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HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF *ABROMA AUGUSTA* AGAINST NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN SD RATS

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Keywords:

Abroma augusta, Hepatoprotective, Antioxidant, NASH, HCC, Sprague-Dawley

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ABSTRACT: Objective: To study the hepatoprotective activity of ethanolic leaves extracts of *Abroma augusta* against non-alcoholic fatty liver disease to validate ethnobotanical claim regarding the plant used in the above mentioned disease. **Materials and Methods:** The hepatoprotective potential of *Abroma augusta* leaves extract (AALE) (250 and 500 mg/kg/ body weight) was studied on Methionine and Choline deficient diet (MCD diet), High Fat Diet (HFD), Cholesterol and Cholate diet (CCD) and streptozotocin + HFD induced non-alcoholic fatty liver disease. In the last treatment, blood was collected from direct cardiac puncture and analyzed for various parameters like alanine aminotransferase (ALT), aspartate transaminase (AST), triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), free fatty acid (FFA) and malondialdehyde (MDA). **Results:** The present study showed for the first time that AALE reduced level of ALT, AST, TG, TC LDL, FFA, MDA and possessed hepatoprotective activity as evidenced by its significant inhibition in the formation of hepatoxicity induced by diet and chemical agents with maximum curation (500mg/kg/b.w.) against non-alcoholic fatty liver disease. It enhances the level of SOD, HDL and also reverses hepatic damage towards normal, which further supports the hepatoprotective activity of leaves extracts of *A. augusta*. **Conclusions:** The ethanolic leaves extract of *Abroma augusta* have a significant effect at a higher dose of 500mg/kg.b.w. These findings could justify, at least partially, the inclusion of this plant in the management of Hepatic disorders in traditional medicine.

INTRODUCTION: *Abroma augusta*, commonly known as Devils's cotton, is mainly used for the treatment of various types of disorder in the traditional system of medicine. *A. augusta* is a plant which is found in all over in India [1-2]. The whole plant contains several alkaloids, including saponins, tannins, flavonoids and amino acid [3]. Abromine, the active constituent of the *A. augusta* identified as betaine is mainly responsible for antihyperglycemic activity [4]. The liver is the most vital organ of the body, which plays a major role in the metabolism of protein, lipids and carbohydrates. NAFLD has evolved into the most common liver disease in industrialized countries [5-6].

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It is estimated that one billion subjects had NAFLD worldwide [7]. The majority of the patient will have a benign evaluation up to 25% develop potentially progressive liver damage non-alcoholic steatohepatitis (NASH). NASH is characterized by liver cell injury, death, inflammation and increased

risk for fibrosis and carcinogenesis [8]. NAFLD and NASH is the third leading indication for liver transplantation in the world and the second cause of hepatocellular carcinoma-related liver transplantation [9]. The term NAFLD encompasses a spectrum of hepatic pathologies ranging from simple steatosis to NASH, which may progress to liver cirrhosis.

NAFLD is a very slowly progressive disease that hinders prospective observational study. NAFLD is also associated with hepatocellular carcinoma (HCC) [10]. HCC is commonly thought of as occurring in the setting of cirrhosis or after decades of chronic hepatitis B infection, and only occasionally in the setting of chronic liver disease that has not yet progressed to cirrhosis. Importantly, a recent survey demonstrated that the preponderance of non-cirrhotic HCC occurs in patients with NAFLD compared to other causes of chronic liver disease [11]. Whereas NAFLD can be diagnosed by imaging studies such as ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI), the presence of NASH still requires a liver biopsy to identify the presence and location of its features such as inflammation, hepatocyte ballooning, Mallory-Denk bodies and early fibrosis [12].

Data from animal studies support the concept that the hepatocellular injury that characterizes NASH is driven by an overload of primary metabolic substrates (glucose, fructose, and fatty acids) in the liver resulting in the diversion of fatty acids into pathways that promote cellular injury and dysfunctional response to that injury [13-18]. In this study, we performed hepatoprotective and antioxidant activity of Abroma augusta against non-alcoholic fatty liver disease.

