Research Article

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LC-HRMS Analysis and Antihyperlipidemic Effect of Ethanolic Leaf Extract of *Momordica charantia* L.

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ABSTRACT

Momordica charantia L. (Bitter melon) has been used to treat hyperglycemia and hyperlipidemia in many parts of the world. The present study revealed antihyperlipidemic activity of ethanolic leaf extract of M. charantia L. (named as EMC). The ethanolic extract of leaves was prepared and phytochemical constituents were identified using liquid chromatography linked with mass spectrophotometry. LC-HRMS study indicates the presence of phenolic compound (m-hydroxy benzoic acid, octyl gallate, 3-hydroxycoumarin), triterpenoids (momordicin II, momordicoside E and momordicoside K), saponin E and fatty acids were the major constituents in EMC. Whereas, Triton X-100 induced hyperlipidemic rat model was used to evaluate antihyperlipidemic activity of EMC at a dose of 50, 100 and 200 mg/kg, b.w. or atorvastatin (10 mg/kg, b.w.). The plasma total cholesterol, triglycerides, HDL, LDL and VLDL level, hepatic cholesterol and triglyceride, fecal cholesterol and triglycerides level were checked. Triton X-100 significantly ($P < 0.01$) increased the serum total cholesterol, triglycerides and LDL with a concomitant reduction in HDL cholesterol. These alterations were ameliorated by EMC at dose dependant manner. EMC (200 mg/kg, b.w) showed significant ($P < 0.01$) reduction in lipid level among three doses of extracts in comparison with the standard drug atorvastatin. Overall results findings suggest that bitter melon may have potential to use as supplementary ingredient for the prevention of hyperlipidemia and related conditions.

Keywords: Hyperlipidemia, *Momordica charantia* L., Triton X-100, HDL, LC-HRMS, SGOT, SGPT

Introduction

Hyperlipidemia is associated with an increase in plasma concentrations of cholesterol (CH), triglycerides (TG), low density lipoprotein (LDLc), and very low density lipoprotein (VLDLc), as well as a decrease in high density lipoprotein concentrations (HDLc). Hyperlipidemia is a major risk factor for the progression and development of cardiovascular illnesses, which can result in serious complications such as coronary artery disease, myocardial infarction, atherosclerosis, and ischemic stroke [1, 2]. In humans, the *de novo* pathway produced around 70% of total cholesterol, with absorption from diet providing the remaining 0.5 g/day [3]. To regulate blood cholesterol levels, several strategies have been used, including dietary fat balance, the use of statins, nicotinic acid derivatives, fibrates, and cholesterol absorption inhibitors [4]. While there are many synthetic medications available to treat hyperlipidemia, many of them have multiple adverse effects and interfere with drug metabolism [5]. As a result, the search for natural sources of plasma cholesterol lowering goes underway. Plants having hypolipidemic qualities are being explored more and more for their possible utility in the management of cardiovascular health. Plants produce a variety of bioactive compounds with varying properties that are effective, have few or no side effects, and are less expensive than synthesized drugs [6].

Momordica is one of the most numerous genera in the Cucurbitaceae family, with roughly 59 species. *M. charantia* L. is a taxonomic group that has been extensively explored for its anti-hyperlipidemic and anti-obesity properties [7]. In nature, several phenotypes of *M. charantia* exist, with notable variability in fruit size and shape. *M. charantia* L. is widely used in traditional Chinese, Indian, and Sri Lankan medicine to treat a range of diseases [8]. *M. charantia* has been demonstrated in recent medical studies to have hypoglycemic, hypolipidemic, anti-tumor, antibacterial, antioxidant, anti-viral, hepatoprotective, immunological regulator, and other effects [9]. Flavonoids, saponins, triterpenes, phenolics, steroids, polysaccharides, proteins, peptides, and alkaloids are among the active constituents identified from *M. charantia* [10]. In India, Sri Lanka, and other countries, *M. charantia* was utilized to make nourishing meals, hypoglycemic medications, emetics, and laxatives [11]. Several investigations have demonstrated that *M. charantia* extract has anti-obesity effect and consequently has excellent therapeutic value [12-16]. The growing body of research points to the lipid-lowering properties of *M. charantia* L.'s fruit, root, seeds, and stem; however the effect of the leaves on hyperlipidemia or dyslipidaemia remains unknown. To the best of our knowledge, this is the first study to look into the antihyperlipidemic activity of *M. charantia* L. leaf extract.

In this study, we use LC- HRMS profiling to identify phytoconstituents in *M. charantia* L. leaves and examine the antihyperlipidemic potential in Triton X-100-induced hyperlipidemia.

Materials and Methods

Chemicals and drugs

Triton X-100 and ethanol were purchased from Fine-chem industries, Mumbai. Atorvastatin used was a product of Emcure pharmaceutical Pvt. Ltd., Bari-Bramhana, Jammu. All other chemicals were of analytical grade and procured from S. D. Fine Chemicals, Mumbai. The biochemistry kits were purchased from Pathozyme diagnostics, MIDC Kagal, Maharashtra.

Plant material and preparation of extract

Momordica charantia L. leaves were harvested in the Latur district of Maharashtra. The leaves were identified and authenticated by the Botanical Survey of India's Western Regional Centre in Pune, Maharashtra. The voucher specimen (BSI/WRC/100-1/TECH./2019/76-1) was stored in the Department's herbarium.

M. charantia dried leaves were ground into a coarse powder using an electric blender. The powdered leaves were defatted with petroleum ether and macerated in 70% ethanol three times with occasional shaking for 48 hours. The extract was filtered and concentrated under reduced pressure to provide 52 g of ethanolic extract (EMC), which was stored in a refrigerator at 4 ^{0}C for later use.

