https://doi.org/10.31925/farmacia.2023.2.14 **ORIGINAL ARTICLE**

ROLE OF NITRIC OXIDE, IL-6, TNF-α AND PROSTAGLANDINS IN HEPATOPROTECTIVE EFFECT OF *ILLICIUM VERUM* ESSENTIAL OIL

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Abstract

The present study investigated the role of inflammatory cytokines, prostaglandins and nitric oxide in hepatoprotective effect of *Illicium verum* (*I. verum*) essential oil in CCl_4 induced hepatotoxicity. The levels of LFTs, SOD, CAT, nitric oxide, total protein contents, and inflammatory markers such as TNF-α, IL-6, lipid peroxidation and COX-2 were determined. Plasma proteins, blood lipid profile and liver biomarkers were estimated. GC/MS analysis of essential oil was carried out. Pre-treatment with *I. verum* enhanced SOD, CAT and plasma proteins levels while a reduction in LFTs, NO, TNF-α, IL-6, lipid peroxidation, COX-2 and blood lipid profile was observed in CCl_4 induced hepatotoxicity. Histopathological studies supported the biochemical findings. GC/MS showed the presence of trans-anethole (97.485%) along with other anti-oxidant and anti-inflammatory constituents. Thus, *I. verum* presented hepatoprotective potential *via* down-regulation of inflammatory mediators and up-regulation of anti-oxidant enzymes.

Rezumat

Studiul a investigat efectul hepatoprotector al uleiului volatil de *Illicium verum* (*I. verum*) într-un model experimental de hepatotoxicitate indusă cu CCl_4. A fost evaluat rolul citokinelor inflamatorii, al prostaglandinelor și al oxidului nitric. Au fost determinate nivelurile de LFT, SOD, CAT, NO, conținutul total de proteine și markeri inflamatori precum TNF-α, IL-6, peroxidarea lipidică și COX-2. S-a efectuat analiza GC/MS a uleiului esențial. Tratamentul inițial cu *I. verum* a îmbunătățit SOD, CAT și nivelul proteinelor plasmatice și a fost observată o reducere a LFT, NO, TNF-α, IL-6, a peroxidării lipidice, a COX-2 și a profilului lipidic. Studiile histopatologice au susținut constatările biochimice. GC/MS a evidențiat prezența trans-anetolului (97,485%) împreună cu alți constituenți antioxidanți și antiinflamatori. *I. verum* a prezentat un potențial hepatoprotector prin reducerea mediatorilor inflamatori și creșterea expresiei enzimelor antioxidante.

Keywords: *Illicium verum*, hepatoprotective, TNF-α, IL-6, lipid peroxidation

Introduction

Liver has the pivotal role in the metabolism of various molecules, transportation and elimination of xenobiotics and is therefore highly vulnerable to toxicity. Generally, hepatotoxicity is idiosyncratic, involving genetic variations, environmental exposures, lifestyle along with pre-existing pathological conditions [1]. The pathophysiology of chemically induced liver damage is still under investigations, however alleged to be associated with conversion of xenobiotics to reactive oxygen species (ROS) through metabolic pathway, that induce oxidative stress and cellular damage [2]. Oxidative stress is defined as disrupted balance between endogenous generated free radicals and antioxidant system, which is supposed to be a key factor associated with various kind of liver injuries like hepatitis, liver cirrhosis, carcinoma and inflammatory necrosis [3-5].

Carbon tetra chloride (CCl_4) is a well-known chemical for induction of acute hepatotoxicity in animals [6]. CCl_4 converts readily to its free radical form i-e, trichloromethyl (CCl_3*) by Cytochrome P450 enzyme system (P450-2E1) in liver. CCl_3* and its metabolite Cl_3COO* react with lipids in the membranes leading to peroxidation, which in turn is associated with disruption of membrane integration, necrosis and release of membrane associated enzymes [7, 8]. In addition to lipid peroxidation, loss of liver enzymes activities, increase in liver function tests (LFT) and pro-inflammatory mediators' activity because of inflammation, have also been reported in earlier studies. Tissue damage associated with hepatic inflammation can be mediated by the pro-inflammatory cytokines such as inducible nitric oxide synthase, interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α). TNF-α and IL-6 are considered major hepatotoxicity mediators in several experimental models of liver injuries [11].