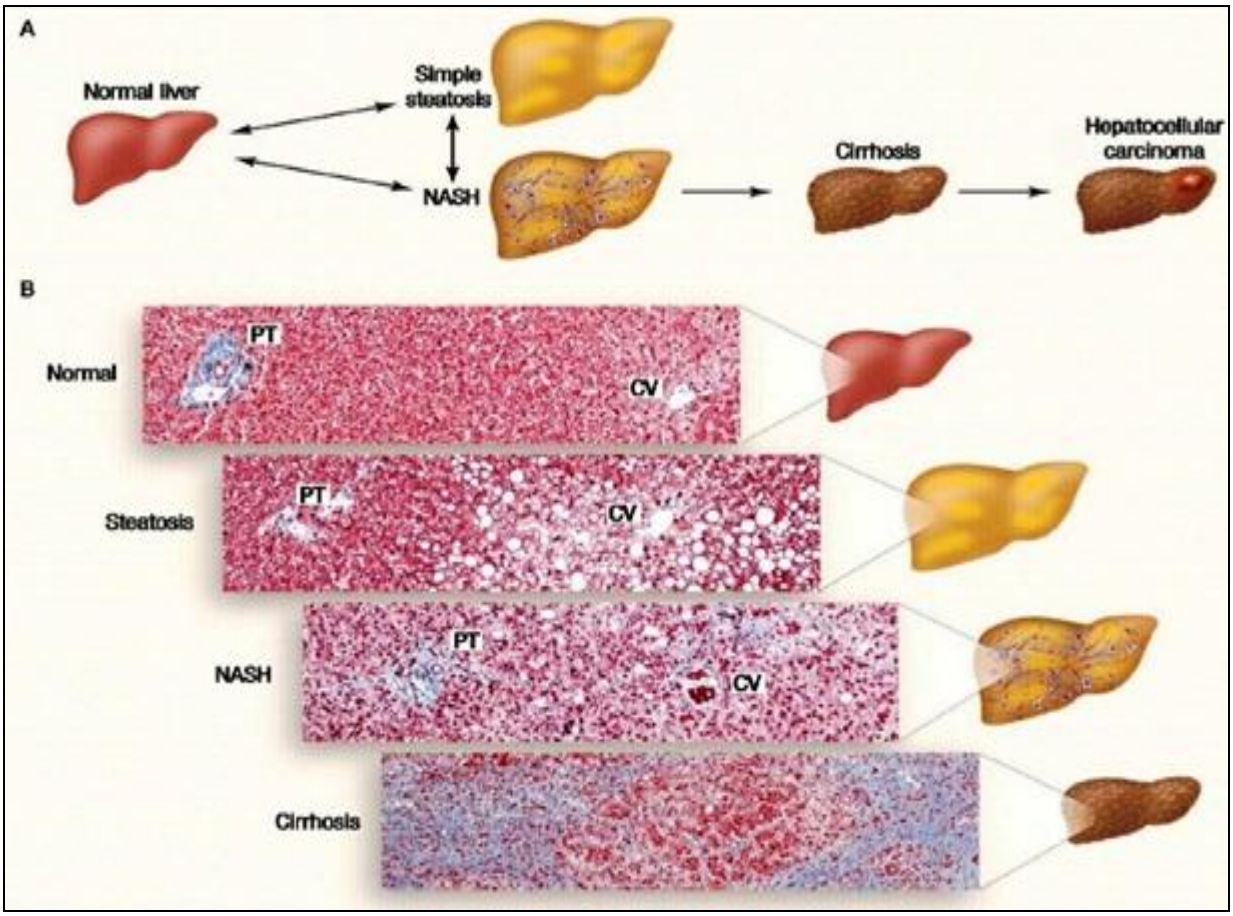

FIG. 1: PROGRESSION OF LIVER DAMAGE IN DIABETES MELLITUS, INSULIN RESISTANCE, AND HYPERINSULINEMIA CAUSE NON-ALCOHOLIC FATTY LIVER DISEASE AND PROGRESS TO NON-ALCOHOLIC STEATOHEPATITIS, WHICH MANIFESTS AS INFLAMMATION AND NECROSIS. PROLONGED NASH WILL LEAD TO LIVER FIBROSIS, KNOWN AS CIRRHOSIS, AND FINALLY HEPATOCELLULAR CARCINOMAS AND END-STAGE LIVER DISEASE. NASH- NON-ALCOHOLIC STEATOHEPATITIS; PT- PORTAL TRIAD; CV- CENTRAL VEIN [19]

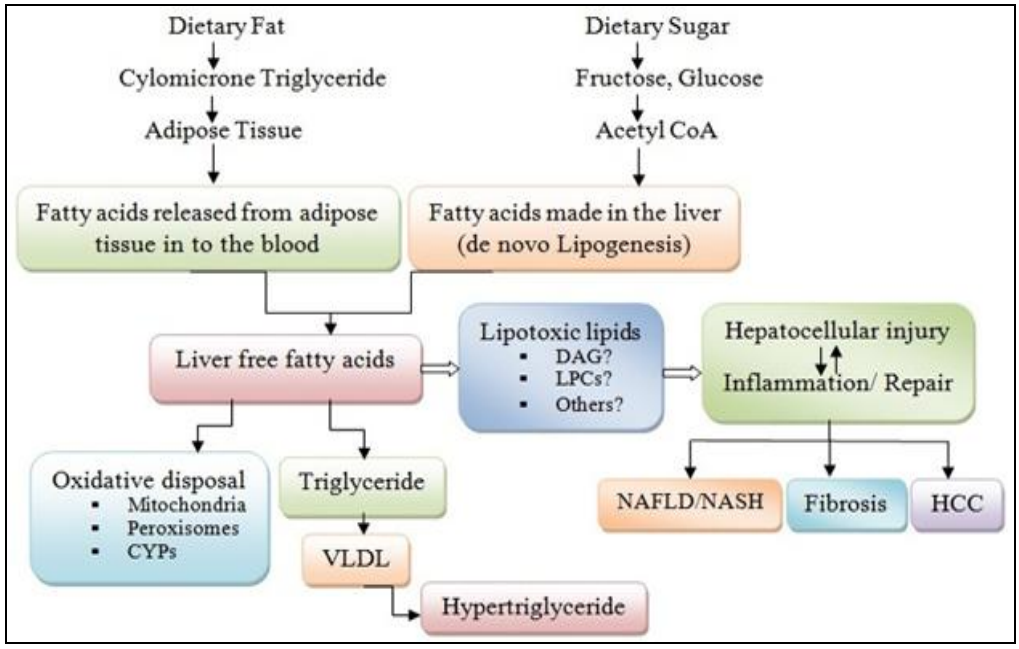

FIG. 2: SUBSTRATE OVERLOAD LIPOTOXIC LIVER INJURY NAFLD/NASH

MATERIALS AND METHODS:

Plant Material: The leaves of *Abroma augusta* were collected from Kundari Rakabganj Lucknow, India. The herbarium sheet was prepared, and it was authenticated by Dr. Navin K. Ambasht, Head and Associate Professor, Botany Department, Christ Church College, Kanpur, and voucher specimens were deposited in the departmental herbarium of Botany, Christ Church College, Kanpur, India, for future reference. The dried leaves materials were mechanically powdered, sheaved using 80 meshes, and stored in an air-tight container.

Preparation of Extract: The dried crushed leaves 2.5kg powder material was soaked in 50% ethanol for 72 hours at room temperature. The ethanol extract was filtered and concentrated under pressure in a rotary evaporator at 60 °C and then evaporated to dryness by vacuum evaporation under low pressure. The dried extract gave a yield of 22.15% (w/w) and was stored in an air-tight container at about 20 °C until used.

Animals: Healthy male and female Sprague - Dawley (SD) of aged 5-8 weeks (140 ± 15gm) was purchased from the local market and resided in a polyethylene cage in a room of 23 ± 2 ^{o}C with a 12 hr light and 12 h dark cycle, were acclimatized for one week at normal diet in the institute animal house of PSIT, Institute of Pharmacy. All experiments involving animals comply with the ethical standards of animal handling and are approved by the institutional animal ethical and welfare committee of the Institute of Pharmacy, PSIT (1273/AC/09/ CPCSEA). The animals were randomly divided into the following five groups (n=6) for each experimental model.

Experimental Design: In the experiment, the animals were divided into five groups (n = 6), group I - Served as normal control and received only as of the vehicle (1 mL/kg/day of 1% CMC; p.o.), Group II – served as negative control and not provide any treatment, Group III received standard 100mg/kg body weight and Groups IV and V received AALE in a dose of 250 and 500 mg/kg body weight. These were administered orally twice daily at 10:00 and 16:00 hr respectively, for 8-24 weeks, as per experimental models for acute hepatoprotective activity against non-alcoholic fatty liver disease.