Identification of phytoconstituents using LC-HRMS

LC- HRMS was used to determine the chemical components of EMC (Agilent Technologies, USA). The HPLC was linked to a mass spectrometer equipped with an electro spray ionization (ESI) source via a

Q-TOF (quadrapole time of flight) interface. For analysis, a Hypersil GOLD C18 column (100 × 2.1 mm i.d.) was employed. A total flow rate of 0.3 mL/min was used to supply the solvents. The extracts were injected into the analytical column in a volume of 5 µL for analysis. Full scan mode was used from 150 to 1000 m/z with a source temperature of 250 ^{0}C. The MS spectra were obtained in positive ion mode, with the drying gas at 300 ^{0}C, a gas flow rate of 13 mL/min, a nebulizing pressure of 35 psi, and a total run period of 30 minutes. The spectrum database for organic molecules was used to identify the mass fragmentation.

Acute oral toxicity

The acute oral toxicity of EMC was evaluated in accordance with OECD recommendations 423. The study included nine (9) female rats at doses of 300 mg/kg and 2000 mg/kg and was monitored for clinical symptoms of toxicity and mortality for up to 14 days [17].

Animals

Thirty-six (36) Wistar albino male rats weighing 180-200 g were acclimatized to the laboratory at a temperature of 22 ± 3 ^{0}C, relative humidity of 55 ± 5 %, and an illumination cycle of 12 hours light and 12 hours dark. They were housed in standard polypropylene cages of six rats each, fed standard rat pellets, and given water ad libitum. The ethical committee approves the experimental study and having details as CPCSEA Registration No. 2030/PO/RcBiBt/S/18/CPCSEA and study code was CRY/2021/018.

Induction of hyperlipidemia and Animal treatment

After an overnight fast of 18 hrs, thirty (30) experimental rats were given a single intraperitoneal injection of freshly prepared Triton X-100 (100 mg/kg b.w.) solution in normal saline [18]. Six (6) groups of six rats each were formed using a random number generator. Five groups of rats were given Triton-X 100 to produce hyperlipidemia, whereas the remaining group was given normal saline. The rats with hyperlipidemia were given either the conventional medication atorvastatin or a single dosage of *M. charantia* extract orally for seven days.

Group I: Control (received normal saline)

Group II: Hyperlipidemic control (HC; Triton X-100 + treated with normal saline)

Group III: Hyperlipidemic rats treated with 50 mg/kg, b.w./day ethanolic extract of *M. charantia* (EMCL)

Group IV: Group V: Hyperlipidemic rats treated with 100 mg/kg, b.w./day ethanolic extract of *M. charantia* (EMCI)

Group V: Hyperlipidemic rats treated with 200 mg/Kg b.w./day ethanolic extract of *M. charantia* (EMCH)

Group VI: Hyperlipidemic rats administered with 10 mg/kg, b.w. atorvastatin (ATV)

Biochemical analysis

The rats were sacrificed 24 hours after the last dose, and blood was taken from the jugular vein into ethylenediaminetetraacetic acid (EDTA) for hematological examination. The protocol specified in commercial kits was used to determine plasma total cholesterol (TC), triglycerides (TG), HDL cholesterol and LDL cholesterol, SGPT, and SGOT (Pathozyme Diagnostic kits). VLDL was determined using the well-known formula of triglyceride/5. The formula was used to calculate the cardiac index (CI) is TC/HDLc, atherogenic index, and coronary artery index was calculated by formulae below [19].

Atherogenic index (AI) = (Total cholesterol −HDLc) / HDLc

Coronary artery index (CAI) = LDLc / HDLc

Liver tissues were extracted overnight using a chloroform: methanol (2:1 v/v) combination. The mixture was filtered, and 6 mL of 0.7 % NaCl was added to the filtrate, vortexed, and centrifuged at 2000 rpm for 10 minutes to obtain supernatants, which were stored in bottles and kept frozen until further analysis, was required. Pathozyme Kits were used to calculate hepatic total cholesterol (TC) and triglycerides (TG). All rats' feces were collected during the first three days and the last three days of the investigation. The feces were dried and pulverized to a fine powder at 40 ^{0}C. The faecal cholesterol and triglycerides content were measured using the same procedure described for liver.

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Histopathological studies

The liver sample was stored in 10% formalin. The specimen was dehydrated in ethanol, cleaned in xylene, and paraffinized. Sections 3-5 mm thick were cut and stained with hematoxylin and eosin. It was examined under a light microscope at a magnification of 40x to demonstrate hepatic pathological alterations and the extract's efficacy in alleviating these pathological features.

Statistical analysis

Data represented as mean of six replicates ± SD. The Graph Pad Prism v5.0 (Graph Pad Prism Inc., La Jolla, CA, USA) was used to analyze and present the data. The data were subjected to one way analysis of variance (ANOVA) followed by Dunnett multiple comparison test. Significant level tested at $p < 0.05$.

Results

Identification of phytoconstituents of EMC

Table 1 listed the compounds found in an ethanolic leaf extract of *M. charantia*. The main components were phenolics (m-hydroxy benzoic acid, octyl gallate, 4-methylumbelliferyl acetate), saponin E and cucurbitane triterpenes, momordicin II, momordicoside K, and momordicoside E. The mass spectrum of several phytoconstituents found in *M. charantia* L was depicted in Figure 1. Other substances included unsaturated fatty acids, 4, 5-dihydrovomifoliol, 19-Methoxypomolic acid 3-arabinoside, mycinamicin III, and 3-hydroxycoumarin.

