



Original Research Article

Arq ajwain protects nonalcoholic fatty liver disease on high-fat diet-induced obese rat

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ABSTRACT

In the present study, we investigated the safety and therapeutic efficacy of arq ajwain (AA) against nonalcoholic fatty liver disease (NAFLD) on a high-fat diet (HFD) induced obese rat model. After 4 weeks of the treatment, AA (4 mL/kg, twice a day) and AA1 (7.75 mL/kg, twice a day) treatments reduced body weight, serum triglycerides (TG), total cholesterol (TC), glucose, insulin, leptin, pancreatic lipase activity and alanine aminotransferase (ALT) levels ($p < 0.001$). AA, AA1, and thymol (T) treatments also decreased lipids (TG and TC) levels and increased thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), and catalase (CAT) levels in hepatic tissue ($p < 0.001$). Moreover, treatment groups considerably reduced hepatic inflammation, steatosis, hepatocellular ballooning, and fibrosis. These results recommend that arq ajwain has a strong protective effect against NAFLD in HFD induced obese rat models.

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1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is more common in people who have certain conditions, including obesity and conditions that may be related to obesity, such as type 2 diabetes, dyslipidemia, and hypertension. Nonalcoholic steatohepatitis (NASH), the most severe form of the NAFLD, is characterized by steatosis, hepatic inflammation, and hepatocellular ballooning and may include varying degrees of fibrosis. The hallmark of NAFLD is TG accumulation in the cytoplasm of hepatocytes (Caliguri et al., 2016). The understanding of the pathophysiology of NAFLD or NASH has evolved significantly from the original 2-hit assumption wherein a first hit, such as insulin resistance, resulted in hepatic steatosis, and a subsequent second hit, such as oxidative stress, was required to develop NAFLD

(Cohen et al., 2011). Many studies have reported that the pathophysiology of NAFLD is complex and numerous intermediaries are involved. Every year, new and exciting developments as pieces of the pathophysiologic conundrum are put together or new pieces are elucidated (Manne et al., 2018). Nonetheless, the task of completing the conundrum seems far in the future. But as the prevalence of this disease continues to increase at an alarming rate, the need for continued research is critical for the innovation of suitable and novel drug targets as well as the development of effective therapies. Most of the recent trials have focused on screening natural sources that have been reported to protect NAFLD without any significant side effects (Shetty et al., 2010; Yang et al., 2018; Li et al., 2018; Haque and Ansari, 2019). This may be an excellent alternative strategy for developing effective and safe anti-NAFLD drugs in the future. AA, is a distillate product that is prepared from a single eminent herb,

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namely *Trachyspermum ammi* (L.) Sprague. AA is much used as a medicinal product in Unani (UPI-Part 2, Volume 1) medicine for its antispasmodic, carminative, anti-phlegmatic, antidiarrheal, hepatoprotective, and body fat reducing properties (Panda, 2002; Anonymous, 2009). It has been reported that AA is a very stable formulation without showing any adverse microbial contamination (Khan et al., 2018). Bairwa et al. (2012) reported that *T. ammi* is used traditionally as an important remedial agent for flatulence, diarrhea, atonic dyspepsia, abdominal tumors, abdominal pains, bronchial problems, galactagogue, asthma, and amenorrhoea. *T. ammi* is a rich source of essential oils and has been widely investigated for its chemical composition and biological activities. Singh et al. (2004) investigated that the volatile oil of *T. ammi* L. contains thymol (39.1%), *p*-cymene (30.8%), γ -terpinene (23.2%), and β -pinene (1.7%) as the major components (Singh et al., 2004). It has been investigated that *T. ammi* fruits yield 4-6% volatile oil containing thymol (45.0-55.0%) (Aftab et al., 1995). Thymol is considered as one of the major bioactive constituents and has a wide spectrum of biological activity. It has been investigated that thymol from *T. ammi* has strong antioxidant (Singh et al., 2004), antimicrobial (Sepahvand et al., 2021), hepatoprotective (Alam et al., 1999), and blood pressure-lowering action (Aftab et al., 1995). Recently, Oskouei et al. (2018) have investigated that thymol has anti-hyperglycemic, anti-hyperlipidemic, and antioxidant activity in STZ-induced diabetic rats. In triton WR1339-treated rats, γ -terpinene has been reported to have an antihyperlipidemic effect (Takahashi et al., 2003). Aside from these reported activities of *T. ammi* volatile components, *T. ammi* itself has been observed with many activities, namely hypolipidemic (Javed et al., 2006), antidiabetic (Patil et al., 2011) and antiplatelets (Srivastava, 1988). Ajwain fruit is also being used as an ingredient for the preparation of antiobesity polyherbal formulation (arq-e-zeera and safoof mahazzil) in the traditional system of Indian medicine (Gupta et al. 2012; Haque and Ansari, 2018). There is no scientific preclinical work reported on Unani formulation AA to validate its anti-NAFLD action up till now. Therefore, we selected a rat model of diet-induced obesity and NAFLD based on numerous experimental studies which indicated that high in fat diets are known to increase fat mass, induce alterations in carbohydrate and lipid level, lead to insulin resistance, exert oxidative stress, increase production and release of leptin in rodents (Shetty et al., 2010; Xu et al., 2010). For this reason, the current study was planned to evaluate the safety and therapeutic efficacy of AA formulation against NAFLD on HFD induced obese rat model.

2. Experimental

2.1. Plant raw material

T. ammi fruits were obtained from Chittorgarh (Rajasthan), India, in December 2012 and identified in the National Institute of Science Communication and Information Resources (NISCAIR), New Delhi on the

basis of morphological (The fruits are about 1.6-3.1 mm long and 1.3-2.9 mm wide, ovoid, mainly occur as entire cremocarps with pedicel attached or detached with bifid stylopod and glabrous cremocarps. Dorsal surface convex with 5 equally distinct, longitudinal primary ridges, 5 primary ridges) and microscopical characters (Endocarp consists of narrow elongated cells having a parquetry arrangement, Vittae are 6 in number, 4 on the dorsal surface at the mesocarpic region below the secondary ridges and 2 on the commissural surface of the mericarp). Voucher specimen and identification certificate reference number NISCAIR/RHMD/Consult/2011-12/1753/53 was obtained and a voucher specimen was deposited in the laboratory of School of Pharmaceutical and Education Research.

2.2. Preparation of AA

Cleaned and dried *T. ammi* (L.) fruits (1000 g) were crushed in an iron mortar to obtain coarse powder and soaked in 12 L of purified water. Then, the soaked *T. ammi* (L.) was transferred to the distillation apparatus along with purified water and distilled at 100 °C for about five and a half hour. Finally, 4000 mL of distillate (AA) was collected (Khan et al., 2018).