Methionine and Choline Deficient Diet (MCD Diet): The male and female Sprague - Dawley rats were used as 5 weak of age. For the elicitation of NAFLD in mice. Methionine - choline-deficient diet model has a high sucrose content and moderate fat content (40% sucrose and 10% fat) but is deficient in methionine and choline, which are essential nutrients in hepatic β-oxidation and the production of very-low-density lipoproteins (VLDL). All the diet was γ- irradiated, which made the diet safer by reducing the number of harmful

bacteria and parasites. Choline deficiency leads to an impaired hepatic very-low-density lipoprotein (VLDL) secretion, resulting in hepatic lipid deposition, oxidative stress and change in cytokines and adipokines, culminating to a liver injury [20]. Increased serum ALT level and steatohepatitis occurred at day 10 in MCD diet fed diet rats and perisinusoidal fibrosis developed after 8-10 weeks [21]. Development of liver injury was followed by intraperitoneal injection (ip) of 30 mg/kg UDCA-LPE solubilized in 0.5% carboxymethylcellulose (CMC) two times a week for three week on the diet in methionine-choline deficient rats. All the animals had free access to diet and drinking water for the duration of the study. The rats were anesthetized and killed through cervical dislocation at the end of feeding duration. Livers were collected, and a portion of fresh tissue was fixed in 10% buffered formalin.

High Fat Diet (HFD): The high-fat diet is widely used to develop NAFLD animal models. In high-fat animal models, the diet of groups consists of a variety of regimens with fat content varying between 45 – 75% kcal [22]. The animal's total calorie intake is derived from fat and animals are fed ad libitum predominantly. The classic High fat diet used rats fed a diet composed of 71% of calories from fat, 11% from carbohydrate and 18% from protein) [23]. Similar to human NAFLD patients, rats developed IR, as shown by elevated plasma insulin levels, marked pan lobular steatosis, inflammation, and fibrogenesis [24-26]. The rats fed high-fat diet showed a similar result after 16 weeks [27]. Feeding SD rats with the help of gastrostomy tube with high-fat diet up to 86% in excess of standard intake for 14 weeks. Obesity, hepatic steatosis, histopathological features similar to NASH in human, as verified by the presences of increased liver triglyceride levels, hepatocyte ballooning, Mallory bodies, higher fasting serum glucose levels and decreased adiponectin levels suggesting hyperglycemia and IR [28]. Their plasma alanine aminotransferase (ALT) levels showed 9 – 10 fold increases.

Cholesterol and Cholate Diet (CCD): Cholesterol in the diet is an important risk factor for Non-alcoholic steatohepatitis (NASH) because it makes the liver sensitive to tumor necrosis factor- and Fas-induced steatohepatitis [29].

The cholic acid presence promotes the absorption of cholesterol and fat and impedes the conversion of cholesterol to bile acids, hence reducing the removal of cholesterol and increasing the cholesterol levels, particularly low-density lipoprotein cholesterol [30]. The cholesterol and Cholate diet containing 1.25% cholesterol and 0.5% Cholate induces the progressive formation of steatosis, inflammation, and fibrosis over 6–24 weeks: steatosis and inflammation (after 6 weeks), hepatocellular ballooning, and fibrosis (after 24 weeks) [31].

Streptozotocin + High Fat Diet: Low dose of streptozotocin (STZ) intraperitoneal administration in newborn Sprague - Dawley (SD) rats lead to a chemical inflammation and destruction of the pancreatic islets, thus inducing diabetes [32]. This model combined with high-fat diet can establish a model of NAFLD.

In this model, newborn two days after birth Sprague - Dawley (SD) rats were given *streptozotocin* (200μg) and then surviving Sprague - Dawley (SD) rats were started the high-fat diet at four weeks old. These rats developed simple steatosis at six weeks, NASH with inflammatory foci and ballooning at eight weeks, progressive pericellular fibrosis at twelve weeks, and multiple HCC at twenty weeks of age [33]. The transaminase and fasting glycemia level is elevated at six weeks of age. This model recapitulates several important histological features of human NAFLD and is also relevant to oxidative stress. But streptozotocin recreating beta (β) cell a function rather than a systemic inflammatory insulin-resistant milieu, this is different from the human state [27]. However, in a similar model where rats were given streptozotocin followed by a high-fat diet, an investigator failed to demonstrate concordance between rats and humans regarding differentially expressed gene [34].

Evaluation of Serum Biochemical Variables: The serum was separated by centrifugation at 3000 rpm for 10 min at 4 ^{o}C from a collected blood sample and stored at -22 ^{o}C for further biochemical analysis. The alanine aminotransferase (ALT), aspartate transaminase (AST), triglycerides (TG), total cholesterol (TC), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) was analyzed by using a commercial kit according to

the manufacturer instruction and using the multifunctional analyzer (AU600, Olympus). At 505 nm the absorbance of alanine aminotransferase (ALT) and aspartate transaminase (AST) were read, and the enzyme activity was calculated as U/L. Triglyceride (TG) and total cholesterol (TC) absorbance were read at 510 nm, and the content was calculated as mmol/L.

MDA Formation Measurement in Lipid Peroxidation: The liver homogenate (10% w/v) were produced by homogenizing the liver tissue in 150 mmol/L tris – HCL buffered saline at (pH 7.2) with polytron homogenizer.

At 532 nm the level of MDA in the liver tissue was measured with the help of a spectrophotometer (U-2001 Hitachi Ltd). The data are expressed as nmol/mg protein of liver tissue.

Evaluation of Liver LDL/HDL and FFA Activity: For the evaluation of liver tissue, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and free fatty acid (FFA) had used the commercial kit and follow the instructions of protocols provided by the manufacturer. The absorbance of LDL, HDL, and FFA reactions were read at 546nm, and the data was expressed as mmol/L.

RESULTS:

Acute Toxicity test: The 50% aqueous alcoholic leaves extract of *Abroma augusta AALE* does not show any sign and symptom of toxicity up to 2000 mg/kg body weight, and hence it was considered to be safe and effective.

Effect of *Abroma augusta* on Body Weight and Liver Coefficient: After being fed with Methionine and Choline deficient diet, High Fat Diet, Cholesterol and Cholate diet, and streptozotocin + HFD, the bodyweight of rats in the model groups was notably increased compared to that of rats in the control group $P < 0.01$, **Fig. 3A**. Meantime after AALE treatment for six (6) weeks, the gain in the bodyweight for the rats in the 250 and 500 mg/kg b.w., AALE treated groups was lower than for the rats in the standard and model group $P < 0.01$, **Fig. 3A**, which indicates AALE treatment could inhibit the occurrence of obesity in Methionine and Choline deficient diet, High Fat Diet, Cholesterol and Cholate diet and streptozotocin + HFD administrated rats. Furthermore, consistent with these modifications, the liver coefficient was also reduced markedly in the AALE treated rats $P < 0.05$, $P < 0.01$, **Fig. 3B**, compared to the control group.

