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Research Article
Protective Effect of *Byrsonima sericea* Extract on Non-alcoholic Fatty Liver Disease Model in Rats

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Abstract

Background and Objective: Non-alcoholic Fatty Liver Disease (NAFLD) is a disease of excessive lipid accumulation which sensitizes the liver cells and leads to hepatic insulin resistance, inflammation and oxidative stress. This study was aimed to evaluate the hepato-protective effect of *Byrsonima sericea* extract (BSE) against High fat emulsion diet (HFED) induced NAFLD in rats. **Materials and Methods:** The NAFLD was induced by administration of HFED (10 mL kg^{-1}/p.o.), along with micro dose of carbon tetra chloride (CCl_4) (0.5 mL kg^{-1}, i.p.). The experiment includes 5 groups with 6 rats in each group. Group 1 was normal control, Group 2 was negative control, where rats received HFED (10 m kg^{-1} p.o, B.W) and CCl_4 (0.5 mL kg^{-1}, i.p.). Group 3, 4 and 5 were BSE treated rats in a dose of 75, 150 and 300 mg kg^{-1}, respectively. All rats were tested for physical as well as biochemical estimations like ALP, blood glucose, insulin and bilirubin content. Histopathological examination of liver was also performed in trial groups of rats. **Results:** The extract at a dose of 300 mg kg^{-1} (i.p.) showed significant protective effect by reducing the higher content of cholesterol, triglycerides, LDL and VLDL level in serum of rats. It also showed a potential decrease in ALP, total bilirubin and SGOT content in serum. The rats treated with extract showed less hepatic damage in liver cells as compare with negative control group rats. **Conclusion:** This study concluded that the rats treated with BSE have significant hepatoprotective action and acts by modulating the hepatic enzymes secretion, via reducing weight gain and organ fat deposition in rats.

Key words: *Byrsonima sericea* leaves, hepatic damage, hepatoprotective, high fat emulsion diet, fat deposition, liver disease

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The NAFLD is a metabolic disorder, affects entire public and it leads to fatty liver sickness, which turned into the most widely recognized reason for liver illness[1]. Early discovery of hepatotoxicity is critical in light of the fact that proceeded with ingestion of the medication is frequently connected with a poor health[2]. Insulin resistances play an important role in the pathogenesis of NAFLD; in this way, obesity, diabetes and the metabolic disorder are often times connected with the illness[3]. Thus, as these metabolic conditions develop as real medical issues in western culture[4], it is presently acknowledged that NAFLD is the most widely recognized incessant liver condition in the western world[5]. The drugs with severe hepatotoxicity or unfavorable responses to drugs utilized in remedial dose might be the principle guilty party in the etiology of liver treatment[6].

Early recognition of hepatotoxicity is critical in light of the fact that proceeded with ingestion of the medication is regularly connected with a poor anticipation[7]. The NAFLD is viewed as the liver dysfunction which leads to metabolic disorder and is emphatically connected with weight, insulin resistance, hypertension and dyslipidaemia[8]. The pathogenesis of non-alcoholic fatty liver disease isn't totally comprehended and regardless of whether insulin obstruction is a main pathogenic key, numerous different variables are ensnared in both liver fat accumulation and slow movement to non-alcoholic steatohepatitis[9]. There is, as up till now no evidence based treatment for NAFLD is available. Treatment is right now coordinated at treating symptoms of the metabolic disorder which may not recover the functioning of liver.

As of now, approaches have concentrated on herbal sources that have been accounted for treatment to NAFLD with less symptoms[10]. Different investigations have uncovered that bioactive constituents, for example, steroids, flavonoids, alkaloids, phenolic corrosive, saponins and tannins have promising effects in NAFLD[11]. As dietary enhancements some herbal therapeutic plants have been utilized for NAFLD like *Silybum marianum* (milk thistle), *Picrorhiza kurroa* (kutkin), *Curcuma longa* (turmeric), *Camellia sinensis* (green tea), *Chelidonium majus* (greater celandine), *Glycyrrhiza glabra* (licorice) and *Allium sativa* (garlic). Some complex Chinese herbal formulae, such as Pro-liver Pill (Yang Gan Wan), Liver Care (Himalaya Drug Co., Bangalore, India), Liv-52, Jianpi Wenshen Pill (Jianpi Wenshen Wan), Binggan capsules (Binggan Jiaonang), Binggan Tang, Yizhu decoction (Yizhu Koufuye), Yiergan Tang and Xiao Chai Hu Tang (Sho-saiko-to or SST) have been reported to have significant therapeutic effects on liver protection or treatment of liver diseases[5,10,11].