2.3. Phytochemical study of AA by GC and GC-MS

Diethyl ether (1 L) was added to AA and frequently shaken for 1 h and allowed to stand for 24 h in a separating funnel, resulting in the formation of two layers, organic (ether) and aqueous layer. Then, ether layer was dried in desiccator (anhydrous P_2O_5). The solvent was evaporated under the nitrogen stream and concentrated into a 10 mL solution, 0.2 μ L of which was injected into GC and GC-MS instruments for the identification of chemical components. For estimation of volatile components, the ether layer phase was introduced to a Shimadzu QP-2010 GC-MS system equipped with AB-Innowax 7031428 WCOT column (60 m x 0.025 cm x 0.25 μ m) directly coupled to the MS. Helium gas was used as the carrier gas with a flow rate of 1.21 mL/min. The oven temperature was programmed as 50 °C for 60 s and subsequently held isothermal for 2 min, injector port: 250 °C, detector: 280 °C, and split ratio was adjusted at 1: 50 ratio. The recording was carried out at 70 eV, scan time 1.5 s, mass to charge (m/z) range of 40-750 amu. Software adapted to knob mass spectra and chromatograph was an Agilent Chemstation (Agilent 6850 Gas Chromatograph). GC analysis of volatile chemical components in ether layer phase was performed on Shimadzu 2010 Gas Chromatograph (Japan) equipped with a flame ionization detector (FID) and AB-Innowax 7031428 WCOT fused capillary column (60 m x 0.025 cm x 0.25 μ m). The injector and detector (FID) temperatures were kept up at 250 and 270 °C, respectively. The carrier gas used was nitrogen at a flow rate of 1.21 mL/min with a column pressure of 155.1 kPa. The sample was inserted into the column with a split ratio of 80:1. The component separation

was accomplished following a linear temperature program from 60-230 °C at a rate of 3 °C/min and then held at 230 °C for 9 min, with a total run time of 55.14 min. The percentage of the chemical components was calculated by electronic integration of FID peak areas. The chemical components were recognized with the help of NIST and Wiley library software and KI values against a mixture of normal alkanes.

2.4. Quantification of thymol in AA by HPTLC

Thymol content present in AA was determined by HPTLC aluminum plates pre-coated with silica gel 60 F254 as stationary phase. Three samples of AA extract along with five standard stock solutions of thymol were spotted in the form of a band (3.0 mm) with a Camag microlitre syringe (width 8 mm) on a TLC plate (20 × 10 cm with 0.2 mm thickness, E. Merck, Berlin, Germany) using a Camag Linomat V sample applicator (CAMAG, Muttenz, Switzerland) attached to CAMAG HPTLC system and TLC scanner III, controlled by win CATS-IV software. A constant application rate of 120 nL s⁻¹ was employed and the space between the two bands was 0.8 cm. The slit dimension was kept at 0.02-0.3 cm and a scanning speed of 20 mm s⁻¹ was employed. The development was carried out using a linear ascending manner in a twin through glass chamber (20 × 10 cm) saturated with the mobile phase composed of toluene:ethyl acetate (9:3 v/v). The optimized chamber saturation time for the mobile phase was 30 min at room temperature and a chromatogram was developed up to the length of 8.5 cm. Then, HPTLC plate was dried and sprayed with vanillin sulphuric acid. Photodensitometric analysis was performed by UV-Visible spectrophotometry at 513 nm after heating (for 5-10 minutes) the sprayed HPTLC plate. For sample preparation, chloroform was added to the AA formulation, frequently shaken for 1-h and allowed to stand for 24-h in a separating funnel. Then, the chloroform layer was separated and the rest of the solution was again macerated with more quantities of chloroform at least three times. The combined chloroform extract was evaporated by a rotary evaporator and the relevant residue was redissolved in methanol. Finally, the methanolic extract of AA (10 mg/mL) was used for the analysis of thymol. A stock solution of thymol (1 mg/mL) was made in methanol and 1, 2, 3, 4, and 5 µL of the stock solution were subsequently spotted on TLC plate six times to obtain the final concentration range of 1000-5000 ng/spot. Thymol standard (98.5% purity) was procured from Sigma Aldrich. The statistics of peak area versus drug concentration were treated by linear least-square regression. *R* value for thymol was found to be 0.72 ± 0.03. The scanning wavelength was chosen at 513 nm for the maximum absorption of the thymol spot.

2.5 Experimental animals

Wistar Albino male rats, weighing 150-200 g (8-12 weeks old), were obtained from the Central Animal House Facility, Hamdard University, New Delhi, India. Before the

beginning of the experiment, the rats were accustomed for one week to the laboratory circumstances. They were maintained in polycarbonate cages, under restrained temperature (25 ± 2 °C) and 12 h light/12 h dark rhythm. Rats were permitted to have free access to a normal pellet diet and water ad libitum. The procedure employed in this experiment for the use of rats was approved by the Institutional Animal Ethics Committee (IAEC) of Hamdard University (Registration Number 173/GO/RE/S/2000/CPCSEA and Approval Number 607).

2.6. Kits, chemical, and experimental diets

Total cholesterol (TC), total triglyceride (TG) (Span Diagnostics Ltd, Surat, Gujarat, India), lactate dehydrogenase (LDH), high-density lipoprotein-cholesterol and glucose (Reckon Diagnostics Pvt Ltd, Baroda, Gujarat, India), rat leptin ELISA kit (BioVendor, Czech Republic), rat insulin ELISA kit (Alpco Diagnostics, Salem, USA), Quanti Chrom™ lipase assay kit (DLPS-100), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) Span Diagnostics Ltd, Surat, Gujarat, India) kits were procured from different suppliers. Normal pellet diet (16% fat, 64% carbohydrate, and 20% protein) and HFD (55% fat, 20% protein, and 25% carbohydrate) were obtained from National Centre for Laboratory Animal Science (NCLAS), National Institute of Nutrition (NIN), Hyderabad, India. Silica gel 60F-254 pre-coated HPTLC aluminum sheets (0.2 mm thick) were purchased from E. Merck Ltd., Mumbai. All other chemicals used were of analytical grade. Double distilled water was used for all biochemical estimations.

2.7. Induction of obesity

HFD was used for the induction of obesity or NAFLD in Wistar Albino male rats.

2.8. Selection and calculation of dose

The human dose of arqajwain (75 mL twice a day, oral) has been mentioned in the Unani Pharmacopoeia of India for the management of disorders (flatulence, diarrhea and asthma, etc.). The dose needed for rats was calculated based on its human dose by using the following formula (Reagan-Shaw et al., 2007; Arora et al., 2016).

$$\text{Animal dose (mL/kg)} = \frac{\text{Human dose (75 mL twice a day)}}{\text{Average body wt (60 kg)} \times 6.2 \text{ (Conversion factor)}} = 7.75 \text{ mL/kg, twice a day for rats (AA1)} \quad (\text{Eqn. 1})$$

A smaller dose (4 mL/kg, twice a day) of AA was also selected for the study of the dose depending on action compared with a higher dose (7.75 mL/kg, twice a day for rats (AA1)).

2.9. Treatment's schedule and serum biomarkers measurements

After one week of acclimation, rats were randomized into 6 groups (n = 6/group).



Group 1 (Control group): Animals have received NPD for 2 weeks. After 2 weeks, 0.5 mL carboxymethylcellulose (CMC) sodium aqueous solution, the vehicle was administered orally along with the same diet for 4 weeks.

Group 2 (HFD group): Animals were received HFD orally for 6 weeks.

Groups 3 (AA + HFD): Animals were received HFD for two weeks. After two weeks, along with HFD, AA (4 mL/kg, twice a day) was given orally (by gavage) for 28 days.

Groups 4 (AA1 + HFD): Animals were received HFD for two weeks. After two weeks, along with HFD, AA1 (7.75 mL/kg, twice a day) was given orally (by gavage) for 28 days.

Groups 5 (T + HFD): Animals were received HFD for two weeks. After two weeks, along with HFD, thymol (12 mg/kg, twice a day) was given orally (by gavage) for 28 days.

