinda tinctoria* leaves extract therapy in Methionine and Choline deficient diet, High Fat Diet, Cholesterol and Cholate diet and Streptozotocin + HFD administrated SD-rats. Moreover, as a result of these changes, the liver coefficient was notably reduced in the *MTLE* treated rats ($P < 0.05$, $P < 0.01$, Fig. 1B), compared to the control group.

3.3 Effect of *Morinda tinctoria* on Serum ALT and AST Levels

The function of liver failure indirectly reflected by the serum level of alanine aminotransferase and aspartate transaminase, As shown in Table 1, serum alanine aminotransferase and aspartate transaminase activates were notably increased after the administration of Methionine and Choline deficient diet, High Fat Diet, Cholesterol and Cholate diet and Streptozotocin + HFD, as compared with the standard group, the level of alanine aminotransferase and aspartate transaminase was notably deceased in a dose dependent fashion after *MTLE* treatment of 150 and 300 mg/kg b.w, ($P <0.05$, $P<0.01$, Table 1).

3.4 Effects of *Morinda tinctoria* on Blood Lipid Levels

Methionine choline deficient diet, High fat diet, Cholate and choline diet and Streptozotocin+High fat diet cause non-alcoholic fatty liver disease and produced a notable increased change in triglycerides and total cholesterol levels are compared with the normal group ($P<0.01$, Table 1), which indicating the successful initiation of the non-alcoholic fatty liver disease models in the Sprague-Dawley rats. Nevertheless, after *MTLE* treatment the concentration of both triglycerides and total cholesterol in blood was remarkably decreased in dose dependent manners, as compared to the non-alcoholic fatty liver disease standard groups ($P <0.05$, $P<0.01$, Table 1). All of these findings point to *MTLE* having lipid-lowering properties in the treatment of non-alcoholic fatty liver disease.

3.5 Effects of *Morinda tinctoria* on Liver Tissue Malondialdehyde (MDA) Levels

Malondialdehyde is an end product of the breakdown of the polyunsaturated fatty acid and related esters is an important index of lipid peroxidation in many organ homogenates [35]. The malondialdehyde concentration notably enhanced by the nourishing with Methionine and Choline deficient diet, High Fat Diet, Cholesterol and Cholate diet and Streptozotocin+High Fat Diet models as compared with the control group ($P<0.01$, Table 1). Although the treatment with *MTLE* 150 and 300 mg/kg b.w. notably reduce the levels of the malondialdehyde level in the live homogenate respectively ($P<0.01$, Table 1).