The star designed fruit of Star Anise (*Illicium verum*) belonging to *Illiaceae* family, is indigenous to China. *Illicium verum* (*I. verum*) has reported pharmacological activities such as anti-inflammatory, antioxidant, gastro-protective [9] antiviral [10], antibacterial, antiseptic, expectorant, diuretic, anti-emetic, and for insomnia, stomach aches, rheumatic pain, constipation, flatulence, spasmodic pain, scabies and bedbugs [11, 12].

Based on the study performed previously for hepato-protective potential of *I. verum*, the current study has been designed to evaluate its *in-vivo* antioxidant and anti-inflammatory effects along with underlying mechanisms of action establishing its hepatoprotective effect in CCl_4 induced hepatotoxicity in rats.

Materials and Methods

Plant collection and essential oil preparation

The shade dried star shaped seeds of Star anise were purchased from local market (Lahore, Punjab, Pakistan). The seeds were washed with water, dried and powdered. Specimen has been kept in laboratory for reference purposes. Powdered mixture was placed in distillation flask and essential oil (EO) was extracted by the process of steam distillation with 4.0% yield.

Chemicals Used

Carbon tetra chloride (CCl_4) was purchased from Merck, Germany and formalin was obtained from local laboratory (analytical grade). Distilled water was also obtained from the laboratory unit. Analytical Kits for estimation of superoxide dismutase (SOD) activity, catalase (CAT) activity, total nitric oxide (NO) value, total proteins value, lipid peroxidation was purchased from Nanjing Jincheng Bioengineering Institute, China. The Assay Kits for TNF-α, IL-6 and cyclooxygenase-2 (COX-2) were purchased from Melson Medical Corporation Ltd., Shanghai, China.

Animals

Total thirty-five male Wister rats, weighing 200 ± 10 g were purchased from the Animal House of Faculty of Pharmacy, The University of Lahore. Rats were provided with food and water ad libitum. Temperature was maintained at 25 ± 5ºC and humidity 55 - 60% with a 12-hour light and dark cycle. Approval from Institutional Research Ethics Committee (IAEC), Faculty of Pharmacy, University of Lahore was obtained (PHMY-10A/2017).

Study Design

The animals, distributed into seven groups with five rats in each, were treated as follows: (1) Negative Control – Normal Saline (1 mL/kg); (2) Positive Control – Silymarin (50 mg/kg daily) + Normal Saline (1 mL/kg); (3) Diseased Control – Normal Saline (1 mL/kg) + single dose of CCl_4 (1 mL/kg); (4) EO100 – Star Anise essential oil (100 mg/kg) + Normal Saline (1 mL/kg); (5) EO100 + CCl_4 – Star Anise essential oil (100 mg/kg) + single dose of CCl_4 (1 mL/kg); (6) EO200 – Star Anise Essential oil (200 mg/kg) + Normal Saline (1 mL/kg); and (7) EO200 + CCl_4 – Star Anise Essential oil (200 mg/kg) + Single dose of CCl_4 (1 mL/kg).

Star Anise essential oil was administered by per oral gavage, suspended in normal saline in 1:1. Star Anise essential oil and normal saline were administered every day for 7 days. On the 7th day, 1 hour after the last dose administration of star anise essential oil and normal saline, animals were treated intraperitoneal with CCl_4 dissolved in olive oil in 1:1. After 24 hours, on the 8th Day, all the rats were anesthetized by chloroform (1 mL/kg) and then sacrificed.