Groups 6 (Orlistat + HFD): Animals were received HFD for two weeks. After two weeks, along with HFD, orlistat (30 mg/kg, once a day) was given orally (by gavage) for 28 days.

Normal control group received 0.5% CMC sodium aqueous solution. Orlistat, thymol, and AA were suspended in 0.5% CMC sodium aqueous solution for animal treatment.

2.10. T (Thymol) content in AA1 (7.75 mL/kg) and AA (4 mL/kg)

Thymol (T) dose (12 mg/kg) was determined from the contents present in the AA1. Thymol content in AA1 and AA were about 12 mg and 7 mg, respectively. In addition to thymol, arq ajwain formulation (AA1 and AA) also contained various volatile components (Table 1). AA also contained less quantity of volatile components in comparison to AA1. Thus, AA1 contained 12 mg of thymol plus other volatile components, while AA contained 7 mg of thymol plus other volatile components.

Table 1

Chemical composition of arq ajwain (AA).

S. No.	Compounds	Molecular formula	Molecular weight	RI	Area%
1.	α -Pinene	C ₁₀ H ₁₆	136.24	935	0.0126
2.	1-Allyl cyclopropane carboxylic acid 2,6-di-tert-butyl-4-methyl-phenyl ester	C ₂₂ H ₃₂ O ₂	328.496	967	0.024
3.	(+)-Sabinene	C ₁₀ H ₁₆	136.24	973	0.0735
4.	β -Pinene	C ₁₀ H ₁₆	136.24	978	2.7345
5.	Myrcene	C ₁₀ H ₁₆	136.24	989	0.5774
6.	(+)-4-Carene	C ₁₀ H ₁₆	136.24	1003	0.0354
7.	α -Phellandrene	C ₁₀ H ₁₆	136.24	1004	0.7345
8.	3-Carene	C ₁₀ H ₁₆	136.24	1011	0.0419
9.	α -Terpinene	C ₁₀ H ₁₆	136.24	1017	0.4415
10.	D-Limonene	C ₁₀ H ₁₆	136.23	1029	0.1708
11.	β -Phellandrene	C ₁₀ H ₁₆	136.24	1030	0.324
12.	<i>ortho</i> -Cymene	C ₁₀ H ₁₆	134.222	1041	19.8987
13.	γ -Terpinene	C ₁₀ H ₁₆	136.23	1059	24.133
14.	<i>trans</i> -Sabinene	C ₁₀ H ₁₈ O	154.253	1098	0.0326
15.	<i>cis-p</i> -Menth-2-en-1-ol	C ₁₀ H ₁₈ O	154.253	1123	0.1394
16.	4-Terpineol	C ₁₀ H ₁₈ O	154.253	1177	0.1731
17.	α -Terpineol	C ₁₀ H ₁₈ O	154.253	1189	0.0195
18.	Myrtenal	C ₁₀ H ₁₄ O	150.221	1192	0.1893
19.	1-(Furan-2-yl)-2,3 dimethylbutane-1,2-diol	C ₁₀ H ₁₆ O ₃	184.235	1231	0.0093
20.	<i>p</i> -Mentha-1,4-dien-7-ol	C ₁₀ H ₁₆	152.233	1240	0.038
21.	Cuminol	C ₁₀ H ₁₄ O	150.221	1251	0.0109
22.	Thymol	C ₁₀ H ₁₄ O	150.22	1290	48.97
23.	Carvacrol	C ₁₀ H ₁₄ O	150.217	1301	0.097
24.	Piperitenone	C ₁₀ H ₁₄ O	150.2176	1340	0.0201
25.	(<i>Z</i>)-Caryophyllene	C ₁₅ H ₂₄	204.357	1407	0.0619
26.	(<i>E</i>)-Caryophyllene	C ₁₅ H ₂₄	204.357	1421	0.3212

Table 1 Continued

S. No.	Compounds	Molecular formula	Molecular weight	RI	Area%
27.	Azulene	C ₁₀ H ₈	128.174	1504	0.0699
28.	Cuminaldehyde	C ₁₀ H ₁₂ O	148.21	1504	0.0056
29.	1-(1,4-Dimethyl-3-cyclohexen-1-yl)-2-buten-1-one	C ₁₂ H ₁₈ O	178.275	1632	0.0238
30.	2-Caren-10-al	C ₁₀ H ₁₄ O	150.221	1711	0.2413
31.	Pentadecanoic acid	C ₁₅ H ₃₀ O ₂	242.3975	1867	0.0832

RI = Determined retention index against a mixture of alkanes; 31 components in arq ajwain were identified. Thymol (1041), γ -terpinene (1059), *ortho*-cymene (1041), and β -pinene (978) were the major components in the formulation.

2.11. Food intake, water intake, body weights, and serum biomarkers measurements

Food, water intake, and body weights were recorded daily during the feeding period and finally, the average value of these parameters was calculated after 6 weeks. At the end of the treatment period, rats were anesthetized with ether, following a 12-h period of fasting. Blood was drawn from the retro-orbital plexus. Then, serum was obtained by centrifuging the blood at 4000 rpm for 10 min. Livers and visceral fat-pads were removed from rats, rinsed with phosphate-buffered saline (PBS), and then weighed. Liver samples were stored in 10% formalin solution at room temperature. While serum samples were stored at -70°C until they were analyzed. TC, TG, HDL-C, AST, ALT, LDH, glucose, insulin, leptin, and pancreatic lipase activity in serum were measured with commercial kits. Estimations of serum low-density lipoprotein-cholesterol (LDL-C) were measured by using Friedewalds equation (Friedewald et al., 1972). Liver samples were used for histopathology, hepatic lipids estimation, thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) assay. A portion of the liver sample was minced and homogenized (10% w/v) for determination of TBARS, GSH, SOD, and CAT activities (Marklund and Marklund, 1974; Ohkawa et al., 1979).

2.12. Hepatic lipid profiles

Hepatic lipids were extracted using the method developed by Folch et al. (1957) and the dried lipid residues were dissolved in 1 mL of ethanol. TC and TG concentrations in the hepatic lipid extracts were measured using the same enzymatic kits that were used for the serum analysis,

2.13. Hepatic tissue study

Rats were sacrificed and rat liver tissue samples were collected, fixed in 10% formalin buffered solutions, cut into 4-5 μm sections, and stained with hematoxylin/eosin. The sections of liver tissues were studied to determine the level of steatosis, hepatocellular ballooning, lobular inflammation, and fibrosis according to the standard criteria for grading of

NASH, with minor modifications (Kleiner et al., 2005). Steatosis was estimated based on the percentage of lipid droplets in stained sections as follows: score 0, no steatosis; score 1, up to 33% steatosis; score 2, 33 to 66% steatosis; and score 3, > 66% steatosis. Hepatocyte ballooning was graded 0 to 2 based on the number of balloon cells observed on stained sections as follows: score 0, no balloon cells; score 1, scattered balloon cells; and score 2, panacinar balloon cells. Lobular inflammation was graded 0-3 based on inflammatory foci observed on stained sections as follows: score 0, no inflammation; score 1, <2 foci/ $\times 200$ field; score 2, 2 to 4 foci/ $\times 200$ field; and score 3, >4 foci/ $\times 200$ field. Fibrosis was scored on stained sections as follows: score 0, no fibrosis; score 1, pericellular and perivenular fibrosis; and score 2, focal bridging fibrosis.

