Assessment of the Anticonvulsant Effect of *Thymus vulgaris* Plant

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Key words: *Thymus vulgaris*, Anticonvulsant Potentiality, MES, PTZ

The Abstract

1. The study objectives: this study was constructed to investigate the anticonvulsant, Sedative effects and possible hepatotoxic effects of ethanolic extract of *Thymus vulgaris* (Thyme).
2. Methods and materials: Pharmacological experiments were as follows; anticonvulsant activity against pentylenetetrazole- (PTZ) and maximal electroshock- (MES)- induced convulsions and sedative potential using simple activity meter. All tests were done on rats.
3. The results: In this study, ethanolic extract of *Thymus vulgaris* have no effect on MES- and PTZ- induced convulsions. Regarding sedative activity test using simple activity meter; ethanolic extracts of *Thymus vulgaris* showed high sedating effect ($P<0.0001$) in comparison with control group. In one part of this investigation, the combined treatment of plant extract with valproic acid (VPA) was studied. Combination treatment of (Thyme + VPA) produced full protection against convulsions induced by PTZ. In MES- induced

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convulsions combined (Thyme +VPA) was found to cause 33% protection. On the other hand, sedative potential of the combination treatment was also increased. Hepatotoxicity studies showed that *Thymus vulgaris* affected the serum level of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST).

4. Conclusion: It could be concluded that the combination treatments of ethanolic extract of *Thymus vulgaris* with VPA have anticonvulsant on PTZ and MES models. Plant extract have sedative effect.

مستخلص البحث:

هدفت هذه الدراسة للتعرف علي التأثير الدوائي للمستخلص الكحولي لنبات الزعتر كمضاد للتشنجات، ولمعرفة تأثيره المهدئ. بجانب دراسة تأثيره السميِ علي الكبد.

التجارب الدوائية كانت كالأتي: الأثر المضاد للتشنجات العصبية المسببة بواسطة مادة البنتايلين تترازول PTZ، والصدمات الكهربائية القصوي MES، التأثير المهدئ بواسطة جهاز قياس الحركة البسيط. أجريت جميع التجارب علي الجرذان.

في هذه الدراسة، وجد ان المستخلص الكحولي لنبات الزعتر ليس لديه تأثير مضاد للتشنجات العصبية المسببة بواسطة مادة البنتايلين تترازول والتشنجات العصبية المسببة بواسطة الصدمات الكهربائية القصوي. فيما يتعلق بدراسة التأثير المهدئ، تبين أن مستخلص نبات الزعتر لديه تأثير عالي كمهدئ. في الجزء الذي يتعلق بدراسة العلاج المشترك بكل من مستخلص النبات والفالبوريت (VPA) يمكن القول بأن العلاج المشترك أدي إلي حماية الجرذان من التشنجات المسببة بواسطة مادة PTZ بنسبة 100%. بينما وجد انه لديه تأثير مضاد للتشنجات المسببة بواسطة MES يصل إلي 33%. في الجزء الأخير من الدراسة، تمت دراسة التأثيرات السمية لمستخلص نبات الزعتر علي الكبد، والتي أوضحت أن الزعتر له تأثير سمي علي الكبد.

نستطيع من هذه الدراسة أن نخلص إلي الأتي: المستخلص الكحولي لنبات الزعتر لديه تأثير عالي كمهدئ. كما تبين ان العلاج المشترك مع الفالبروات له تأثير مضاد للتشنجات العصبية المسببة PTZ و التشنجات العصبية المسببة بواسطة MES.

INTRODUCTION

Thymus vulgaris (Thyme) is a flowering medicinal herb belongs to the mint family *Lamiaceae.* It is growing up to 15-30 cm tall by 40 cm wide. Thyme is cultivated worldwide for culinary, cosmetic, and medical purposes [1]. Traditionally, *Thymus vulgaris* has been used for chronic gastritis, diarrhea in children and for asthma. Thyme also used in various combinations with some plants oil for diseases of the upper respiratory tract (laryngitis, tonsillitis and specifically for bronchitis) [2, 3].

Thyme contains flavonoids, polysaccharides, and phenol compounds such as carvacrol and thymol. Thymol is the predominant compound among the essential oil components (51.34%) while the amount of all other components of the oil was less than 19% [2].