Byrsonima sericea is a traditional Brazil plant with Malpighiaceae family. The utilization of plants of this variety in northeastern Brazil is regular in people drug for the treatment of fever, gastrointestinal issues (the runs and gastric ulcer), asthma, skin diseases and snake chomps[11-13]. An ethanolic extricate from the leaves of *B. sericea* gathered in the province of Ceara (additionally in northeastern Brazil) has shown gastro-protective properties and the phytochemical examination recognized triterpenes, tannins and flavonoids as the primary classes of constituents had been reported on *Byrsonima sericea*[10]. However; its hepatoprotective action has not been investigated. Based on different chemical constituents present in *Byrsonima sericea* and literature it ought to be tried for its impact in NAFLD[11]. The plant may have the possibility to repress the diverse catalyst and decline the illnesses of metabolic issue like hepatic damage. Along these lines, present investigation was aimed to assess its belongings in HFED prompted liver damage in rats.

MATERIALS AND METHODS

All the work related to this study was performed on affiliated institutes. The analytical part and animal study were conducted in month of July-December, 2018.

Plant material: The hydro-alcoholic extract of *Byrsonima sericea* was procured from Amstar Pvt. Ltd., Indore (M.P).

Chemicals: All the chemicals used in this study were of analytical grade. Methanol, chloroform, sesame oil, NaCl, $CaCl_2$, phosphate buffer solution, copper reagent, diethyl dithio carbamate sodium, oleic acid, porcine pancreatic lipase and bovine serum albumin serum were obtained by Sigma-Aldrich and Serum Cholesterol kit, Serum Triglyceride kit, Serum HDL-Cholesterol were provided from High media pvt., Ltd.

Preliminary phytochemical screening

Qualitative phytochemical screening: Phytochemical screening was carried out for various constituent such as; flavonoids, alkaloids, carbohydrates, proteins, tannins, amino acid, acidic compound, volatile oil, glycosides and saponins[14].

Quantitative phytochemical screening

Determination of total flavonoids content: Total flavonoids content of plant extract was evaluated by the method described by Bais *et al.*[15]. The absorbance of the solution was measured at 415 nm using a spectrophotometer against blank. Methanol served as

blank. The total content of flavonoids compound in plant extract was expressed in mg/g Quercetin Equivalent (QE).

Determination of total phenolic content: Total phenolic content was estimated by Folin Ciocalteu's method[15]. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve. Total phenolic content was expressed as milligrams of Gallic Acid Equivalent (GAE) per gram of extract by using the following equation:

$$C = \frac{cV}{M}$$

Where:

C = Total content of phenolic compounds in mg g^{-1} GAE
c = Concentration of gallic acid (μg mL^{-1}) established from the calibration curve
V = Volume of extract
m = Weight of pure plant extract

Determination of total tannin content: The tannin contents were determined by method earlier described by Bais and Prashar[16] with slight modification[17]. A volume of 400 μL of extract is added to 3 mL of a solution of vanillin (4% in methanol) and 1.5 mL of concentrated hydrochloric acid. After 15 min of incubation the absorbance was read at 500 nm.

Determination of total alkaloid content: The total alkaloid contents were determined by method of Bais and Prashar[16] with few modifications[17]. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract.

Determination of hydrogen peroxide scavenging activity: Scavenging of hydrogen peroxide was measured by the method described by McCullough[18]. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank containing phosphate buffer without hydrogen peroxide:

$$H_2O_2 \text{ radical scavenging } (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control and A_1 was the absorbance of sample.

***In vitro* enzyme assay**

α-glucosidase inhibition assay: α-glucosidase inhibitory activity was described by Zhang *et al.*[19] with slight modification. Maltose was used as a standard. The absorbance was measured at 405 nm using a UV-visible spectrophotometer. The inhibition (%) was determined as follows:

$$\text{Inhibition } (\%) = \frac{1 - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Thin Layer Chromatography (TLC): The TLC was done on glass plates (10×20 cm) coated with silica gel G. Solvent system was used like[11,14] Butanol:Acetic acid:Water (4:1:5), Butanol:Ethanol:water (4:1:2.2), Ethyl acetate:Chloroform (1:9). Detecting Reagents were Iodine vapor, ammonia vapor and UV chamber by using the retention factor equation:

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Experimental animal: Experiments were carried out on Healthy Swiss Albino rat of either sex weighing 180-200 g. The animals were housed in grouping of six per polypropylene cages kept under controlled room temperature (22-24°C) in a 12 h light dark cycle. The animals were kept in paddy husk. The animals were allowed to free access to food and water. All procedure were approved and carried out as per the Guidelines of Experiments on Animals (Reg. No. IAEC/15/2018).