2.14. Data analysis

Data analysis was carried out using GraphPad Prism software (San Diego, California, USA). All data were presented as mean \pm SEM. Groups of data were compared with the analysis of variance (ANOVA) followed by Dunnett's t-test to identify significance among groups. The value was considered statistically significant when $p < 0.05$ and highly significant when $p < 0.001$.

3. Results and Discussion

Humans are currently facing a variety of persistent diseases and undoubtedly one of the best and most straightforward strategies to combat these illnesses is the potential use of a wide number of medicinal and herbal plants which often involve many valuable bioactive compounds relating to different classes of natural products and secondary metabolites like essential oils (Hussain et al., 2021; Mohammadhosseini et al., 2021; Mouthe Kemayou et al., 2021; Nahar et al. 2021). Arq ajwain (AA) is widely used for the treatment of spasmodic, flatulence, diarrheal, and body asthma (Panda, 2002). In the current study, we investigated the effect of AA against NAFLD on HFD-induced obesity in a rat model and their possible therapeutic effect.

3.1. Phytochemical analysis of AA

AA is a distillate product that is why it contains only

volatile compounds. The volatile compounds in AA are shown in Table 1.

3.2. Chemical composition of AA by GC and GC-MS

The phytochemical composition of AA was investigated by GC-FID and GC-MS and 31 components were identified. Thymol (48.97%), γ -terpinene (24.13%), *ortho*-cymene (19.90%) and β -pinene (2.73%) were the major components (Table 1).

3.3. Estimation of thymol content in AA

A major component (thymol) in AA was quantified by

HPTLC photodensitometric method and its content was found to be 1.8 ± 0.33 (w/w%).

3.4. Food intake, water intake, bodyweight gain (BWG), visceral fat-pad weights (VFPW), and liver weight

HFD, AA, AA1, orlistat, and thymol alone treatment group showed an increase in FI ($p < 0.001$) as compared to NPD-fed rats. It was observed that differences in food and water intake among the test groups (AA, AA1, and T) throughout the study were not altered significantly as compared to HFD-fed rats. But orlistat treatment reduced food and water intake ($p < 0.05$) as compared to HFD-fed rats (Table 2).

Table 2

Effect of AA, AA1, T and orlistat on different parameters.

Variables	Control	HFD	AA + HFD	AA1 + HFD	T + HFD	Orlistat + HFD
Physical						
FI (g/150g rat/day)	14.13 \pm 0.98	20.20 \pm 2.09 ^a	20.86 \pm 1.09 ^{a, D}	20.54 \pm 0.99 ^{a, D}	19.86 \pm 0.78 ^a	18.34 \pm 1.07 ^{b, a}
WI(g/150g rat/day)	15.54 \pm 0.94	20.94 \pm 2.08 ^a	20.78 \pm 1.08 ^{a, D}	20.89 \pm 1.54 ^{a, D}	19.87 \pm 1.01 ^a	18.09 \pm 1.04 ^{b, a}
BWG(g)	70.21 \pm 2.65	128.89 \pm 5.98 ^a	85.43 \pm 5.32 ^{c, a, D}	75.22 \pm 5.43 ^{c, E}	87.61 \pm 6.43 ^{c, a, D}	71.09 \pm 4.54 ^c
VFPW(g)	8.5 \pm 0.98	22.42 \pm 1.65 ^a	12.32 \pm 1.98 ^{c, A, D}	9.98 \pm 1.88 ^c	15.81 \pm 1.99 ^{c, a, d}	8.97 \pm 1.69 ^c
Liver weight(g)	4.96 \pm 0.93	11.47 \pm 0.65 ^a	7.19 \pm 0.65 ^{c, a, D}	5.76 \pm 0.87 ^c	8.09 \pm 0.96 ^{c, a, D}	5.02 \pm 0.89 ^c
Serum lipidic						
TG (mg/dl)	48.6 \pm 4.01	109.14 \pm 7.98 ^a	75.18 \pm 4.98 ^{c, a, d}	53.45 \pm 6.12 ^{c, A, e}	86.84 \pm 5.96 ^{c, a, d, E}	50.98 \pm 3.87 ^c
TC (mg/dl)	62.82 \pm 3.01	98.59 \pm 2.09 ^a	80.19 \pm 2.43 ^{c, a, d}	74.54 \pm 2.87 ^{c, A, D, E}	83.84 \pm 3.09 ^{c, a, d}	65.98 \pm 2.01 ^c
LDL-C (mg/dl)	18.96 \pm 3.43	54.89 \pm 3.21 ^a	30.98 \pm 2.34 ^{c, a, d}	24.23 \pm 3.21 ^{c, E}	37.87 \pm 2.43 ^{c, a, d, E}	20.98 \pm 2.09 ^c
HDL-C (mg/dl)	34.1 \pm 3.32	25.07 \pm 4.12 ^a	30.98 \pm 2.54 ^{c, A, d}	34.65 \pm 2.76 ^{c, E}	29.56 \pm 3.21 ^{c, a, d}	35.78 \pm 2.3 ^c
Glycemic						
Glucose (mg/dl)	78.19 \pm 3.36	131.96 \pm 7.38 ^a	98.08 \pm 8.7 ^{c, a, d}	79.98 \pm 6.27 ^{c, e}	106.64 \pm 7.19 ^{c, a, d}	79.58 \pm 9.5 ^c
Insulin (μ g/ml)	0.92 \pm 0.05	1.79 \pm 0.09 ^a	1.11 \pm 0.02 ^{c, a, d}	0.97 \pm 0.094 ^{c, E}	1.27 \pm 0.06 ^{b, a, d}	0.94 \pm 0.03 ^c
Leptin (ng/ml)	0.7 \pm 0.15	3.1 \pm 0.26 ^a	1.41 \pm 0.21 ^{c, a, d}	0.89 \pm 0.29 ^{c, E}	1.51 \pm 0.16 ^{c, a, d}	0.82 \pm 0.32 ^c
Hepatic						
AST (1U/L)	62.44 \pm 2.6	87.75 \pm 6.77 ^a	73.87 \pm 5.21 ^{c, a, d}	68.68 \pm 6.39 ^{c, E}	77.83 \pm 4.17 ^{b, a, d}	64.77 \pm 9.56 ^c
ALT (IU/L)	42.46 \pm 2.92	67.09 \pm 3.39 ^a	52.92 \pm 3.2 ^{c, A, D}	46.22 \pm 2.38 ^{c, E}	56.61 \pm 3.84 ^{b, a, d}	43.39 \pm 2.56 ^c