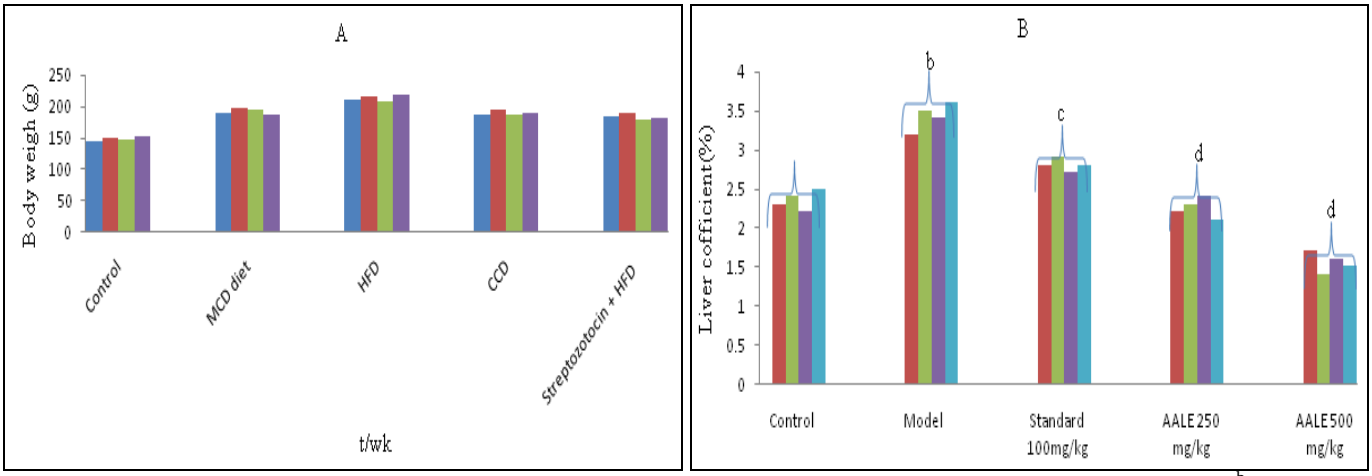

FIG. 3: EFFECT OF *ABROMA AUGUSTA* ON BODY WEIGHT (A) AND LIVER COEFFICIENT (B). $^{b}P < 0.01$ *vs* control group; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* model group.

Effect of *Abroma augusta* on Serum ALT and AST Levels: Serum level of ALT and AST indirectly reflects the failure of liver function; as shown in **Table 1**, serum ALT and AST activates were significantly 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 ALT and AST was significantly decreased in a dose-dependent manner after *AALE* treatment of 250 and 500mg/kg b.w, ($P < 0.05$, $P < 0.01$, **Table 1.**

Effects of *Abroma augusta* on Blood Lipid Levels: Methionine choline-deficient diet, High fat diet, Cholate and choline diet, and Strepto-zotocin+High fat diet-induced NAFLD produced a marked incremental change in triglycerides (TG) and total cholesterol (TC) levels compared with those in the normal group (P<0.01, **Table 1**, which indicates the successful establishment of the NAFLD models in the rats. However, after AALE exposure, the concentration of both TG and TC in blood was remarkably decreased in dose-dependent manners, as compared to the NAFLD standard groups (P <0.05, P<0.01, **Table 1**. All of these findings indicate that *AALE* shows lipid-lowering effects against NAFLD.

Effects of *Abroma augusta* on Liver Tissue MDA Levels: MDA 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 feeding significantly enhanced the MDA concentration with Methionine and Choline deficient diet, High Fat Diet, Cholesterol and Cholate diet, and streptozotocin + HFD models compared with the control group P<0.01, **Table 1**. Although the treatment with AALE 250 and 500 mg/kg b.w. Significantly reduce the levels of the MDA level in the live homogenate respectively P<0.01, **Table 1**.

TABLE 1: EFFECTS OF 50% AQUEOUS-ALCOHOLIC EXTRACT OF *ABROMA AUGUSTA* (*AALE*) 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	17.82±3.72	61.63±7.38	0.63±0.06	0.82±0.07	0.38±0.01
MCD diet	62.35±14.52^{b}	165.18± 36.43^{b}	1.48±0.31^{b}	3.06±0.72^{b}	0.55±0.02^{b}
Silymarin (100mg/kg)	32.91±4.74^{c}	97.82±12.65^{c}	0.70±0.12^{c}	3.21±0.27^{c}	0.43±0.01^{c}
AALE (250mg/kg)	28.33±3.07^{d}	86.53±8.17^{d}	0.60±0.7^{d}	2.12±0.16^{d}	0.24±0.01^{d}
AALE (500mg/kg)	22.62±4.42^{d}	74.43±7.68^{d}	0.42±0.4^{d}	1.73±0.10^{d}	0.12±0.01^{d}
High Fat Diet (HFD)					
Control	16.73±4.74	63.53±6.28	0.65±0.07	0.85±0.08	0.36±0.01
HFD	66.24±12.53^{b}	170.17± 46.42b	1.45±0.32b	3.07±0.71b	0.53±0.02b
Silymarin (100mg/kg)	20.53±3.22^{c}	72.42±8.58c	0.73±0.5c	1.63±0.12c	0.44±0.01c
AALE (250mg/kg)	37.62±5.73^{d}	98.72±13.65d	0.52±0.13d	4.22±0.26d	0.22±0.01d
AALE (500mg/kg)	26.42±5.08^{d}	87.63±9.17d	0.40±0.6d	2.14±0.14d	0.11±0.01d
Cholesterol and Cholate diet (CCD)					
Control	19.76±4.72	63.63±8.37	0.62±0.07	0.81±0.07	0.39±0.01
CCD diet	66.34±15.52^{b}	155.17± 35.42^{b}	1.47±0.32^{b}	3.05±0.73^{b}	0.55±0.02^{b}
Silymarin (100mg/kg)	34.81±4.73^{c}	98.72±12.64^{c}	0.67±0.13^{c}	4.22±0.26^{c}	0.45±0.01^{c}
AALE (250mg/kg)	29.35±3.06^{d}	84.43±8.17^{d}	0.50±0.6^{d}	2.14±0.16^{d}	0.26±0.01^{d}
AALE (500mg/kg)	23.52±5.42^{d}	73.33±7.68^{d}	0.41±0.5^{d}	1.76±0.12^{d}	0.14±0.01^{d}
Streptozotocin + HFD					
Control	17.72±3.62	60.64±6.28	0.63±0.04	0.81±0.06	0.36±0.01
Streptozotocin+ HFD	61.35±13.55^{b}	162.18± 33.53^{b}	1.38±0.21^{b}	3.04±0.74^{b}	0.52±0.02^{b}
Silymarin (100mg/kg)	30.91±3.84^{c}	95.92±13.65^{c}	0.72±0.11^{c}	2.41±0.24^{c}	0.40±0.01^{c}
AALE (250mg/kg)	26.33±2.07^{d}	88.53±9.17^{d}	0.56±0.7^{d}	2.00±0.12^{d}	0.22±0.01^{d}
AALE (500mg/kg)	20.66±3.52^{d}	78.43±9.68^{d}	0.43±0.4^{d}	1.83±0.15^{d}	0.10±0.01^{d}