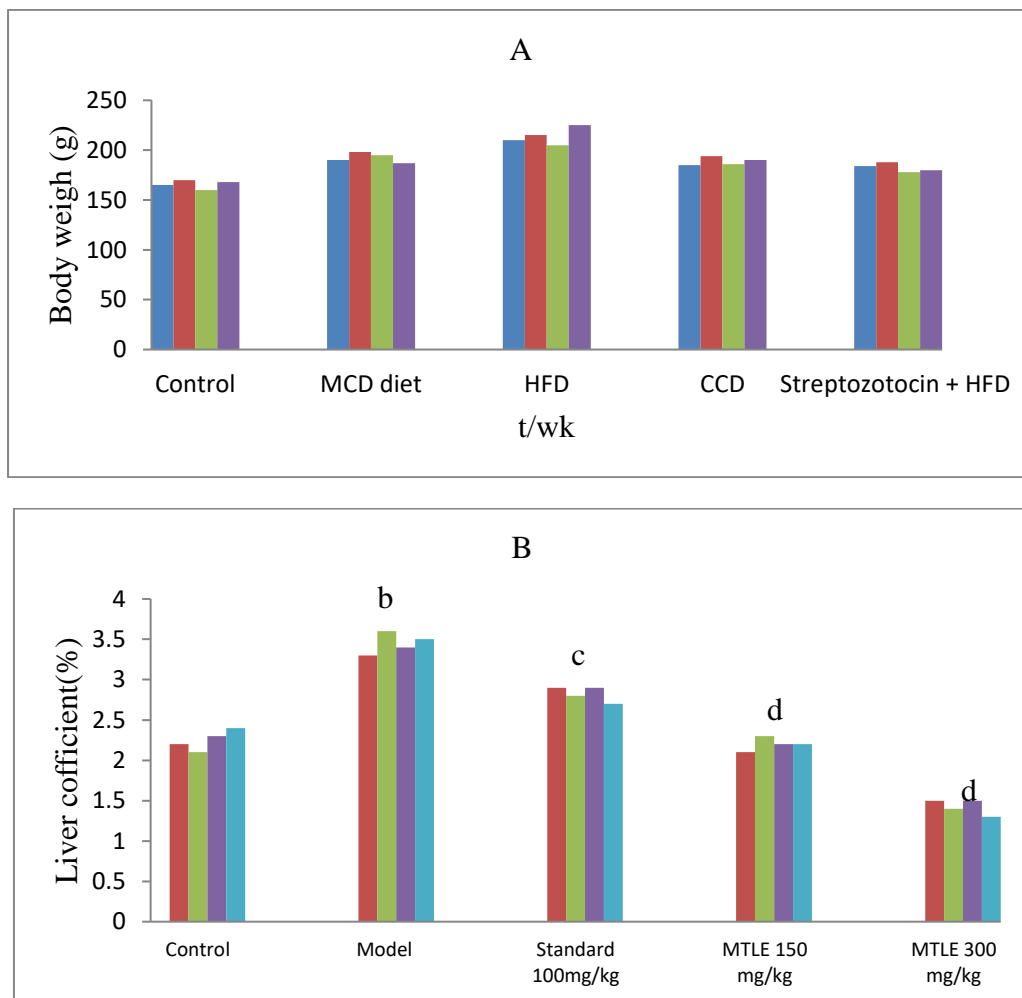**Fig. 1. Effect of *Morinda tinctoria* [b]P *<0.01 vs* control group; [c]P *<0.05*, [d]P< *0.01 vs* model group for Body weight (A) and Liver coefficient (B)**

Table 1. Effects of 50% aqueous alcoholic extract of *Morinda tinctoria* (*MTLE*) on alanine aminotransferase (ALT), aspartate transaminase (AST), triglycerides (TG), total cholesterol (TC), and malondialdehyde (MDA)

Treatment	ALT (IU/L)	AST (IU/L)	TG nmol/L	TC nmol/L	MDA
Methionine and Choline deficient diet (MCD)					
Control	18.83±2.61	62.53±8.37	0.64±0.07	0.83±0.06	0.37±0.02
MCD diet	64.35±14.52[b]	168.17± 36.43[b]	1.50±0.31[b]	4.06±0.72[b]	0.65±0.01[b]
Silymarin (100mg/kg)	36.81±5.63[c]	112.72±11.75[c]	0.74±0.12[c]	3.31±0.27[c]	0.53±0.02[c]
MTLE (150mg/kg)	29.43±4.06[d]	87.52±7.16[d]	0.62±0.7[d]	2.24±0.16[d]	0.34±0.01[d]
MTLE (300mg/kg)	17.52±3.41[d]	52.43±7.58[d]	0.38±0.4[d]	1.36±0.10[d]	0.22±0.01[d]
High Fat Diet (HFD)					
Control	19.73±2.61	64.33±8.36	0.63±0.08	0.82±0.06	0.39±0.01
HFD	66.45±14.42[b]	165.16± 37.42[b]	1.52±0.32[b]	5.06±0.72[b]	0.66±0.02[b]
Silymarin (100mg/kg)	35.86±5.62[c]	110.75±11.85[c]	0.76±0.14[c]	4.32±0.27[c]	0.54±0.01[c]
MTLE (150mg/kg)	28.44±4.07[d]	86.54±7.14[d]	0.63±0.7[d]	2.65±0.16[d]	0.36±0.01[d]
MTLE (300mg/kg)	16.53±3.42[d]	51.33±6.58[d]	0.36±0.5[d]	1.32±0.10[d]	0.23±0.01[d]
Cholesterol and Cholate diet (CCD)					
Control	18.73±2.61	62.33±7.36	0.64±0.07	0.83±0.07	0.36±0.02
CCD diet	65.45±14.42[b]	167.16± 37.32[b]	1.53±0.32[b]	4.06±0.72[b]	0.76±0.02[b]
Silymarin (100mg/kg)	38.86±5.62[c]	114.75±12.85[c]	0.72±0.14[c]	5.32±0.27[c]	0.59±0.01[c]
MTLE (150mg/kg)	27.44±4.07[d]	83.54±8.14[d]	0.68±0.7[d]	2.85±0.16[d]	0.38±0.01[d]
MTLE (300mg/kg)	16.63±3.52[d]	52.33±7.58[d]	0.34±0.5[d]	1.14±0.10[d]	0.24±0.01[d]
Streptozotocin + HFD					
Control	17.72±3.62	61.64±6.27	0.61±0.05	0.82±0.06	0.34±0.01
Streptozotocin+ HFD	67.35±13.55[b]	162.18± 33.51[b]	1.37±0.22[b]	3.54±0.74[b]	0.54±0.02[b]
Silymarin (100mg/kg)	37.91±3.84[c]	94.92±13.64[c]	0.76±0.12[c]	2.43±0.23[c]	0.43±0.01[c]
MTLE (150mg/kg)	28.33±2.07[d]	87.53±9.16[d]	0.52±0.6[d]	2.02±0.12[d]	0.24±0.01[d]
MTLE (300mg/kg)	20.66±3.52[d]	72.43±9.78[d]	0.32±0.5[d]	1.23±0.14[d]	0.12±0.01[d]