Table 2 Continued

Variables	Control	HFD	AA + HFD	AA1 + HFD	T + HFD	Orlistat + HFD
LDH (IU/L))	43.48 ± 7.64	208.82 ± 12.46 ^a	93.64 ± 9.32 _{c, A, D}	59.56 ± 8.32 _{c, n, E}	126.54 ± 7.8 _{c, a, d, E}	56.46 ± 12.34 ^c
Inflammation Biomarkers						
TBARS(nmol MDA/mg protein)	0.22 ± 0.03	0.73 ± 0.03 ^a	0.35 ± 0.5 _{c, a, d}	0.24 ± 0.06 _{c, E}	0.43 ± 0.04 ^c _{a, d, E}	0.20 ± 0.04 ^c
GSH(μmol of P liberated/min/mg/protein)	28.98 ± 0.72	8.99 ± 0.22 ^a	22.15 ± 0.71 _{c, A, D}	26.78 ± 0.61 _{c, E}	20.95 ± 0.56 _{c, a, d}	27.78 ± 0.61 ^c
SOD(U/mg protein)	4.11 ± 0.32	2.20 ± 0.14 ^a	3.13 ± 0.12 ^c _{A, D}	3.92 ± 0.06 _{c, E}	3.07 ± 0.13 _{b, a, d}	3.93 ± 0.12 ^c
CAT(n mol of H ₂ O ₂ /min/mg/protein)	58.16 ± 0.46	26.56 ± 0.47 ^a	37.86 ± 0.41 _{c, a, d}	56.16 ± 0.31 _{c, e}	35.42 ± 0.36 _{c, a, d}	57.86 ± 0.41 ^c
Hepatic lipidic						
Hepatic TG (mg/g liver)	1.33 ± 0.12	3.32 ± 0.29 ^a	2.01 ± 0.29 _{c, A, D}	1.43 ± 0.36 _{c, e}	2.47 ± 0.13 _{b, a, d, E}	1.29 ± 0.21 ^c
		0.29 ^a				
Hepatic TC (mg/g liver)	0.47 ± 0.09	0.99 ± 0.08 ^a	0.78 ± 0.09 _{c, a, d}	0.54 ± 0.08 _{c, e}	0.85 ± 0.05 _{b, a, d}	0.51 ± 0.06 ^c

All values were expressed as mean ± SEM (n= 6). ^a*p* < 0.05, ^{ap}*p* < 0.001 as compared to control group. ^b*p* < 0.05. ^{cp}*p* < 0.001 as compared to HFD group. ^d*p* < 0.05, ^{dp}*p* < 0.001 as compared to orlistat group. ^e*p* < 0.05, ^{ep}*p* < 0.001 as compared to AA group. Positive control and treated rats were fed the HFD for 42 days, while control rats received standard rat chow (NPD).

AA and AA1 treatment group showed an increase (*p* < 0.05) in FI and WI as compared to the orlistat group. HFD, AA, and thymol alone treatment group showed an increase in BWG (*p* < 0.001) as compared to NPD-fed rats. But AA1 and orlistat treatment groups showed non-significant changes in BWG as compared to the control group. AA, AA1, T, and orlistat treatment decreased BWG (*p* < 0.001) as compared to HFD-fed rats (Table 2). AA and T alone treatment group increased BWG (*p* < 0.05) as compared to orlistat group, while AA1 did not show a significant increase in BWG as compared to orlistat treatment group. AA1 treatment group decreased BWG (*p* < 0.05) as compared to AA treatment group, while T alone treatment group did not show a significant increase in BWG as compared to AA treatment group. HFD-fed rats and the thymol alone treatment groups showed a rise in VFPW (*p* < 0.001) as compared to NPD-fed rats. AA showed less significant (*p* < 0.05) alteration in VFPD as a comparison to the control group. But AA1 and orlistat treatment groups showed non-significant changes in VFPW as compared to the control group. AA, AA1, T, and orlistat treatment decreased VFPW (*p* < 0.001) as compared to HFD-fed rats. AA and T alone treatment group showed a significant increase in VFPW as compared to orlistat group, while AA1 treatment did not increase VFPW significantly as compared to orlistat treatment group. AA1 and T alone treatment groups did not alter VFPW significantly as compared to the AA treatment group. Liver weight was about 2.31 times larger in the HFD group than that in the control group (Table 2). AA, AA1,

T, and orlistat treatment decreased liver weight (*p* < 0.001) as compared to HFD-fed rats. AA and T alone treatment groups an increased liver weight (*p* < 0.05) as compared to the orlistat group, while AA1 treatment did not show a significant increase in liver weight as compared to the orlistat treatment group. AA1 and T alone treatment groups did not alter liver weight significantly as compared to the AA treatment group. Administration of AA, AA1, T, and orlistat for 28 days remarkably lowered body weight compared with that of the HFD group. Administration of AA, AA1, and T did not alter food and water intake. Hence, arq ajwain would be supportive in the prevention of obesity-induced NAFLD without any change in food and water intake.

3.5. Serum lipids

HFD-fed rats and thymol alone treatment group showed increases in serum TG, TC, and LDL-C levels (*p* < 0.001) and decreases in HDL-C level (*p* < 0.001) as compared to NPD-fed rat. AA1 and orlistat treatment groups showed non-significant changes in serum lipids as compared to the control group. The AA group showed significant (*p* < 0.001) changes in the case of serum TG, TC, and LDL-C levels, while in the case of HDL-C, AA showed less significant (*p* < 0.05) alteration as a comparison to the control group. AA, AA1, T, and orlistat treatment decreased serum TG, TC, and LDL-C levels (*p* < 0.001) and increased HDL-C level (*p* < 0.001) as compared to the HFD group. AA and T showed significant changes in TG, TC, LDL-C, and HDL-C levels as compared to the orlistat



treatment group, while the AA treatment group did not alter TG, TC, HDL-C, and HDL-C levels significantly as compared to the orlistat group. AA1 treatment group decreased TG, TC, HDL-C, and HDL-C significantly as compared to the AA treatment group, while T alone treatment group increased TG and LDL-C levels ($p < 0.05$) as compared to AA treatment group. HFD-induced hyperlipidemia, oxidative stress, hyperglycemia, and increased levels of leptin and insulin are well reported (Petersen et al., 2002). Our data also showed that rats in the HFD group exhibited significantly higher TG, TC, and LDL-C levels, and lower HDL-C. However, administration of AA, AA1, T, or orlistat significantly lowered TG, TC, and LDL-C levels and higher HDL-C levels in serum (Table 2). It has been reported that NAFLD, is associated with dyslipidemia, characterized by elevated TG and reduced HDL-C concentrations. TGs are involved in the ectopic accumulation of lipid stores in the liver and are associated with several diseases such as metabolic syndrome. Diminutions in TG in serum could be attributed to the inhibition of lipid absorption in the GI tract, such as gastrointestinal lipase inhibition (Petersen et al., 2002). Thus, this result suggested that arq ajwain would be helpful in the prevention of NAFLD complications through improving hyperlipidemia.

3.6. Serum glucose, insulin, and leptin

HFD-fed rats, AA, and T group showed increases in serum glucose, insulin, and leptin ($p < 0.001$) against NPD-fed rats. But AA1 and orlistat treatment groups showed non-significant changes in serum glucose, insulin, and leptin as compared to the control group. AA, AA1, and orlistat treatment decreased serum glucose, insulin, and leptin level ($p < 0.001$) as compared to the HFD group. However, T alone treatment reduced serum glucose ($p < 0.001$), insulin ($p < 0.05$) and leptin levels ($p < 0.001$) as compared to HFD group (Table 2). AA and T alone treatment group increased glucose, insulin, and leptin levels ($p < 0.001$) as compared to orlistat treatment group, while AA1 treatment did not show significant changes in glucose, insulin, and leptin levels as compared to orlistat treatment group. AA1 treatment group significantly decreased glucose, insulin, and leptin levels as compared to AA treatment group, while T alone treatment group did not increase glucose, insulin, and leptin levels significantly as compared to AA treatment group. It is manifested that AA, AA1, T or orlistat treatment significantly lowered serum glucose, insulin, and leptin levels in HFD-induced NAFLD rats (Table 2). Numerous publications have proved that resistance to both leptin and insulin actions has been implicated in the accumulation of hepatic TG (Kostula et al., 2020). Leptin is an adipocyte-derived antiobesity hormone that in rodents protects "lipotoxicity" by limiting TG accumulation and also regulates matrix deposition (fibrosis) during wound healing. Leptin is one of the important factors that can regulate oxidative stress as well as enhance proinflammatory and profibrogenic responses in the liver (McCullough et al., 1996). Many research data have shown that the

