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ANTIOXIDANT AND ANTITUMOR ACTIVITIES OF THE ESSENTIAL OIL AND ETHANOLIC EXTRACT OF THYME (*Thymus vulgaris L.*) LEAVES

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ABSTRACT

The present study was conducted to evaluate the antioxidant and antitumor activities of the essential oil and ethanolic extract of thyme (*Thymus vulgaris*) leaves The antioxidant test results showed that the essential oil strongly reduced the thiobarbituric acid (TBA) values at 100, 200, 400 and 1000 ppm concentrations and it was more effective than thymol and BHT at a concentration of 1000 ppm. The ethanolic extract was able to reduce the TBA values, which were lower than those of essential oil, thymol and BHT The viability of Ehrlich ascites carcinoma cells (EACC) with essential oil and ethanolic extract at different concentrations was examined. It was found that incubation of tumor cells with essential oil reduced the viability of these cells and the dead cells produced by this oil reached 100% by concentrations over 200 ppm, while the ethanolic extract was lower than essential oil compared with thymol. Also, effect of essential oil and thymol at concentrations of 200 and 400 ppm on the survival of tumor bearing mice in the same time and after a week of injection with EACC was studied. The essential oil gave a higher cytotoxic effect than thymol whereas different treatment showed significant increase in the life span of tumor bearing mice compared with untreated tumor control.

The chemical composition of essential oil and ethanolic extract were analyzed by GC and HPLC. The essential oil comprised of 18 constituents representing 91.42% of the oil. Carvacrol (28.96%), limonene (25.17%), γ - terpinene (10.86%), terpeniol (9.73%), β- caryophyllene (7.81%), linalool (2.83%) and thymol (2 12%) were the major components comprising 84.64% of the oil. The ethanolic extract was analyzed by HPLC system. Data showed that *T vulgaris* characterized by the presence of a high content of p-coumaric acid (39.80%), vanillin (24.34%) and moreover, caffic acid (5.09%) and Ferulic acid (4.04%) were also detected Results presented here may suggest that the essential oil of *T. vulgaris* can be used in pharmaceutical industries for its potential medicinal properties including antioxidant, protective and anticarcinogenic effects.

Keywords: *Thymus vulgaris*, antioxidant, antitumor, anticancer, essential oil, thymole, phenolic compounds.

INTRODUCTION

Numerous physiological and biochemical processes in the body may produce oxygen-centered free radicals and other reactive oxygen species as byproducts. Overproduction of such free radicals can cause oxidative damage to biomolecules, eventually leading to many chronic diseases, such as atherosclerosis, cancer, diabetes, aging and other degenerative diseases in humans (Niki, 1997 and Poulson *et al.*, 1998).

The genus *Thymus* (lamiaceae) consists of over 300 evergreen species of herbaceous perennials and subshrubs, native to Southern Europe and Asia (konemann, 1999). Medicinal plants have received growing

attention in recent years as potential chemopreventive agents. Thyme (*Thymus vulgaris L.*) has been used for its potential medicinal properties including antioxidant (Dorman *et al.*, 2000; Young-Ok *et al.*, 2004; Seung-Joo *et al.*, 2005), antimicrobial (Gianni *et al.*, 2005); hepatoprotective and anticarcinogenic effects (Yizhong *et al.*, 2004 and Gianni *et al.*, 2005). This plant may contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites which are rich in antioxidant activity (Shahidi and Naczk, 1995; Cotelle *et al.*, 1996; Velioglu *et al.*, 1998; Zheng and Wang, 2001 and Cai *et al.*, 2003). These antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumer, antimutagenic, anticarcinogenic, antibacterial or antiviral activities to a greater or lesser extent (Owen *et al.*, 2000 and Sala *et al.*, 2002).

As mentioned before most of the studies were focused on the antibacterial, antifungal and antioxidant effects of essential oil and ethanolic extract of thyme plant but up to our knowledge, no work has been done concerning its effects on the tumor cells. The present study was carried out to investigate the chemical composition of the essential oil and ethanolic extracts of *Thymus vulgaris*, as well as the bioactivity of these extracts as antioxidant and antitumor agents in order to evaluate their medicinal potential.

MATERIAL AND METHODS

Plant material

Samples of *Thymus vulgaris L.* were collected from Experimental Station of Medicinal plants, Faculty of Pharmacy, Cairo University, Giza.