Data are reported as mean ± SD (n = 6) with a, [b]$P < 0.01$ vs control group [c]$P < 0.05$, [c]$P < 0.05$, [d]$P < 0.01$ vs model group

3.6 Effects of *MTLE* on Low Density Lipoprotein, High Density Lipoprotein, Superoxide Dismutase and Free Fatty Acid Levels in the Liver Tissue

The volume of lipid production was notably raised as a result of Methionine choline deficient diet, High fat diet, Cholate and choline diet and Streptozotocin+High fat diet nourishing in the model group correlated with the control group (*P<0.01,* Table 2). Its results shows that the low density lipoprotein was notably raised in the model groups correlated with the normal group (*P<0.01,* Table 2) and dramatically reduced in the *MTLE* treated groups correlated with the standard groups (*P <0.05, P<0.01,* Table 2). The volume of high density lipoprotein was notably reduced at the end of the experiments and the *MTLE* treatment notably improved the high density lipoprotein volume correlated with that in the standard groups (*P <0.05, P<0.01,* Table 2). Although the treatment with 150 and 300 mg/kg b.w. of *MTLE* notably increase the levels of the antioxidant enzyme SOD in the dose dependent manners respectively (*P <0.05, P<0.01,* Table 2) Similarly the amount of the free fatty acid was notably increased after Methionine choline deficient diet, High fat diet, Cholate and choline diet and Streptozotocin+High fat diet administration and treatment with *MTLE* notably

reduced the content of free fatty acid at a dose dependent manner (*P <0.05, P<0.01,* Table 2).