main physiological role of leptin is to prevent lipid accumulation in nonadipose sites, such as the heart, skeletal muscle, pancreas, and liver, a concept referred to as "lipotoxicity" (Zhao et al., 2021). Several factors could contribute to increased serum leptin levels in NAFLD (McCullough et al., 1996). First, hyperleptinemia that occurs in inflammatory disorders has been attributed to cytokines such as tumor necrosis factor α (TNF- α), which is also increased in NAFLD. However, TNF- α is not obligatory for the pathogenesis of hepatic steatosis, insofar as leptin-deficient ob/ob mice that are also nullizygous for TNF- α still develop fatty liver (Memon et al., 2001). Second, hyperinsulinemia is a major determinant of circulating leptin. Whereas acute insulin administration produces only transient changes in leptin, chronic hyperinsulinemia is associated with persistent hyperleptinemia (Wauters et al., 2000). In obese conditions, leptin and insulin concentrations are boosted resulting in leptin and insulin resistance either centrally or locally at the level of the liver (Petersen et al., 2002). Our data also demonstrated that rats in the HFD group showed significant increases in leptin and insulin levels in serum. However, administration of AA, AA1, T, or orlistat significantly reduced leptin and insulin levels (Table 2). The results from current studies suggest that arq ajwain caused the considerable reduction of hepatic TG accumulation, indicated by reduced leptin and insulin levels. Thus, this result suggested that arq ajwain would be helpful in the protection against nonalcoholic steatohepatitis through improvement in leptin and insulin sensitivity.

3.7. Serum AST, ALT and LDH levels

HFD-fed rats and the T group showed an increase in AST, ALT, and LDH levels ($p < 0.001$) as compared to the control group. AA group showed significant ($p < 0.001$) changes in AST and LDH, while in the case of ALT, AA showed less significant ($p < 0.05$) alteration as a comparison to the control group. However, AA1 and orlistat treatment groups showed non-significant changes in AST, ALT, and LDH as compared to the control group. AA, AA1, and orlistat treatment decreased AST, ALT, and LDH ($p < 0.001$) as compared to the HFD group. Moreover, T treatment decreased serum LDH ($p < 0.001$), AST and ALT ($p < 0.05$) levels as compared to HFD group (Table 2). AA and T alone significantly increased AST, ALT, and LDH levels as compared to the orlistat group, while AA1 did not alter AST, ALT, and LDH levels significantly as compared to the orlistat group. AA1 treatment group significantly decreased AST, ALT, and LDH levels ($p < 0.05$) as compared to the AA treatment group, while T alone treatment group significantly increased LDH level only as compared to AA treatment group. The results of our study showed that HFD-treated rats were more prone to hepatotoxicity as evidenced by an increased level of serum AST, ALT, and LDH. AA, AA1, thymol, and orlistat treatment significantly reduced the increased AST, ALT, and LDH levels in obese rats which could be attributed to the protective effect on hepatic tissue (protective

against nonalcoholic steatohepatitis).

3.8. Inflammation biomarkers

HFD, AA, and T groups increased pancreatic lipase activity level ($p < 0.001$) as compared to the normal healthy group. However, AA1 and orlistat treatment groups showed non-significant changes in pancreatic lipase activity level as compared to the control group. AA, AA1, T, and orlistat treatment groups reduced pancreatic lipase activity level ($p < 0.001$) as compared to the HFD group (Table 2). HFD-fed rats and the T group showed a decrease in hepatic GSH, SOD, and CAT ($p < 0.001$) and augmentation in MDA ($p < 0.001$) as compared to the control group (Table 2). AA group showed significant ($p < 0.001$) changes in TBARS, CAT levels, and GSH, while in the case of SOD, AA showed less significant ($p < 0.05$) alteration as a comparison to the control group. But AA1 and orlistat treatment groups showed non-significant changes in TBARS, GSH, SOD, and CAT as compared to the control group. In contrast, AA, AA1, and orlistat treatment augmented hepatic GSH, SOD, and CAT level ($p < 0.001$) and reduced MDA level ($p < 0.001$) as compared to the HFD group. Moreover, T treatment augmented hepatic GSH ($p < 0.001$), SOD level ($p < 0.05$), CAT ($p < 0.001$) and reduced MDA level ($p < 0.001$) as compared to HFD group. AA and T alone treatment groups showed significant changes in pancreatic lipase, MDA, GSH, SOD, and CAT activity levels as compared to the orlistat treatment group, while the AA1 treatment group did not show significant alteration in pancreatic lipase, MDA, GSH, SOD and CAT activity levels as compared to orlistat group. The AA1 treatment group showed significant changes in pancreatic lipase, MDA, GSH, SOD, and CAT activity levels as compared to the AA treatment group, while T alone treatment group significantly increased pancreatic lipase activity level ($p < 0.05$) as compared to the AA treatment group. Pancreatic lipase is the main enzyme for fat absorption that hydrolyzes triglycerides in the GI tract. Pancreatic lipase inhibitor which assists to limit intestinal fat absorption at the initial stage has been proved as helpful medication for the treatment of obesity and a great promise as anti-NAFLD agents (Kim et al., 2020). AA, AA1, T, and orlistat significantly inhibited pancreatic lipase activity on obese rats and thus it prevents lipid absorption that hydrolysis triglycerides in the GI tract. NAFLD synergistically promotes systemic oxidative stress-imbalance between tissue free radicals, reactive oxygen species (ROS), and antioxidants (Dallio et al., 2021). ROS could react with polyunsaturated fatty acids, which leads to lipid peroxidation. Possible mechanisms that generate oxidative stress in NAFLD include elevated lipid levels, inadequate antioxidant defenses, and an increase in insulin and leptin concentration. Accumulation of hepatic lipids is correlated with an increase in the lipid peroxidation product, malondialdehyde (MDA). MDA is a by-product of lipid peroxidation and reflects the degree of oxidation in hepatic tissue. As noted, MDA

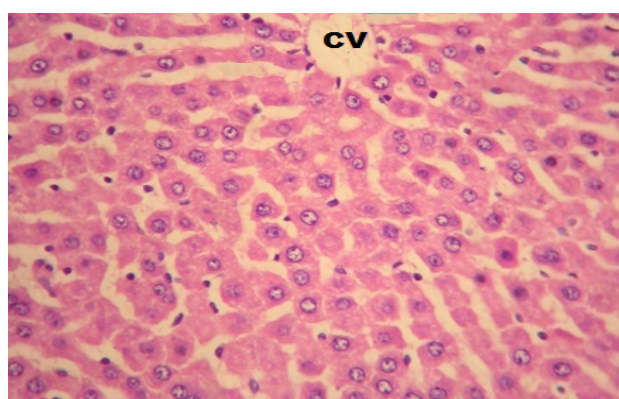
level in hepatic tissue decreased significantly in the group treated with AA, AA1, T, or orlistat. Reduction in lipid peroxidation could be related to the antioxidant and free radical scavenging properties of the thymol present in arq ajwain (Yildiz et al., 2021). In our present study, AA, AA1, T, and orlistat treatment significantly increased GSH, SOD, and CAT (free radicals scavenger) in HFD-fed rats (Table 2). Thus, this result suggests that the antioxidant activity of AA may, at least partly, contribute to the reduction of hepatic lipids. GC and GC-MS study of AA demonstrated that thymol, β -pinene, γ -terpinene, and *ortho*-cymene were major components. It has been reported that thymol, γ -terpinene, and *ortho*-cymene possess strong antioxidant potential (Hassan et al., 2010). Therefore, it is reasonable to assume that AA and AA1 treatment improves oxidative stress balance in HFD-fed NAFLD rats because arq ajwain was able to reduce the level of TBARS and free radical generation.