High fat diet formula: The HFED that consists of 58% fat, 25% protein, 17% carbohydrate, 13% lard, 1% cholesterol, 0.6% vitamin and minerals, respectively, was administered every day. Food intake was calculated everyday and body weight was measured every week.

Dose selection: In the present study three doses of the *Byrsonima sericea* extract were selected as 75, 150 and 300 mg kg^{-1}. These dosed were selected on the basis of previous reports[13] of the acute toxicity LD_{50} is 3.65 g kg^{-1}.

Methodology: The Wistar rats (220-250 g) were used for the study. Rats were housed in polyacrylic cages in temperature controlled rooms (25°C) with steady moistness and 12 h/12 h light/dark cycle. Rats were enabled free

access to normal pellet diet and water. The NAFLD was induced by administration of High fat emulsion diet (HFED) (10 mL kg^{-1}/p.o.) along with CCl_4 (0.5 mL kg^{-1}, i.p.). The CCl_4 were administered once in a weak for the initial 14 days and then on 24th day of experimental protocol.

The experiment includes 5 groups with 6 rats in each group. Group 1 was considered as normal control group in which rats were on normal chow diet and free access to water. Group 2 was considered as negative control group, in which the rats were received High Fat Emulsion Diet (HFED) (10 m kg^{-1} p.o., b.wt.) and CCl_4 (0.5 mL kg^{-1}, i.p.) to produce Non-alcoholic Fatty Liver Disease (NAFLD). Group 3, 4 and 5 were considered as extract treated as a dose of 75, 150 and 300 mg kg^{-1}, respectively through oral route. On the last day of the experiment all the animals were sacrificed by cervical dislocation. Blood sample were collected. The clear serum was separated at 2500 rpm for 15 min using centrifuge. The liver homogenate was prepared for the estimations of hepatic parameters. The levels of total cholesterol, triglyceride, HDL-C, LDL-C, VLDL-C, alkaline phosphatase (ALP), total bilirubin, direct bilirubin SGPT and SGOT in liver tissue homogenate were determined through span automatic analyzer. The glucose, insulin level and Free Fatty Acid (FFA) in serum were detected by commercials kits (Span diagnostic Pvt., Ltd., China) available in institute.

In vivo pharmacological evaluation parameters

Body weight and feed intake: Body weight of all the animals in the groups was recorded on day one and then weekly throughout the study using digital balance. Feed intake of each group was measured daily using analytical balance.

Weight of organs: Organs of rat like kidney, liver and fat pads were dissected out carefully and weighed using electronic digital balance.

Biochemical estimation: On the 36th day of the experiment all the animals were sacrificed by cervical dislocation. Blood samples were collected. The clear serum was separated at 2500 rpm for 15 min using centrifuge. The levels of total cholesterol, triglyceride, HDL-C, LDL-C, VLDL-C, alkaline phosphatase (ALP), total bilirubin, direct bilirubin SGPT and SGOT in liver tissue homogenate were determined through span automatic analyzer[14]. The glucose , insulin level and Free Fatty Acid (FFA) in serum were detected by commercials kits (Span diagnostic Pvt. Ltd., China) available in institute.

Statistical analysis: The data was analyzed on Graph Pad Prism 7.0 software and the data obtained from various parameters were expressed as Mean±SEM (n = 6) followed by one way analysis of variance (ANOVA). In all the tests, the criterion for the statistical significance was set at $p<0.05$. The data for all studies was analyzed using one-way ANOVA followed by Tukey-Kramer Multiple Comparisons test.

RESULTS

Qualitative analysis: The results of the qualitative analysis of hydro-alcoholic extract of *Byrsonima sericea* leaf showed the presence of alkaloids, acidic compounds, flavonoids, tannins, glycoside, amino acids, volatile oil, protein, steroids, fat and fixed oil except saponins (Table 1).

