PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES OF ETHANOLIC EXTRACT OF *THYMUS VULGARIS*

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ABSTRACT

The medicinal plants have been played an important role in the preparation of many drugs. *Thymus vulgaris* has been extensively used in folk medicine for the treatment of headache, fevers, ulcers, arthritis, microbial infections even cancers. The current study was designed to investigate some phytochemical and pharmacological aspects of ethanolic extract of *Thymus vulgaris* as indicated by experimental tests. The results showed that *Thymus vulgaris* leaves contain alkaloids, carbohydrates and glycosides, flavonoids, resins, saponins, tannins and unsaturated sterols and triterpenes. Acute toxicity study of the extract showed no toxicological symptoms, mortality and post mortem changes at the maximum dose of 7000 mg/kg body weight. Pre-treatment with ethanolic extract of *Thymus vulgaris* at dose levels of (250-500 mg/kg, orally) significantly decreased (P < 0.05) the ulcer index and increased the gastroprotective activity at dose dependant manner in comparison with control group. Also, topical application of ethanolic extract of *Thymus vulgaris* ointment (10 %) once daily on wound area exerted rapid closure of the wound and enhanced the wound healing process. In conclusion, this study has established the antiulcerogenic and wound healing activities of ethanolic extract of *Thymus vulgaris* and thus, justifies the ethnic uses of the plant. However, further studies are required to isolate active compounds from the potent extract and to elucidate the exact mechanism of action.

KEY WORDS: *Thymus vulgaris*, phytochemical tests, antiulcerogenic effect, wound healing.
2. INTRODUCTION
Greater attention has been paid to the herbal medicine even in developed countries. Medicinal plants, or their extracts, have been used in the prevention and treatment of several chronic diseases such as cardiovascular diseases, inflammatory diseases, arthritis, diabetes, and others.\textsuperscript{[1]} Gastric ulcer is one of the most common multifactorial gastrointestinal disorders which widely distributed all over the world causing a high rate of morbidity and substantial mortality.\textsuperscript{[2]} Gastric ulcer treatment includes many synthetic drugs such as sucralfate, amoxicillin, ranitidine and omeprazole.\textsuperscript{[3, 4]} but most of these drugs produce several side effects including hypersensitivity, arrhythmia, potential interference with drug metabolism, headache and confusion upon prolonged administration.\textsuperscript{[5, 6]} Wounds are common events of life and defined as loss of skin continuity.\textsuperscript{[7]} which arise because of chemical or physical injury and/or microbial infections following rupture and break the protective barrier of the skin.\textsuperscript{[8]} Wound healing process includes haemostasis; inflammation; proliferation; and wound remodeling with scar tissue formation.\textsuperscript{[9]} The problems of high cost of synthetic drugs, residual effects on livestock products, adverse effects and development of drug resistance;\textsuperscript{[10]} have led ours to find safe, potent, unconventional and economic natural drug sources. Thymus vulgaris (T. vulgaris) is a permanent, herbaceous shrub that belongs to Lamiaceae family which has been extensively used in traditional medicine.\textsuperscript{[11]} Thyme was used as antibacterial, antiviral.\textsuperscript{[12, 13]} potent antihypertensive,\textsuperscript{[14]} anti fungal,\textsuperscript{[15]} anti-inflammatory,\textsuperscript{[16]} antispasmodic and analgesic agent.\textsuperscript{[17]} To our knowledge, there are no studies done to determine antiulcerogenic and wound healing activities of ethanolic extract of T. vulgaris. Therefore, the current study was designed to investigate the antiulcerogenic and wound healing activities of ethanolic extract of T. vulgaris in rats.

3. MATERIALS AND METHODS
3.1. Plant
Dried leaves of Thymus vulgaris were purchased from Faculty of Pharmacy, Cairo, Egypt. The plant was botanically identified by Dr. Mohamed Abd Elhalim, Department of Plant Systematic, Agricultural Research Center, Egypt.

3.1.1. Preparation of ethanolic extract of T. vulgaris L.
The powdered plant was soaked in adequate volume of ethyl alcohol 70 %. Extraction was carried out by intermittent shaking at room temperature for 3 days. After filtration through filter paper (Whatman No. 4), the residue was re-extracted twice, and then the extract was
evaporated under reduced pressure using a rotatory evaporator and dried to obtain a constant weight. The residue was stored at 4°C until use.\textsuperscript{[18]}

3.2. Experimental design: 3.2.1. Phytochemical screening
Some preliminary phytochemical tests were carried out on the \textit{Thymus vulgaris} plant as stated by Claus.\textsuperscript{[19]}

3.2.2. Toxicological study

\textbf{Determination of Median Lethal Dose (LD50)}

The acute toxicity of the extract was estimated in mice following the procedure described by Lorke.\textsuperscript{[20]} The evaluation of median lethal dose (LD50) in mice was investigated in two phases. Mice in the first five groups (three mice of each) were given ethanolic extract of \textit{T. vulgaris} orally at doses of 3000, 3500, 4000, 4500, 5000 mg/kg body weight. Similarly, three groups of three mice each were later administered with the extract at graded doses of 5000, 6000 and 7000 mg/kg body weight in the second stage. After that, the animals were kept under observation for 24 hours during which symptoms of toxicity and rate of mortality were recorded.