Sample Collection

Blood samples were collected using cardio puncture for analysis of biochemical parameters. Liver samples were dissected and preserved in 10% formalin solution for histopathological studies. The liver homogenates were prepared by weighing accurately 1 g liver tissue and dissolving in 100 mL normal saline solution and mixing with the help of homogenizer in a cool water bath with temperature maintained around 4ºC (10% liver homogenate solution). 1 mL of 10% liver homogenate solution was taken and mixed with 100 mL normal saline to prepare 1% liver homogenate solution. 1% and 10% liver homogenates solutions were used in analysing various biochemical parameters of liver.

Serum Biochemical Parameters

Total lipids, cholesterol, triglycerides, high density lipoproteins (HDL), very low-density lipoproteins (VLDL), low density lipoproteins (LDL), total bilirubin, enzyme markers of hepatocellular damage including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, proteins including albumin and globulin were determined in serum samples. All experiments were performed in triplicate by Olympus AU 400 auto-analyser (Hamburg, Germany).

Oxidative Stress and inflammatory markers

Oxidative stress was estimated by measuring superoxide dismutase (SOD), catalase (CAT), total nitric oxide value and total protein contents, and inflammatory markers such as TNF-α, IL-6, lipid peroxidation, COX-2 levels were estimated using analysing Kits (Nanjing Jincheng Bioengineering Institute, China).

Histopathological Studies

Liver samples were cut into 5 µm section and stained with Haematoxylin and Eosin (H&E) to estimate hepatocytes necrosis and vacuolization in cytoplasm. Morphological changes were also observed including ballooning degeneration, apoptotic bodies in hepatocytes, cirrhotic nodules, lymphocytic infiltration in portal areas and propelling nucleus in periphery.

GC/MS Analysis of star anise EO

GC-MS Analysis was carried out using Agilent Technologies. GC Model # 7890A & MS model # 5975C with capillary column # HP-MS (30 m x 250 µm x 0.25 µm) was used with manual injection

source. Helium was used as inert gas, at the flow rate of 0.8 mL/min and the injection port temperature was 450ºC. The oven temperature for GC-MS was at 60ºC for 0 minute, then it was raised to 5ºC/min to 80ºC for 2 minutes, then 10ºC/min to 200ºC for 2 minutes and then finally raised to 10ºC/min to 310ºC for 4 minutes. Total run time was 35 minutes. Split mode was used with heater at 240ºC. Solvent delay was 2 min. for MS acquisition parameters with low mass 50.0 and high mass 650.0. MS source was 240ºC maximum 250ºC while MS Quad was 150ºC maximum 200ºC. Identification of constituents were made by comparison of obtained spectra and retention indexes with NIST mass spectra Library and literature data. Quantitative analysis provided percentage composition of each constituent.

Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). The intergroup variations were measured by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Results were considered statistically significant if $P < 0.05$. Data were analysed using Graph Pad Prism software, LLC, Version 6.01 (San Diego, CA 92108, USA).

Results and Discussion

Effect of I. verum EO on Liver Function Markers

Serum levels of proteins (Albumin, globulin), liver enzymes and total bilirubin were measured to determine the liver function. The liver damage induced by CCl_4 significantly elevated the serum levels of SGOT, SGPT, ALP, proteins and total bilirubin. Treatment with *I. verum* significantly ($P < 0.05$) lowered the levels of these enzymes and total bilirubin level (Table I).

Table I.

Effect of *I. verum* on ALP, ALT, AST, albumin, globulin, total proteins and bilirubin