Quantitative analysis

Determination of total phenolic, flavonoids, tannin and alkaloid content: Result demonstrates that phenolic content was found higher as compared with other constituents. The alkaloidal content was lesser as contrast with all. The order of content was like Total Alkaloidal content<Total Tannin content<Total Flavonoid content<Total Phenolic content (Table 2).

Determination of hydrogen peroxide scavenging activity: The scavenging ability of hydroalcoholic extract of *Byrsonima sericea* leaf on hydrogen peroxide assay is shown in Table 3. The extract showed a higher scavenging property against hydrogen peroxide as compare to vehicle.

***In vitro* α-glucosidase inhibition assay:** α-glucosidase inhibitory activity of the hydro-alcoholic extract of *Byrsonima sericea* leaf is presented in Table 4 (IC_{50} = 84.54 µg mL^{-1}). The inhibition of plant extract against α-glucosidase had shown maximum activity in a concentration of 1000 µg mL^{-1}.

Thin layer chromatography: The TLC of hydro-alcoholic extract of *Byrsonima sericea* leaf was done for identification of secondary metabolites (Table 5). The extract shown spot that have different R_f value. The extract showed the presence of some important constituents like Scopolin, Caffeoylquinic acid and Vanillic acid.

Effect of *Byrsonima sericea* leaf extract on serum lipid profile: The rats on high fat diet regimen with micro-dose of CCl_4 for 28 days showed significant increase in the lipids

level like Total Cholesterol (TC), Triglyceride (TG), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and decline the content of HDL-High density lipoprotein when compared with control group rats. Treatment with *Byrsonima sericea* extract showed increase HDL content and decline raised content of Total Cholesterol (TC), Triglyceride (TG), LDL, VLDL level as compared with negative control group (Table 6).

Table 1: Phytochemical screening result

Extract constituents	Present (+) /Absent (-)
Alkaloids	+
Acidic compounds	+
Volatile oils	+
Steroids	+
Phenolic/Tannins	+
Proteins	+
Glycosides	+
Flavonoids	+
Saponins	-
Fat	+
Fixed oil	+
Amino acids	+

Table 2: Total phenolic, flavonoid, tannin and alkaloidal content

Extract	Parameters/Test of extract (%)	Values
Hydro-alcoholic extract of *Byrsonima sericea* leaf	Total phenolic content (mg GAE/g)	128.10±1.45
	Total flavonoid content (mg QE/g)	56.45±1.12
	Total tannin content (mg GAE/g)	41.14±2.30
	Total alkaloidal content(mg AE/g)	21.10±1.45

Table 3: Hydrogen peroxide scavenging activity of BSE

S. No.	Inhibition (%)	IC_{50} value (μg mL^{-1})
Vehicle	0	0
BSE Ex.	94.3	97.45

Values are expressed in Mean±SEM, where, n = 3, *Concentrations of extracts and standard were in the range of 0.001-1000 μg mL^{-1}, BSE Ex.: *Byrsonima sericea* extract

Table 4: α-glucosidase inhibition activity of BSE

S. No.	Inhibition (%)	IC_{50} value (μg mL^{-1})
Vehicle	0	0
Standard (Acarbose)	92.1	150.75±0.74
BSE Ex.	94.3	84.54

Values are expressed in Mean±SEM, where n = 3, *Concentrations of extracts and standard were in the range of 0.001-1000 μg mL^{-1}, BSE Ex.: *Byrsonima sericea* extract

Effect of *Byrsonima sericea* leaf extract on other biochemical parameters: The rats treated with HFED and CCl_4 showed the elevated level of ALP, blood glucose level, insulin level, total bilirubin content and direct bilirubin content in rats when compared with normal control rats. The extract treated groups found decrease contents of ALP, blood glucose level, insulin level, total bilirubin content and direct bilirubin which was very close to normal values observed in control rats. The observed effect was dose dependent when given to HFED rats (Table 7).

Effect of *Byrsonima sericea* leaf extract on liver enzymes: It was observed that rats treated with HFED (Group 2) consecutively for 28 days without treatment exhibited increase ($p<0.001$) in the component of serum SGOT and SGPT level when compared with normal control rats (Group 1). Treatment of rats with *Byrsonima sericea* leaf extract showed significant reduction ($p<0.001$) in SGOT and SGPT level which directly correlated with its hepatoprotective effect (Table 8).