Preparation of ethanolic extract

Freshly collected leaves of thyme (25 g) were extracted under reflux with ethanol (70%) at 80°C in water bath for 6hs. The ethanolic extract was evaporated under reduced pressure to remove the solvent. The residue of aqueous extract was completed to 50 ml with distilled water (Mabry *et al.*, 1970).

Extraction of volatile oil

Freshly collected leaves of thyme were submitted to hydrodistillation for 3 h using a Clevenger- type apparatus (yield 2% v/w). The resultant essential oil was dried over anhydrous sodium sulphate, weighed and the oil content was calculated. This oil was stored at – 20°C in a deep freezer for subsequent analysis.

Identification and determination of leaves essential oil and ethanolic extract composition by gas liquid chromatography:

The essential oil and ethanolic extract were analyzed by Hewlett Packard 5890 series II gas chromatograph equipped with a dual FID unit. The chromatograph was fitted with a capillary column (30 m × 0.25 nm) coated with carbwax 20 M (0.25 µm film thickness). Separation conditions were: initial temperature 50°C for 3 min, program rate 3°C / min, final temperature 240°C; injector and detector temperatures were 220°C and 290°C, respectively. Flow rates: N_2 (carrier), 1.5 ml min^{-1}, H_2, 30 and air, 300 ml /

min^{-1}, respectively. Peak identification was performed by comparing the retention time of each peak with those of known compounds. Also, the identification of each major component was made by injection of the oil along with those of authentic samples. Quantitative determination was conducted using a computing integrator (Hewlett Packard (hp) Vectra 486/33 vl).

Identification and determination of thyme leaves phenolic compounds composition:

Identification of individual phenolic compounds of thyme plant was performed on a JASCO HPLC, using a hypersil C_{18} reversed- phase column (250 × 4.6 mm) with 5µm particle size. Injection by means of a Rheodyne injection value (Model 7125). A constant flow rate of 0.3 ml/ min was used with two mobile phases: (A) 0.5% acetic acid in distilled water at pH 2.65; and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear starting with (A) and ending with (B) over 35 min, using an UV detector set at wavelength 254 nm. Phenolic compounds of sample were identified by comparing their relative retention times with those of the standard mixture chromatogram. Quantitative determination was conducted using a computing integrator (Ben- Hammouda *et al.*, 1995).

***In vitro* antioxidant activity**

Antioxidant activity of thyme extracts was determined in microsomes which were isolated from liver of male Wistar rats weigh 120 ± 5g using Fe^{++}/H_2O_2 system. Lipid peroxidation of microsomes has been shown to be absolutely depended on concentration of ferrous ion added. The liver microsmes were incubated with different concentrations of thyme extracts (50, 100, 200, 400 and 1000 ppm) as well as butylated hydroxytoluene (BHT) and thymol at the same concentration at 37 ± 1°c for 15, 30 and 60 min. lipid peroxidation products were measured by quantitation of thiobarbituric acid reactive substances (TBARS) during incubation period as described by Haraguchi *et al.* (1997). After incubation at different periods, the lipid peroxidation was determined by rapid addition of 2.5 ml of TCA- TBA reagent (15%, w/v trichloroacetic acid and 0.37% thiobarbituric acid) to the reaction mixture, then heated for 20 min in boiling water bath. After cooling, the flocculent was removed by centrifugation at 1000 xg for 5 min. The absorbency of TBARS in the supernatant was recorded at 532 nm against a blank containing all the reagents expect liver microsomal.

The extinction coefficient of TBARS was taken as 1.56×10^5 to convert the absorbance values into concentration (Joseph *et al.*, 1988). The TBARS concentration in liver microsomes was normalized with protein concentration.

Antitumar activity of thyme extracts on Ehrlich ascites carcinoma cells (EACC)

Source of tumor cells

The study was performed using a line of Ehrlich ascites carcinoma resistant to Endoxan (El- Merzabani and Tewfik, 1976). The tumor line was maintained in the National Cancer Institute (NCI) in female swiss albino mice weighing 22-25g at weekly intraperitoneal (i.p.) by transplantation of 2.5×10^6 cells. For the *in vitro* and *in vivo* studies, the cells were taken from tumor

transplantation and centrifuged at 1000 rpm for 5 min, then washed with saline and counts adjusted so that 1 ml contained 4×10^6 cells.

***In vitro* cytotoxicity**

The viability percentage of tumor cells was measured by the modified cytotoxic trypan blue – exclusion technique of Bennett *et al.* (1976).