Data are expressed as mean ± SD (n = 6) for each group, ^{b}P < 0.01 vs control group ^{c}P < 0.05, ^{c}P < 0.05, ^{d}P < 0.01 vs model group.

Effects of AALE on LDL, HDL, SOD, and FFA Levels in the Liver Tissue: The volume of lipid products were significantly increased after Methionine choline-deficient diet, High fat diet, Cholate and choline diet and Streptozotocin+HFD feeding in the model group as compared to the control group P<0.01, **Table 2**. Its results show that the LDL was significantly increased in the model groups compared to the normal group P<0.01, Table 2 and dramatically decreased in the AALE treated groups compared with the standard groups P <0.05, P<0.01, **Table 2**. In contrast, the volume of HDL was significantly decreased at the end of the experiments. The AALE treatment significantly improved the HDL volume compared with that in the standard groups P <0.05, P<0.01, **Table 2**.

Although the treatment with 250 and 500 mg/kg b.w. of AALE significantly increased 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 (FFA) was notably increased after Methionine choline-deficient diet, High fat diet, Cholate, and choline diet and Streptozotocin+HFD administration and treatment with AALE significantly decreased the content of free fatty acid (FFA) in a dose-dependent manner P <0.05, P<0.01, **Table 2**.

TABLE 2: EFFECTS OF 50% AQUEOUS-ALCOHOLIC EXTRACT OF *ABROMA AUGUSTA* (AALE) ON LOW-DENSITY LIPOPROTEIN (LDL), HIGH-DENSITY LIPOPROTEIN (HDL), SUPEROXIDE DISMUTASE (SOD) AND FREE FATTY ACID (FFA) LEVEL IN THE LIVER TISSUE

Methionine and Choline deficient diet (MCD)				
Treatment	**LDL mmol/L**	**HDL mmol/L**	**SOD (U/mgprot)**	**FFA mmol/L**
Control	0.33±0.08	0.97±0.10	132.52±17.28	0.83±0.13
MCD diet	2.44±0.13[b]	0.54±0.02[b]	78.72±7.53[b]	2.06±0.15[b]
Silymarin (100mg/kg)	1.36±0.10[c]	0.72±0.04[c]	92.90±8.64[c]	1.72±0.12[c]
AALE (250mg/kg)	0.92±0.05[d]	0.76±0.05[d]	112.52±12.33[d]	1.54±0.10[d]
AALE (500mg/kg)	0.58±0.08[d]	0.82±0.05[d]	128.25±10.68[d]	1.32±0.08[d]
High Fat Diet (HFD)				
Control	0.34±0.07	0.98±0.12	131.52±16.28	0.82±0.14
HFD	2.48±0.12[b]	0.55±0.03[b]	77.72±7.53[b]	2.03±0.16[b]
Silymarin (100mg/kg)	1.32±0.11[c]	0.70±0.05[c]	90.92±9.64[c]	1.73±0.14[c]
AALE (250mg/kg)	0.95±0.08[d]	0.80±0.08[d]	109.52±11.33[d]	1.56±0.12[d]
AALE (500mg/kg)	0.62±0.06[d]	0.87±0.04[d]	121.25±10.68[d]	1.34±0.04[d]
Cholesterol and Cholate diet (CCD)				
Control	0.36±0.08	0.96±0.12	133.41±18.38	0.81±0.12
CCD diet	2.52±0.13[b]	0.53±0.02[b]	78.62±8.52[b]	2.05±0.14[b]
Silymarin (100mg/kg)	1.38±0.10[c]	0.74±0.04[c]	94.82±7.63[c]	1.74±0.13[c]
AALE (250mg/kg)	0.97±0.05[d]	0.85±0.05[d]	110.42±13.32[d]	1.51±0.11[d]
AALE (500mg/kg)	0.60±0.08[d]	0.92±0.05[d]	128.35±11.67[d]	1.30±0.07[d]
Streptozotocin + HFD				
Control	0.37±0.07	0.94±0.10	138.52±15.28	0.82±0.13
Streptozotocin+ HFD	2.40±0.13[b]	0.55±0.02[b]	79.72±7.53[b]	2.07±0.15[b]
Silymarin (100mg/kg)	1.35±0.10[c]	0.73±0.04[c]	93.92±7.64[c]	1.75±0.10[c]
AALE (250mg/kg)	0.94±0.05[d]	0.84±0.05[d]	113.52±12.33[d]	1.52±0.12[d]
AALE (500mg/kg)	0.63±0.08[d]	0.90±0.05[d]	125.25±14.68[d]	1.33±0.07[d]

Data are expressed 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: MAIN FEATURES OF COMMONLY USED SD-RATS MODELS OF NON-ALCOHOLIC FATTY LIVER DISEASES (NAFLD)

S. no.	Model	Obesity	IR	Steatosis	NASH	Fibrosis	Ballooning	Carcinoma
1	MCD diet	Weight Loss	Hepatic IR Only	Yes	Yes	Yes	No	No
2	HFD	Yes	Yes	Yes	Yes	Yes (Slight)	No	No
3	CCD	Weight Loss	Hepatic IR Only	Yes	Yes	Yes	Yes	No
4	(STZ+HFD)	Yes	Yes	Yes	Yes	Yes	Yes	Yes

NAFLD - Non-alcoholic fatty liver disease; MCD diet - Methionine and choline-deficient diet; HFD - High-fat diet; CCD - Cholesterol and cholate diet; STZ – Streptozotocin; IR - Insulin resistance; NASH – Non-alcoholic steatohepatitis.