Groups	ALP (U/L)	ALT (U/L)	AST (U/L)	Albumin (mg/dL)	Globulin (mg/dL)	Total proteins (U/mg)	Bilirubin (mg/dL)
(-) Control	21.2 ± 2.86	74.6 ± 10.09	149.8 ± 9.60	3.24 ± 0.23	2.49 ± 0.22	1.92 ± 0.15	3.22 ± 0.19
(+) Control	38.0 ± 7.11**	89.56 ± 2.84***	208.8 ± 12.68*	4.36 ± 0.36	2.88 ± 0.64	2.52 ± 0.22***	4.24 ± 0.27***
Diseased Control	77.0 ± 6.12***	161.40 ± 38.74*	291.0 ± 6.44***	2.30 ± 0.45	4.50 ± 1.13***	4.74 ± 1.11	7.82 ± 0.88
E0-100 mg/kg	28.40 ± 4.39$^{###}$	112.20 ± 10.18***, $^{###}$	162.0 ± 27.72$^{###}$	4.34 ± 1.12$^{##}$	2.20 ± 0.43$^{###}$	2.88 ± 0.58$^{##}$	3.76 ± 0.59***, $^{###}$
E0-100 mg/kg + CCl_4	62.8 ± 5.67***, $^{#}$	95.8 ± 6.3***, $^{#}$	231.0 ± 56.77**, $^{#}$	3.94 ± 0.61$^{#}$	1.84 ± 0.73$^{###}$	3.14 ± 0.09$^{#}$	6.54 ± 0.46$^{#}$
E0-200 mg/kg	33.0 ± 8.31$^{###}$	134.8 ± 3.63	173.8 ± 37.59$^{###}$	4.52 ± 0.48$^{##}$	1.98 ± 0.50$^{###}$	2.40 ± 1.30$^{###}$	4.20 ± 0.70***, $^{###}$
E0-200 mg/kg + CCl_4	59.80 ± 6.76***, $^{##}$	124.20 ± 8.14$^{###}$	217.60 ± 14.01*, $^{##}$	3.86 ± 4.30$^{#}$	1.38 ± 0.46$^{###}$	2.90 ± 0.16$^{##}$	5.52 ± 0.19$^{###}$

The results were represented as mean ± SEM (n = 5). ***, **, * signifies difference *vs.* (-) Control group, $P < 0.001$, $P < 0.01$ & $P < 0.05$ respectively. $^{###, ##, #}$ signifies difference *vs.* Diseased Control group, $P < 0.001$, $P < 0.01$ & $P < 0.05$ respectively (ALP; alkaline phosphatase, ALT; alanine transaminase, AST; aspartate transaminase, EO; essential oil of *I. verum*)

Effect of I. verum EO on antioxidant enzymes, NO and lipid peroxidation

The activity of the antioxidant enzymes (SOD, CAT), nitric oxide and lipid peroxidation were analysed. Among CCl_4 treated rats, a fall in SOD, CAT while a rise in NO and lipid peroxidation was observed, indicating severe cellular damage. Upon *I. verum* EO administration, significant ($P < 0.05$) enhancement in the levels of these antioxidant enzymes, and decrease in NO and lipid peroxidation was found, indicating the survival of hepatocytes (Table II).

Table II.

Effect of *I. verum* on SOD, CAT, total NO and lipid peroxidation of hepatocytes

Groups	SOD (U/mgprot)	CAT (U/mgprot)	Total NO (U/mgprot)	Lipid peroxidation (U/mgprot)
(-) Control	40.08 ± 2.84	4.01 ± 0.73	0.37 ± 0.06	4.81 ± 0.59
(+) Control	62.22 ± 2.32***	3.43 ± 0.53	0.28 ± 0.04	3.46 ± 0.42
Diseased Control	29.2 ± 2.81***	1.90 ± 0.270***, $^{#}$	0.59 ± 0.11**	9.71 ± 1.19***
E0-100 mg/kg	50.71 ± 6.916**, $^{###}$	2.15 ± 0.66*, $^{##}$	0.29 ± 0.06$^{###}$	6.25 ± 0.69$^{###}$
E0-100 mg/kg + CCl_4	36.5 ± 2.6$^{#}$	2.82 ± 0.407**, $^{#}$	0.27 ± 0.04$^{#}$	2.79 ± 0.60*, $^{#}$
E0-200 mg/kg	33.54 ± 2.12*	2.07 ± 0.28***	0.40 ± 0.11	7.26 ± 1.34**, $^{##}$
E0-200 mg/kg + CCl_4	62.08 ± 0.87***, $^{###}$	3.06 ± 0.184*, $^{##}$	0.35 ± 0.06$^{###}$	3.67 ± 0.86$^{###}$