The culture medium used was prepared using RPMI 1640 media, 10% fetal bovine serum and L- glutamine (Gibco). The medium was then filtered through 0.22 Millipore filters and one ml was transferred into appropriate tubes, two ml of the cell suspension containing 4×10^6 cells. The different concentrations (50, 100, 200, 400 and 1000 ppm) of examined extracts and thymol were added to five of these tubes, while the other tube served as control (saline plus tween 80 was used instead of extract). The tubes were then incubated at 37°C in the presence of 5% CO_2 for 2, 5 and 6 hours, then the tubes were centrifuged at 1000 rpm for 5 min and the separated cells were suspended in saline. The content of each examined tube and control was transferred into new clean and dry small test tubes and added to each 10 µl of cell suspension, 80µl saline and 10µl trypan blue and mixed. The number of dead cells was calculated using a haemocytometer slide and the lethal doses for 50% of cells (LC50) were subjected to probit analysis by using method of Finney, (1952).

***In vivo* cytotoxicity of essential oil**

Swiss albino mice (60), weighing 22 – 25 g were obtained from the Research Institute of Ophtalmology, Giza, Egypt. The animals were fed on a standard diet and water was available ad – libitum for one week as an adaptation period.

After the adaptation period, animals were injected intraperitoneal by Ehrlich ascites carcinoma cells suspension (2×10^7 cells/ml) and randomly divided into two main groups. The first main group was randomly divided into five subgroups and injected at the same time with the tumor cells. The first subgroup of the first main group (6 animals) was injected by sun flower oil and considered as a tumor control. The 2 nd and 3 rd subgroups were injected by 160 µl of essential oil at concentrations of 200 and 400 ppm, respectively while 4 th and 5 th subgroups were injected with thymol at the same concentrations, respectively. After one week, the animals in all treated subgroups were divided into two groups (each 3 rats) and one group from them were injected once again with mentioned before and the other group was maintained as it is until the end of the experiment.

The same design was adopted for the second main group but after one week from injection with tumor cells.

Antitumor activity of treated groups was evaluated by the increase of life span percentage (ILS %) comparing with the tumor control group according to Rajkapoor *et al.* (2004), as the following formula:

$$ILS\ \% = (T - C/C)\ 100$$

where T = number of days the treated animals survived and C = number of days tumor control animals survived.

Statistical analysis

The data obtained from the present work were statistically analyzed according to the procedure outlined by Steel and Torrie (1980). The means of the results were compared by the least significant differences test (L. S. D.).

RESULTS AND DISCUSCION

Chemical composition of the essential oil and ethanolic extract

GC analysis resulted in the identification of eighteen compounds representing 91.42% of the oil. Carvacrol (28.98%), limonene (25.17%), γ - terpinene (10.86%), terpeniol (9.73%), β - caryophyllene (7.81%), linalool (2.83) and thymol (2.12%) were the main components comprising 87.50% of the oil (Table 1). Ethanolic extract did not identify any components. These data are in agreement with those obtained by Dorman *et al.* (2000). Compared to other *Thymus species*, carvacrol content of the oil presented here is lower than that of *T. revolutus* (43.13%) but higher than that of *T. pectinatus* (3.7%) (Vardar – Unlu *et al.*, 2003). It would also be noteworthy to point out that the composition of any plant essential oil studied is influenced by the presence of several factors, such as local, climatic, seasonal and experimental conditions (Daferera *et al.*, 2000); thereby altering the biological activities is expected (Vardar – Unlu *et al.*, 2003).

Table (1): Chemical composition of essential oil of *Thymus vulgaris* (GLC).

Component	RT*	%
α - pinene	5.683	0.66
Camphene	6.867	0.41
β - pinene	7.450	0.87
Myrcene	8.467	1.65
Limonene	9.383	25 17
γ - terpinene	9.950	10.86
P- Cymene	10.783	1.62
Linalool	11.650	2.83
Thymol	13.800	2.12
Borneiol	14.417	0 62
Terpniol	15.200	9.73
α - Terpinene	16.150	0.61
Carvacrol	16.883	28.98
β - caryophyllene	17.900	7.81
Unknown	19.217	1.91
Unknown	19.967	1.13
Unknown	21.783	2.12
Unknown	22.267	0.90
Identified compounds		93.48

*RT: refers to retention time (min).