3.9. Hepatic lipids (TG and TC)

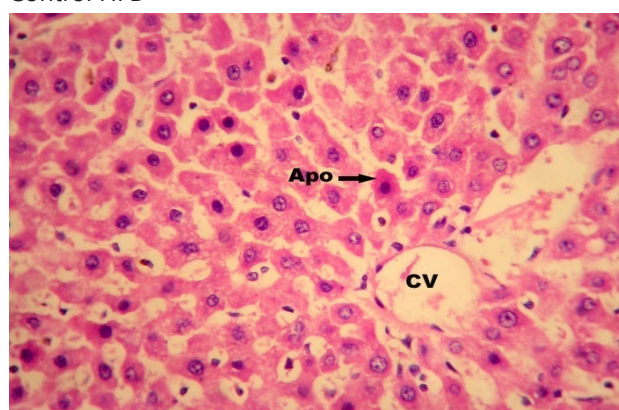
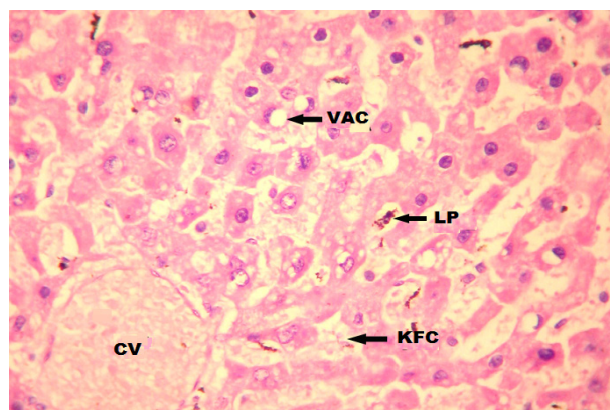
HFD and T alone groups showed an increase in hepatic TG and TC levels ($p < 0.001$) as compared to the control group. The AA group showed significant ($p < 0.001$) changes in the case of hepatic TC, while in the case of hepatic TG, AA showed less significant ($p < 0.05$) alteration as a comparison to the control group. But AA1 and orlistat treatment group showed non-significant changes in hepatic TG and TC levels as compared to the control group. AA, AA1, and orlistat treatment groups decreased hepatic TG and TC ($p < 0.001$) as compared to the HFD group, while T treatment decreased hepatic TG and TC levels ($p < 0.05$) as compared to the HFD group (Table 2). AA and T alone treatment groups significantly increased hepatic TG and TC levels as compared to the orlistat group, while the AA1 treatment group did not show a significant increase in hepatic TG and TC levels as compared to the orlistat treatment group. AA1 treatment group significantly decreased hepatic TG and TC levels as compared to the AA treatment group, while T alone treatment group significantly increased hepatic TG level as compared to AA treatment group.

3.10. Histopathological analysis of liver

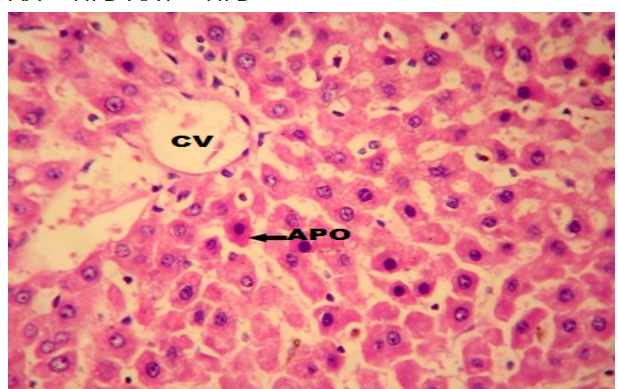
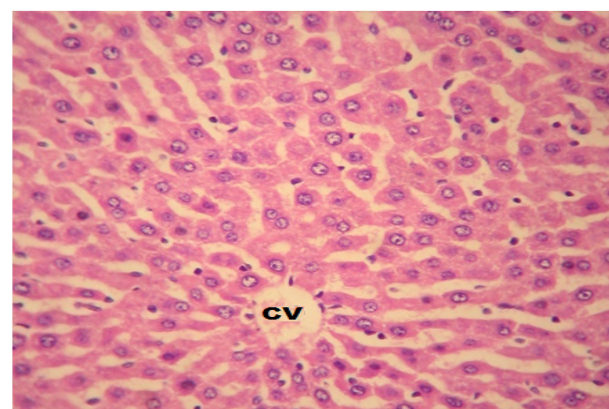
Our histological examination of the liver demonstrated steatosis, hepatocellular ballooning, lobular inflammation, and fibrosis in liver tissues of the HFD group as a comparison to the control group. Hepatocytes vacuolation, the larger size of central vein, Kupffer cell hypertrophy, lipofuscin pigment, and apoptotic hepatocytes were also observed in the HFD group (Fig. 1 and Table 2). AA, AA1, T, and orlistat treatment prevented or attenuated the level of steatosis, hepatocellular ballooning, lobular inflammation, and fibrosis significantly in liver tissues as a comparison to the HFD group. AA, AA1, T, and orlistat treatment prevented or attenuated hepatocytes vacuolation, the larger size of central vein, Kupffer cell hypertrophy, lipofuscin pigment, and apoptotic hepatocytes (Fig. 1 and Fig. 2).



Control HFD



AA + HFD AA1 + HFD



T + HFD Orlistat + HFD

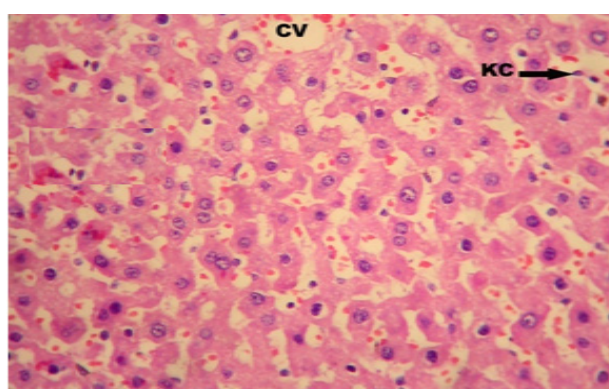


Fig. 1. Photomicrograph (hematoxylin and eosin, Magnification $\times 400$) of the hepatic tissue from the various experimental groups. Positive control and treated rats were fed the HFD for 42 days, while control rats received standard rat chow (NPD).

