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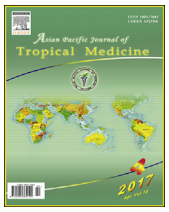

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The protection of *Thymus vulgaris* leaves alcoholic extract against hepatotoxicity of alcohol in rats

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ABSTRACT

Objective: To investigate the protective effect of *Thymus vulgaris* (*T. vulgaris*) leaves 70% alcoholic extract against alcohol-mediate hepatotoxicity rats.

Methods: The protective effect of *T. vulgaris* extract was investigated at dose of 500 mg/kg/day (as 0.1 of LD_{50}) orally against alcohol-mediate hepatotoxicity using adult male Wister albino rats during 21 days. Protective effect of *T. vulgaris* extract was evaluated comparing with silymarin standard drug at recommended dose (25 mg/kg/day) orally for 21 days. Serum liver and kidney functions, serum lipid profile, liver antioxidant enzymes activities, liver glutathione concentration (GSH), liver oxidative parameters and histopathological study of liver and kidney were estimated to find out protective effect of *T. vulgaris* extract.

Results: Alcohol-mediate hepatotoxicity rats (alcohol-control) showed hepatocytes distortion represented as marked increment on liver biomarkers; alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) activities, as well as pronounced reduction on total protein and its fractions albumin and globulin production corresponding to normal ranges. Oxidative stress status was appeared on alcohol-control evident as significant depletion on GSH concentration, antioxidant enzymes activities; catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione- *S*- transferase (GST) and glutathione peroxidase (GPx) recorded significant dwindling, concurrence with significant augmentation on oxidative stress parameters; malondyaldehyde (MDA) and hydrogen peroxide (H_2O_2) concentrations with respect to normal values. Serum lipid profile was affected by alcohol administration, total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) were significantly reduced, meanwhile high density lipoprotein cholesterol (HDL-C) was raised comparing to normal ranges. Co-administration of *T. vulgaris* extract with alcohol showed protective effect on hepatocytes manifested as remarkable minimizing on ALP, AST and ALT activities and marked increment on total protein, albumin and globulin production compared to alcohol-control. Amelioration was achieved on oxidative stress status on rats co-administrated *T. vulgaris* extract with alcohol. Accordingly, antioxidant enzymes activities; CAT, SOD, GR, GST and GPx were significantly magnified, while oxidative stress parameters; MDA and H_2O_2 concentration were significantly lessened corresponding to alcohol-control. Also, lipid profile was markedly improved and risk ratio was lowered by *T. vulgaris* extract co-administrated in comparison with alcohol-control. All these obvious results were confirmed by histopathological examination, which illustrated that extract showed normalization of degenerated and fibrotic liver tissue as of alcohol-control.

Conclusion: *T. vulgaris* extract protected hepatocytes from damaging by alcohol reflecting improvement on liver performance and inhibition of oxidative stress status of liver. *T. vulgaris* extract appeared hepatoprotective, hypolipidemic and antioxidant activities on alcohol-mediate hepatotoxicity rats compared to silymarin.

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1. Introduction

Alcohol administration causes oxidative stress leads to alcohol-mediated hepatotoxicity. Many stressful processes as changes in redox state of liver concurrence with increase acetaldehyde free radical production, induction of CYP2E1, mobilization of iron, damaging in the cell mitochondria and cell membrane, hypoxia, changed cytokine production and immune system disturbance are associated with alcohol consumption [1]. Liver disease like steatosis, alcoholic hepatitis and liver cirrhosis are the most common complication of ethanol administration [2]. Alcohol consumption generates reactive oxygen species (ROS) like superoxide anion ($\bullet^{-}O_2$), hydroxyl radical (•OH) and hydrogen peroxide (H_2O_2) and accumulates these in the hepatic cells that oxidize the glutathione resulting lipid peroxidation of cellular membranes, protein and DNA oxidation lead to hepatic damage [3]. Chronic alcohol administration leads to fat accumulation in the liver, hyperlipidemia and ultimately cirrhosis [4]. Decreasing fatty acid oxidation and increased lipogenesis which is altered the [NADH]/[NAD+] redox balance in the liver causing fatty liver. By alcohol dehydrogenase (ADH) enzyme alcohol is metabolized into acetaldehyde, followed by aldehyde dehydrogenase (ALDH) enzyme acetaldehyde is metabolized into acetic acid, which is finally oxidized into carbon dioxide (CO_2) and water [2]. Alcohol metabolism generates NADH, and increases the NADH/NAD^+ ratio. A higher NADH concentration induces fatty acid synthesis while a decreased NAD level results in decreased fatty acid oxidation [4]. Alcohol consumption have some benefits as increasing high density lipoprotein (HDL-C) causing protection against atherosclerosis [2].

The synthetic drugs of liver diseases such as corticosteroids, antiviral and immunosuppressant agents may be lead to serious adverse effects up to hepatic damage Eg: cholestatic jaundice with azathioprine and elevation of serum transaminases by interferon and virazole [5]. It is therefore pressing to investigate alternate sources for the treatment of liver disease more efficacy and safety.

Thymus vulgaris L. (*T. vulgaris*) is only cultivated specie of *Thymus* genus, which contains 300 species and other species are grow widely. *T. vulgaris* L. (Lamiaceae) known by its essential oils which consist of borneol, carvacrol, linalool, and thymol [6]. *T. vulgaris* contains polyphenol, flavonoids, tannin, saponins and triterpenes. Flavonoids are including luteolin, apigenin, naringenin, eriodictyol, cirsilineol, salvigenin, cirsimaritin, thymoine, thymusine. Triterpenes are including ursolic and oleanolic acids [7]. In folk medicine, *T. vulgaris* has been used as anti-asthmatic, bronchodilator, expectorant, antiseptic, antispasmodic, antitussive, carminative, secretomotor, anthelmintic, astringent, antibacterial, antifungal, antiviral, antiprotozoan and antioxidant. Additionally, it has been used for dyspepsia, chronic gastritis, diarrhea in children, enuresis in children and tonsillitis [8]. Also, *T. vulgaris* extracts documented as immunomodulator and anti-inflammatory agents [9], antioxidant and free radical scavenging, anti-inflammatory, vasorelaxant, anti-platelet, anti-thrombin, anti-hyperlipidemic and anti-diabetic [10,11] antihypertensive [12]. On the other hand, Vetvicka and Vetvickova [13] revealed to the very limited effects of thymus derived essential oils as immunomodulator, anti-inflammatory, proliferation of the human breast cancer cell line ZR-75-1 and liver protection. Moderate dose of *T. vulgaris* medicine is generally regarded as safe, but high doses may cause intestinal problems such as diarrhea and bloating [14]. *T. vulgaris* use is not safe during pregnancy, excessive doses should be avoided if there is possibility of pregnancy.

This research aimed to achieve the protection role of *T. vulgaris* leaves 70% alcoholic extract against alcohol-mediated hepatotoxicity rats.