Chemical composition of phenolic compounds of ethanolic extract

The phenolic compounds of ethanolic extract of thyme were identified by high performance liquid chromatography (HPLC) against standard compounds and the results are listed in Table (2). The identified material

represented 86.88% of thyme ethanolic extract. The lack of certain standard phenolic compounds did not allow the complete identification of the phenolic composition. The most abundant components of thyme phenolic compounds were p – coumaric acid (39.80%), vanillin (24.34%). 3, 5 dimethoxy benzyl alcohol (8.51%), caffeic acid (5.09%) and ferulic acid (4.64%). These results are in agreement with Yanishlieva and Marinova (1995) who found that the *Thyme* species containe 3.4 dihydroxy benzoic acid, ferulic, sinapic and caffeic acids by higher concentrations which were responsible for antioxidant properties.

Table (2): Phenolic compounds composition of ethanolic extract of *Thymus vulgaris* (HPLC).

Component	RT*	(%)
Pyrogallic acid	7.92	1.87
Hydroquinone	9.10	0.80
Unknown	21.72	2.65
p- Hydroxybenzoic acid	23.23	1.22
Caffeic	24.82	5.09
Vanillin	28.45	24.40
p- Coumaric acid	31.48	39.80
3,5 Dimethoxy benzyl alcohol	33.88	8.51
Ferulic acid	34.77	4.64
Unknown	37.58	6.71
Eugenol	49.47	0.45
Unknown	52.98	0.19
Chrysin	54.07	1.41
Unknown	58.73	2.35
Unidentified compounds		13.12

*RT: refers to retention time (min).

In vitro antioxidant activity

It is of great interest to investigate the antioxidant activity of the essential oil and ethanolic extract of *Thymus vulgaris* in order to evaluate their potential medicinal importance. Subsequently, we examined the effect of essential oil and ethanolic extracts of this plant on lipid peroxidation by measuring TBARS using Fe^{++} / H_2O_2 system in the hepatic sells. The results in Figure (1) shows that there was a significant increase of the TBA values in control during incubation times (15, 30 and 60 min) at different concentrations (50, 100, 200, 400 and 1000 ppm).

Mixing hepatic extract with various concentrations of essential oil or ethanolic extract (50, 100, 200, 400 and 1000 ppm) and incubation for 15, 30 and 60 min results in gradual and significant decrease in the TBA values as the concentration and incubation time increase compared with control. Upon addition of both thymol and essential oil at different concentrations (50, 100, 200, 400 and 1000 ppm), it was clear that essential oil is more effective than thymol at 400 and 1000 ppm. There is an exception at concentrations of 50, 100 and 200 ppm, the effect of essential oil approaches that of thymol for all incubation periods.