AA and T alone treatment groups showed a significant increase in steatosis, hepatocellular ballooning, lobular inflammation, and fibrosis score as compared to the orlistat group, while the AA1 treatment group did not show a significant increase in steatosis, hepatocellular ballooning, lobular inflammation, and fibrosis score as compared to orlistat group. The AA1 treatment group showed a significant decrease in steatosis, hepatocellular ballooning, and fibrosis score as compared to the

AA treatment group, while T alone treatment group showed an increase in hepatocellular ballooning and lobular inflammation score as compared to the AA treatment group. Macrovesicular steatosis (hepatocytes vacuolation), the larger size of central vein, Kupffer cell hypertrophy, lipofuscin pigment, and apoptotic hepatocytes were observed in the HFD group (Fig. 1). The hallmark of NAFLD is TG accumulation in the cytoplasm of hepatocytes (Shetty et al., 2010). AA, AA1, thymol, and

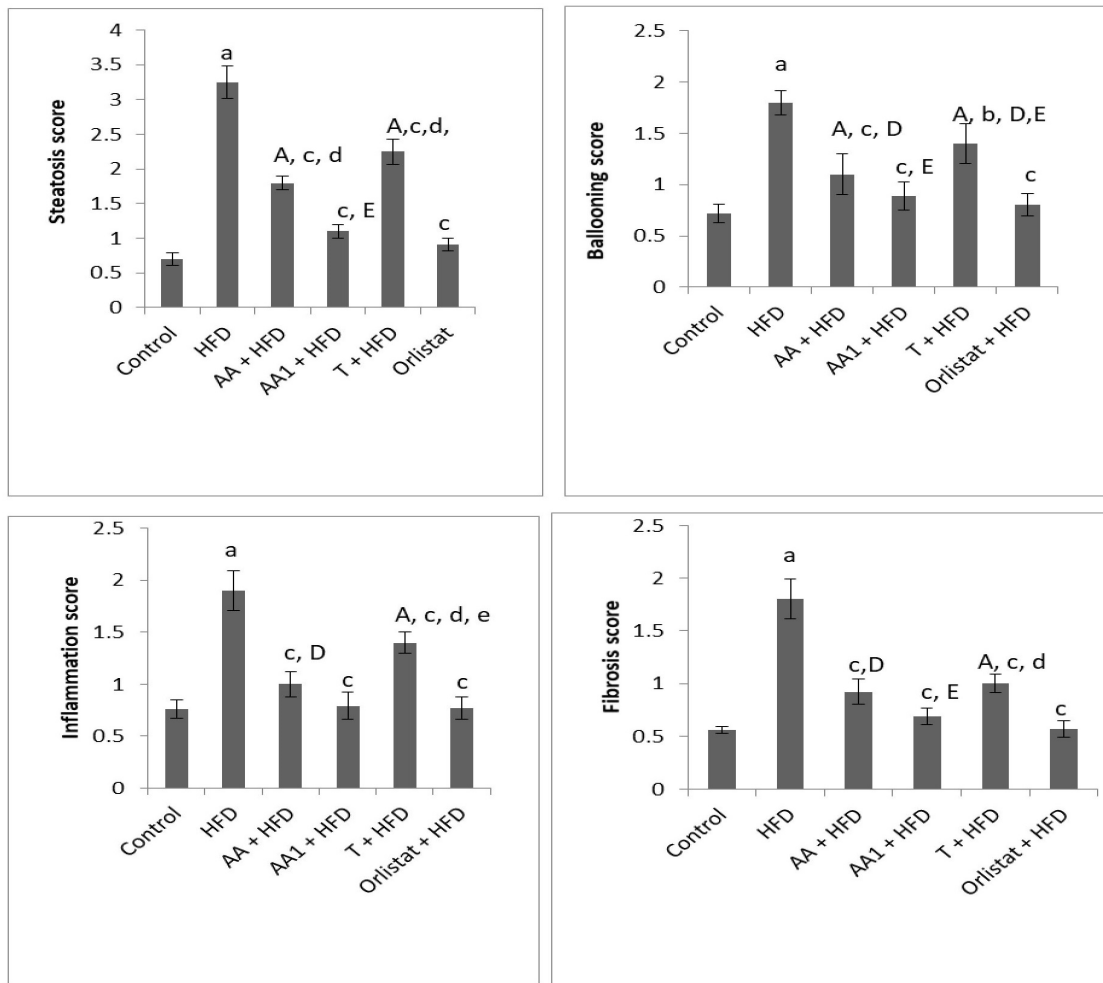


Fig. 2. Effect of AA, AA1, T, and orlistat on liver histology and pathological scores concerning steatosis, hepatocellular ballooning, inflammation, and fibrosis. All values were expressed as mean \pm SEM (n= 6). ^a $p < 0.05$, ^a $p < 0.001$ as compared to control group. ^b $p < 0.05$. ^c $p < 0.001$ as compared to HFD group. ^d $p < 0.05$, ^d $p < 0.001$ as compared to orlistat group. ^e $p < 0.05$, ^e $p < 0.001$ as compared to AA group. Positive control and treated rats were fed the HFD for 42 days, while control rats received standard rat chow (NPD).

orlistat treatment significantly decreased hepatic lipids levels as compared to the HFD group (Table 2). Moreover, AA, AA1, T, and orlistat administration noticeably attenuated and prevented the extent of macrovesicular steatosis, Kupffer cell hypertrophy, and development of lipofuscin pigment and apoptotic hepatocytes. AA, AA1, T, and orlistat treatment also significantly decreased hepatocellular ballooning, lobular inflammation, and fibrosis, suggesting that arq ajwain may be considered as potential promising candidate for the protection of nonalcoholic steatohepatitis (Figs 1-2 and Table 2). But thymol alone administration attenuated and prevented the less extent of macrovesicular steatosis, Kupffer cell hypertrophy, and development of lipofuscin pigment and apoptotic hepatocyte as compared to AA1 and AA treatment. AA1 showed more therapeutic efficacy as compared to AA (Table 2). While AA showed more therapeutic efficacy as compared to thymol alone treatment on obese rats (Table 2). GC study demonstrated that thymol comprises approximately 49% of the

phytochemical composition of the AA test material (Table 1). Additionally, β -pinene (2.7%), *ortho*-cymene (19.9%), γ -terpinene (24.1%), and thymol comprise a total of 95.7% of the identified phytochemicals. Thus, AA contained 14 mg of these 4 compounds, a value closer to the 12 mg T in the thymol-only treatment. While AA1 contained 19 mg of these 4 compounds. That is why AA showed more therapeutic efficacy as compared to T alone treatment and AA1 showed more therapeutic efficacy as compared to AA treatment. It has also been reported that β -pinene, *ortho*-cymene, γ -terpinene has a strong protective effect against liver injuries and NAFLD (Rasooli et al., 2016; Sinha et al., 2020). Thus, the obtained results showed that arq ajwain and thymol have a potential anti-NAFLD effect on HFD induced obese rat model. Our results also suggest that the traditional herbal preparations have good quality and therapeutic efficacy without any risk of significant adverse effects compared to synthetic medicine.



4. Concluding remarks

Based on the outcomes of our current study, it can be concluded that arq ajwain has a strong protective effect against obesity and nonalcoholic fatty liver disease without showing any adverse effects. This protective effect of arq ajwain against NAFLD on HFD induced obese rats is achieved through regulating serum and hepatic lipids via improvement in leptin and insulin sensitivity, inhibition of pancreatic lipase action, promoting antioxidant defense, and decreasing enzyme activities. In addition, arq ajwain did not alter food and water intake and hence it may be considered as an excellent alternative strategy for developing effective and safe antiobesity and hepatoprotective drugs in the future against dietary-induced obesity and NAFLD without showing any change in food and water intake.

Conflict of interest

The authors declared that there is no conflict of interest.

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