2. Material and methods

2.1. Chemicals

Silymarin was obtained from CID Company (Egyptian Chemical Industries Development Company, El Haram, Giza, Egypt). Kits of liver and kidney function; total protein, albumin, liver enzymes activities; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Alkaline phosphatase (ALP), uric acid, urea and creatinine and others of antioxidant enzymes activities; glutathione reductase (GR), glutathione peroxidase (GPx), glutathione -*S*-transferase (GST), superoxide dismutase (SOD) and catalase (CAT), as well as oxidative parameters; malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) were obtained from BIODIGONESTIC diagnostics Egypt, Dokki, Giza, Egypt. Other chemicals and reagents used for extraction were of analytical grade.

2.2. Preparation of plant material

Plant material used in this study was the leaves of *T. vulgaris*. The seedlings of *T. vulgaris* were kindly received from Elmizan company at the SEKM Company Farm at Bilbase, El- Sharkya Governorate, Egypt during winter season of 2015. The seedlings were transplanted in the permanent field of the experimental Farm of SEKM at Bilbase, Sharkya, Egypt in February and treated with the normal agricultural practices usually applied with thyme plants in Egypt. The leaves were collected in April and were air dried. Leaves were macerated at 70% ethanol solution several time. The mixture was filtered and the filtrate was evaporated under reduced pressure with evaporator to obtain crud extract. Resides extract was lyophilized and remained powder was kept in −20 °C until using.

2.3. Assay of total polyphenol of T. vulgaris extract

Total phenols of *T. vulgaris* leaves crude extract was assayed spectrophotometrically (Schimadzu UV/Vis-240IPC) according to the methods of Gorinstein *et al.*, [15] using the Folin Ciocalteu with slight modification. Total phenols concentrations were expressed as gallic acid equivalents (GAE).

2.4. Assay of total flavonoids of T. vulgaris extract

The total flavonoids of *T. vulgaris* leaves extract was determined spectrophotometrically according to the method described by Lin and Tang [16] using the aluminum chloride. Total flavonoids concentrations were expressed as g quercetin/100 g dry weight.

2.5. HPLC analysis of phenolic compounds of T. vulgaris extract

HPLC analysis was carried out according to Kim *et al.*, [17] with slight modifications using an Agilent Technologies 1100 series liquid chromatography equipped with an auto sampler and a diode-array detector. The analytical column was Agilent Eclipse XDB C18 (150 × 4.6 μm; 5 μm) with a C18 guard column. The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 mL/min for a total run time of 70 min and the gradient program was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. There was 10 min of post-run for reconditioning. The injection volume was 10 μL and peaks were monitored simultaneously at 280, 320 and 360 nm for the benzoic acid, cinnamic acid derivatives and flavonoids compound, respectively. All samples were filtered through a 0.45 μm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectrum and compared with those of the standards.

2.6. Assay of acute oral toxicity (LD_{50})

The acute oral toxicity of alcoholic extract of *T. vulgaris* leaves were determined by using Swiss albino mice (n = 8) weighing between 25 and 40 g. LD_{50} was carried adopting the method of Bruce [18] as up and down procedure. The animals were fasted 12 h before to the experiment and were administered with single dose of extract dissolved in distilled water. Dosing protocol of investigated extract were 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 g/kg b. wt. orally. Negative control group received distilled water only. All groups were noticed during 24 h to observe changes in behavioral parameters for assessing toxicity. After 24 h, the survival animals were kept for a further 14 days and noticed daily for behavioral and body weight changes. Obtained results showed that, LD_{50} of the extract was 5.0 g//kg body weight.

2.7. Hepatoprotective experiment

2.7.1. Experimental animal

Experiment performed in animal house of National Research Centre, Dokki, Giza, Egypt. All the protocols and the experiments were carried out in compliance according to the institution's guideline for the care and use of laboratory animals National Research Centre, Dokki, Giza, Egypt. This study was confirmed by Medical Research Ethics Committee, National Research Centre, Egypt, under registration No. 16/092.

A total of 36 adult male albino rats Wister strain (150–200 g) were maintained and acclimatized for two days under standard animal house conditions, i.e. room temperature of (25 ± 1) °C; relative humidity 55%–65% and a 12:12 h light/dark cycle. The animals had free access to standard rat pellet, with water supplied *ad libitum*.

2.7.2. Extract and drug doses protocol

One dose of *T. vulgaris* leaves extract was tested as the results of LD_{50} (500 mg/kg/day as 0.10 of the LD_{50}) for 21 days. Silymarin was tested with recommended dose; 25 mg/kg b. wt./day for 21 days. The extract and silymarin were dissolved in distilled water. Ethanol as a hepatotoxicity agent was used at concentration 40% (v/v) [19]. The dose of ethanol was 2.00 mL/100 g b. wt./day for 21 days. Rats administrated these solutions orally by gavage for 21day experimental period.

2.7.3. Experimental design and samples

After 48 h of acclimatization, animals were habituated to laboratory conditions for prior to experimental protocol. Animals were divided into three main groups; negative control group (6 rat), positive control groups (18 rats) and treated group (12 rat). Negative control group, rats received distilled water for 21 days and kept as negative control. Positive control group, classified into three subgroups; (A) rats received silymarin at recommended dose 25 mg/kg b. wt./day orally for 21 days and kept as positive silymarin, (B) rats received *T. vulgaris* extract at dose 500 mg/kg b. wt./day orally for 21 days and kept as positive extract, (C) rats received 40% ethanol only at dose 2.00 mL/l00 g b. wt./day for 21 days and kept as alcohol-control. Treated groups were divided into two subgroups: (A) rats received 40% ethanol at dose 2.00 mL/l00 g b. wt./day orally and co-administrated with recommended dose of silymarin (25 mg/kg b. wt./day orally) at the same time for 21 days, (B) rats received 40% ethanol at dose 2.00 mL/100 g b. wt./day orally and co-administrated with *T. vulgaris* extract at dose 500 mg/kg b. wt./day orally at the same time for 21 days.

At the end of the experimental period (21 days), animals were fasted overnight and blood samples were obtained from the experimental and control rats by puncturing retro-orbital plexus. Blood samples were centrifuged by using Sigma labor zentrifugen at 4000 rpm for 10 min, and serum was separated and kept at −20 °C. Livers and kidneys were collected then immediately excised, rinsed in ice cold normal saline and then weighted. Liver homogenate was prepared in phosphate buffer (pH 7.4) using an ultrasonic tissue homogenizer. The resulting homogenate in each case was centrifuged at 4000 rpm, 15 min, 4 °C by using Sigma labor zentrifugen [20] for antioxidant enzymes analysis and oxidative stress parameters.

2.7.4. Biochemical assessment

Liver function of rats were determined in sera samples. Total protein (TP) and Albumin were measured spectrophotometrically according to methods described by Henry, [21] and Doumas *et al.*, [22]. Globulin was calculated by the difference between total protein and albumin according to Reinhold, [23]. Liver enzyme biomarkers; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) activities were estimated spectrophotometrically according to the methods of Reitman and Frankel, [24] and BelfIeld and Goldberg, [25]. Kidney functions of rats assayed in sera samples. Urea, uric acid and creatinine were estimated according to the methods of Tabacco *et al.*, [26], Gochman and Schmitz, [27] and Faulkner and king, [28] respectively.