The results were represented as mean ± SEM (n = 5). ***, **, * signifies difference *vs.* (-) Control group, $P < 0.001$, $P < 0.01$ & $P < 0.05$ respectively. $^{###, ##, #}$ signifies difference *vs.* Diseased Control group, $P < 0.001$, $P < 0.01$ & $P < 0.05$ respectively (SOD; superoxide dismutase, CAT; catalase, NO; nitric oxide, EO; essential oil of *I. verum*)

Effect of I. verum EO on Lipid Profile

The total lipids, cholesterol, triglycerides, LDL and VLDL were increased significantly ($P < 0.05$) except HDL which was decreased in CCl_4 treated rats as compared to normal control group. *I. verum* EO administration decreased these parameters significantly ($p < 0.05$) with increased HDL level as shown in Table III.

Table III

Effect of *I. verum* on cholesterol, triglycerides, VLDL, LDL, HDL and total lipids

Groups	Cholesterol (mg/dL)	TGs (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	Total lipids (mg/dL)
(-) Control	60.2 ± 1.92	60.2 ± 1.92	13.2 ± 2.77	64.6 ± 3.05	43.62 ± 2.70	350.8 ± 4.44
(+) Control	70.2 ± 13.92	70.2 ± 13.92	25.6 ± 8.05	72.6 ± 8.41	79.2 ± 6.38	400.8 ± 16.93
Diseased Control	107.4 ± 14.08***	107.4 ± 14.08***	43.4 ± 7.77***	90.8 ± 7.82***	29.6 ± 5.81***	548 ± 40.12***
E0-100 mg/kg	67 ± 13.55 ###	67 ± 13.55 ###	25.4 ± 8.38 ###	65.6 ± 4.83 ###	49.6 ±.7.30**, ###	337.6 ± 40.19 ###
E0-100 mg/kg + CCl_4	84.2 ± 9.78*, #	84.2 ± 9.78*, #	39.4 ± 5.77**, #	75 ± 3.24 #	40.8 ± 0.84 #	465 ± 48.22***, #
E0-200 mg/kg	95 ± 13.25##	95 ± 13.25##	40.6 ± 14.59**, #	70.4 ± 9.76##	49 ± 2.12###	385.4 ± 43.96###
E0-200 mg/kg + CCl_4	77.6 ± 12.05##	77.6 ± 12.05##	35.20 ± 8.87*, ##	70.6 ± 7.86##	42.4 ± 5.18##	456.4 ± 36.49**, ##

The results were represented as mean ± SEM (n = 5). ***, **, * signifies difference *vs.* (-) Control group, $P < 0.001$, $P < 0.01$ & $P < 0.05$ respectively. ###, ##, # signifies difference *vs.* Diseased Control group, $P < 0.001$, $P < 0.01$ & $P < 0.05$ respectively (VLDL; very low-density lipoproteins, LDL; low density lipoproteins, HDL; high density lipoproteins, EO; essential oil of *I. verum*)

Effect of I. verum EO on Inflammatory Markers

CCl_4 treatment enhanced COX-2, TNF-α, nitrite and IL-6 whereas supplementation of *I. verum* EO lowered ($P < 0.05$) all these parameters as compared to CCl_4 treated rats (Table IV).