Effect of *Byrsonima sericea* leaf extract on body weight: On starting period there was slight increase in weight of HFED rats (Group 2) which was continuously raised over the 7 weeks of the study. The extract treated rats showed (Group 3, 4 and 5) less reduction in weight gain of rats over the period of study (Table 9).

Effect of *Byrsonima sericea* leaf extract on organ body weight: On the last day, all animals were dissected and their organs were isolated and weighted. The inducer

Table 5: Thin layer chromatography of extract

Solvent system	Visualization technique	R_f value	Compounds
Butanol: Acetic acid: Water	UV chamber	0.54	Scopolin
Butanol: Ethanol: Water	Iodine tank	0.46	Caffeoylquinic acid
Ethyl acetate: Chloroform	Iodine tank	0.86	Vanillic acid

Table 6: Effect of *Byrsonima sericea* leaf extract on serum lipid parameter

Groups	TC (mg dL^{-1})	TG (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})	VLDL (mg dL^{-1})
Control (0.9% NaCl)	165.14±2.25	112.27±1.52	42.58±1.58	66.10±1.89	32.85±1.52
Inducer ((HFED)+CCl_4)	232.42±1.15^{a***}	161.85±1.92^{a***}	21.52±1.29^{a***}	91.15±1.58^{a***}	58.37±1.18^{a***}
BSLD (75 mg kg^{-1}, p.o)	184.30±1.45^{a*b**}	149.25±1.21^{a**b**}	25.25±1.54^{a**bns}	87.98±2.27^{a***bns}	51.09±1.178^{a*b*}
BSMD (150 mg kg^{-1}, p.o)	171.05±1.91^{a**b**}	135.50±1.76^{a**b**}	27.42±0.27^{a***b*}	68.40±1.64a^{*b**}	41.50±1.153^{a**b**}
BSHD (300 mg kg^{-1}, p.o)	152.35±1.58^{a***b***}	121.84±2.53^{a**b***}	34.17±0.36^{a**b**}	58.57±2.09^{a***b***}	33.96±0.3^{a***b***}

Data are Mean±SEM values, n = 6, data were analyzed by one-way ANOVA followed by Tukey Kramer Multiple comparisons test *$p<0.005$, **$p<0.01$, ***$p<0.001$, ns: $p>0.05$, [a]When compared with normal control group, [b]When compared with inducer group, BSLD: *Byrsonima sericea* low dose, BSMD: *Byrsonima sericea* medium dose, BSHD: *Byrsonima sericea* high dose

Table 7: Effect of *Byrsonima sericea* leaf extract on other biochemical parameters

Groups	ALP (U L^{-1})	Blood glucose (mmol L^{-1})	Insulin (µlU mL^{-1})	Total bilirubin (mg dL^{-1})	Direct bilirubin (mg dL^{-1})
Control (0.9% NaCl)	89.56±7.25	5.27±1.52	22.58±1.28	0.86±0.89	0.55±0.52
Inducer ((HFED)+CCl_4)	450.42±8.15^{a***}	8.85±1.92^{a***}	34.12±2.29^{a***}	9.21±1.58^{a***}	6.37±1.08^{a***}
BSLD (75 mg kg^{-1}, p.o)	284.30±8.45^{a*b**}	7.5.25±3.12^{a*b**}	32.87±2.54^{a**bns}	8.98±1.07^{a***bns}	5.09±1.11^{a*b*}
BSMD (150 mg kg^{-1}, p.o)	171.05±7.91^{a***b**}	5.50±1.45^{a***b**}	26.52±2.27^{a***b*}	4.40±0.64a^{*b**}	2.50±0.15^{a**b**}
BSHD (300 mg kg^{-1}, p.o)	98.35±5.58^{a***b***}	4.84±2.53^{a**b***}	21.17±1.66^{a**b**}	2.57±0.95^{a**b***}	1.05±0.3^{a***b***}

Data are Mean±SEM values (n = 6), data were analyzed by one-way ANOVA followed by Tukey Kramer Multiple comparisons test *p<0.005, **p<0.01, ***p<0.001, ns: p>0.05, [a]When compared with normal control group, [b]When compared with inducer group, BSLD: *Byrsonima sericea* low dose, BSMD: *Byrsonima sericea* medium dose, BSHD: *Byrsonima sericea* high dose