Glutathione -L- reduced concentration and antioxidant enzymes activities of liver were determined in liver homogenate spectrophotometrically. Glutathione -L- reduced (GSH) concentration was measured at 405 nm [29] and the unit of concentration was mmol/g tissue using Ellman's reagent (5,5′-dithiobis 2-nitrobenzoic acid; DTNB), which was reduced by thiol groups to form 1 mol 2-nitro 5-mercaptobenzoic acid/mol thiol and with maximal absorption at 412 nm. Glutathione reductase (GR) activity was determined at 340 nm according to

the methods of Goldberg and Spooner, [30] and the amount of the enzyme reducing 1 μmol GSSG per min per mg protein was regarded 1 activity unit. Glutathione -*S*- transferase (GST) activity was assayed at 340 nm according to the methods of Habig *et al.*, [31] and the amount of the enzyme that conjugate 1, chloro-2, 4- dinitrobenzene with reduced glutathione per min per mg protein was regarded 1 activity unit. Glutathione peroxidase (GPx) activity was measured at 340 nm according to the methods described by Paglia and Valentine, [32] and the amount of the enzyme converting 1 μmol GSH per min per mg protein was taken as 1 activity unit. Catalase (CAT) activity was estimated at 510 nm according to the methods described by Beers and Sizer, [33]. Superoxide dismutase (SOD) was determined at 560 nm according to the methods of Fridovich, [34] as the decrease suppression rate of nitrotetrazolium blue and for 1 unit of activity.

Oxidative stress parameters; lipid peroxidation biomarker; malondyaldehyde (MDA) and hydrogen peroxide (H_2O_2) were measured by spectrophotometric method described by Ohkawa *et al.*, [35] and Chance and Maehly, [36] respectively.

2.7.5. Histopathological assessment

Specimens of liver and kidney from all animals were dissected immediately after death. All the specimens were fixed in 10% neutral-buffered formal saline for 72 h at least, washed in distilled water and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Serial sections of 6 μm thick were cut and stained with Hematoxylin and eosin [37] for histopathological investigation. Images were captured and processed using Adobe Photoshop version 8.0.

2.8. Statistical analysis

Data were analyzed by one-way ANOVA test for comparisons among means at $P \leq 0.05$ using Co-Stat 6.303 Software Computer Program 2004 (COSTAT-C program 1988).

3. Results

3.1. Total phenols, flavonoids and HPLC analysis of phenolic compounds

T. vulgaris leaves 70% alcoholic extract have high amount of total phenol; (214.55 ± 15.65) mg gallic/g extract the most of it is flavonoids; (143.75 ± 13.45) mg catechin/g extract. The main separated phenolic compounds separated with HPLC are shown in Table 1. Total phenol compounds of *T. vulgaris* leaves extract ranged between Scoplatine the minor component (0.001 mg/g extract) to Rosmarinic acid the major component (48.419 mg/g extract). Rosmarinic acid the major component followed by hesperidin (3.016 mg/g extract) and myrectin (1.165 mg/g extract).

Table 1
Phenolic components of *T. vulgaris* leaves 70% extract as separated with HPLC.

Compound	Rt	conc (mg/g)
Pyrogallol	4.7	0.255
Gallic acid	5.7	0.033
Protocatechuic	9.9	0.274
p-hydroxybenzoic	15.1	0.166
Gentisic	16.8	0.324
Chlorgenic acid	20.6	0.214
Caffeic acid	21.4	0.496
Syrngic	23	0.158
Vanillic acid	24.8	0.219
Scoplatine	30.9	0.001
Ferulic acid	32.4	0.077
Sinapic	33.8	0.013
Rutin	36.2	0.670
p-coumaric	37.2	0.567
Naringin	37.8	0.087
Hesperidin	38.5	3.016
apeginin-7-glucoside	38.8	0.105
Myricetin	39.5	1.165
Rosmarinic acid	40	48.419
Cinnamic acid	42.8	0.082
Quercetin	43.5	0.983
Apennine	46	0.201
Kaempferol	46.5	0.132
Chrysin	52	0.668

3.2. Effect of T. vulgaris extract on liver functions

Alcohol administration caused pronounced disturbance on liver performance represented as significant reduction of serum total protein and its fractions; albumin and globulin by about 52.02%, 29.40% and 76.61% respectively corresponding to negative control (Table 2). In comparison with alcoholic control, co-administration *T. vulgaris* extract significantly magnified total protein concentration (164.45%) and its fractions; albumin (44.13%) and globulin (548.28%). The increment in the total protein was in favor of the globulin. No significant differences were observed between the effect of co-administration *T. vulgaris* extract and silymarin on total protein and its fractions. The same observation noticed in positive control of extract and recorded significant increment on total protein and its fractions, compared with negative control.

When the liver cell is damaged, the AST and ALT in liver cells will be released to serum. Therefore, levels of AST and ALT are the most commonly used biochemical indexes for evaluating the damage of liver. According to the biochemical indexes (Table 2), the administration alcohol caused significant raise on the AST, ALT and ALP activities of alcoholic control 33.26%; 44.70% and 42.21% respectively high than negative control ($P < 0.05$). Compared to alcoholic control, both of *T. vulgaris* extract and silymarin significantly reduced AST, ALT and ALP activities of alcoholic-induced hepatotoxicity rats ($P < 0.05$). On AST, both groups treated with *T. vulgaris* extract and silymarin recorded AST activity close up that of negative control and positive control. On ALT, both of *T. vulgaris* extract and silymarin significantly decreased ALT activity with equal rate, ALT of both groups were lower than that of negative control and positive controls. On ALP activity, *T. vulgaris* extract caused significant increment (53.04%) on ALP of alcoholic-induced hepatotoxicity rats higher than that caused by silymarin (18.80%). ALP activity of positive controls did not significantly change with respect of negative control.

3.3. Effect of T. vulgaris extract on kidney functions

Alcohol ingestion to rats lead to a significant elevation in kidney biomarker included urea and creatinine (103.75 and 37.29% respectively) when compared to negative control reflecting renal functions disturbance ($P < 0.05$). Data shown in

Table 2
Liver functions of alcohol-induced rat's liver injury treated with *T. vulgaris* leaves alcoholic extract.