Histopathological Changes in the Liver Tissue: The histopathological changes in the liver were observed with naked eyes, the liver of the control groups were deep red, moist, glossy, and resilient **Fig. 4A/C/E/G I** while those of the model groups showed yellow necrotic foci, grey red colour, loss of luster and tumescent **Fig. 4A/C/E/G II**. But in the AALE treated SD-rats, the liver injuries were attenuated dramatically in dose-dependent manner as compared with the standard **Fig. 4A/C/E/G III-V**. In **Fig. 4B/D/F/H HE**- stained sections are shown. With the help of the photomicroscope, the liver section from the normal control groups showed normal lobular architecture; liver cells are with well-preserved cytoplasm and well-defined nucleus **Fig. 4B/D/F/H I.** For the present, the liver sections from the model groups showed full-fat vacuoles in the lobule cells, inflammatory cells infiltration, cell swelling, and lipid degeneration in the central region of the lobules **Fig. 4B/D/F/H II**.

But in the liver section of AALE treated Sprague Dawley - rats, inflammatory response and lipid degeneration, were remarkably reduced compared with the standard groups, and the liver cell volume became smaller, the droplet numbers of fat were reduced, and the hepatic lobules were clearly represented **Fig. 4B III-V.**

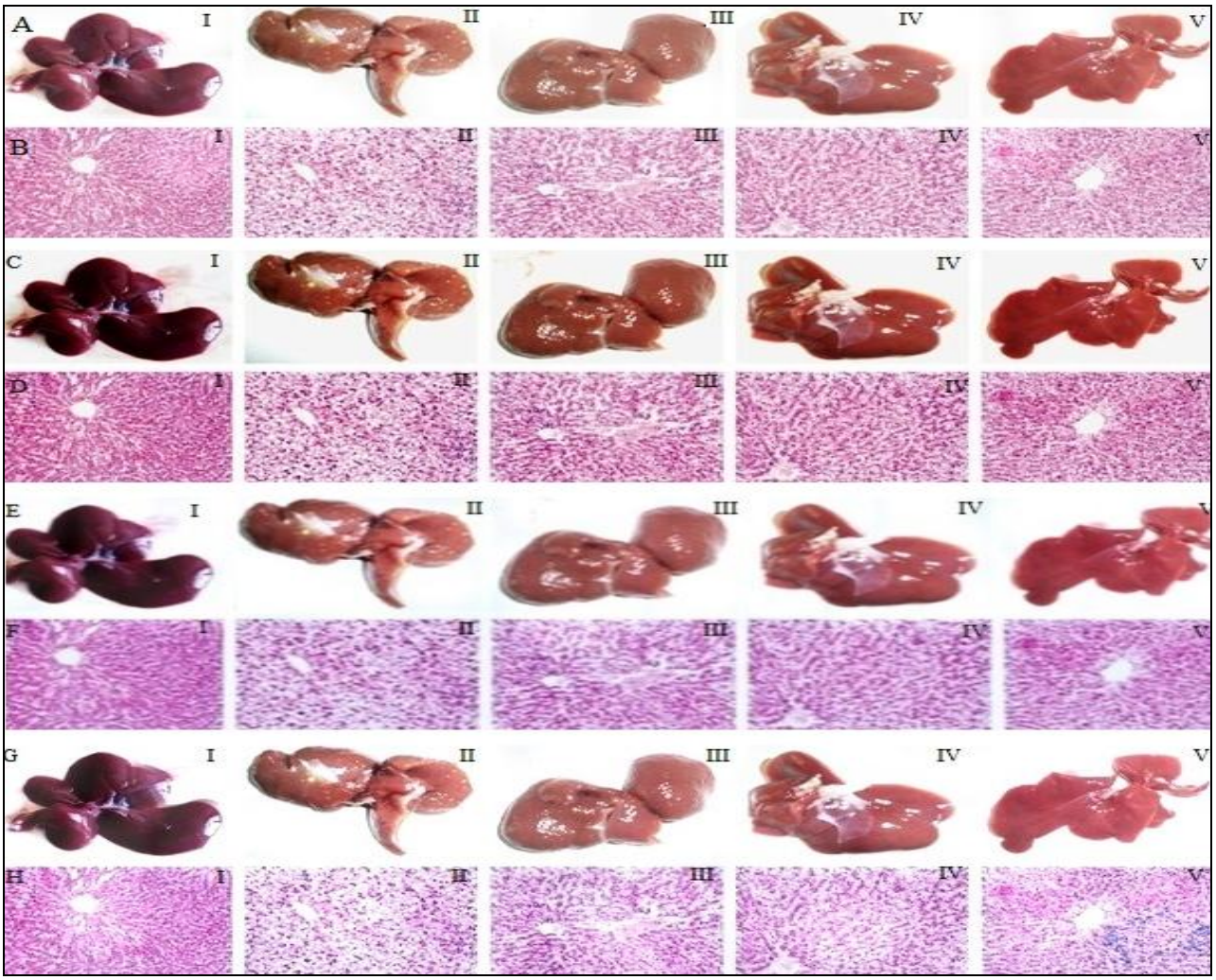

FIG. 4: APPEARANCE OF SD-RATS LIVER TISSUE (A/C/E/G) AND HISTOPATHOLOGICAL EXAMINATION BY HE (B/D/F/H, 200X). I: CONTROL GROUP; II: MODEL GROUP; III: 100MG/KG B.W STANDARD GROUP; IV AND V: AALE 250 AND 500MG/KG B.W GROUP