Acute oral toxicity

According to the findings of the study, EMC did not produce mortality in female rats given doses of 300 mg/kg and 2000 mg/kg. All of the animals showed no clinical indicators of toxicity immediately after treatment and no clinical signs of intoxication during daily assessments for up to 14 days. According to OECD Guidelines 423, the LD_{50} value of EMC was found to be in Globally Harmonized System (GHS) Category 5 (LD_{50} > 2000-5000 mg/kg body weight) and safe for usage.

Effect of EMC on Lipid profile

As indicated in Table 2, plasma TC, TG, LDL, and VLDL levels were considerably (P<0.01) raised in Triton X-100-induced rats, whereas HDL levels decreased abruptly when compared to the control group. In comparison to hyperlipidemic rats, oral treatment with EMC (50,100, and 200 mg/kg, b.w.) and atorvastatin (10 mg/kg, b.w.) for seven days after Triton X-100 treatment showed that TC, TG, LDL, and VLDL levels were significantly reduced and HDL levels were significantly increased in the groups.

In Triton X-100-treated rats, a rise in the hepatic levels of TC and TG was observed as shown in Table 3. After treatment with atorvastatin 10 mg/kg b.w., 50, 100, and 200 mg/kg bodyweight of the extract, the change of both plasma and hepatic lipid profiles was reduced in hyperlipidemic rats. The antihyperlipidemic activity of the ethanolic extract of *M. charantia* was concentration dependent, with the extract at 200 mg/kg b.w. having the highest (P< 0.01) antihyperlipidemic potential.

When compared to hyperlipidemic rats, the CAI, AI, and CI indices of rats treated with atorvastatin,50,100, and 200 mg/kg bodyweight of ethanol extract were considerably (P< 0.01) lower (Figure 2A-B-C). When compared to control group rats, CAI, AI, and CI of Triton X-100 induced rats increased significantly (P< 0.05).

In addition, when Triton X-100 treated rats were compared to control rats, fecal cholesterol and triglyceride levels were considerably lower (Table 4). In hyperlipidemic rats, atorvastatin and 50, 100, and 200 mg/kg bodyweight of ethanol extract increased cholesterol and triglyceride excretion rates in the feces. In all of the treatment groups, fecal excretion of cholesterol and triglycerides increased significantly in the last three days compared to the first three.

Effect of EMC on hepatic enzymes

The serum antioxidant activity of enzymes SGOT and SGPT are also used to assess liver injury. As shown in Figure 3A-B, the activities of SGOT and SGPT were significantly higher in Triton X-100 induced hyperlipidemic rats compared to the control groups, implying that major oxidative damage had occurred in the liver. These enzyme levels were dramatically lowered in hyperlipidemic rats treated with ethanolic extract at a dose of 200 mg/kg b.w. In compared to hyperlipidemic rats, hyperlipidemic rats given atorvastatin and ethanolic extract at a dose of 200 mg/kg had significantly lower SGOT and SGPT activity.