Groups	Subgroups	Total protein	Albumin	Globulin	ALP	AST	ALT	AST/ALT
Negative control group	Negative control	7.67 ± 0.34^{c}	3.98 ± 0.16^{a}	3.72 ± 0.33^{b}	156.22 ± 3.85^{c}	65.40b ± 4.69	32.57 ± 2.52^{b}	2.02 ± 0.26^{c}
Positive control group	Silymarin positive	7.23 ± 0.14^{c}	3.60 ± 0.17^{a}	3.63 ± 0.27^{c}	144.34 ± 5.31^{d}	63.08b ± 4.18	26.05 ± 1.79^{c}	2.43 ± 0.30^{b}
	T. vulgaris positive	8.43 ± 0.23^{b}	3.36 ± 0.08^{b}	5.07 ± 0.30^{b}	160.09 ± 3.55^{c}	62.97b ± 3.50	20.70 ± 1.39^{d}	3.05 ± 0.12^{a}
	Alcohol-control	3.68 ± 0.19^{d}	2.81 ± 0.18^{c}	0.87 ± 0.10^{d}	222.16 ± 4.05^{a}	77.15a ± 2.16	47.13 ± 1.84^{a}	1.64 ± 0.11^{c}
Treated group	Silymarin	9.35 ± 0.46^{a}	3.43 ± 0.32^{b}	5.92 ± 0.32^{a}	180.41 ± 9.80^{b}	63.50 ± 2.91^{b}	21.31d ± 2.32	3.00 ± 0.33^{a}
	T. vulgaris	9.75 ± 0.24^{a}	4.05 ± 0.18^{a}	5.64 ± 0.15^{a}	104.32 ± 2.17^{d}	66.39 ± 5.43^{b}	20.30d ± 0.98	3.27 ± 0.15^{a}

Data are presented as the means ± S.D of three replicates. Data analyzed by ANOVA, $P \leq 0.05$, Value with the same letter has no significant but value with different letter has significant at 0.05. AST; aspartate aminotransferase, ALT; alanine aminotransferase, ALP; alkaline phosphatase.

Table 3
Kidney functions of alcohol-induced rat's liver injury treated with *T. vulgaris* leaves alcoholic extract.

Groups	Subgroups	Uric acid	Urea	Creatinine
Negative control group	Negative	5.31 ± 0.04^{a}	73.85 ± 3.77^{d}	0.59 ± 0.07^{b}
Positive control group	Silymarin positive	5.51 ± 0.25^{a}	73.36 ± 2.97^{d}	0.62 ± 0.08^{b}
	T. vulgaris positive	4.92 ± 0.34^{a}	75.06 ± 4.08^{d}	0.64 ± 0.05^{b}
	Alcohol control	5.51 ± 0.25^{a}	150.47 ± 8.24^{a}	0.81 ± 0.05^{a}
Treated group	Silymarin	4.80 ± 0.27^{a}	105.53 ± 4.29^{b}	0.83 ± 0.02^{a}
	T. vulgaris	4.91 ± 0.15^{a}	95.11 ± 4.22^{c}	0.64 ± 0.03^{b}

Data are presented as the means ± S.D of three replicates. Data analyzed by ANOVA, $P \leq 0.05$, Value with the same letter has no significant but value with different letter has significant at 0.05.

Table 4
Lipid profile of alcohol-induced rat's liver injury treated with *T. vulgaris* leaves alcoholic extract.

Groups	Subgroups	TC	HDL-C	TG	VLDL-C	LDL-C	RR
Negative control group	Negative control	96.66 ± 3.13^{b}	49.86 ± 2.14^{bc}	102.56 ± 3.72^{a}	20.51 ± 0.74	26.28 ± 2.31^{bc}	0.53 ± 0.03^{ab}
Positive control group	Silymarin positive	96.22 ± 5.64^{b}	46.69 ± 1.05^{c}	96.77 ± 2.58^{a}	19.35 ± 0.52	30.18 ± 2.27^{ab}	0.65 ± 0.05^{a}
	T. vulgaris positive	90.97 ± 2.38^{b}	56.08 ± 4.27^{ab}	105.53 ± 4.18^{a}	21.11 ± 0.84^{a}	13.78 ± 1.72^{e}	0.22 ± 0.04^{d}
	Alcohol-control	109.53 ± 2.35^{a}	56.35 ± 2.35^{ab}	100.71 ± 7.55^{a}	20.14 ± 1.51^{a}	32.71 ± 2.79^{a}	0.59 ± 0.06^{a}
Treated group	Silymarin	103.61 ± 5.55^{ab}	63.43 ± 2.43^{a}	99.95 ± 2.36^{a}	19.99 ± 0.47^{a}	21.86 ± 1.49^{d}	0.38 ± 0.06^{cd}
	T. vulgaris	100.70 ± 7.96^{ab}	57.02 ± 6.88^{ab}	99.40 ± 8.82^{a}	19.88 ± 1.77^{a}	23.79 ± 1.92^{cd}	0.43 ± 0.06^{bc}

Data are presented as the means ± S.D of three replicates. Data analyzed by ANOVA, $P \leq 0.05$, Value with the same letter has no significant but value with different letter has significant at 0.05. TC; total cholesterol, HDL-C; high density lipoprotein cholesterol, TG; triglycerides, VLDL-C; very low density lipoprotein cholesterol, LDL-C; low density lipoprotein cholesterol and RR; risk ratio.

Table 3 indicated that neither alcohol nor by *T. vulgaris* extract and silymarin application changed uric acid concentration. No significant difference was noticed between uric acid of all experiment groups. Urea and creatinine concentrations were significantly declined when rats co-administrated alcohol and *T. vulgaris* extract by about 36.80 and 21.00% respectively corresponding to alcoholic control. An improved effect was showed to *T. vulgaris* extract on alcohol-induced hepatotoxicity rats, was higher than silymarin. kidney functions of positive controls still like those of negative control.

3.4. Effect of T. vulgaris extract on lipid profile

Lipid profile of rat's force fed with alcohol was significantly influenced. Alcohol-control rats were characterized by slight significant increment on TC (13.32%), HDL-C (13.02%) and

Table 5
Antioxidant enzymes activities and glutathione concentration of alcohol-induced rat's liver injury treated with *T. vulgaris* leaves alcoholic extract.

Groups	Subgroups	GSH	GR	GST	GPx	SOD	CAT
Negative control group	Negative control	4.73 ± 0.36^{a}	6.02 ± 0.27^{b}	3.25 ± 0.25^{d}	2.50 ± 0.24^{c}	15.68 ± 1.74^{b}	30.16 ± 1.95^{a}
Positive control group	Silymarin positive	4.67 ± 0.35^{a}	6.86 ± 0.26^{a}	4.56 ± 0.15^{c}	3.18 ± 0.23^{b}	15.72 ± 1.04^{b}	29.81 ± 0.64^{a}
	T. vulgaris positive	4.80 ± 0.32^{a}	7.10 ± 0.35^{a}	5.49 ± 0.10^{b}	3.60 ± 0.19^{b}	19.37 ± 1.28^{a}	30.03 ± 1.28^{a}
	Alcohol control	2.68 ± 0.19^{b}	2.30 ± 0.14^{c}	1.17 ± 0.11^{e}	1.50 ± 0.15^{d}	8.58 ± 0.49^{c}	16.08 ± 1.23^{b}
Treated group	Silymarin	4.45 ± 0.17^{a}	7.35 ± 0.35^{a}	5.97 ± 0.33^{a}	4.66 ± 0.42^{a}	14.93 ± 1.00^{b}	29.38 ± 2.09^{a}
	T. vulgaris	4.76 ± 0.18^{a}	6.81 ± 0.25^{a}	5.23 ± 0.16^{b}	3.35 ± 0.17^{b}	17.62 ± 1.66^{ab}	31.42 ± 2.2^{a}

Data are presented as the means ± S.D of three replicates. Data analyzed by ANOVA, $P \leq 0.05$, Value with the same letter has no significant but value with different letter has significant at 0.05. GSH; reduced L glutathione, GR; glutathione reductase; GST; glutathione- *S*- transferase, GPx; glutathione peroxidase, CAT; catalase and SOD; superoxide dismutase.