3.2.3. Pharmacological studies

\textit{Antiulcerogenic activity of ethanolic extract of \textit{T. vulgaris}}

Twenty eight rats of both sexes weighing 200-250 g body weight were randomly divided into four equal groups as follows: group (I) was served as a control group, whereas group (II) received RANITIDINE\textsuperscript{®} (50 mg/kg body weight). Groups (III) and (IV) received ethanolic extract of \textit{T. vulgaris} at dose levels of (250 and 500 mg/kg body weight). All drugs were given orally once daily for two weeks. At 15th day, animals were starved for 24 hours but allowed free access to water except for the last hour before ulcer induction. Gastric ulcer was induced by oral administration of absolute ethanol then after one hour, oral administration of RANITIDINE\textsuperscript{®} for the second group and the ethanolic extract of \textit{T. vulgaris} for third and fourth groups. The rats were killed by cervical dislocation 1 hour later. The abdominal cavity was opened and the stomach was excised, opened along the greater curvature, rinsed with physiological saline solution and pinned flat on a cardboard to be exposed for gross lesions evaluation. The long lesions in the glandular part of the stomach were counted with the aid of an illuminated magnifying lens (10x) according to Khayyal.\textsuperscript{[21]} and measured along the greater diameter using transparent ruler. The percentage of protection was calculated according to the method described by Hano.\textsuperscript{[22]}
Percentage of protection = (ulcer index of control − ulcer index of treated)  
\[ \text{ulcer index of control} \]  \times 100  

☐ **Wound healing activity of ethanolic extract of *T. vulgaris***

The wound healing activity of *T. vulgaris* ointment was determined according to the method stated by Farahpour and Habibi.[7] For this purpose 18 rats of both sexes weighing 150-200 grams body weight were used. After induction of anesthesia by intraperitoneal injection of ketamine 5% (90 mg/kg body weight) and xylazine hydrochloride 2% (5 mg/kg body weight), the fur of each rat was aseptically removed and predetermined area marked on the back of animals. Surgical wound of (1 cm x 1 cm) was made on the back of each rat after keeping them on ventral posture. All rats were randomly divided into three groups. Group I served as control. FUCIDINE® ointment was applied once daily as standard drug to Group II and ethanolic extract of *T. vulgaris* ointment (10 gm) based on Vaseline (100 gm), previously prepared, was applied once daily to Group III. Rats were kept individually under hygienic and controlled conditions. The ointments were topically applied daily, starting 24 hours post-operation, on the wound area until wound completely healed. All rats were monitored daily and any wound fluid or evidence of infection or other abnormalities were noted. Wound contraction percentage and wound closure time were used to assess wound-healing activity. The wound size was computed on days 4, 8, 12 and 16 day post-operation. The wound healing percentage was calculated by the Walker formula.[23]

Percentage of wound size = \[ \frac{\text{Wound area on day X}}{\text{Wound area on day zero}} \]  X 100

Percentage of wound healing=100 − Percentage of wound size.

**Statistical analysis**

Results were expressed as mean ± standard error of mean. Statistical significance was determined by one-way analysis of variance (ANOVA) according to Snedecor and Cochran,[24] followed by Tukey’s post-hoc test for multiple comparisons using SPSS (version 20.0) software (IBM SPSS Statistic 20.0, Armonk, NY, USA). The *P* values less than 0.05 were considered statistically significant.
4. RESULTS

4.1. Preliminary Phytochemical Screening
The results of phytochemical screening carried out on *T. vulgaris* leaves revealed that the presence of alkaloids, carbohydrates and glycosides, flavenoids, resins, saponins, tannins and unsaturated sterols and triterpenes.

4.2. Determination of LD50
Oral administration of ethanolic extract of *T. vulgaris* up to 7000 mg/kg body weight of mice showed no toxicological symptoms, mortalities and post-mortem changes.