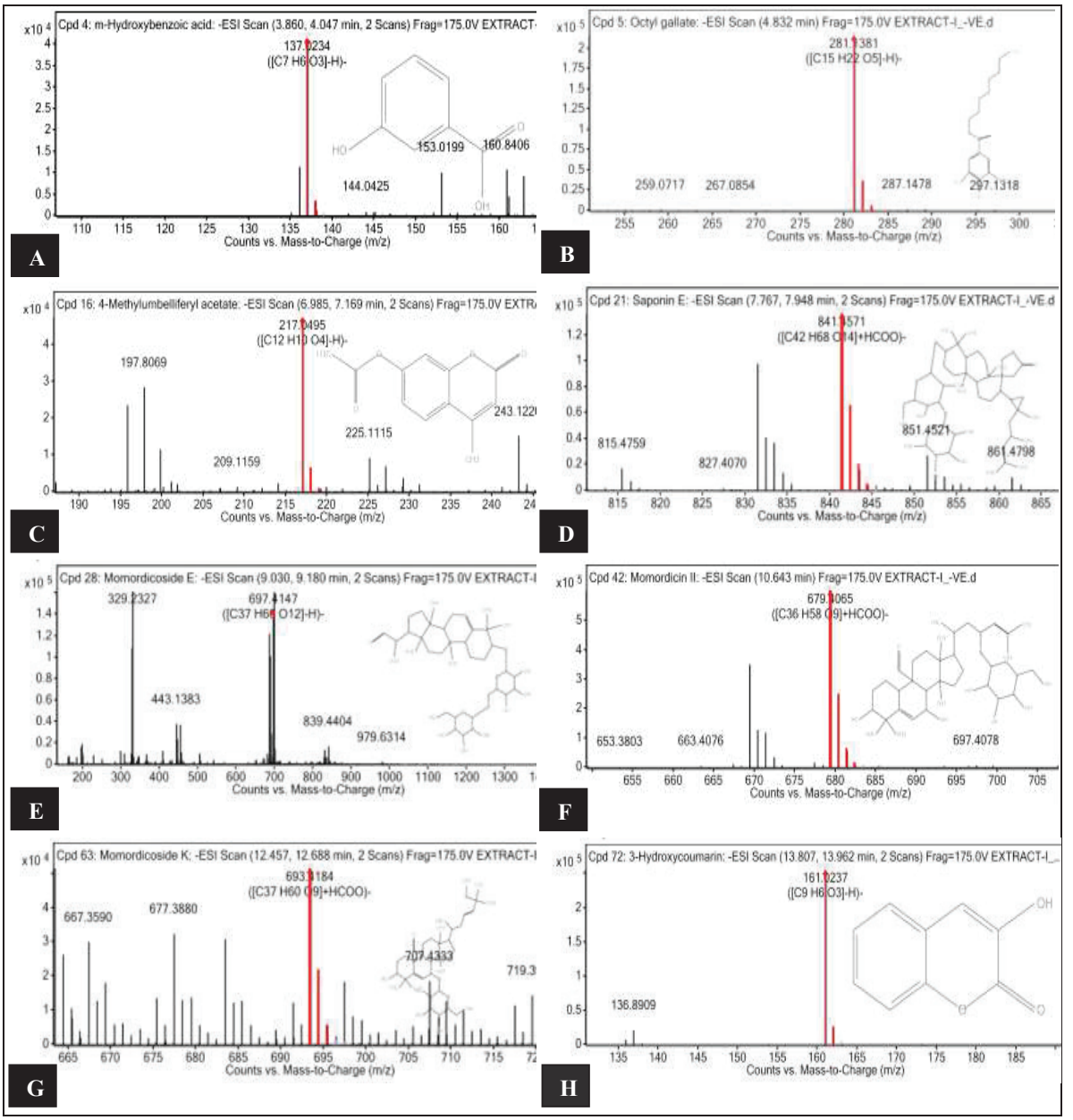

Figure 1. High resolution mass spectrum (HRMS) of identified phytoconstituents of ethanol leaf extract of *M. charantia* L. (A): m-hydroxy benzoic acid; (B): octyl gallate; (C): 4-methylumbeliferyl acetate; (D): saponin E; (E): momordicoside E; (F): momordicin II; (G): Momordicoside K; (H): 3-hydroxycoumarin.

Histological evaluation of liver and kidney

The hypolipidemic effects of EMC were validated in this investigation by histological evaluation of liver and kidney tissues. In the control, atorvastatin (10 mg/kg, b.w.) and ethanolic extract (200 mg/kg, b.w.) groups, the liver sections exhibited normal hepatocellular morphology and no histological abnormalities (Figure 4A-F). The livers of hyperlipidemic rats given EMC and atorvastatin at a dose of 200 mg/kg showed normal histology of hepatic cells, less serious loss of liver histoarchitecture, and well preserved cytoplasm when compared to the control group. Triton X-100 (mg/kg, b.w.) treatment, on the other hand, resulted in a significant loss of hepatocytes, hepatic necrosis, and inflammatory cell infiltration. The hyperlipidemic animals given 50 and 100 mg/kg b.w.

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Table 1. Identified phytochemical constituents of ethanolic leaf extract of *M. charantia* L. by LC- HRMS

Phytoconstituents	RT (min)	Abund	Mass	Formula	Diff (ppm)	Ion
m-hydroxy benzoic acid	3.971	41289.72	138.0307	$C_7H_6O_3$	7.29	$(M-H)^-$
Octyl gallate	4.844	215032.16	282.1453	$C_{15}H_{22}O_5$	4.84	$(M-H)^-$
4,5-Dihydrovomifoliol	6.305	37069.68	226.1551	$C_{13}H_{22}O_3$	6.67	$(M+HCOO)^-$
4-Methylumbelliferyl acetate	7.095	47356.01	218.0567	$C_{12}H_{10}O_4$	5.44	$(M-H)^-$
Saponin E	7.877	135831.13	796.4587	$C_{42}H_{68}O_{14}$	2.41	$(M+HCOO)^-$
Momordicoside E	9.118	136483.45	696.4118	$C_{37}H_{60}O_{12}$	2.22	$(M-H)^-$
Momordicin II	10.655	599800.63	634.4077	$C_{36}H_{58}O_9$	-0.26	$(M+HCOO)^-$
Mycinamicin III	10.733	1004442.94	680.4132	$C_{36}H_{59}NO_{11}$	1465.25	$(M-H)^-$
9Z-Octadecenedioic acid	11.637	44309.75	312.229	$C_{18}H_{32}O_4$	3.3	$(M-H)^-$
19-Methoxypomolic acid 3-arabinoside	12.186	60292.59	618.4109	$C_{36}H_{58}O_8$	3.53	$(M+HCOO)^-$
Momordicoside K	12.589	51259.62	648.4194	$C_{37}H_{60}O_9$	5.15	$(M+HCOO)^-$
3-Hydroxycoumarin	13.9	254565.97	162.031	$C_9H_6O_3$	4.29	$(M-H)^-$
3,5-Dichloro-4-hydroxy 2-methoxy-6-methylbenzoic acid	20.477	98584.28	249.9797	$C_9H_8C_{12}O_4$	1.1	$(M-H)^-$

Table 2. Effect of ethanolic leaf extract of *M. charantia* L. on lipid profile of control and experimental rats

Groups	TC (mmol/l)	TG (mmol/l)	LDL (mmol/l)	VLDL (mmol/l)	HDL (mmol/l)
Control	79.54 ± 1.80^{c}	87.83 ± 2.72^{c}	27.16 ± 1.13^{c}	17.57 ± 0.54^{c}	33.68 ± 1.29^{c}
Hyperlipidemic	113.93 ± 3.19^{b}	188.70 ± 2.89^{b}	50.46 ± 1.77^{b}	37.80 ± 0.62^{b}	26.83 ± 1.17^{b}
Hyperlipidemic+EMCL	90.84 ± 1.79^{bc}	117.47 ± 4.47^{bc}	39.09 ± 1.37^{bc}	23.49 ± 0.89^{bc}	29.18 ± 0.5^{bc}
Hyperlipidemic+EMCI	88.04 ± 2.32^{bc}	114.74 ± 5.65^{bc}	37.37 ± 1.17^{bc}	22.95 ± 1.13^{bc}	30.09 ± 1.01^{bc}
Hyperlipidemic+EMCH	84.68 ± 2.76^{ac}	103.04 ± 3.09^{bc}	33.14 ± 1.25^{bc}	20.61 ± 0.62^{bc}	32.14 ± 1.1^{nsc}
Hyperlipidemic+ATV	82.01 ± 4.20^{nsc}	95.86 ± 3.42^{bc}	31.67 ± 1.46^{bc}	19.17 ± 0.68^{bc}	28.84 ± 0.84^{bc}

Values were in mean±SD (N=6), values with different superscript letters indicates significant differences compared with control group (a = $P^{\#}<0.05$; b = $P^{\#\#}<0.01$; ns = $P> 0.05$), and hyperlipidemic group (c = $P^{**}<0.01$) by ANOVA followed by Dunnett test.