Table 6
Oxidative stress parameters of alcohol-induced rat's liver injury treated with *T. vulgaris* leaves alcoholic extract.

Groups	Subgroups	MDA nmol/g tissue	H_2O_2 (uM/g tissue)
Negative control group	Negative	4.87 ± 0.17[b]	6.75 ± 0.15[c]
Positive control group	Silymarin positive	4.54 ± 0.15[b]	6.40 ± 0.20[c]
	T. vulgaris positive	3.21 ± 0.38[c]	3.50 ± 0.28[e]
	Alcohol control	5.44 ± 0.23[a]	8.32 ± 0.13[a]
Treated group	Silymarin	3.20 ± 0.08[c]	7.76 ± 0.37[b]
	T. vulgaris	3.10 ± 0.22[c]	4.49 ± 0.27[d]

Data are presented as the means ± S.D of three replicates. Data analyzed by ANOVA, $P \leq 0.05$, Value with the same letter has no significant but value with different letter has significant at 0.05. MDA; Malondyalde-hyde the biomarker of lipid peroxidation, H_2O_2; hydrogen peroxide.

LDL-C (24.47%), while both TG and VLDL-C did not change significantly in comparison with negative control ($P < 0.05$).

Neither *T. vulgaris* nor silymarin significantly changed TC, TG, VLDL-C and HDL-C with respect of alcohol-control as shown in Table 4. The mentioned results reflected on the risk ratio, which did not influence compared with negative control ($P < 0.05$). On contrary, LDL-C concentration of rat's force fed with alcohol and treated with *T. vulgaris* extract or silymarin was significantly lessened by about 27.27 and 33.17% respectively in comparison with alcohol-control.

In the light of the above mentioned results, risk ratio significantly lowered by co-administration *T. vulgaris* extract or silymarin (27.12 and 35.60% respectively) corresponding to alcohol-control ($P < 0.05$). Lipid profile of positive control of *T. vulgaris* extract showed significant reduction on LDL-C and risk ratio, while other parameters did not alter with respect of negative control.

3.5. Effect of T. vulgaris extract on antioxidant status of liver

The activities of liver antioxidant enzymes and glutathione concentration were significantly declined as a response to alcohol administration evident on oxidative stress status. GSH; non-enzymatic antioxidant was reduced (43.34%), and enzymatic antioxidant activities; GR (61.80%), GST (64.00%), GPx (40.00%), CAT (46.68%) and SOD (45.28%) conformable with negative control (Table 5).

GSH concentration was significantly augmented when rats co-administrated *T. vulgaris* extract and alcohol by about 77.61%

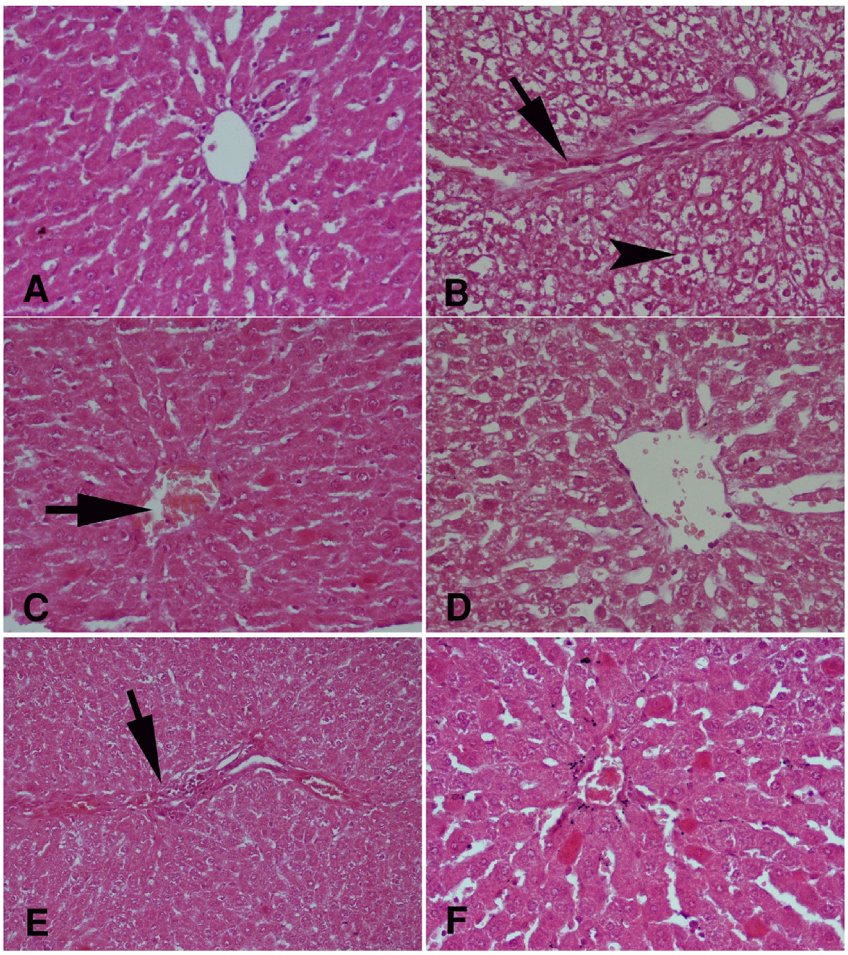

Figure 1. A photomicrograph of liver sections from: A) negative control rat showed normal structure of liver tissue; B) An alcohol-control rat showed a band of fibrous tissue extending in between the cells (arrow), while most of cells suffering from vacuolar degeneration (arrowhead); C) positive silymarin control rat showed only slight congestion of central vein (arrow); D) positive extract control rat showed only slight dilatation of central vein and blood sinusoids. E) Treated rats with silymarin and ingested alcohol showed reduction of fibrosis of liver tissue, although it is still observed (arrow) with slight congestion of blood vessels. No vacuolar degeneration is noticed in hepatocytes; F) Treated rats with extract and ingested alcohol showed normalization of liver tissue but with fine dilatation of main blood vessels and sinusoids. (H& E × 200, (E) × 100).

compared to alcohol-control ($P < 0.05$). *T. vulgaris* extract was similar with silymarin effect on GSH represented as a lack significant difference between GSH concentration of both groups. GSH concentration of positive control did not be alter compared to negative control. No significant differences were observed between all experimental groups except alcohol-control.