4.3. Pharmacological studies

- **Antiulcerogenic effect of ethanolic extract of *T. vulgaris***
Oral administration of ethanol caused gastric ulcer formation in the stomach. Pretreatment with ethanolic extract of *T. vulgaris* at dose levels of (250 and 500 mg/kg body weight) exerted significant (p < 0.05) reduction in ulcer index in a dose-dependent manner. Gastroprotective activity was recorded as 58.63 and 86.98 %, respectively. Also, standard ranitidine (50 mg/kg body weight) significantly inhibited (P<0.05) ulcer index compared with control group as recorded in table (1), graph (1) and (Fig.1).

| Table (1): Antiulcerogenic activity of ethanolic extract of *T. vulgaris* on rats. (n=7). |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|
| **Group** | **Dose (mg/kg, body weight)** | **Mean± Standard error** | **Percentage of protection** |
| Control | - | Ulcer number | Ulcer index | Percentage of protection |
| Standard (Ranitidine®) | 50 | 23.14±1.37a | 78.14±3.14a | 0 |
| Ethanolic extract of *T. vulgaris* | 250 | 9.14±0.70b | 22.43±1.09c | 71.30 |
| | 500 | 10 ±0.73b | 32.33±2.62b | 58.63 |
| | 4±0.26c | 10.17±0.6d | 86.98 |

Means with different letters (a, b, c, d) within the same column are significantly different at P value ≤ 0.05.
Chart (1): Antiulcerogenic activity of ethanolic extract of *T. vulgaris* on rats.

Figures (1-4): Antiulcerogenic activity of ethanolic extract of *T. vulgaris* on rats.

Fig. (1): The stomach of control group shows severe injuries in the gastric mucosa Fig. (2): The stomach of standard (ranitidine®) treated group shows moderate injuries in the gastric mucosa Fig. (3): The stomach of *T. vulgaris* (250 mg/kg body weight) treated group shows mild injuries in the gastric mucosa Fig. (4): The stomach of *T. vulgaris* (500 mg/kg body weight) treated group shows very mild injuries in the gastric mucosa *The arrow point to ulcers.*

**Effect of ethanolic extract of *T. vulgaris* on wound healing**

Topical application of ethanolic extract of *T. vulgaris* in the form of ointment (10 %) daily on wound area, induced significant reduction (P < 0.05) in wound size starting from eight day onwards in comparison with the control non treated group. Similarly, the standard fucidine ointment significantly reduced the wound size as recorded in table (2), graph (2) and (Fig.2). Complete wound closure was observed in the group treated with the *T. vulgaris* ethanolic extract and standard group on day 16 compared to control group whereas it took about 26 days for complete healing in the control non treated animals.
Table (2): Wound healing activity of ethanolic extract of *T. vulgaris* on rats. (n=6).

<table>
<thead>
<tr>
<th>Group</th>
<th>Wound size (mm) and Percentage of wound healing at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4th day</td>
</tr>
<tr>
<td>Control</td>
<td>83.83±3.24a (7.03%)</td>
</tr>
<tr>
<td>Standard (Fucidine® Ointment)</td>
<td>81.00±2.32a (10.33%)</td>
</tr>
<tr>
<td><em>Thymus vulgaris</em> Ointment</td>
<td>85.5±2.01a (5.18%)</td>
</tr>
</tbody>
</table>

Means with different letters (a, b) within the same column are significantly different at P value ≤ 0.05.

Chart (2): Wound healing activity of ethanolic extract of *T. vulgaris* on rats.

Figures (5-16): Wound healing activity of ethanolic extract of *T. vulgaris* on rats.
5. DISCUSSION

The present research work emphasis on phytochemical, toxicological and pharmacological studies of T. vulgaris. The preliminary phytochemical screening of T. vulgaris leaves revealed that the presence of alkaloids, carbohydrates and glycosides, flavonoids, resins, saponins, tannins and unsaturated sterols and triterpenes. These results are come in accordance with,[25] they found that flavonoids, saponins, steroid compounds were the major phytochemicals of T. vulgaris plant. It had been reported that the percentage of tannins, saponins and volatile oils of thyme are (9.2 %, 23.1 %, 50.7 %) respectively, in addition, flavonoids, carbohydrates, condensed tannins, catechol, loquanthucyandin, saponins and phenolic acids.[26] Moreover, thyme contains saturated fatty carbohydrates, proteins, vitamins (A, B, C), iron and manganese elements, and high concentrations of chromium, nickel elements and fiber.[27] The result of acute toxicity study (LD50) of ethanolic extract of T. vulgaris showed no mortality at the maximum dose of 7000 mg/kg body weight. From earlier studies it has been found that, feeding thyme leaves to male Wister rats at 2 or 10 % of standard diet for 6 week showed no toxicity to rats.[28] Other study revealed that the median lethal dose value of thyme alcoholic extract was 6125 mg/kg for white mice.[26] whereas the hexanic and methanolic extracts of T. vulgaris were safe in mouse at dosage as high as 5 g/kg.[30] In addition, the median lethal dose of ethanolic extract of thyme in laboratory mice was about (4220 mg/kg).[31] The estimation of LD50 may differ in its value among other studies due to the difference in thyme sources and consequently difference in chemical composition of the extract, the difference in laboratory animals regarding for their number and species. We suggested that the tested extract is considered highly safe, since substances possessing LD50 higher than 50 mg/kg body weight are considered non toxic.[32]