Previous pharmacological studies showed that the essential oil of *Thymus vulgaris* has antiseptic, antifungal, antimicrobial and tonic properties. This strongly antiseptic and antifungal activity is mainly due to presence of phenolic compounds [2-5]. Today thyme is used as a respiratory (antitussive) remedy, as well as in cases of variety of other ailments [4].

Epilepsy, a chronic and often progressive disorder, is a serious and one of the most common brain disorders that affecting at least 50 million persons worldwide with an estimation of incidence of 34 to 76 new cases per year per 100,000 people [6]. 5-10% of patients are needed to be stabilized by the addition of another antiepileptic drug (AED) but there remains over 20% of newly diagnosed epilepsy patients will remain resistant to both drug

monotherapy and polytherapy and will continue to experience seizures [6-8].

Although there are a number of synthetic anticonvulsant drugs currently available for use in the management epilepsy, most of these pharmaceutical anticonvulsant drugs are not only inaccessible and unaffordable, but many patients have undesirable effect and experience toxic adverse effects from these drugs (e.g. teratogenicity and liver toxicity). However, some patients have seizures that are refractory to medical therapy. Therefore, this study can potentially play an important role in the development of cost-effective and less toxic alternatives to standard AEDs.

This study was constructed to investigate the anticonvulsant, Sedative effects and possible hepatotoxic effects of ethanolic extract of *Thymus vulgaris* (Thyme).

MATERIAL AND METHODS

Experimental animals

Albino rats of both sexes weighing 80 – 130 g were used. Animals were housed in standard cages under controlled conditions at temperature (25°C) and relative humidity (~40%) with a 12 hours light/dark cycle beginning at 7 am. The rats were provided with standard diet (laboratory rodent's chow) and tap water.

Plant materials

Thyme (Leaves) was purchased from Khartoum local market, Sudan. The plant was authenticated by Taxonomy Department of Medicinal and Aromatic Plants Research Institute, National Centre for Research, Ministry of Science and Technology, Khartoum, Sudan.

The plant material was first washed, air dried, and then milled into a coarse powder. Hundred grams of the powdered plant material was macerated in five hundred milliliters of ethanol 95% for 24 hours with occasional shaking [9]. The mixture obtained was filtered using filter papers. Then, the solvent (ethanol 95%) was evaporated at 70 °C using a rotary evaporator. The resultant residue was dried by dry air to a constant weight. The resulting solid mass was collected and stored in a refrigerator until use. The resulting solid mass was dissolved in 20% $Tween_{80}$ (SD Fine-Chem Limited, Mumbai, India) solution.

The chemical solutions were freshly prepared. Pentylenetetrazole (Sigma Chemical Co., USA) was dissolved in distilled water, picrotoxin (Sigma Chemical Co., USA) was dissolved in warm distilled water, and strychnine (Sigma Chemical Co., USA) was dissolved in a small quantity (1-2ml) of warm 0.1N HCl. All this chemicals were used to induce seizers. Sodium valproate (Sanofi-aventis, France), used as standard anticonvulsant drug, was dissolved in 0.9% NaCl. Intraperitoneal and subcutaneous routes of administration were used in this study.

PHARMACOLOGICAL METHODS

1. Measurement of the sedative activity:

Evaluation of sedative effect of *Thymus vulgaris* was determined by using modified simple activity meter (locally manufactured) [10]. The simple activity meter is a box composes of two glassy and two wooden sides stand on 625 cm2 wooden plane board divided into 25 squares (10 cm X 10 cm for each square). A rat was placed on the center of the board and left to move freely for a period of 5

minutes. The number of squares crossed by the rat was counted in the consecutive 5 minutes. Decrease in number of movements/5 minutes was taken as an indication of sedative activity [11].

2. Methods for assessment of the anticonvulsant activity:

2. 1. The chemical test:

Pentylenetetrazole (PTZ) seizure threshold test was used as well-known chemical test to determine anticonvulsant activity. The dose of PTZ (85 mg/kg) administered subcutaneously was found to be approximate minimal dose that induced 100% convulsion in SWR rats. The rats that received PTZ (s.c) were observed for thirty minutes. A single five seconds episode of clonic spasms and fallen down was taken as threshold seizure [12].