GR activity of rats co-administrated *T. vulgaris* extract was magnified by 196.09%, while silymarin increased with 219.57% with respect of alcohol-control ($P < 0.05$). In comparison with negative control, *T. vulgaris* extract like silymarin significantly elevated GR activity. There were no significant differences between GR activity of alcohol-induced hepatotoxicity rats treated with extract or silymarin and positive controls of the same compounds.

GST activity was significantly raised when alcohol-induced hepatotoxicity rats treated with *T. vulgaris* extract (347.01%) compared to alcohol-control ($P < 0.05$). The ameliorated effect of *T. vulgaris* extract was lower than that of silymarin. Although, GST activity of rats co-administrated alcohol and *T. vulgaris* extract was higher than GST activity of negative control and positive of silymarin and close to positive of extract.

GPx activity was significantly increased by about 123.33 and 210.67% respectively by co-administration rats *T. vulgaris* extract or silymarin and alcohol for 21 days with respect of alcohol-control ($P < 0.05$). *T. vulgaris* extract improved GPx activity nearly to that of silymarin compared to alcohol-control. GPx activity of positive controls was significantly declined corresponding to negative control.

SOD activity was significantly raised by co-administration *T. vulgaris* extract either on alcohol-induced hepatotoxicity rats or positive extract control rats (105.36 and 23.53% respectively) comparing to each control ($P < 0.05$). *T. vulgaris* extract more effective on SOD activity of alcohol-induced hepatotoxicity rats or positive control than silymarin.

CAT activity followed the same trend of SOD and was significantly elevated by force feeding *T. vulgaris* extract or silymarin in comparison with alcohol-control. There are no significant differences were recorded between CAT activity of all experimental groups, except for alcohol-control ($P < 0.05$).

3.6. Effect of T. vulgaris extract on oxidative stress status of liver

In comparison with negative control, alcohol administration resulted in pronounced oxidative stress status. So, oxidative stress parameters; MDA and H_2O_2 concentrations significantly elevated on rat's force fed alcohol by about 11.70 and 23.26% respectively (Table 6). Meanwhile, *T. vulgaris* extract protected liver from lipid peroxidation represented as significant reduction on MDA

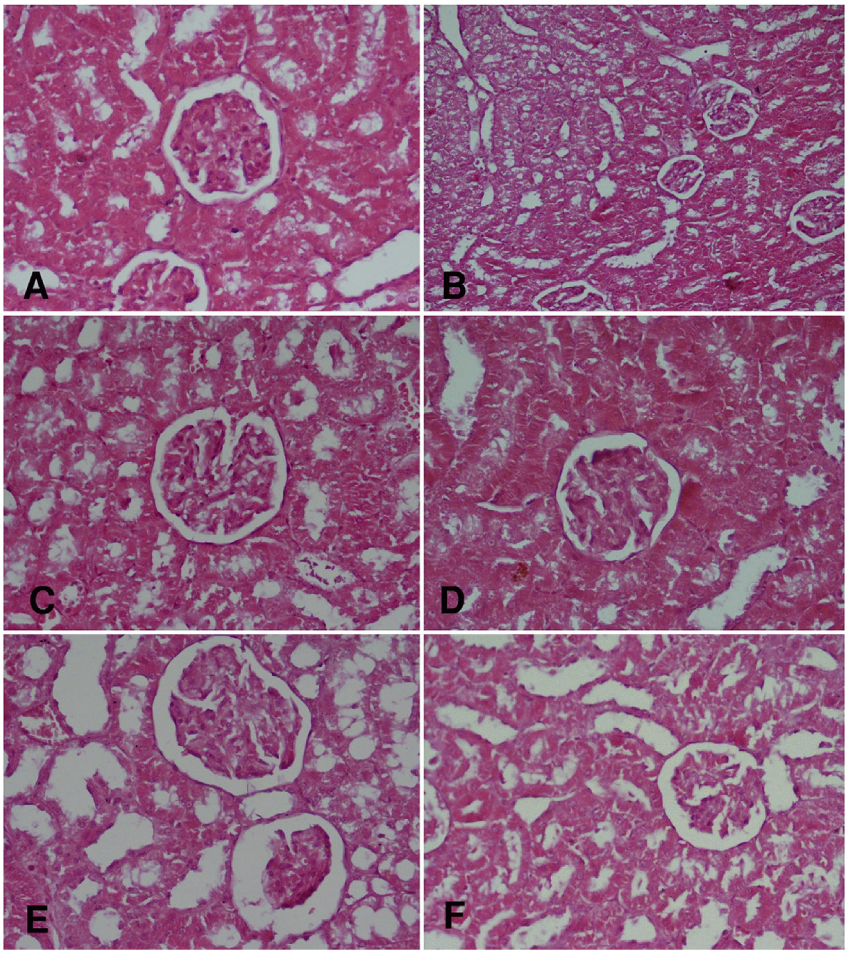

Figure 2. A photomicrograph of renal tissue sections from: A) negative control rat showed normal structure of renal tissue; B) An alcohol-control rat showed dilatation of many tubules with vacuolar degeneration in most of cells lining them; C) positive silymarin control rat showed normal renal tissue; D) Positive extract control rat showed normal structure of renal tissue; E) Treated rats with silymarin and ingested alcohol showed reduction of vacuolar degeneration of epithelial cells but dilatation of tubules is still noticed; F) Treated rats with extract and ingested alcohol showed normal renal tissue with slight dilatation of some tubules. (H&E, × 200, (B) × 100).

concentration of alcohol-induced hepatotoxicity rats (43.01%) compared to alcohol-control. MDA concentration of group treated with extract close to that of group treated with silymarin. MDA concentration of positive control extract was significantly decreased with respect of negative control ($P < 0.05$).

As well as, significant reduction was recorded on H_2O_2 concentration of rats co-administrated alcohol and *T. vulgaris* extract (46.03%) corresponding to alcohol-control. H_2O_2 concentration of positive control of extract was significantly reduced by about 48.15% comfortable to negative control. Extract showed improvement on oxidative stress parameter H_2O_2 higher than silymarin either in alcohol-induced hepatotoxicity rats or positive control extract ($P < 0.05$).

3.7. Effect of T. vulgaris extract on histopathological examination of liver and kidney

The histopathological examination of liver section of negative control rats appeared with a relatively dark-red color (Figure 1A). It revealed normal hepatic lobules with normal central veins, hepatic cords; sinusoids; and portal tracts. Liver of positive silymarin rats showed slight congestion of main blood vessels (Figure 1C), while that of positive extract rats showed slight dilatation of blood vessels and sinusoids denoting slight edema (Figure 1D).

Alcohol ingestion to rats lead to damaging effect on liver tissue, represented as a marked vacuolar degeneration for most cells with formation of fibrous tissue as bands extending in between hepatocytes (Figure 1B). On contrary, using silymarin in treating the damaging effect of ethanol shows disappearance of vacuolar degeneration in cells with reduction of fibrosis and congestion of blood vessels (Figure 1E). The effect of extract in treating the damaging effect of ethanol was better than that of silymarin, as normalization of liver tissue was observed but with signs of slight edema (dilatation of blood vessels and sinusoids) (Figure 1F).