Table 8: Effect of *Byrsonima sericea* leaf extract on liver enzymes

Groups	SGPT	SGOT
Control (0.9% NaCl)	54.33±0.51	119.23±1.52
Inducer ((HFED)+CCl_4)	92.47±1.45^{a***}	191.21±0.52^{a***}
BSLD (75 mg kg^{-1})	88.48±1.25^{a*b**}	174.21±2.35^{a*b**}
BSMD (150 mg kg^{-1})	68.90±0.99^{a**b*}	149.08±1.68^{a*b**}
BSHD (300 mg kg^{-1})	56.95±0.82^{ansb***}	139.84±2.29^{ansb***}

Data are Mean±SEM values, n = 6, data were analyzed by one-way ANOVA followed by Tukey Kramer Multiple comparisons test *p<0.005, **p<0.01, ***p<0.001, ns: p>0.05, [a]When compared with normal control group, [b]When compared with inducer group, BSLD: *Byrsonima sericea* low dose, BSMD: *Byrsonima sericea* medium dose, BSHD: *Byrsonima sericea* high dose

Table 9: Effect of *Byrsonima sericea* plant extract on body weight

Weeks	Control (0.9% NaCl)	Inducer ((HFED)+CCl_4)	BSLD (75 mg kg^{-1})	BSMD (150 mg kg^{-1})	BSHD (300 mg kg^{-1})
WK-00	220.09±0.07	220.55±0.20	220.76±0.11	220.76±0.14	220.54±0.12
WK-01	220.77±0.20	224.51±0.49	222.84±0.21	223.11±0.19	223.51±0.24
WK-02	220.93±0.16	227.60±0.30	225.42±0.52	225.72±0.51	225.78±0.54
WK-03	221.11±0.18	230.68±0.30	227.94±0.52	229.34±0.53	229.15±0.64
WK-04	221.39±0.19	233.76±0.68	231.03±0.43	230.71±0.27	230.99±0.78
WK-05	221.59±0.18	235.12±0.71	228.87±0.28	229.05±0.35	228.53±0.58
WK-06	221.87±0.15	237.78±0.50	227.28±0.21	227.24±0.20	226.90±0.64
WK-07	220.11±0.30	239.34±0.39	225.94±0.27	225.26±0.36	224.85±0.54

All values were expressed as Mean±SEM, (n = 6), BSLD: *Byrsonima sericea* low dose, BSMD: *Byrsonima sericea* medium dose, BSHD: *Byrsonima sericea* high dose

Table 10: Effect of *Byrsonima sericea* plant extract on organ weight

Organs	Control (0.9% NaCl)	Inducer ((HFED)+CCl_4)	BSLD (75 mg kg^{-1})	BSMD (150 mg kg^{-1})	BSHD (300 mg kg^{-1})
Liver (g)	2.257±0.41	5.75.26±0.12^{a***}	3.14±1.15^{a*b*}	2.63±0.64^{a**b*}	1.18±0.25^{a***b***}
Right kidney (g)	1.14±0.21	1.93±0.25^{a***}	1.41±0.64^{a*b*}	1.31±0.44^{a*b**}	1.12±0.23^{a***b***}
Left kidney (g)	1.15±0.28	1.95±0.36^{a***}	1.54±0.67^{a*b*}	1.34±0.78^{a**b**}	1.23±0.74^{a***b***}
Mesenteric fat (g)	0.23±0.12	2.45±0.45^{a***}	1.45±0.24^{a*b*}	1.35±0.73^{a*b**}	1.14±0.12^{a***b***}

Data are Mean±SEM values (n = 6), data were analyzed by one-way ANOVA followed by Tukey Kramer Multiple comparisons test *p<0.005, **p<0.01, ***p<0.001, ns: p>0.05, [a]When compared with normal control group, [b]When compared with inducer group, BSLD: *Byrsonima sericea* low dose, BSMD: *Byrsonima sericea* medium dose, BSHD: *Byrsonima sericea* high dose

group 2 showed significantly higher organ weight (Liver, kidney and mesenteric fat) in comparison to other groups. *Byrsonima sericea* extract treated rats were not showed hyper trophy in isolated organs when compared with HFED rats (Table 10).

Effect of *Byrsonima sericea* leaf extract on liver histology: The histopathological results acquired from the present investigation in comparison of control. Figure 1a demonstrated that HFED causes hepatocellular damage and increased level of hepatocellular enzymes like SGPT and SGOT (Fig. 1b). Histopathological investigations of *Byrsonima sericea* treated rats showed less hepatic damage and improved vitality of cells (Fig. 1c-e).