Table IV

Effect of *I. verum* on TNF-α, COX-2 and IL-6 levels

Groups	IL-6 (pg/mL)	TNF-α (pg/mL)	COX-2 (U/L)
(-) Control	78.70 ± 8.40	2.20 ± 0.06	33.35 ± 4.43
(+) Control	98.66 ± 5.66**	3.08 ± 0.15**	22.08 ± 0.86**
Diseased Control	197.79 ± 4.63***	5.34 ± 0.23***	44.36 ± 7.46**
E0-100 mg/kg	91.86 ± 10.64###	1.93 ± 0.09***, ###	24.54 ± 3.62*, ###
E0-100 mg/kg + CCl_4	80.56 ± 8.54**, #	3.31 ± 0.19***, #	13.27 ± 1.03#
E0-200 mg/kg	92.84 ± 2.04###	2.28 ± 0.22###	31.04 ± 3.22###
E0-200 mg/kg + CCl_4	103.88 ± 3.97***, ###	4.00 ± 0.08***, ##	20.0 ± 1.19***, ###

The results were represented as mean ± SEM (n = 5). ***, **, * signifies difference *vs.* (-) Control group, $P < 0.001$, $P < 0.01$ & $P < 0.05$ respectively. ###, ##, # signifies difference *vs.* Diseased Control group, $P < 0.001$, $P < 0.01$ & $P < 0.05$ respectively (TNF-α; tumour necrosis factor, COX-2; cyclooxygenase-2; IL-6; interleukin-6, EO; essential oil of *I. verum*)

Liver Histopathology

Normal liver morphology was observed among the control rats. There was no evidence of inflammation/ necrosis/haemorrhage or cholestasis. In CCl_4 treated rats, irregular, swollen hepatocytes with mild fatty change containing fat droplets as well as fatty degeneration were noticed. Some of the hepatocytes showed degenerative changes (DH) and bi-nucleated. In *I. verum* EO treated rats, hepatocytes of normal morphology, but were multinucleated indicating cellular regeneration. No evidence of inflammation/necrosis/ haemorrhage or cholestasis was observed among *I. verum* EO treated rats (Figure 1).

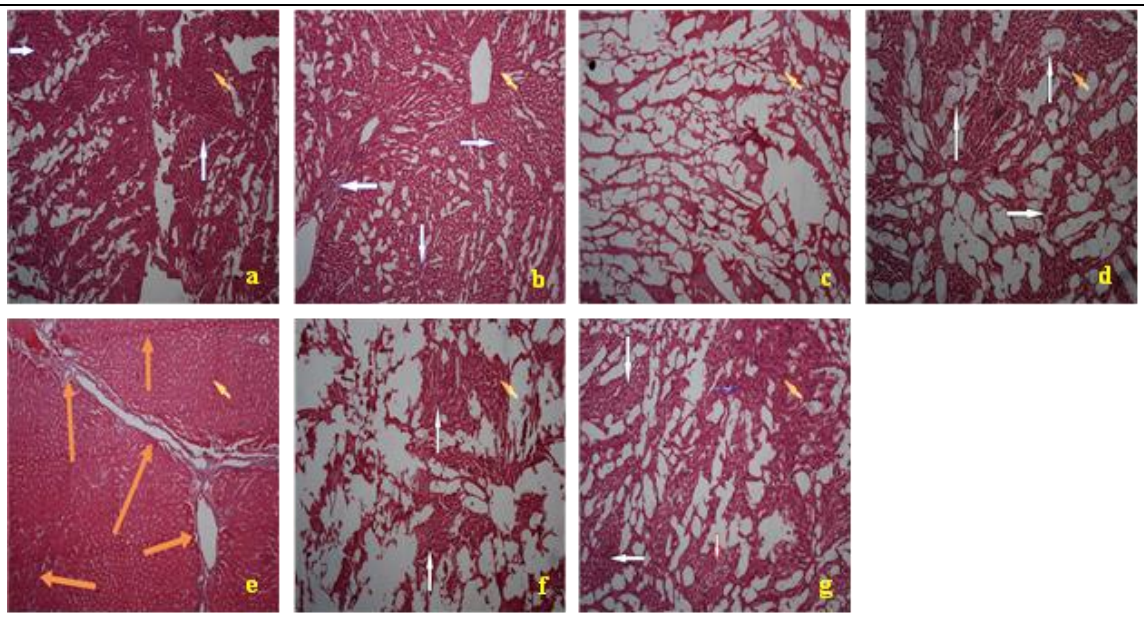

Figure 1.
Histopathological studies of liver tissues from Negative Control (a), Positive Control (b), Diseased Control (c), *I. verum* EO 100 mg/Kg (d), *I. verum* EO 100 mg/kg and CCl_4 (e), *I. verum* EO 200 mg/Kg (f) and *I. verum* EO 200 mg/kg and CCl_4 (g) treated groups of rats