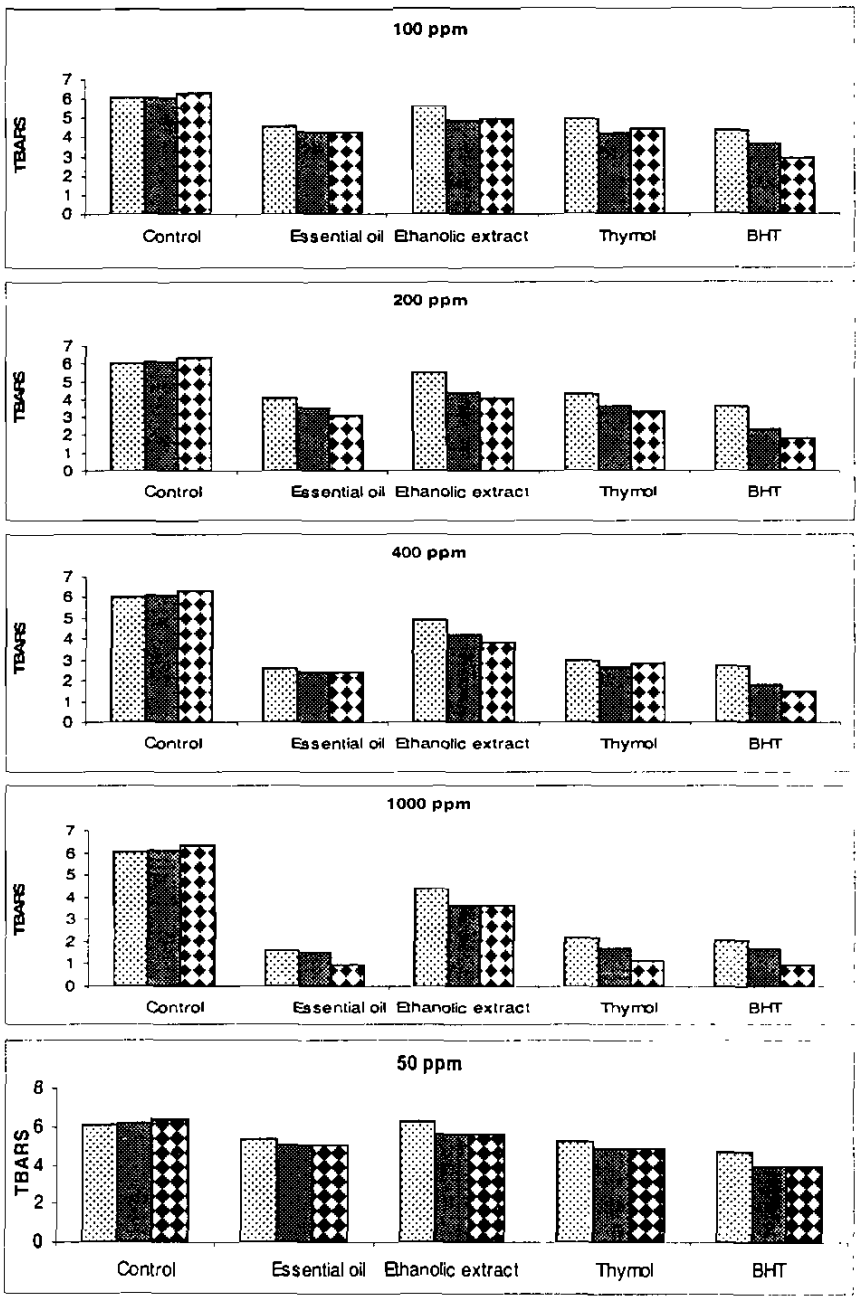

Figure (1): *In vitro* antioxidant activity of *Thymus vulgaris* essential oil and ethanol extract of various concentrations and times.

Comparing the antioxidant efficiency of BHT at various concentrations (50, 100, 200, 400 and 1000 ppm) with the various levels of essential oil and ethanolic extract, one would observe that the essential oil at 100, 200, 400 and 1000 ppm exhibited antioxidant activity and the concentration at a 1000 ppm of essential oil was superior to that of BHT in retarding lipid peroxidation in living systems. In addition, it is of interest to note that essential oil at 50, 100, 200 and 400 ppm possessed nearly the same antioxidant activity as those of BHT at the same concentrations. It's recommended to add 1000 ppm of the essential oil to oils to increase stability. On the other hand, the results demonstrated that the ethanolic extract was less efficient as antioxidant than the essential oil, thymol and BHT. This is matching the opinion of Atalay *et al.* (2004) who suggested that the essential oil and ethanolic extracts of *Thymus spathulifolium* possess antimicrobial and antioxidant properties and therefore, they can be used as a natural preservative ingredient in food and pharmaceutical industry. Bektas *et al.* (2005) concluded that antioxidant capacity of the essential oil of *T. sipyleus* could be attributed to the presence of high amount of thymol and carvacrol, since the sesquiterpenes have slight or no antioxidant activity potential.

Peroxidation or oxidation of lipids exposed to oxygen may cause damage to the tissues and may lead to cancer and inflammatory diseases (Yagi, 1987). Lipid peroxidation is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation. In the present study, treatment with *T. vulgaris* oil decreased the level of TBA values. So, it may be concluded that *T. vulgaris* essential oil can be used as a free radical suppressor, and it is recommended as an excellent natural source of essential oil that combats the oxidative stress produced by chemical or physical factors. Consequently, it can be considered as an antimutagenic agent.

In vitro **cytotoxicity of thyme essential oil and ethanolic extracts**

Effect of thyme leaves ethanolic extract and essential oil as well as thymol as standard was tested for their antitumor activity. The viability of Ehrlich ascites carcinoma cells (EACC) after incubation for 2, 5 and 6hs with the extracts was evaluated. The obtained results are given in Table (3). It was found that the incubation of tumor cells with essential oil and ethanolic extract and thymol at all concentrations (50 – 1000 ppm) for 2, 5 and 6 hs reduced the viability of these cells. The dead cells were increased by increasing the concentrations of essential oil, ethanolic extracts and thymol during incubation times. The addition of essential oil at a concentration of 50 ppm cell media and incubated for 2, 5 and 6 h to reduced the viable cells from 90.45% to 66.45%; 90.28% to 66.2% and 80.87% to 62.21%, respectively, while the dead cells produced by this oil reached 100% by 400 and 1000 ppm after the incubation for 2h and 200 to 1000 ppm after the incubation for 5h and 6h, respectively compared to control (9.55%, 9.72% and 19.13% after 2, 5, 6 h from incubation, respectively). Also, the addition of thymol reduced the viability from 90.28% and 80.87% to 52.36 and 47.51% by 100 ppm after incubation for 5 and 6h, respectively and reached to 3.09% and 0% by 200 ppm thymol.