Table 3. Hepatic cholesterol and triglyceride concentration of hyperlipidemic rats following oral administration of ethanolic leaf extract of *M. charantia*

Groups	Hepatic TC (mmol/l)	Hepatic TG (mmol/l)
Control	20.95 ± 0.84^{c}	0.33 ± 0.02^{c}
Hyperlipidemic	35.08 ± 2.12^{b}	0.73 ± 0.02^{b}
Hyperlipidemic + EMCL	28.97 ± 1.67^{bc}	0.48 ± 0.04^{bc}
Hyperlipidemic + EMCI	24.23 ± 2.01^{bc}	0.47 ± 0.03^{bc}
Hyperlipidemic + EMCH	21.08 ± 1.67^{nsc}	0.46 ± 0.02^{bc}
Hyperlipidemic + ATV	21.19 ± 1.41^{nsc}	0.40 ± 0.02^{bc}

Values are in mean±SD (N=6), values with different superscript letters indicates significant differences compared with control group (a = $P^{\#}<0.05$; b = $P^{\#\#}<0.01$; ns = $P> 0.05$), and hyperlipidemic group (c = $P^{**}<0.01$) by ANOVA followed by Dunnett test.

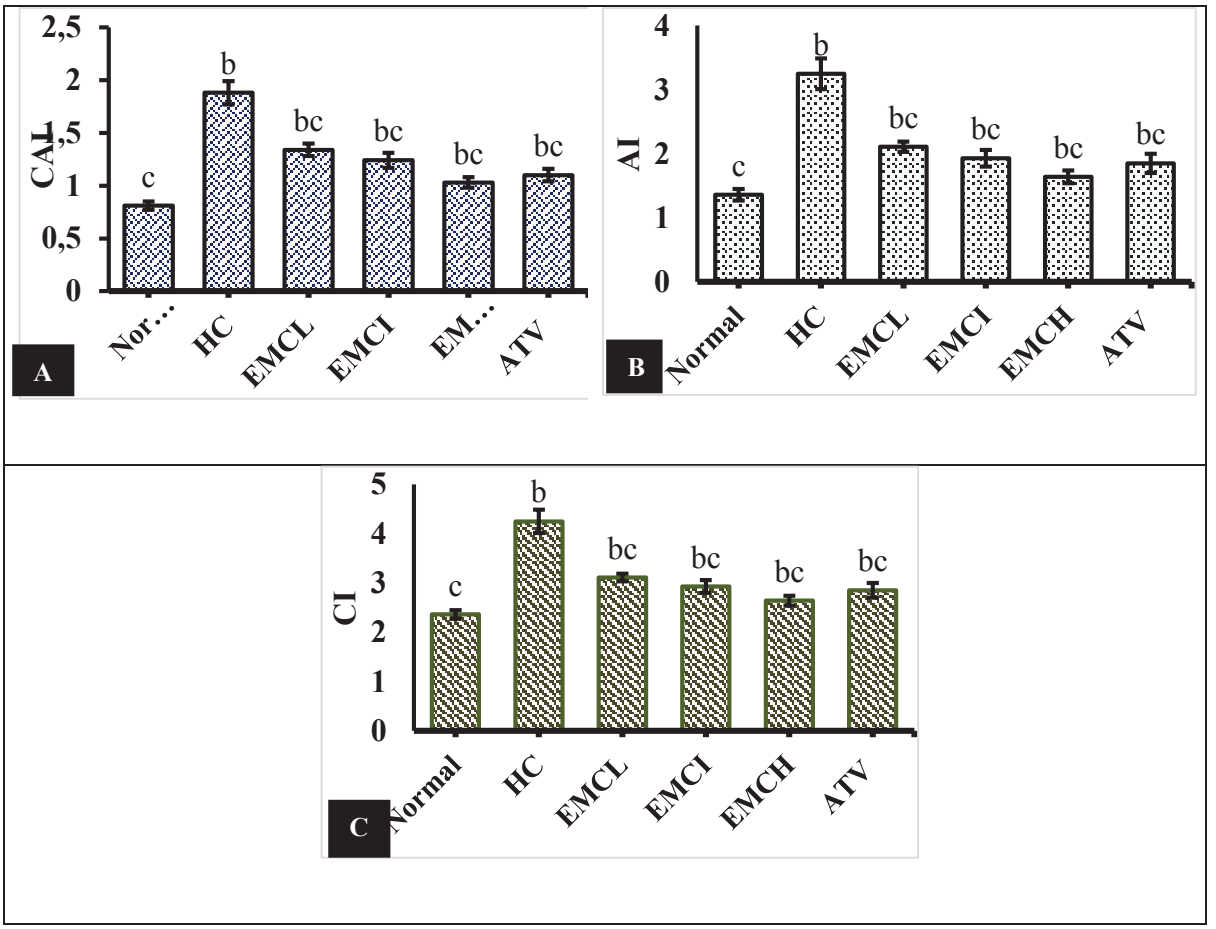

Figure 2. Effect of ethanol leaf extract of *M. charantia* on cardiac indices [Coronary artery index (A); Atherogenic index (B); Cardiac index (C)] in Triton X-100 induced hyperlipidemic rats. Values are in mean±SD (N=6), values with different superscript letters indicates significant differences compared with control group (a = $P^{\#}$<0.05; b = $P^{\#\#}$<0.01; ns = P> 0.05), and hyperlipidemic group (c = P^{**}<0.01) by ANOVA followed by Dunnett test. HC: Hyperlipidemic control, EMCL: EMC- low dose; EMCI: EMC- intermediate dose; EMCH: EMC- high dose.