The histopathological examination of renal section of negative control rats showed healthy glomeruli by light microscopy. The glomerular capillary loops were thin and delicate. Endothelial and mesangial cells were healthy and the surrounding tubules were normal (Figure 2A). Both of positive of silymarin or extract rats showed normal structure of renal tissue (Figure 2C & D respectively).

The same results of liver were obtained in case of renal tissue as ethanol caused marked vacuolar degeneration of epithelial cells lining the tubules with dilatation of these tubules (Figure 2B). Silymarin markedly reduced the vacuolar degeneration of epithelial cells, but the signs of edema (dilatation of tubules) are still observed (Figure 2E), while using extract in treating the damaging effect of ethanol gave better results as renal tissue regained its normal structure but with slight dilatation of tubules (Figure 2F).

Generally, the used extract showed better results than those of silymarin in treating the damaging effect of ethanol on both liver and kidney tissues, although it caused retention of water that appeared in the form of dilatation of blood vessels and tubules even if it is used to normal rats.

4. Discussion

Alcohol administration caused liver injury dependent on oxidative metabolites of alcohol in liver reflecting oxidative stress status. Free radicals the metabolites of alcohol metabolism formed radicals covalently to the proteins and macromolecules causing peroxidative degradation in unsaturated fatty acids of lipids membrane of endoplasmic reticulum. Previous process lead to lipid peroxidation and accumulation of lipids in liver caused fatty liver [38].

When rats force fed 40% alcohol for 21 days, hepatotoxicity was happened represented as significant reduction in total protein and its fractions albumin and globulin production. As well as hepatocytes distortion represented as significant increment in ALP, AST and ALT activities of rats administrated alcohol. Additionally, antioxidants liver enzymes activities (GR, GST, GPx, CAT and SOD) and reduced glutathione concentration were significantly minimized, while oxidative stress parameters (MDA and H_2O_2) were significantly maximized, when rats force fed alcohol for 21 days, reflecting oxidative stress status. On alcohol-control, significant elevation in TC and LDL-C was recorded without significant changes on risk ratio, because alcohol did not reduce HDL-C significantly. Obtained results were in accordance of Arun and Balasubramanian, [38], Sharma *et al.*, [19], Karwani and Sisodia, [39] and Begum and Kiran, [40].

Elevated activities of AST, ALT, and ALP enzymes indicate liver damage, this is because of higher concentration of alcohol dehydrogenase in liver, which catalyzes alcohol to its corresponding aldehyde [41]. In the present study *T. vulgaris* extract at a dose of 500 mg/kg b. wt. showed a significant inhibition in the AST and ALT activities towards the respective normal range and the extract was more effective on ALT than AST. These results are indicating that stabilization of plasma membrane and repair of hepatic tissue damage caused by alcohol. In addition, significant decrease in elevated ALP activities synchronous with decrement in total protein and its fractions; albumin and globulin levels suggests the stability of biliary dysfunction in alcohol-induced hepatotoxicity rats [42]. These results indicate that *T. vulgaris* extract protected the structural integrity of the hepatocellular membrane and liver cell structure, which destroyed by alcohol and was confirmed by histopathological examination. These results agreed with those of Arun and Balasubramanian, [38] on *Phyllanthus amarus and Eclipta prostrata*, Sharma *et al.*, [19] on *Adina cordifolia*, Padmanabhan and Jangle, [43] on 80% alcoholic extract of leaves of *Aloe vera*, *Bacopa monniera*, *Moringa oleifera and rhizome of Zingiber officinale*, Karwani and Sisodia, [39] on *tagetes erecta and* Begum and Kiran, [40] on *Cleome chelidonii*. While, our results disagreed with the results obtained by Vetvicka and Vetvickova [13], they demonstrated that derivatives of essential oils of thymus showed a small reduction on AST, ALT and ALP activities. The differences were found between our study and that of Vetvicka and Vetvickova [13] may be due to the difference between two extracts, we used alcoholic extract of leaves, while them used derivatives from essential oil extract. The main components found in the used extract was polyphenols and flavonoids which have been known as hepatoprotective agents [19].

Significant reduction in an antioxidant enzymes activities of SOD, CAT, GR, GST and GPx in tissues on the chronic alcohol treatment in rats were recorded concurrent with significant increment in oxidative parameters; MDA and H_2O_2. The alcohol was metabolized by CYP2E1 and produced 1-hydroxy ethyl radicals. 1-hydroxy ethyl radicals have been shown to inactivate several proteins including antioxidant enzyme system [44]. The inhibition of antioxidant system may cause the accumulation

of H_2O_2 or products of its decomposition [45]. Meanwhile, administration *T. vulgaris* extract converted obvious decrease in activities of antioxidants enzymes to significant increment. Researchers have reported results like obvious results as Arun and Balasubramanian, [38] on *P. amarus and E. prostrata*, Munusamy and Johnson, [46] on resveratrol and El-Newary *et al.*, [47] on *Tagetes lucida* leaves extract all these plants improved antioxidants enzymes activities and suppressed oxidative stress parameters. The observed increase in the activities of antioxidant enzymes on *T. vulgaris* extract administration may be due to stimulatory effect of its active constituents like polyphenols and flavonoids. As well as the decrease in the tissue lipid peroxidation on *T. vulgaris* extract treatment can also be correlated with the elevation in the activities of antioxidant enzymes.

Alcohol ingestion augments lipid peroxidation of cellular membrane, while co-administration of *T. vulgaris* extracts decreased it. Poly unsaturated fatty acids in cell membranes of alcohol-induced hepatotoxicity livers rats is main target of oxidative stress causing lipid peroxidation and excessive formation of MDA which may leads to damage of the cell structure evident excessive formation of MDA levels [48,49]. Lipid peroxidation leading to tissue damage and suppression the antioxidant defense system, which prevents the formation of excessive free radicals [38]. *T. vulgaris* co-administration significantly restored antioxidant defense system and lowered lipid peroxidation.

Chronic administration of alcohol depletes glutathione concentration (GSH) of liver. Alcohol catalyzes into acetaldehyde, which conjugates with sulfhydryl groups of GSH. GSH is the coordinator the body's antioxidant defense processes. GSH acts as free radical scavenger and supports in the conservation of protein sulfhydryl groups. GSH is an important source for reducing power during oxidative stress generated by ROS [50]. *T. vulgaris* extract significantly raised GSH concentration, which resulted in increasing in an antioxidants reserve of cells.

Superoxide dismutase (SOD) is the most susceptible enzyme to liver cells damage. SOD scavenges the superoxide anion ($O^{2\cdot}$) and converts it to hydrogen peroxide to reduce the toxic effects causing by the free radical [51]. The reduced activity of SOD in alcohol toxicity may cause the accumulation of $O^{2}\bullet$, H_2O_2 or the products formed by their degradation [42]. Elevation in superoxide anions radical ($O^{2\cdot}$) production forcing the cell to synthesize increases mitochondrial SOD through enhancing gene transcription. In this investigation *T. vulgaris* and silymarin showed significant increase in SOD activities of enzymes.