Table (3): Effect of *Thymus vulgaris* leaves essential oil and ethanol extracts on the viability of Ehrlich Ascites Carcinoma Cell (EACC)*.

Treatment		Incubation time (hour) [men ± S.E. (n =3)]											
		2			5			6					
		% Viable cells	% Dead cells	LC_{50} ppm	% Dead cells	% Viable cells	LC_{50} ppm	% Dead cells	% Viable cells	LC_{50} ppm
		90.45± 3.59	9.55± 0.35		9.72± 0.44	90.28± 4.71		19.13± 0.73	80.87± 4.59	
Tumor + Essential oil	50ppm	66.45± 2.32	33.55± 1.16	86.17	33.8± 1.82	66.20± 2.19	82.45	37.79± 1.65	62.21± 2.98	88.87
	100ppm	51.77± 1.65	48.23± 1.67		52.36± 1.70	47.64± 1.15		54.77± 2.56	45.23± 1.42	
	200ppm	2.18± 0.06	97.82± 4.31		100.00 ± 4.91	0.0		100.00 ± 5.31	0.0	
	400ppm	0.0	100.00± 5.08		100.00 ± 5.23	0.0		100.00 ± 5.71	0.0	
	1000ppm	0.0	100.00± 4.61		100.00± 3.97	0.0		100.00 ± 5.01	0.0	
Tumor + Ethanolic extract	50ppm	90.39± 4.66	9.61± 3.24	5754.56	18.44± 0.72	81.56± 3.62	21865.28	20.18± 1.04	79.82± 3.75	12019.34
	100ppm	89.97± 3.57	10.03± 0.49		22.32± 1.20	77.68± 3.50		26.24± 1.24	73.76± 3.00	
	200ppm	88.05± 4.14	11.95± 0.56		22.66± 0.73	77.34± 2.97		27.93± 1.41	72.07± 3.75	
	400ppm	83.89± 2.78	16.11± 0.53		29.22± 0.79	70.78± 2.87		32.21± 1.72	67.79± 2.00	
	1000ppm	79.44± 3.17	20.56± 0.63		31.28± 1.53	68.72± 2.22		34.36± 1.04	65.64± 3.52	
Tumor + Thymol	50ppm	62.42± 2.51	37.58± 0.84	94.93	38.27± 1.25	61.37± 3.06	92.62	40.01± 2.23	59.99± 2.41	85.50
	100ppm	53.65± 1.70	46.53± 1.60		47.64± 2.17	52.36± 2.92		52.49± 2.94	47.51± 2.19	
	200ppm	17.24± 0.05	82.76± 4.11		96.91± 4.44	3.09± 0.90		100.00 ± 5.61	0.0	
	400ppm	4.88± 0.14	95.12± 3.28		100.00 ± 4.61	0.0		100.00 ± 4.66	0.0	
	1000ppm	0.0	100.00± 5.17		100.00± 5.62	0.0		100.00 ± 5.31	0.0	
The value of L. S. D. at 5%		7.179	8.491		9.040	6.985		10.156	7.048	

*: 2ml of EACC solution containing 4×10^6 cell.
± refers to standard error.
LSD refers to least significant difference test.

On the other hand, the ethanolic extract has lower efficacy than essential oil whereas, it reduced the viable cells from 96.45%, 90.28%, 80.87% to 79.44%, 68.72% and 65.64% at a concentration of 1000 ppm of cell media after incubation for 2, 5 and 6h, respectively. Furthermore, it was noticed that 50% death (LD_{50}) of tumor cells occurred by 86.17, 82.45 and 86.87 µg/ml (ppm) essential oil, while this value was obtained by 94.93, 92.62 and 85.50 µg/ml (ppm) thymol at different incubation times (2, 5 and 6 h, respectively). This may be due to that essential oil contains other compounds beside thymol which may have also antitumor activity.