Table 4. Fecal cholesterol and triglyceride of hyperlipidemic rats after treatment of ethanolic leaf extract of *M. charantia* L.

Groups	Fecal cholesterol (mmol/l)		Fecal triglycerides (mmol/l)	
	First 3 days	Last 3 days	First 3 days	Last 3 days
Control	50.13±1.92^{c}	50.88±1.82^{c}	0.83±0.03^{c}	0.84±0.04^{c}
Hyperlipidemic	36.43±1.35^{b}	33.74±1.56^{b}	1.16±0.04^{b}	1.17±0.03^{bc}
Hyperlipidemic + EMCL	37.72±3.24^{bns}	46.33±2.51^{nsc}	1.19±0.02^{bns}	1.65±0.03^{bc}
Hyperlipidemic + EMCI	38.02±2.21^{bns}	46.60±2.63^{nsc}	1.16±0.04^{bns}	1.71±0.03^{bc}
Hyperlipidemic + EMCH	36.88±3.30^{bns}	48.55±4.25^{nsc}	1.17±0.04^{bns}	1.81±0.03^{bc}
Hyperlipidemic + ATV	36.61±2.15^{bns}	54.36±4.02^{nsc}	1.15±0.04^{bns}	1.86±0.04^{bc}

Values are in mean±SD (N=6), values with different superscript letters indicates significant differences compared with control group (a = $P^{\#}$<0.05; b = $P^{\#\#}$<0.01; ns = P> 0.05), and hyperlipidemic group (c = P^{**}<0.01) by ANOVA followed by Dunnett test.

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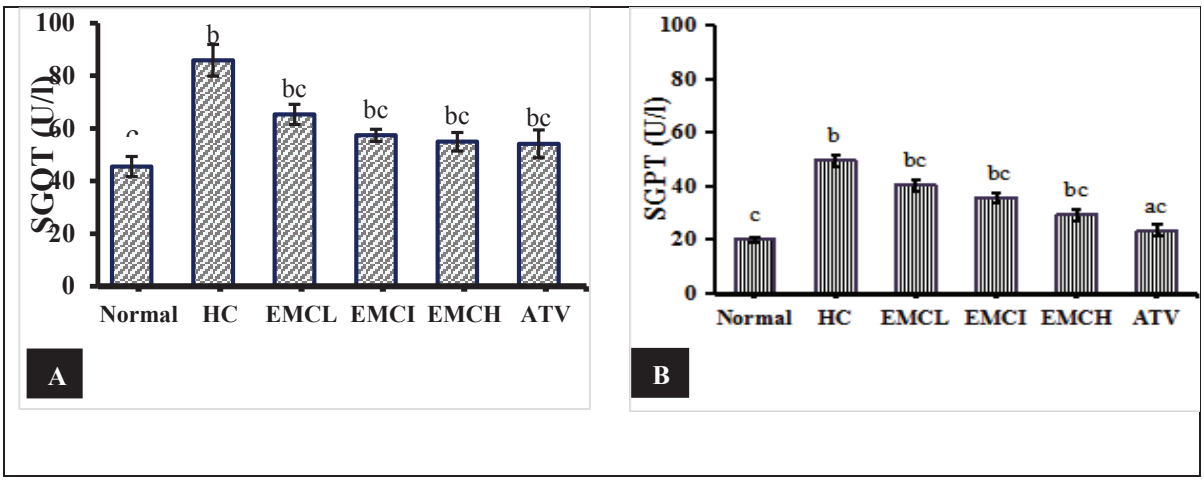

Figure 3. Effect of ethanol leaf extract of *M. charantia* on hepatic enzymes [SGOT (A); SGPT (B)] in Triton X-100 induced hyperlipidemic rats. Values are in mean±SD (N=6), values with different superscript letters indicates significant differences compared with control group (a = $P^{\#}$<0.05; b = $P^{\#\#}$<0.01; ns = P> 0.05), and hyperlipidemic group (c = P^{**}<0.01) by ANOVA followed by Dunnett test. HC: Hyperlipidemic control, EMCL: EMC- low dose; EMCI: EMC- intermediate dose; EMCH: EMC- high dose; ATV: atorvastatin.