2. 2. The electrical test:

Maximal electroshock seizure (MES) was used to evaluate anticonvulsant activity of *Thymus vulgaris***.** Seizures were induced in rats by delivering alternating current with certain adjustable intensity (mA), frequency (Hz) and duration (seconds) through a pair of ear clip electrodes (moistened with normal saline) by means of an electro- convulsiometer (UGO BASILE ECT unit, Model 57800-001). Rats were restrained by hand and subjected to electrical shock through their ears, and released immediately following electrical stimulation, to permit observation of the maximal seizure. Various frequencies, current intensities and duration of stimulation were tried on group of SWR rats of both sexes. The minimal electroshock that induced 100% maximal seizures was

found to be 50 mA alternating current of 100 Hz frequency for 0.3 seconds duration.

3. Assessment of serum liver enzymes:

Increase in serum liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values were used as indicator hepatotoxicity [13].

The potential hepatotoxic effects following i.p administration of extract of Thyme and was investigated in rats by measuring serum level of AST and ALT by mean of URIT-810 (A High performance chemistry analyzer with halogen lamp: 6V/10W, Wavelength: 300~800nm and Absorbance range: -0.3~3.0Abs; Guangzhou Shihai Medical Equipment Co., Ltd. China).

4. Experimental design and treatment protocol

Experimental Design:

For assessment of anticonvulsant, sedating effect and studying hepatotoxicity; Rats of both sexes were divided into 9 groups (3 to 5 rats in each) as follows:

Group 1: Control (10 ml/kg, i.p)

Group 2: Standard drug (Sodium valproate 0.4 g/kg, i.p)

Group 3: Thyme (0.9 g/kg, i.p)

Treatment Protocols:

The control group received NaCl 0.9% (10ml/kg) intraperitoneally. Group of standard drug was given 400mg/kg sodium valproate intraperitoneally. In combination treatment 200 mg/kg of VPA was used which found ineffective as anticonvulsant.

Protocol of the sedative activity study:

Thymus vulgaris was injected intraperitoneally and tested for sedative activity 1 hour post-treatment using simple activity meter as described before.

Regarding combined (thyme + VPA), the sedative effect was studied. Sodium valproate was injected 45 min after injection of thyme.

Protocol of anticonvulsant study:

To assess the anticonvulsant activity of *Thymus vulgaris*; rats were given a dose volume of 10ml/kg intraperitoneally from each extract and tested for anticonvulsant activity. The anticonvulsant activity was tested after 1 hour using chemical and electrical models.

To show anticonvulsant activity of combination of thyme with sodium valproate, 200mg/kg of sodium valproate was used. This dose was injected intraperitoneally 15 min pre-treatment (using chemical and electrical models) found to be non-protective dose.

Firstly rats were given a dose volume of 10ml/kg intraperitoneally from thyme extract and then sodium valproate was injected intraperitoneally 15 min pre-treatment in chemical and electrical models. Injected rats were tested for anticonvulsant activity 1 hour post-treatment using chemical and electrical models.

Protocol of hepatotoxicity study:

Presence of hepatotoxicity was determined by intraperitoneal administration of Thyme, (10ml/kg/day), VPA (0.4 g/kg/day) and NaCl 0.9% (10ml/kg/day) for a period of 7 consecutive days. After Twenty four hours of the last administration, the animals were anaesthetized and dissected. Blood was obtained into the sample tubes and

thereafter centrifuged to separate serum from the blood cells. The blood serum obtained was used for assay of ALT and AST.

Statistical analysis:

Data of the results of sedative activity measurement and hepatotoxicity assessment are presented as mean ± SEM and analyzed for statistical significance between different groups means were done by one way analysis of variance (ANOVA). A *P*- value less than 0.05 were considered statistically significant. Statistical evaluation was performed with Prism 5.0 computer program. The results of assessment of anticonvulsant activity are expressed as percentage of animals protected.