DISCUSSION: Non-alcoholic fatty liver disease now one of the very serious problems in 21st century. Obesity enhances the risk of non-alcoholic fatty liver disease. The biochemical variations occur mainly in ALT, AST, TG, TC, LDL and MDA *etc*. this entire biochemical changes occur because of altered structure and function of liver and enzyme activity. When the level of (TG) exceeds 5% liver weight and often show a histological spectrum ranging from simple steatosis to NASH. NASH is characterized by hepatocellular damage, fibrogenesis and lobular necro-inflammation [36-37]; this may evolve to hepatic cirrhosis and HCC [38-39]. Although MCD diet, HFD, CCD and streptozotocin + HFD induced non-alcoholic fatty liver disease animals models required a long feeding period and they are more close to humans non-alcoholic fatty liver disease in pathophysiology, including induced obesity, insulin resistance (IR) and hepatic steatosis in Sprague Dawley – rats [40]. Emotional disorder and poor diet with the key point of blood stasis and phlegm is regarded as the etiology of non-alcoholic fatty liver disease, and these etiologies are related to the organs of the liver, spleen, and kidney, according to the traditional medicine theory [41]. Promoting blood circulation to remove meridian obstruction, reduce phlegm, remove dampness, and liver-kidney-

tonifying is an effective approach for treating non-alcoholic fatty liver disease. Hence development and exploring a novel agent to detain or reverse the pathogenesis progression of non-alcoholic fatty liver disease are very important objectives. The AALE 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 natural and herbal medicine market. Lipid metabolism plays an important role in energy dissipation and is responsible for maintaining a steady state in the body [42]. Disruption in the metabolism of lipids may lead to life-threatening situations 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]. When we compared to normal control groups, it was demonstrated that the liver coefficient and the levels of serum ALT, AST, TG and TC were significantly increased, the levels of LDL and FFA in liver was markedly increased and HDL were markedly reduced in the MCD diet, HFD, CCD and Streptozotocin+HFD induced non-alcoholic fatty liver disease. After treatment with AALE showed that *Abroma augusta* is able to inhibit the incremental changes in ALT and AST, to decrease the TG, TC, LDL, and FFA levels, and to increase HDL levels.

In addition, the histopathological changes from the microscopy observation correlated with the examination of the 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 AALE prevents these histopathological changes in the Sprague Dawley - rats with MCD diet, HFD, CCD, and streptozotocin + HFD induced non-alcoholic fatty liver disease. Thus, these results 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 AALE against MCD Diet, CCD, and Streptozotocin + HFD induced non-alcoholic fatty liver disease. AALE decreases the level of MDA against the MCD diet, HFD, CCD, and streptozotocin + HFD induced non-alcoholic fatty liver disease in the Sprague Dawley - rats and produces hepatoprotective activity. AALE enhanced the activity of SOD and decreased MDA rats with MCD diet, HFD, CCD, and Streptozotocin+HFD 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 present study results showed that for the first time, the AALE possessed hepatoprotective activity as evidenced by its significant inhibition in the formation of non-alcoholic fatty liver disease by diet and chemical agents. These findings could justify, at least partially, the inclusion of this plant in the management of the hepatic disorder in ethnomedicine. Since the role of free radicals and antioxidants for showing the hepatoprotective effect is very clearly defined, the potential of *Abroma augusta* 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.

CONCLUSION: In conclusion, the result of the present study showed that *Abroma augusta* has a potent hepatoprotective action upon Methionine and Choline deficient diet (MCD diet), High Fat Diet (HFD), Cholesterol and Cholate diet (CCD), and streptozotocin + HFD induced non-alcoholic fatty liver disease in rats. Further studies relating to the separation of the active compound responsible for this activity and confirmation of its hepatoprotective and mechanism of action are needed for further work. This indicates that the extract may be used as an effective hepatoprotective agent.

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REFERENCES:

1. Nahar N, Hazra BK, Mosihuzzaman M and Rahman MM: Andersson Restructure studies of mucilage from *Abroma augusta* root bark. Carbohydr Polym 2016; 277-280.
2. Gupta N, Ganeshpurkar A, Jatav N, Bansal D and Dubey N: Invitro prevention of chick pancreatic lipase activity by Abroma augusta extract. Asian Pac J Trop Biomed 2017; 2(2): 712-715.
3. Saikot FK, Khan A and Hasan MF: Antimicrobial and cytotoxic activities of *Abroma augusta* Lnn. Leaves extract. Asian Pac J Trop Biomed 2018; 1418-1422.
4. Mir SH, Darzi MM and Mir MS: Efficacy of Abroma augusta on biochemical and histomorphological features of alloxan-induced diabetic rabbits. Iranian J Pathol 2015; 153-158.
5. Angulo P: Non-alcoholic fatty liver disease. N Engl J Med 2002; (16)346: 1221-31.
6. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD and Cohen JC: Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004; 40(6): 1387-95.
7. Loomba R and Sanyal AJ: The global NAFLD epidemic. Nat Rev Gastroenterol Hepatol 2013; 10(11): 686–90.
8. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH and Loomba R: Fibrosis progression in non-alcoholic fatty liver *vs*. non-alcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. Clin Gastroenterol Hepatol 2015; 13(4): 643–54.
9. Wong RJ, Cheung R, Ahmed A Non-alcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. Hepatology.2014- 59 (6):- 2188– 95. doi: 10.1002/hep.26986, PMID 24375711.
10. Reeves HL, Zaki MY and Day CP: Hepatocellular carcinoma in obesity, type 2 diabetes and NAFLD. Dig Dis Sci 2016; 61: 1234–45.
11. Mittal S, El-Serag HB, Sada YH, Kanwal F, Duan Z, Temple S, May SB, Kramer JR, Richardson PA and Davila JA: Hepatocellular carcinoma in the absence of cirrhosis in United States veterans is associated with non-alcoholic fatty liver disease. Clin Gastroenterol Hepatol 2016; 14: 124–31.
12. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016; 64: 1388–402.
13. Neuschwander-Tetri BA: Hepatic lipotoxicity and the pathogenesis of non-alcoholic steatohepatitis: the central role of no triglyceride fatty acid metabolites. Hepatology 2010; 52: 774–88.
14. Cusi K. Role of obesity and lipotoxicity in the development of non-alcoholic steatohepatitis: pathophysiology and clinical implications. Gastroenterology 2012; 142: 711–25.
15. Szabo G and Petrasek J: Inflammasome activation and function in liver disease. Nat Rev Gastroenterol Hepatol 2015; 12: 387–400.
16. Machado MV and Diehl AM: Pathogenesis of non-alcoholic steatohepatitis. Gastroenterology 2016; 150: 1769-77.
17. Hirsova P, Ibrahim SH, Gores GJ and Malhi H: Lipotoxicity lethal and sub lethal stress signaling in hepatocytes: relevance to NASH pathogenesis. J Lipid Res 2016; 57: 1758–70.
18. Ertunc ME and Hotamisligil GS: Lipid signaling and lipotoxicity in metabolic inflammation: indications for metabolic disease pathogenesis and treatment. J Lipid Res 2016; 57: 2099–114.
19. Cohen JC, Horton JD and Hobbs HH: Human fatty liver disease: Old questions and new insights. Science 2011; 332: 1519–23.
20. Van Herck MA, Vonghia L and Francque SM: Animal models of non-alcoholic fatty liver disease-A starter's guide. Nutrients 2017; 9(10): 1072.
21. Takahashi Y, Soejima Y and Fukusato T: Animal models of non-alcoholic fatty liver disease non-alcoholic steatohepatitis. World J Gastroenter 2012; 18(19): 2300-8.
22. Jacobs A, Warda AS, Verbeek J, Cassiman D and Spincemaille P: An overview of mouse models of non-alcoholic steatohepatitis: from past to present. Curr Protoc Mouse Biol 2016; 6(2): 185-200.
23. Lieber CS, Leo MA, Mak KM, Xu Y, Cao Q, Ren C, Ponomarenko A and DeCarli LM: Model of nonalcoholic steatohepatitis. Am J Clin Nutr 2004; 79(3): 502-9.
24. Asgharpour A, Cazanave SC, Pacana T, Seneshaw M, Vincent R, Banini BA, Kumar DP, Daita K, Min HK, Mirshahi F, Bedossa P, Sun X, Hoshida Y, Koduru SV, Contaifer D, Warncke UO, Wijesinghe DS and Sanyal AJ: A diet-induced animal model of non-alcoholic fatty liver disease and hepatocellular cancer. J Hepatol 2016; 65(3): 579-88.
25. Clapper JR, Hendricks MD, Gu G, Wittmer C, Dolman CS, Herich J, Athanacio J, Villescaz C, Ghosh SS, Heilig JS, Lowe C and Roth JD: Diet-induced mouse model of fatty liver disease and non-alcoholic steatohepatitis reflecting clinical disease progression and methods of assessment. Am J Physiol Gastrointest Liver Physiol 2013; 305(7): 483-95.
26. Fan JG and Cao HX: Role of diet and nutritional management in non-alcoholic fatty liver disease. J Gastroenterol Hepatol 2013; 28(4): 81-7.
27. Santhekadur PK, Kumar DP and Sanyal AJ: Preclinical models of non-alcoholic fatty liver disease. J Hepatol 2018; 68(2): 230-7.
28. Eccleston HB, Andringa KK, Betancourt AM, King AL, Mantena SK, Swain TM, Tinsley HN, Nolte RN, Nagy TR, Abrams GA and Bailey SM: Chronic exposure to a high-fat diet induces hepatic steatosis, impairs nitric oxide bioavailability, and modifies the mitochondrial proteome in mice. Antioxid Redox Signal 2011; 15(2): 447-59.
29. Marí M, Caballero F, Colell A, Morales A, Caballeria J, Fernandez A, Enrich C, Fernandez-Checa JC and García-Ruiz C: Mitochondrial free cholesterol loading sensitizes to TNF- and Fas-mediated steatohepatitis. Cell Metab 2006; 4(3): 185-98.
30. Nishina PM, Verstuyft J and Paigen B: Synthetic low and high fat diets for the study of atherosclerosis in the mouse. J Lipid Res 1990; 31(5): 859-69.
31. Matsuzawa N, Takamura T, Kurita S, Misu H, Ota T and Ando H: Lipid-induced oxidative stress causes steatohepatitis in mice fed an atherogenic diet. Hepatology 2007; 46(5): 1392–403.
32. Lo L, McLennan SV, Williams PF, Bonner J, Chowdhury S, McCaughan GW, Gorrell MD, Yue DK and Twigg SM: Diabetes is a progression factor for hepatic fibrosis in a high fat fed mouse obesity model of non-alcoholic steatohepatitis. J Hepatol 2011; 55(2): 435-44.
33. Fujii M, Shibazaki Y, Wakamatsu K, Honda Y, Kawauchi Y, Suzuki K, Arumugam S, Watanabe K, Ichida T, Asakura H and Yoneyama H: A murine model for non-alcoholic steatohepatitis showing evidence of association

between diabetes and hepatocellular carcinoma. Med Mol Morphol 2013; 46(3): 141-52.
34. Sadi G, Baloğlu MC and Pektaş MB: Differential gene expression in liver tissues of streptozotocin-induced diabetic rats in response to resveratrol treatment. PLOS ONE 2015; 10(4): 0124968.
35. Yen FL, Wu TH, Lin LT and Lin CC: Hepatoprotective and antioxidant effects of *Cuscuta chinensis* against acetaminophen-induced Hepatotoxicity in rats. J Ethnopharmacol 2007; 111(1): 123-8.
36. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ and Sanyal AJ: Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for non-alcoholic fatty liver disease. Hepatology 2005; 41(6): 1313-21.
37. Day CP: From fat to inflammation. Gastroenterology. 2006; 130(1): 207-10.
38. Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M and Rizzetto M: Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002; 123(1): 134-40.
39. Liu YL, Patman GL, Leathart JB, Piguet AC, Burt AD, Dufour JF, Day CP, Daly AK, Reeves HL and Anstee QM: Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. J Hepatol 2014; 61(1): 75-81.
40. Carabelli J, Burgueño AL, Rosselli MS, Gianotti TF, Lago NR, Pirola CJ and Sookoian S: High fat diet-induced liver steatosis promotes an increase in liver mitochondrial biogenesis in response to hypoxia. J Cell Mol Med 2011; 15(6): 1329-38.
41. Dong H, Lu FE and Zhao L: Chinese herbal medicine in the treatment of non-alcoholic fatty liver disease. Chin J Integr Med 2012; 18(2): 152-60.
42. Perez-Martinez P, Garcia-Rios A, Delgado-Lista J, Perez-Jimenez F and Lopez-Miranda J: Metabolic syndrome: evidences for a personalized nutrition. Mol Nutr Food Res 2012; 56(1): 67-76.
43. Graff SK, Kappagoda M, Wooten HM, McGowan AK and Ashe M: Policies for healthier communities: historical, legal, and practical elements of the obesity prevention movement. Annu Rev Public Health. 2012; 33: 307-24. doi: 10.1146/annurev-publhealth-031811-124608, PMID 22224883.
44. Uchiyama S, Taniguchi Y, Saka A, Yoshida A and Yajima H: Prevention of diet-induced obesity by dietary black tea Polyphenols extract *in-vitro* and *in-vivo*. Nutrition 2011; 27(3): 287-92. doi: 10.1016/j.nut.2010.01.019, PMID 20627658.

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