3.7 Histopathological Variations in Liver Tissue

With naked eyes, histopathological variations in liver have been seen: the control groups' liver were deep red, moist, glossy, and robust (Fig. 2A/C/E/G I), but the model groups' liver were yellow necrotic foci, grey red colour, loss of lustre, and tumescent (Fig. 2A/C/E/G II). The liver damage in the MTLE-treated SD-rats, were notably reduced in a dose-dependent manner as compared to the control (Fig. 2A/C/E/G III-V). HE-stained sections are displayed in Fig. 2B/D/F/H. The photomicroscope revealed typical lobular architecture in liver sections from the normal control groups, as well as well-preserved cytoplasm and well-defined nuclei (Fig. 2B/D/F/H I). For the present being, full fat vacuoles in the lobule cells, inflammatory cell infiltration, cell swelling, and lipid degradation in the core region of the lobules were seen in liver sections from model groups (Fig. 2B/D/F/H II). But in the liver section of MTLE treated SD-rats; inflammatory response and lipid degeneration was remarkably reduced as compared with standard groups and liver cell volume became smaller, the droplet numbers of fat were decreased and the hepatic lobules was clearly represent (Fig. 2B III-V).
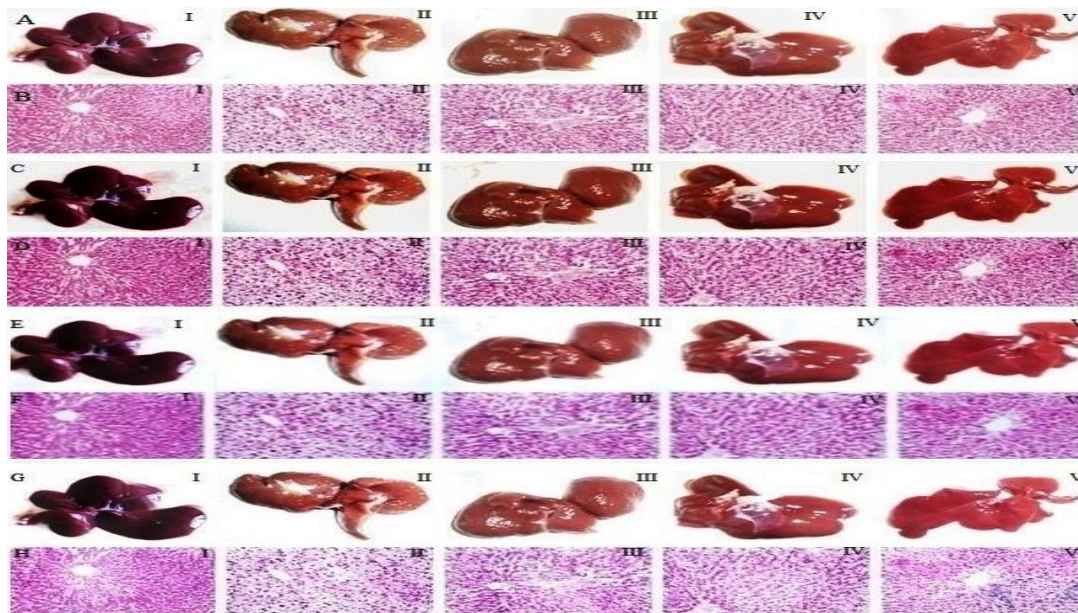

Fig. 2. Histopathological examination of SD-rat liver tissue (B/D/F/H, 200x) and appearance of SD-rat liver tissue (A/C/E/G). I: Control; II: Model; III: Standard group 100mg/kg b.w.; IV and V: MTLE group 150 and 300mg/kg b.w

Table 2. Effects of 50% aqueous alcoholic extract of *Morinda tinctoria* (*MTLE*) on low density lipoprotein (LDL), high density lipoprotein (HDL), Superoxide dismutase (SOD) and free fatty acid (FFA) level in the liver tissue