Characterization of I. verum EO

The essential oil obtained after steam distillation of *I. verum* had a yellow colour with a strong aroma like liquorice. The main constituent identified by GC/MS was benzene, 1-methoxy-4-(1-propenyl)-(Z) (trans Anethole) (97.485%). Other constituents were beta-phellandrene (0.029%), 3-carene (0.016%), 1,3,6-octariene, 3,7-dimethyl-, (Z) (0.058%), estragole (1.09%), 2-Allyl-4-methylphenol (0.404%), 4-Butylbenzyl alcohol (0.469%), caryophyllene (0.39%), 2-ter-Butyl-4-methyl-6-(a-methylbenzyl) phenol (0.039%) and retinol acetate (0.02%) (Table V).

Table V
GC/MS analysis of *I. Verum* EO

No.	Component Name	Mol. Wt.	Mol. formula	Retention time	% of component
1	Beta-Phellandrene	136	$C_{10}H_{16}$	5.054	0.029
2	3-Carene	136	$C_{10}H_{16}$	6.52	0.016
3	1,3,6-Octariene, 3,7-dimethyl-, (Z)	136	$C_{10}H_{16}$	7.029	0.058
4	Estragole	148	$C_{10}H_{12}O$	10.505	1.09
5	2-Allyl-4-methylphenol	148	$C_{10}H_{12}O$	11.516	0.404
6	Benzene, 1-methoxy-4-(1-propenyl)- (Trans Anethole)	148	$C_{10}H_{12}O$	12.399	97.485
7	4-Butylbenzyl alcohol	164	$C_{11}H_{16}O$	13.702	0.469
8	Caryophyllene	204	$C_{15}H_{24}$	13.94	0.39
9	2-ter-Butyl-4-methyl-6-(a-methylbenzyl) phenol	268	$C_{19}H_{24}O$	26.424	0.039
10	Retinol acetate	328	$C_{22}H_{32}O_2$	26.696	0.02

There are several regimens for treating hepatic damage induced by synthetic drugs which produce adverse effects [13]. Hence, this study was aimed to investigate the possibility of food-derived and natural alternative for protection against chronic liver damage, a major reason for chronic liver diseases. People used different herbs and medicinal plants to cure and prevent health issues in ancient times [14, 15]. Carbon tetra chloride (CCl_4) is a renowned hepatotoxin which has been used in our studies to induce hepatotoxicity and oxidative stress [16, 17].

The enzymes (ALT, AST, Alkaline phosphatase) and bilirubin, found in liver naturally, are fundamentals for normal functioning of hepatocytes. In the present study, enzyme levels together with bilirubin were raised by administration of CCl_4 indicating release of enzymes and bilirubin into bloodstream from damaged hepatocytes while total proteins, albumin and globulin levels were also decreased by treatment with CCl_4 in this study possibly due to hepatic cells damage. However, treatment with *I. verum* essential oil significantly reduced the enzyme levels, bilirubin and increased plasma proteins when compared with diseased group. *I. verum* may have hepatocytes membrane stabilizing potential to prevent the leakage of these liver markers into circulation [18].

Damage to cell membrane of liver cells induced by CCl_4 also caused a rise in total lipids, triglycerides,

cholesterol, VLDL and LDL while a fall in HDL level. Deterioration of hepatic cells leads to decreased ability to metabolize lipids [18]. Pretreatment of rats with *I. verum* essential oil reduced total lipids, TG, cholesterol, VLDL and LDL and increased HDL level at dose 100 mg/kg ($P < 0.05$) and 200 mg/kg ($P < 0.01$).