***In vivo* cytotoxicity of thyme essential oil**

The cytotoxicity of the thyme essential oil on survival of tumor bearing mice is demonstrated in Figure 2 and 3. Figure (2) shows that the intraperitoneal administration with 160 µl in concentration of 200 and 400 ppm using one and two doses of thyme essential oil as well as thymol for the comparison in the same time injection with EACC led to a significant increase in life span percentage to be 28.85% and 32.69%; 59.62% and 65.38%, respectively compared with untreated tumor control.

From the data in Figure (3), we found that the administration of the essential oil 160 µl in concentrations of 200 and 400 ppm as well as thymol after one week of injection with EACC results in a significant increase in life span percentage to be 28.85%, 29.38% and 35.16%, 40.88% using one and two doses, respectively compared with untreated tumor control. It was clear that essential oil of thyme plant is more effective than thymol on survival of tumor bearing mice by 1.61 and 1.24 times for 200 ppm concentration, and 1.09 and 1.07 times for 400 ppm concentration. So, we can use the essential oil as potential chemopreventive and therapeutic agents against tumor cells. These results are in agreement with those of Gianni *et al.* (2005) who reported that *Thymus vulgaris* possesses antimicrobial activity, ability to neutralize free radical and prevent unsaturated fatty acid oxidation. Also, natural antioxidants are extensively studied for their capacity to protect organisms and cells from damage induced by oxidative stress which is a result of aging, degenerative diseases and cancer (Cozzi *et al.*, 1997).

Flavonoides in ethanolic extract have been recognized as a dietary chemopreventive agent that might block neoplastic inception or delay tumor progression (Gao *et al.*, 1999; Wong and Mclean, 1999).

Conclusion

Major aroma compounds found in essential oil of *Thymus vulgaris*; in particular, carvacrol, thymol, γ - terpinene, terpniol and myrcene found in this plant; exhibited potent antioxidant activity, comparable to the known antioxidant, BHT. Considering the abundance of these aroma chemicals in natural plants, the total activity may be comparable with, or more, than that of the known antioxidant. Furthermore, ingestion of these aroma compounds may help to prevent *in vivo* oxidative damage, such as lipid peroxidation, which is associated with cancer, premature aging, atherosclerosis and diabetes.

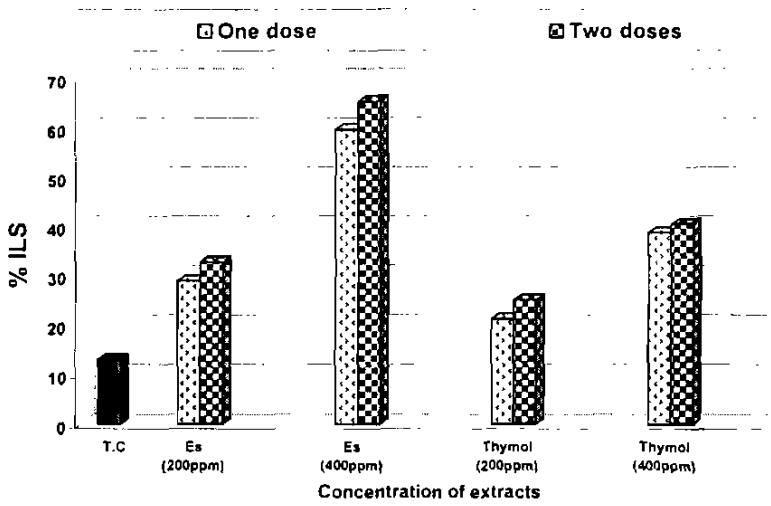

Figure (2): Effect of essential oil and thymol on the survival of tumor bearing mice in the same time injection with EACC.

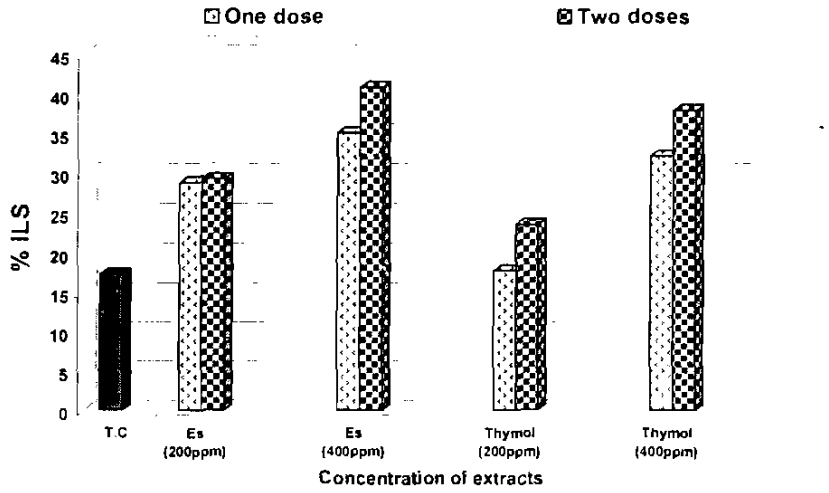

Figure (3): Effect of essential oil and thymol on the survival of tumor bearing mice After a week injection with EACC.