Treatment	LDL mmol/L	HDL mmol/L	SOD (U/mgprot)	FFA mmol/L
Methionine and Choline deficient diet (MCD)				
Control	0.34±0.09	0.96±0.12	133.53±16.24	0.85±0.12
MCD diet	2.43±0.12[b]	0.56±0.03[b]	79.64±8.52[b]	2.07±0.14[b]
Silymarin (100mg/kg)	1.32±0.10[c]	0.72±0.04[c]	94.91±8.63[c]	1.73±0.12[c]
MTLE (150mg/kg)	0.87±0.05[d]	0.84±0.05[d]	110.52±12.33[d]	1.24±0.10[d]
MTLE (300mg/kg)	0.48±0.08[d]	0.90±0.05[d]	129.25±10.68[d]	0.86±0.08[d]
High Fat Diet (HFD)				
Control	0.35±0.07	0.98±0.13	132.52±16.25	0.86±0.12
HFD	2.42±0.13[b]	0.50±0.02[b]	78.63±8.56[b]	2.09±0.15[b]
Silymarin (100mg/kg)	1.34±0.12[c]	0.67±0.06[c]	92.92±8.61[c]	1.73±0.13[c]
MTLE (150mg/kg)	0.86±0.04[d]	0.78±0.07[d]	107.54±10.33[d]	1.26±0.13[d]
MTLE (300mg/kg)	0.44±0.07[d]	0.84±0.04[d]	127.26±12.68[d]	0.92±0.08[d]
Cholesterol and Cholate diet (CCD)				
Control	0.42±0.07	0.98±0.13	132.52±16.25	0.87±0.13
CCD diet	2.43±0.13[b]	0.50±0.02[b]	78.63±8.56[b]	2.08±0.16[b]
Silymarin (100mg/kg)	1.35±0.12[c]	0.67±0.06[c]	92.92±8.61[c]	1.72±0.13[c]
MTLE (150mg/kg)	0.92±0.04[d]	0.76±0.07[d]	110.54±10.33[d]	1.27±0.14[d]
MTLE (300mg/kg)	0.49±0.07[d]	0.87±0.04[d]	126.26±12.68[d]	0.94±0.07[d]
Streptozotocin + HFD				
Control	0.38±0.06	0.97±0.12	135.51±17.25	0.86±0.14
Streptozotocin+ HFD	2.42±0.14[b]	0.52±0.09[b]	78.62±8.56[b]	2.09±0.15[b]
Silymarin (100mg/kg)	1.36±0.13[c]	0.68±0.08[c]	94.90±8.63[c]	1.74±0.12[c]
MTLE (150mg/kg)	0.91±0.05[d]	0.72±0.06[d]	108.53±11.34[d]	1.26±0.14[d]
MTLE (300mg/kg)	0.47±0.06[d]	0.84±0.05[d]	122.25±13.67[d]	0.92±0.07[d]

Data are reported as mean ± SD (n=6) for each group. [b]$P < 0.01$ vs control group; [c]$P < 0.05$, [d]$P < 0.01$ vs model group

Table 3. A preliminary phytochemical study on 50% aqueous leaves extract of *Morinda tinctoria*

S.No.	Test	Observation	Inference
1.	Alkaloids (Mayer`s test)	Positive	Present in extract
2.	Flavonoids (Sodium hydroxide test)	Positive	Present in extract
3.	Amino acid (Ninhydrin test)	Positive	Present in extract
4.	Saponins (Foaming test)	Positive	Present in extract
5.	Tannins and phenolic compounds	Positive	Present in extract
6.	Carbohydrates (Molish`s test)	Positive	Present in extract
7.	Glycoside (Killer-Killani test)	Negative	Absent in extract

Non-alcoholic fatty liver disease now one of the very serious problem in 21st century. Obesity is the measure cause of non-alcoholic fatty liver disease. The biochemical variations occur mainly in alanine aminotransferase, aspartate transaminase, low density lipoprotein, triglycerides, total cholesterol, and malondialdehyde etc. this entire biochemical changes occur because of altered structure and function of liver and enzyme activity. When triglyceride levels exceed 5% of liver weight, a histological spectrum spanning from basic steatosis to non-alcoholic steatohepatitis can be seen. Hepatocellular destruction, fibrogenesis, and lobular necro-inflammation are all symptoms of non-alcoholic steatohepatitis [36-37], which can progress to cirrhosis and hepatocellular carcinoma [38-39]. Although Methionine and Choline deficient diet, High Fat Diet, Cholesterol and Cholate diet, and Streptozotocin+High Fat Diet induced non-alcoholic fatty liver disease animals models required a long nourishing period and they are more close to humans non-alcoholic fatty liver disease in pathophysiology, including induced obesity, insulin resistance and hepatic steatosis in Sprague Dawley rats [40]. According to traditional medicine theory, the etiology of non-alcoholic fatty liver disease includes emotional problem and poor diet, with the essential point of blood stasis and phlegm. These investigations are related to the organs of the liver, spleen, and kidney [41]. Promoting blood circulation to clear meridian obstructions, lowering phlegm, clearing moisture, and tonifying the liver and kidneys are just a few of the benefits. Hence development and examine a new agent to detain or reverse the pathogenesis progression in of non-alcoholic fatty liver disease are very important objective. The *M.tinctoria* leaves extract exhibits the highest and efficient hepatoprotective and antioxidant activity among all the Sprague Dawley - rat models, hence now which have increased their demand in the market of natural and herbal medicine. Lipid metabolism play an important role in energy dissipation and hence