Catalase (CAT) is a common enzyme found in nearly all living organisms exposed to oxygen. CAT is a haemoprotein protects cells from the accumulation of H_2O_2 by decomposing it to form H_2O and O_2 or by using it as an oxidant in which it works as a peroxidase. Alcohol-mediate hepatotoxicity rats significantly minimized levels of catalase could be due to depletion of NADPH, or generation of superoxide radicals, or elevated activity of lipid peroxidation or combination of all [38]. But co-administration *T. vulgaris* extract significantly maximized the levels of CAT.

GPx and GR activities of rat's force fed with 40% alcoholic were significantly diminished due to increase in free radical damage and induced lipid peroxidation [52]. Co-administration with *T. vulgaris* extract showed amelioration in the antioxidant system against free radicals and significantly maximized GPx and GR activities. GPx enzyme is the catalyst of the reaction of hydroperoxides with reduced glutathione to produce glutathione disulphide (GSSG) and hydroperoxides. To convert oxide glutathione; GSSG to reduce form GSH, the GR support the reverse reaction to reduced GSH with the co-enzyme NADPH [42]. *T. vulgaris* extract co-administration maintained GR and GPx activities in normal ranges.

Polyphenol and flavonoids have been known as hepatoprotective plants [19]. Therefore, it has been suggesting that the hepatoprotective activity shown by the *T. vulgaris* extract can be because of these polyphenols and flavonoids. Flavonoids had been proved to increase the intracellular glutathione concentrations through increase the expression of the rate limiting enzyme in the synthesis of glutamylcysteine synthetase [53,54]. ROS- induced hepatotoxicity can be effectively controlled through administration of agents possessing anti-oxidant [55], free radical scavenger [56] and anti-lipid per oxidant activities [57]. Hepatoprotective property depending on *T. vulgaris* extract may be attributed to the polyphenolic compounds particularly flavonoids. *T. vulgaris* leaves extract contains total phenol; (214.55 ± 15.65) mg gallic/g extract and flavonoids; (143.75 ± 13.45) mg catechin/g extract. Quercetin flavonoids reduced alcohol –induced toxicity as indicated by the lowering of marker enzymes activities; ALP, AST and ALT [58]. Saalu *et al.*, [59] and Pari *et al.*, [60] illustrated the protective effects of caffeic acid in alcohol-mediated hepatotoxicity may be referred to the cell membrane stabilizing ability of the caffeic acid banning the hepatic enzymes infiltration into the serum. Quercetin and caffeic acid as flavonoids acts about 0.983 and 0.496 mg/g *T. vulgaris* extract. Rosmarinic acid and caffeic acid oral intubation showed remarkably reduction on *tert-* butyl hydroperoxide-induced oxidative damage *in vitro* and *in vivo*. Rosmarinic and caffeic acids caused significant reduction on liver biomarkers activities (AST and ALT) and lipid peroxidation biomarker (MDA) concurrent with significant increment on GSH concentration and CAT and GPx activities of rats injected with *tert-* butyl hydroperoxide [61]. Rosmarinic acid the major constituent of polyphenols, extract contains 48.419 mg/g extract. Hesperidin administration have hepatoprotective activity against nicotine-induced toxicity rats represented as markedly diminishing on ALT, AST, ALP and LDH activities and amelioration on liver histology [62]. Additionally, hesperidin reduced TC of plasma and liver reflecting to reduction on lipid accumulation on liver. Hesperidin ingestion reversed liver functions; AST, ALT, ALP, LDH, GGT and bilirubin levels as well as, kidney functions; urea, uric acid and creatinine levels of iron-induced hepatotoxicity in normal ranges. Also, hesperidin significantly ameliorated lipid profile as a significant reduction on TC, TG and lipid peroxidation (MDA) on plasma, liver and kidney reflecting on liver and kidney histology. These results may be due to *in vivo* antioxidants activity of hesperidin showed as remarkably elevation on GSH concentration and enzymes activities; CAT, SOD, GPx and GST of iron-induced hepatotoxicity rats *T. vulgaris* extract contains 3.016 mg hesperidin/g extract.

Chronic alcohol intake results hypercholesterolemia and enhanced lipid peroxidation. Chronic lipid accumulation in liver cells caused it fibrotic and lead to impaired liver function. Prevention lipid peroxidation protects cell membrane and is necessity to prevent or delay the aforesaid pathologies [49]. TC levels increment in alcohol-mediates hepatotoxicity rats may be due to stimulate alpha–hydroxyl methyl glutaryl CoA

(HMG CoA) reductase activity, which is the rate limiting step in cholesterol biosynthesis [63]. TC reduction by co-administration *T. vulgaris* extract may be due to HDL-C increment, which transports cholesterol to liver [64]. The results of the current study showed that administration of alcohol caused relative increase in HDL-C level of rats, this increment continued by *T. vulgaris* extract administration. Confirmation that, moderate alcohol consumption reduces coronary heart disease risk through elevation HDL-C [65]. On contrary, alcohol administration maximized LDL-C level of the rats, while co-administration *T. vulgaris* extract significantly minimized LDL-C levels.

The ameliorated effect of *T. vulgaris* on lipid profile of alcohol-induced hepatotoxicity may be due to its phenols and flavonoids content [66]. Flavonoids improves lipid metabolism through inhibition of acyl coenzyme A: cholesterol O-acyltransferase and 3-hydroxy- 3-methylglutaryl-coenzyme A (HMG CoA) reductase in rats [67]. Quercetin as flavonoids has been demonstrated to ameliorate hyperlipidemia, suppression oxidative stress through enhancement lipolysis activity and magnifies the lipids beta oxidation (by upregulation the adipocytes genes expression) [68]. *Cordia dichotoma* fruits extract improved lipid profile of high fat diet-induced hyperlipidemic rats, extract contains rosmarinic acid as a major component (comprises 26.39% of extract) [66]. Rosmarinic acid is the major component of *T. vulgaris* extract (48.419 mg/g extract) and quercetin acts about 0.983 mg/g.

The results of this research indicated that *T. vulgaris* leaves crude alcoholic extract was effective treatment for the control of hepatotoxicity induced by alcohol. Protective effect of *T. vulgaris* extract evident by amelioration in serum liver functions ALP, AST, ALT activities, total protein and its fractions albumin and globulin with respect with alcohol-induced hepatotoxicity rats. Antioxidant defense system was improved concurrence with suppression on lipid peroxidation by oral administration *T. vulgaris* extract. *T. vulgaris* extract showed hypolipidemic effect represented as decreasing on TC and LDL-C and increasing HDL-C levels. In conclusion, *T. vulgaris* extract showed significant hepatoprotective, hypolipidemic and antioxidant activity close to silymarin the standard drug. The pronounced effect of *T. vulgaris* leaves extract could be attribute to its polyphenols and flavonoids contents. Finally, we can report that *T. vulgaris* leaves crude alcoholic extract is a good hepatoprotective agent with a good margin of safety.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgment

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