The antioxidant potential of *I. verum* was estimated by measuring SOD and CAT activities, lipid peroxidation, and total NO levels. SOD catalyses the reduction of peroxides to alcohol or water. Catalase has a vital role in eradication of ROS resulting from oxidation-reduction reactions in the liver and it becomes inactive by the lipid peroxides [19, 20]. In our studies, we demonstrated that CCl_4 caused significant reduction in antioxidant enzymes activities including SOD and CAT indicating oxidative stress and damage to hepatocytes. But treatment of rats with *I. verum* essential oil restored SOD and CAT levels dose dependently presenting decrease in oxidative stress induced by CCl_4.

Lipid peroxidation is a process of oxidative deprivation of lipids whereby free radicals steal electrons from the lipids in cell membranes leading to cell death [21]. This study showed lipid peroxidation levels were significantly lowered ($P < 0.001$) in *I. verum* treated rats when compared to CCl_4 treated group at both doses.

The current investigation found that animals given CCl_4 had considerably greater blood and tissue IL-6 and TNF-α levels than mice in the control group. However, as compared to the CCl_4-treated group, administration of *I. verum* essential oil significantly reduced ($P < 0.001$) IL-6 and TNF-α, indicating a protective effect against CCl_4 damage. This anti-inflammatory effect might be linked to the presence of trans anethole and caryophyllene in *I. verum* essential oil [22].

COX-2 is known to be involved in inflammatory responses and the development of hepatocellular cancer [23]. The levels of COX-2 protein and mRNA expression rose in the CCl_4 group, whereas *I. verum* significantly reduced ($P < 0.001$) these increases. High levels of iNOS and COX-2 promote the synthesis of large amounts of NO and eicosanoids *via* the COX-prostaglandins pathways [26], which results in cellular inflammation, necrosis, and fibrosis.

Furthermore, the findings of this study were supported by histopathological analysis. Control group rats showed intact hepatocytes while in CCl_4 treated groups, hepatocytes were damaged. The ballooning degeneration, vacuolation formation and shifting of nucleus towards periphery were observed. While animals treated with *I. verum* essential oil showed normal morphology. The hepatocytes were regular, though multinucleated showing cells regeneration. No signs of inflammation were observed.

GC-MS analysis of essential oil from *I. verum* presented benzene, 1-methoxy-4-(1-propenyl)-(Z) (trans Anethole) (97.485%), which exhibits anti-inflammatory and antioxidant effects [9]. Other constituents were beta-Phellandrene (0.029%), 3-Carene (0.016%), 1,3,6-Octariene, 3,7-dimethyl-,(Z) (0.058%), Estragole (1.09%), 2-Allyl-4-methylphenol (0.404%), 4-Butyl-benzyl alcohol (0.469%), Caryophyllene (0.39%), 2-ter-Butyl-4-methyl-6-(a-methylbenzyl) phenol (0.039%), Retinol acetate (0.02%) [9]. Caryophyllene, a bicyclic sesquiterpenes also have proven Anti-oxidant ad anti-inflammatory actions [24]. Natural Anetholes exist in high quantity in star anise essential oil (above 90%). It exerts its anti- inflammatory potential by suppressing interleukins and TNF-α production and number of leukocytes migration. It exerts its antioxidant effects by decreasing Total NO levels and PGE_2 levels [25]. Estragole, a polypropanoid, has pronounced anti-inflammatory activity by inhibition of COX-2 [26]. Caryophyllene, a bicyclic sesquiterpenes also have proven anti-oxidant ad anti-inflammatory actions which elicit its actions by inhibition of inflammatory mediators [24].

Conclusions

In conclusion, verdicts of this study confirmed the antioxidant and hepatoprotective potential of *Illicium verum* essential oil. Hence, this essential oil could be a potential source of drug to be used as hepato-protective. However, further studies are needed to authorize these effects at the cellular and molecular levels.

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Conflict of interest

The authors declare no conflict of interest.

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