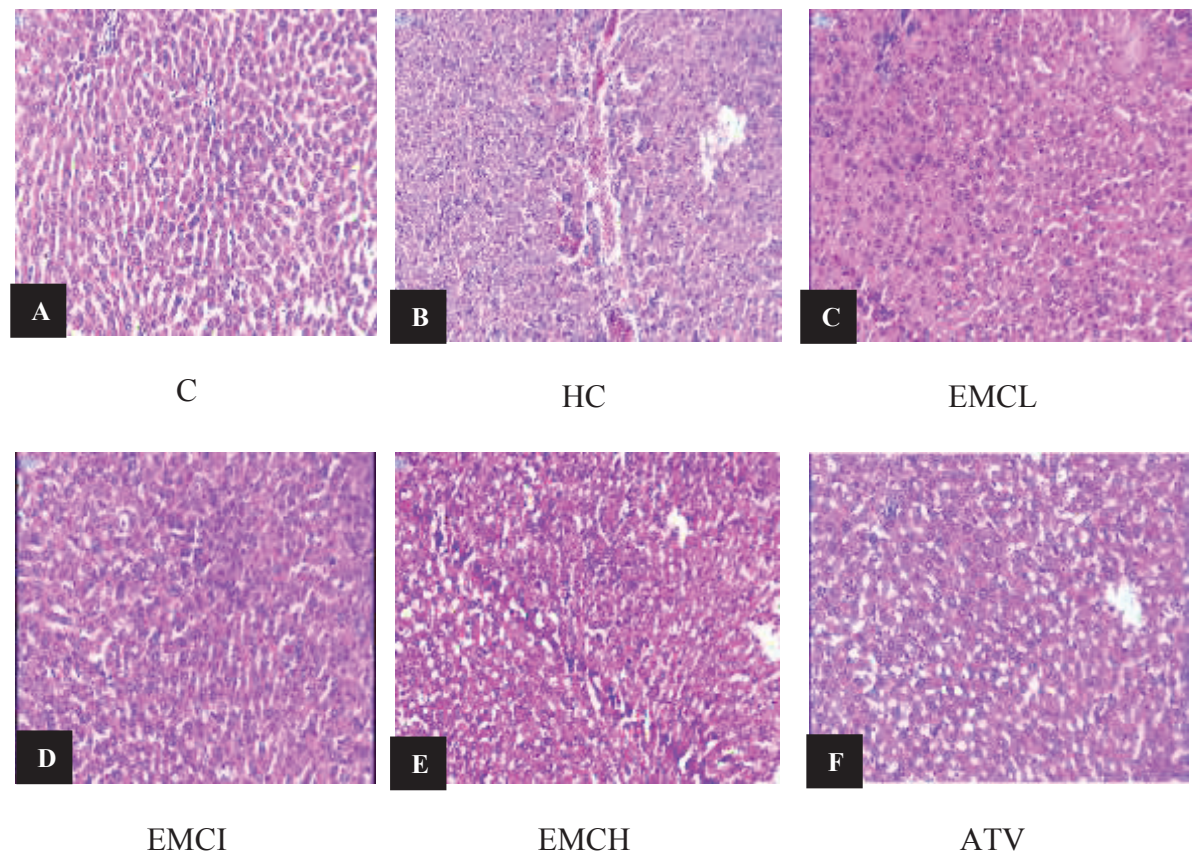

Figure. 4. Histopathological alteration in livers (A-F) of rats treated with Triton X-100 followed by ethanolic leaf extract of *M. charantia* Linn. C: normal control; HC: Hyperlipidemic control; EMCL= EMC- low dose; EMCI: EMC- intermediate dose; EMCH: EMC- high dose; ATV: Atorvastatin.

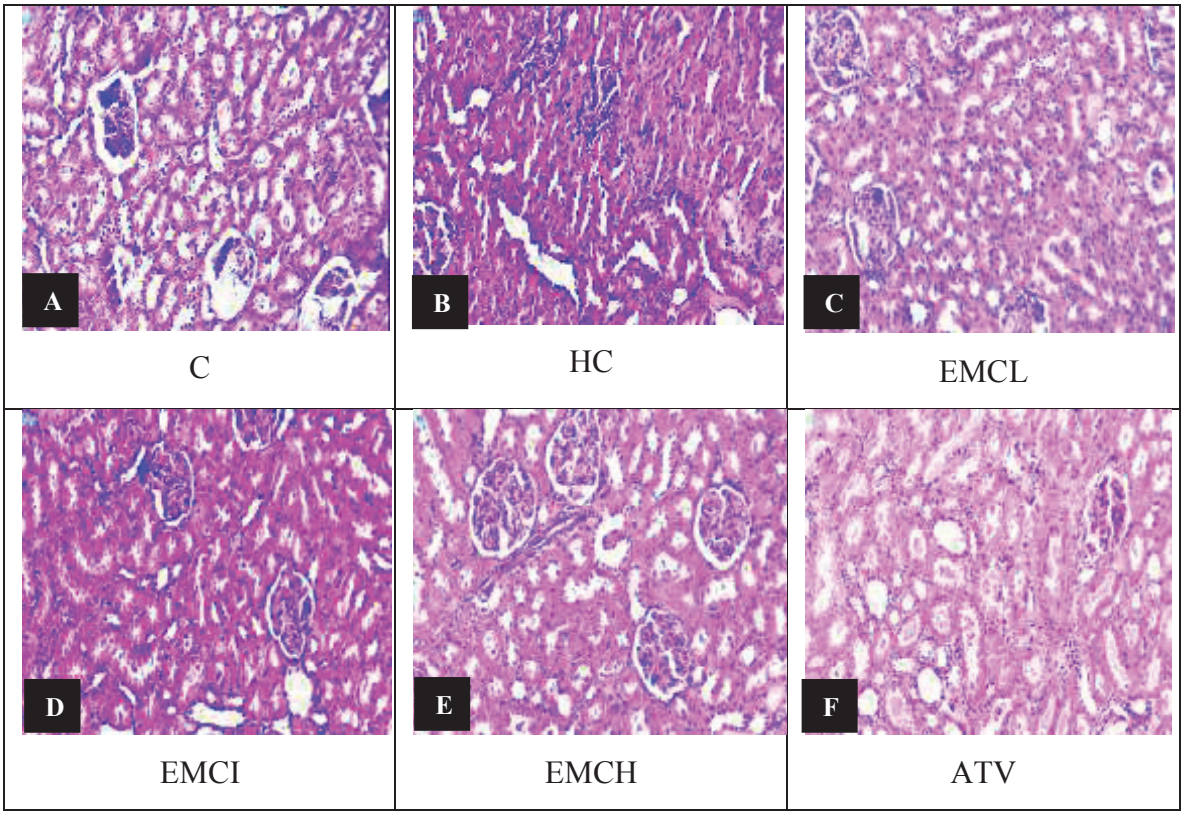

Figure 5. Histopathological alteration in kidneys (A-F) of rats treated with Triton X-100 followed by ethanolic leaf extract of M. charantia Linn. C: normal control; HC: Hyperlipidemic control; EMCL= EMC- low dose; EMCI: EMC- intermediate dose; EMCH: EMC- high dose; ATV: Atorvastatin.

dosages had modest to severe polymorph infiltration and hepatocellular degeneration, although not as much as the diseased animals.