RESULTS AND DISCUSSION

Effect of plant extract on the locomotor activity of the rats:

Table 1 shows effect of ethanolic extract of *Thymus vulgaris* locomotor activity (0.9 g/kg). Results show that thyme caused extremely significant ($P<0.0001$) reduction in motor activity. This finding suggests that the three extracts possess sedative action. **Regarding literature review no published work was found concerning this effect. This result indicates that some components of the plant may elicit these varying degrees of sedation. Enhancing GABA mediating synaptic inhibition could be one of mechanism involve**

Anticonvulsant effect of plant extract against PTZ-MES-induced convulsions:

Table 2 and 3 show anticonvulsant effect of ethanolic extract of *Thymus vulgaris* against PTZ- and

MES-induced convulsions, respectively. The results Illustrate that the ethanolic extract of plant has no effect on both models of convulsions. **No previous studies were found to be compared with our findings. Pentylenetetrazole (PTZ) seizure threshold test, was utilized as a model for petit-mal epilepsy, whereas, the maximal electroshock seizure (MES) one of the electrical tests was employed as a model for grand- mal epilepsy. Different mechanisms were suggested of seizures induced by the two models. Therefore, this studied plant seems to be devoid of constituents that can combat these mechanisms.**

Anticonvulsant effect of combined (thyme + VPA) on PTZ- and MES-induced convulsions:

Data from tables 4 and 5 indicate that combination of Thyme with 200mg/kg sodium valproate produce full protection (100%) against convulsions induced by PTZ and partial protection (33%) in convulsions induced by MES.

The results obtained herein support the claimed folkloric use of *Thymus vulgaris* for their relaxation and calming effect. Their exact mechanism of action as anticonvulsant and sedative agents are unknown. However, flavonoid is one of the constituents. Several guidelines suggest that one or more of the flavonoid constituents may produce relaxation and calming effect by affecting γ-amino butyric acid (GABA), nor adrenalin (NA), dopamine (DA), and serotonin neurotransmission [14, 15], or by modifying hypothalamic-pituitary-adrenocortical axis function [16]. Furthermore, thymol (is chemically related to the anesthetic propofol) has been shown to act as a positive modulator of GABA-A [17].

Effect of plant extract on liver enzymes (ALT, AST):

Data obtained from investigation of hepatotoxicity caused by extract of thyme was showed in Table 6. Means of serum Level of ALT and AST were obtained, compared with that of group treated by sodium valproate and *p* value was found. VPA and extract of thyme were found to cause significant hepatotoxic effects compared to the control group. These results obtained estimated liver toxicity which may due to lipopolysaccharide constituents of thyme [18, 19].

Table 1
Effect of extract on locomotor activity:

Group No.	Treatment groups	Dose (g/kg)	Locomotor activity in sq./5 min.	*P* value
			Mean ± S.E.M	
1.	Control	10ml/kg	90.75 ± 1.652	(C)
2.	Thyme	0.9	↓ 18.75 ± 1.436	P<0.0001

Table 2
Effect of extract on PTZ- induced convulsions:

Group No.	Treatment group	Dose (g/kg)	No. of rats	No. of protected rats	% protection
1.	VPA	0.4	5	5	100
2.	Thyme	0.9	3	0	0

Table 3
Effect of extract on MES- induced convulsions

Group No.	Treatment group	Dose (g/kg)	No. of rats	No. of protected rats	% protection
1.	VPA	0.4	5	5	100
2.	Thyme	0.9	3	0	0

Table 4
Effect of combined (Thyme+VPA) on PTZ- induced convulsions

Group No.	Treatment group	Dose (g/kg)	No. of rats	No. of protected rats	% protection
1.	VPA	0.4	5	5	100
2.	Thyme+ VPA	0.9+0.2	5	5	100

Table 5
Effect of combined (Thyme+VPA) on MES- induced convulsions

Group No.	Treatment group	Dose (g/kg)	No. of rats	No. of protected rats	% protection
1.	VPA	0.4	5	5	100
2.	Thyme + VPA	0.9+0.2	3	1	33

Table 6
Effect of VPA and plant extract on liver enzymes:

Group No.	Treatment group	Liver enzyme	Mean ± S.E.M U/L	*p* value
1.	**Control**	**ALT**	13.50 ± 1.323	
		AST	31.5 ± 1.500	
2.	VPA	**ALT**	57.52 ± 3.250	$p< 0.0001$
		AST	44.75 ± 1.250	$p=0.0005$
3.	Thyme+ VPA	**ALT**	64.75 ± 4.956	$p< 0.0001$
		AST	46.00 ± 1.472	($P=0.0005$)
		AST	32.75 ± 1.315	ns

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