It is evident from the present study that T. vulgaris ethanolic extract has antiulcerogenic effect at a dose levels of (250 and 500 mg/kg body weight), but the higher dose is more potent. There is significant decrease in ulcer index in T. vulgaris and ranitidine treated groups in comparison to control group. Stomachs of rats treated with a higher dose of the extract showed only very mild congestion; otherwise, stomach appearance was normal. These results
are agreed with.[33] They studied the gastroprotective effect of *Thymus algeriensis* (Lamiaceae) essential oil in rats and found that oral administration of *Thymus algeriensis* essential oil at dose levels of (54, 117 and 180 ml/kg) inhibited HCl/ethanol-induced ulcers. Ethanol ingestion has been reported to involve the depletion of gastric defensive mechanisms resulted in gastritis that characterized by congestion, mucosal edema, erosion and multiple hemorrhagic streaks along the long axis of the glandular stomach as recorded by.[34, 35, 36] In addition, *Zataria multiflora* (Lamiaceae) extract exerted gastroprotective effect at a dose level of 800 mg/kg body weight. The exact mechanism of action could not be clearly defined but may be due to the active materials of *T. vulgaris* plant.[37] Several mechanisms accounting for flavonoids include increase of mucosal prostaglandine contents, decrease of histamine secretion and scavenging of free radicals.[38, 39] Luteolin flavonoid exerted gastroprotective effects through inhibiting the growth of *H. pylori* by inhibition of arylamine N-acetyltransferase activity.[40] Co-administration of diacerein with thymol for treatment of gastric ulcer induced by the Rainsford’s cold stress model revealed that higher potent antiulcerogenic activity than usage of everyone alone as developed by.[41] Furthermore, rosmarinic acid is accounted for gastric ulcer treatment. Triterpenoids are potential compounds with antiulcer activity due to their ability to strength the defensive factors of the stomach by stimulation of mucus synthesis or maintenance of the prostaglandins level.[42]

This study showed that, the topical application of ethanolic extract of *T. vulgaris* ointment (10%) enhances wound healing process in rats. These result is come in accordance with.[43] They found that the *T. vulgaris* essential oil improved wound contraction and is useful for wound healing when used at concentrations of (1.5 and 3%). Wound healing is a physiological process depends on collagenation, wound contraction and epithelization thus; intervention at any one of these stages using drugs could eventually either promote or inhibit one or all stages of healing.[44, 45] Recent researches have shown that active ingredients such as triterpenoids and flavonoids promote wound-healing activity against methicillin resistant *S. aureus*, mainly due to their astringent and antimicrobial properties, which seem to be responsible for wound contraction and an increased rate of epithelialization.[46, 47] *T. vulgaris* has anti-oxidant activity which prevents lipid peroxidation followed by atherosclerosis due to contents of thymol and carvacrol.[48, 49] Also, Eugenol showed anti-inflammatory effects.[50] which helps to accelerate wound healing. Neovascularization is a defensive and physiologic process leads to reducing free radicals as well as releasing the metabolites to the cells involving in the healing process.[51] Proliferation and migration of the epithelial cells and
fibroblasts as well as suppressed inflammation, promotes neovascularization in wound area.\cite{52} High rate re-epithelialization was considered as an indicator for best healing. Therefore, shortened epithelialization time in 10% T. vulgaris-treated animals showed its activity on epithelial cells proliferation. In fact, anti-inflammatory, antioxidant and antimicrobial activities are the main properties of remedies which accelerate wound healing. Thus, all these reported characteristics of T. vulgaris ethanolic extract more effective and faster wound contraction and also promote wound healing.

CONCLUSION
According to the obtained results, it could be concluded that ethanolic extract of T. vulgaris had potential antiulcer effect, which was superior to the respective effect observed with ranitidine. The antiulcer curative ratios were dose dependent with no adverse effects. Also, it had beneficial effects on full thickness wound healing. Therefore, thyme extract is recommended for gastrointestinal and skin disorders. However, further studies are required to isolate active compounds from the potent extracts and to elucidate their exact mechanism of action.

CONFLICT OF INTEREST
The author(s) confirm that this article content has no conflicts of interest.

6. REFERENCES