The groups treated with normal saline, EMC, and atorvastatin had no histological abnormalities and normal nephrons that were arranged in a regular pattern. However, as seen in Figure 5A-F, animals treated with Triton-X 100 showed minor inflammation, minimal glomerular fibrosis, and mild tissue necrosis. Interestingly, the glomerular membrane of nephrons from hyperlipidemic rats treated with 100 mg/kg ethanolic extract revealed better nephrons infiltration, despite the presence of mild to moderate bleeding. The nephrons in the kidney segment taken from the high dose of ethanolic extract treated group had a normal appearance with no tissue infiltration. A similar pattern of kidney histoarchitecture was observed in the atorvastatin treated group compared to that of control groups.

Discussion

The Elevated levels of TC, TG, LDLc, and VLDLc, along with a reduction in HDLc, are indications of atherosclerosis and heart diseases [20]. Triton X-100 acts as a surfactant and inhibits lipase activity, resulting in increased blood lipid concentration. The biphasic character of Triton X-induced hyperlipidemia aids in the understanding of hypolipidemic agent mechanisms [21]. Several previous studies have reported an increase in plasma TC, TG, LDLc, and a decrease in HDLc in Triton X-100-induced hyperlipidemic rats [6, 22, 23]. A considerable reduction in HDLc [24] is a positive risk factor for the development of atherosclerosis, and a recent study found that the risk associated with hyperlipidemia can be reduced with *M. charantia*. The use of ethanolic extract may inhibit HMG-CoA reductase in a way similar to atorvastatin and reverse the hyperlipidemic induced lipid profile, demonstrating antihyperlipidemic activity [25]. The previous studies demonstrated that *M. charantia* fruit extract sup-

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plementation significantly modulated the lipid profiles in plasma, liver, and feces compared to mice fed the HFD [26, 27]. In the present investigation, EMC treated rats also had lowering the plasma TG, TC and LDLc along with liver TC and TG concentration. Treatment with the EMC also elevated plasma HDLc and fecal lipid level compared to disease control rats. The similar results have been found in previous studies, *M. charantia* has been shown to possess both hepatoprotective and hypolipidemic effects in rats with alloxan-induced diabetes with an elevation in the concentration of HDLc [28, 29]. The presence of momordicin II, momordicin E, momordicin K, saponin E, and phenolic substances enhanced plasma and hepatic lipid profiles [30].

According to one study, aqueous fruit extracts of *Solanum macrocarpon* decreased lipids in chronic Triton-induced hyperlipidemic rats by limiting lipid absorption and boosting faecal cholesterol excretion [31]. Similarly, an ethanol extract of *M. charantia* L. could account for the correction of hyperlipidemia-induced lipid profile changes by limiting cholesterol uptake. Coronary artery, atherogenic, and cardiac alterations are strong and reliable indicators of cardiovascular, coronary, and ischemic disorders. The greater the value of AI, CAI, and CI, may lead to the greater chances of acquiring cardiovascular disease, and vice versa [32, 33]. Increased indices of AI, CAI, and CI in Triton X-100 caused hyperlipidemic mice have previously been reported [34]. The considerable reduction in CAI, AI, and CI values reported in ethanolic extract-treated hyperlipidemic rats implies that the extract can protect against atherosclerosis, coronary artery disease, and cardiovascular disease.

The Triton-X 100 causes liver abnormalities, which have been linked to a change in hepatocytes membrane permeability, resulting in the release of enzymes into the bloodstream [35]. The findings current study suggested that *M. charantia* leaf extract preserved membrane integrity and prevented hepatic enzyme escape.

Conclusions

Based on the findings, it is possible to conclude that ethanol leaf extract of *Momordica charantia* (Linn.) not only resulted in a significant reduction in cholesterol, triglyceride, LDLc, and VLDLc levels, but also increased HDLc levels at a low dose level. It possesses anti-hyperlipidemic activity, as indicated by an improved lipid profile. More research on the isolated fractions and constituents is needed to isolate the molecule responsible for antihyperlipidemic activity and explain the mechanism by which *Momordica charantia* (Linn.) exerts antihyperlipidemic benefits.

List of abbreviations

EMC: Ethanolic extract of *Momordica charantia* L.; HDLc: High density lipoproteins; LDLc: Low density lipoproteins; VLDLc: Very low density lipoproteins; TC: Total cholesterol; TG: Triglycerides; LC- HRMS: Liquid chromatography- high resolution mass spectroscopy; ESI: Electro spray ionization; EMCL: EMC-Low; EMCI: EMC-intermediate; EMCH: EMC-high; ATV: Atorvastatin; AI: Atherogenic index; CAI: Coronary artery index; CI: Cardiac index; EDTA: Ethylene-diamine tetra acetic acid; OECD: Organization for economic cooperation and development; HFD: High fat diet; CPCSEA: Committee for purpose of control and supervision of experiments on animals; SGPT: Serum glutamic pyruvic transaminase; SGOT: Serum glutamic-oxaloacetic transaminase.

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Conflicts of Interests

The authors declare that they have no competing interests.

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