responsible to maintain a steady state in the body [42]. Disruption in the metabolism of lipids may lead to life threating situation like hypercholesterolemia, obesity, atherosclerosis, heart blocked etc [43]. Thus prevention of lipid absorption could be an alternate strategy to treat obesity and non-alcoholic fatty liver disease [44]. In the Methionine and Choline deficient diet, High Fat Diet, Cholesterol and Cholate diet, and Streptozotocin+High Fat Diet induced non-alcoholic fatty liver disease, the liver coefficient and serum alanine aminotransferase, aspartate transaminase, triglycerides and total cholesterol were notably increased, the levels of low density lipoprotein and free fatty acid in the liver were markedly increased, and high density lipoproteins were markedly reduced when compared to normal control groups. *Morinda tinctoria* leaves extract inhibited gradual changes in alanine aminotransferase and aspartate transaminase, decreased triglycerides, total cholesterol, low density lipoprotein, and free fatty acid levels, and increased high density lipoproteins and superoxide dismutase levels following treatment with *Morinda tinctoria* leaves extract. Furthermore, the histological abnormalities observed under microscopy were related to the study of liver function. The centrolobular hepatic necrosis, ballooning degeneration, fatty changes and infiltrating lymphocytes was observed in non-alcoholic fatty liver disease models groups. The treatment with *M. tinctoria* leaves extract prevents these histopathological changes in the Sprague Dawley rats with Methionine and Choline deficient diet, High Fat Diet, Cholesterol and Cholate diet, and Streptozotocin+High Fat Diet induced non-alcoholic fatty liver disease. Thus, these findings suggested that the inhibition of the elevation of liver function markers, obvious lipid lowering and liver damage may be related to the protective effects of M. tinctoria leaves extract against non-alcoholic fatty liver disease induced by Methionine and Choline deficient diet, High Fat Diet, Cholesterol and Cholate diet, and

Streptozotocin+High Fat Diet. *M. tinctoria* leaves extract decrease the level of malondialdehyde against the Methionine and Choline deficient diet, High Fat Diet, Cholesterol and Cholate diet, and Streptozotocin+ High Fat Diet induced non-alcoholic fatty liver disease in the Sprague Dawley - rats, and produce hepatoprotective activity. *M. tinctoria* leaves extract enhanced the activity of superoxide dismutase and decrease malondialdehyde in rats with Methionine and Choline deficient diet, High Fat Diet, Cholesterol and Cholate diet, and Streptozotocin+ High Fat Diet induced non-alcoholic fatty liver disease in the Sprague Dawley rats, suggesting that the activity of antioxidants may play a role in the mechanism of its hepatoprotective activity. The results of present study showed that for the first time the *M. tinctoria* leaves extract possessed hepatoprotective activity as an evidenced by its significant inhibition in the formation of non-alcoholic fatty liver disease by diet and chemical agents. These finding could justify, at least partially the inclusion of this plant in the management of hepatic disorder in ethnomedicine. Since the role of free radicals and antioxidants for showing the hepatoprotective effect is very clearly defined, the potential of *Morinda tinctoria* leaves extract may be in part due to its potent antioxidant activity of the plant. Further experiments are needed to test the effect of this plant in the treatment of chronic non-alcoholic fatty liver disease.

4. CONCLUSION

In conclusion, the current study found that *Morinda tinctoria* had a substantial hepatoprotective effect in rats when fed a Methionine and Choline deficient diet (MCD diet), a High Fat Diet (HFD), a Cholesterol and Cholate diet (CCD), or Streptozotocin+High Fat Diet. Further research will require studies on the separation of the active molecule responsible for this activity, as well as validation of its mechanism of action. This suggests that the extract has the potential to be an effective hepatoprotective agent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments involving animals complies with the ethical standards of animal handling and approved by institutional animal ethical and welfare committee of the Institute of Pharmacy

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/75186