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النشاط المضاد للأكسدة والأورام السرطانية للمستخلص الإيثانولي والزيت الطيار لأوراق نبات الزعتر

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تهدف هذه الدراسة الى تقييم نشاط الزيت الطيار ومستخلص الإيثانول لأوراق نبات الزعتر كمضادات أكسدة في مستخلص ميكروسومات كبد الفئران باستخدام تركيزات مختلفة منها وكذلك الثيمول (١٠٠٠،٤٠٠،٢٠٠،١٠٠،٥٠ جزء في المليون) مع الاستعانة بمركب BHT كمادة قياسية للمقارنة . أيضا تم دراسة تأثيره على نمو خلايا ورم EACC خارج جسم الفار في بيئة مناسبة وذلك باستخدام نفس التركيزات المذكورة سابقا وأيضا دراسة تأثير مستخلص الزيت الطيار فقط على الزيادة في عمر الفار بعد إصابته بالورم بتركيزات ٢٠٠ و ٤٠٠ جزء في المليون لتقييم تأثيره كمضاد للسرطان.

وقد أظهرت النتائج أن للزيت الطيار فاعلية قوية كمضاد للأكسدة وذلك بالمقارنة بBHT والثيمول والكنترول على جميع التركيزات فيما عدا تركيز ٥٠ جزء في المليون وقد لوحظ أنه أكثر فاعلية من ال BHT والثيمول على جميع فترات التحضين وعلى تركيز ١٠٠٠ جزء في المليون . أيضا أظهر فاعلية قوية لقتل الخلايا السرطانية (EACC) حيث وصلت نسبة الخلايا الميتة إلى ١٠٠% تقريبا على تركيز ٤٠٠ جزء في المليون بعد ساعتين من التحضين . وبالتالي فانه أدى إلى زيادة في عمر الفار المصاب بالورم على تركيزات ٢٠٠ و٤٠٠ جزء في المليون وقد لوحظ أن تأثيره يفوق تأثير الثيمول في زيادة عمر الفأر ويزداد هذا التأثير بزيادة التركيز وذلك بالمقارنة مع الكنترول . على العكس فان مستخلص الإيثانول كان أقل فاعلية بدرجة كبيرة كمضاد للأكسدة ومانع لنمو الخلايا السرطانية وذلك بالمقارنة بالكنترول والمعاملات الأخرى (الزيت الطيار – الثيمول – BHT) .

وعند تحليل مكونات الزيت الطيار بواسطة جهاز التحليل الغازي الكروماتوجرافي (GLC) وجد أنه يتميز بوجود الكارفاكرول بنسبة عالية (٢٨,٩٦ %) وثيمول بنسبة (٢,١٢ %) كذلك يحتوي على ليمونين (٢٥,١٧ %) وجاما –تربينين (١٠,٨٦ %) وتربينول (٩,٧٣ %) وبيتا– كاريوفلين (٧,٨١ %) والتي قد يرجع إليها جميعا التأثير الفعال للزيت والذي يفوق تأثير الثيمول ، وقد لوحظ غياب المركبات المتطايرة عند تحليل المستخلص الإيثانولي وعند تحليل المستخلص الإيثانولي بواسطة جهاز HPLC وجد انه يحتوي على حمض بارا كوماريك (٣٩,٨٠ %) وفانيلين (٢٤,٣٤ %) وكافييك (٥,٠٩ %) و فيروليك (٤,٠٤ %) كفينولات رئيسية به والتي قد يعزى إليها تأثير المستخلص الكحولي .

ولذلك تقترح الدراسة امكانية استخدام الزيت الطيار لنبات الزعتر في الصناعات الصيدلانية لما يتميز به من خواص قوية مضادة للأكسدة ومانعة للإصابة بالسرطان لما له من تأثير واقي ضد الإصابة بالأورام أو إضافة أوراق الزعتر بطريقة علمية للأغذية للوقاية من العديد من الأمراض أهمها الأورام السرطانية علما بانه يستخدم بهذه الطريقة في العديد من الدول العربية.