DISCUSSION

High fat emulsion diet showed similar pathology to human NAFLD, including; steatosis, hepatic inflammation and fibrous tissue development. High fat eating regimen for about 4 months likewise created hyperglycemia and hyperinsulinemia, which impersonates human metabolic disorder process. Hepatic steatosis indicated metabolic disorder issue and is joined by insulin resistance and raised triglycerides[19,20].

In present study, the rats administered with BSE for 4 weeks showed enhanced liver enzymes, insulin obstruction and lipids level content by means of aversion of hepatic inflammation and preventing

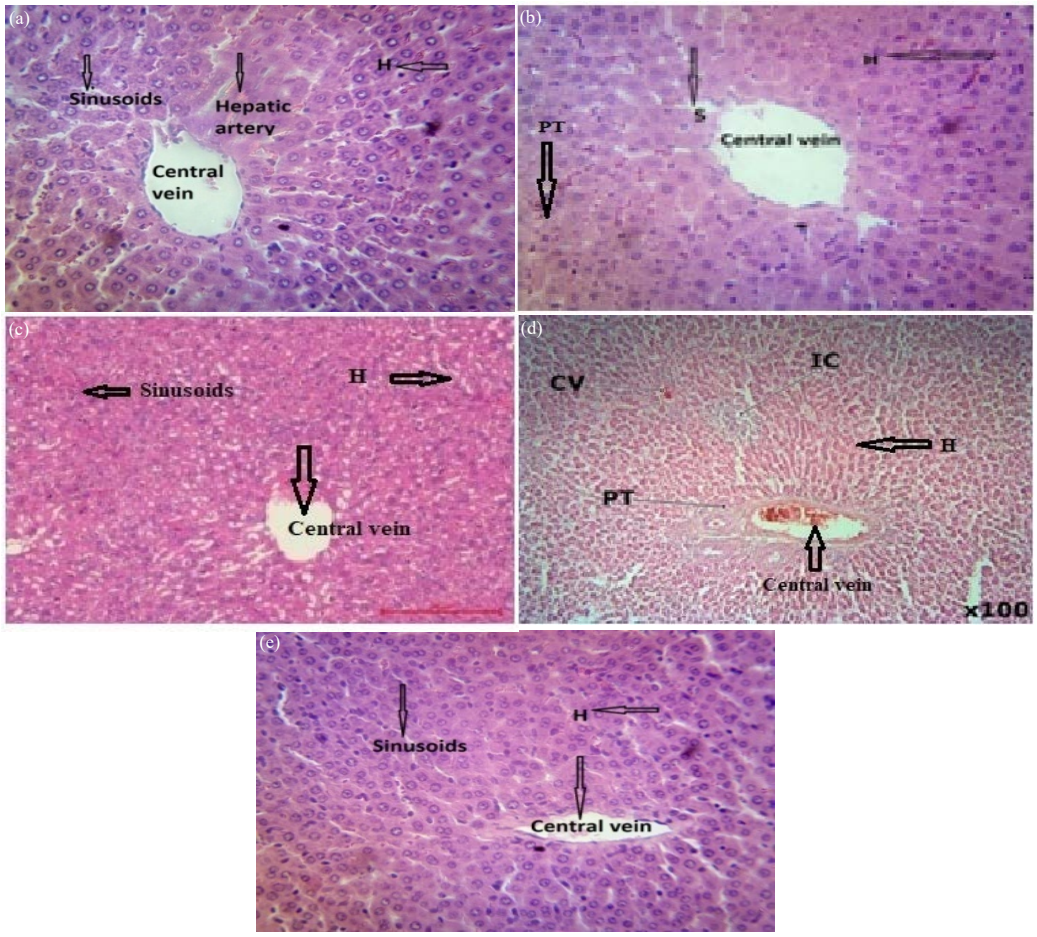

Fig. 1(a-e): Histopathology of liver, (a) Control, (b) Inducer, (c) *Byrsonima sericea* (75 mg kg^{-1}), (d) *Byrsonima sericea* (150 mg kg^{-1}) and (e) *Byrsonima sericea* (300 mg kg^{-1})

insulin resistance. This information is as per a work of Ayala *et al.*[21] who found similar observation.

NAFLD, obesity and the related insulin resistance are associated with an expanded accumulation of lipids (triglycerides) in the liver. Increased quantities of lipid peroxidation markers have been seen in the liver, in animal[22-23] models of diabetes and obesity. The study showed normal content of lipids in control group which was found higher in HFED treated rats. The content of different enzymes like SGOT and SGPT were also changed and observed as per the previous studies published[22]. A connection between increased LDL and low HDL and oxidative stress in animal models is entrenched. The LDL receptor-inadequate mice fed cholesterol-improved eating routine results, in lifted LDL levels and thusly oxidative stress[24].

In the present investigation, the results exhibited that biochemical parameters including serum ALP, cholesterol, triglycerides, LDL, in addition, fasting insulin and fasting blood glucose were essentially higher in NAFLD[25] rats.

Many herbals are now used in NAFLD, because of important phyto-constituents present and isolated for its therapeutic treatment. Number of natural extracts had been utilized in traditional system and experimentally investigated for their evaluation in treatment of liver disorders, like Pro-liver Pill (Yang Gan Wan), Liver Care (Himalaya Drug Co., Bangalore, India), Liv-52, Jianpi Wenshen Pill (Jianpi Wenshen Wan), Binggan capsules (Binggan Jiaonang), Binggan Tang, Yizhu decoction (Yizhu Koufuye), Yiergan Tang and Xiaochaihu Tang (Sho-saiko-to or SST)[10]. This study also showed a significant effect in treatment of NAFLD, because of constituents like scopoline, quinic acid and vanillin which were also proved as hepato-protecting in earlier studies by De Moraes Lira *et al.*[11].

Oral administration of *Byrsonima sericea* leaf extract has decreased food intake and chances of higher weight gain, fat rate and total fat in a dose dependent way. So, liver disease is better connected with increased oxidative pressure and prompts other degenerative ailments, for

example, diabetes, hypertension and hyperlipidemic. In a search of plant which was dual activity like protective and scavenging effects to reduce metabolic dysfunction[25,26]. Present study also showed a similar effect on rats by acting as antioxidant and modulation of metabolism which was a main disturbance caused by NAFLD. The HFED increases free unsaturated fat or immersed unsaturated fat as well as prompts metabolic diseases and chronic activation of inflammation[27]. In present examination, it was uncovered that hydroalcoholic extract of *Byrsonima sericea* is one such therapeutic plant which was professed to have therapeutic action against NAFLD.

The histopathological studies uncovered that NAFLD induced by high fat diet and deposition of unsaturated of lipids are related with inflammation, congestion and non-alcoholic fatty liver disease leading to hepatic failure causing increase level of SGOT and SGPT enzyme level in the serum[26-29]. The HFED induced obesity increase the enzymes of liver and monitor of hepatocellular damage which is correlate with increased liver weight[30,31]. This study also supported the similar observation in NAFLD induced rats and BSE found protective against HFED treated rats. Although, more studies are needed to support this promising mechanism, this research might provide implication in human patients. The compounds with potential alpha glucosidase inhibition activity were proven as to change the gut environment for carbohydrates absorption. This effect was reported as protective against steatohepatitis. The BSE also showed alpha-glucosidase inhibition which additional effect was caused by it in NAFLD. The after effects of starter phytochemical screening of the concentrate affirmed the nearness of different classes of optional metabolites in the *Byrsonima sericea* extract. This study also supported the similar observation by TLC method which distinguished numerous auxiliary metabolites present in plant like Scopolin, vanillic acid, caffeoylquinic acid.

CONCLUSION

The present study concluded that *Byrsonima sericea* leaf extract displayed the hepatoprotective action against the HFED induced weight. The histopathological investigations of the kidney, liver, heart and mesenteric fat likewise upheld that *Byrsonima sericea* lessened the poisonous quality of HFED. Study outcomes recommended that *Byrsonima sericea* may be valuable to keep the improvement of weight. Taking everything into account, the present discoveries proposed that *Byrsonima sericea* leaf extract showed the hepato-protection against the HFED induced NAFLD. Present results suggested that *Byrsonima sericea* might be important to keep the better functioning of liver.

SIGNIFICANCE STATEMENT

This study discovered the mechanism of *Byrsonima sericea* extract in HFED induced NAFLD and balances out the remedial role of it in metabolic disorders that can be advantageous for other researcher to investigate the different constituent's responsible for hepato-protective activity on targeted receptors in liver disorders. This investigation will assist the researchers to uncover the conceivable reason for remedial impact of *Byrsonima sericea* extract and help others scientist to plan the better restorative regimen in metabolic disease.

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