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Promising Antidiabetic and Wound Healing Activities of *Forsskaolea tenacissima* L. Aerial Parts.

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Abstract

The aim of present study was to evaluate the antidiabetic activity of the total methanolic extract (TME) and different fractions of *Forsskaolea tenacissima* L. aerial parts with alloxan-induced diabetic rats and estimate its wound healing activity by excision wound model in rats as well as determine its toxicological activity by lethal dose 50 % (LD_{50}). The ethyl acetate fraction showed a significant decrease in blood glucose level (36.00 %, $P < 0.001$) in comparison with glibenclamide (36.14 %, $P < 0.001$) as standard. The TME group showed marked increase in wound healing activity (97.9 %) compared with gentamycin standard group (96.8 %). The TME is considered safe due to its LD_{50}, which was 7 g/kg body weight.

Key words

Forsskaolea tenacissima, Urticaceae, Antidiabetic, Wound healing, Acute toxicity, LD_{50}.

1. Introduction

Family Urticaceae comprises 54 genera and more than 2000 species of herbs, shrubs, small trees, and a few vines, distributed primarily in tropical regions [1, 2]. A survey of literature on family Urticaceae showed many reports about antidiabetic, anti-inflammatory and antimicrobial activities [3]. *Forsskaolea* is a small genus of six species distributed from the Canary Islands & south-eastward Spain to Pakistan and India [4-6]. *Forsskaolea tenacissima* L. has been used in folk medicine in Pakistan as anti-inflammatory, antispasmodic, antidiabetic and antipyretic [7]. It also showed antioxidant [8], hepatoprotective [9], antinociceptive, antipyretic [10], antiviral and antibacterial activities [11]. Moreover, the preliminary phytochemical screening of aerial parts of *F. tenacissima* L. showed the presence of sterols, triterpenes and polyphenolics such as flavonoids & tannins, in addition to the isolation of sterols such as (*β*-sitosterol, *β*-sitosterol-3-O-*β*-D-glucopyranoside, stigmasterol & stigmast-4-ene-3-one), triterpenes such as (*β*-amyrin, lupeol, maslinic acid & friedelin) and simple phenolic such as 2-hydroxy imino 3-phenyl propionic acid [11, 12]. Moreover, a ceramide (forsskamide) was isolated from the aerial parts of *F. tenacissima* L. It displayed a moderate cytotoxic activity against human colorectal carcinoma cell line [13]. This provoked us to study additional biological activities for this plant *viz.*, antidiabetic and wound healing activities as well as the determination of LD_{50}.

2. Materials and Methods

2.1. Chemicals

Thiopental sodium injection (500 mg) was obtained from Egyptian International Pharmaceutical Industry Co., Egypt (EIPICO). Bees wax, white soft paraffin and normal saline (0.9 %) were obtained from El-Nasr Pharmaceutical and Chemical Co., Egypt (ADWIC). Gentamycin® ointment (5 %) was obtained from Memphis Co. for Pharmaceutical industries, Egypt. Alloxan monohydrate was obtained from Sigma-Aldrich Chemicals Co., Germany.

2.2. Plant materials

F. tenacissima L. aerial parts were collected in the period from April to June 2013 and collected from El-Kawthar city, Sohag, Egypt. Identification of the plant was done by Prof. Salah M. El-Naggar, Department of Botany and Plant Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt. The plant was kept in the Herbarium of Faculty of Pharmacy, Minia University, Minia, Egypt with voucher specimen number (Mn-Ph-Cog-007).

2.3. Preparation of the extract and fractions

The air-dried powdered of *F. tenacissima* L. aerial parts (4.5 kg) were exhaustively extracted by maceration in 90 % MeOH (3x, 10 L each), each time was for a week. The total methanol extract (TME) was concentrated under reduced pressure to give a dark green syrupy residue (390 g). Subsequently, it was suspended in distilled water (500 ml), transferred to a separating funnel and partitioned successively with *n*-hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and *n*-butanol

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(*n*-BuOH), till complete exhaustion in each step to yield (42 g), (15 g), (14 g) and (17 g), respectively.

2.4. Experimental Animals

Wister albino male rats of (150 ± 20 g) were used, obtained from the Animal House, Pharmacology Department, Faculty of Medicine, Assiut University. All animal rules were conducted in stratification with the internationally accepted principles for laboratory animals use and care as found in the European Community Guidelines and Institutional Ethical Committee Approval was obtained. This study protocol (Project number: 021/2018) was accepted by Department of Pharmacology & Toxicology and "The Research Ethics Committee", Faculty of Pharmacy, Minia University, Egypt. Animals were housed under standardized environmental conditions in the Animal House in plastic cages at ambient temperature (25 ± 2 ºC) and relative humidity of 55 - 70 %. A 12:12 h light/dark cycle was well-kept during the experiments. They were fed a standard diet, water was provided *ad libitum* and they were adapted for one week before entry into the later study. Each group was housed separately after recording its animal's body weight and had kept separate marks for identifying the dose level, group and individual number.

LD_{50} determination

The acute toxicity of the TME of *F. tenacissima* L. aerial parts was determined by measuring LD_{50} (lethal dose for 50 % of the laboratory animals) [14]. Rats were divided into ten groups, each containing five animals. The TME was administered orally at doses ranging from (1-10 g/kg, orally) following standard method [15]. Animals were continuously observed for 24 h to detect changes in the autonomic or behavioral responses and then monitored for any signs of toxicity and mortality. Toxicity signs to be observed include diarrhea, paw-licking, stretching, respiratory distress or death [16]. A group of animal treated with the vehicle (1 %, v/v Tween 80) in distilled water served as negative control.

Antidiabetic activity

The antidiabetic activity of the TME and different fractions of *F. tenacissima* L. aerial parts were evaluated using alloxan-induced hyperglycemia method [17]. The rats were allowed to fast for 24 h prior to the experiment and diabetic rats were obtained by the administration of a single dose of intraperitoneal injection of alloxan 120 mg/kg body weight [18]. After 72 h of alloxan injection, diabetes was confirmed by testing the blood sugar level by using Accu-Chek® Glucometer (Germany) to monitor the blood sample from the tail vein. When blood glucose level above 250 mg/dl was considered as diabetic. Rats were divided into seven groups, each group of five animals. The first negative control group treated with (1 %, v/v Tween 80) in distilled water. The second group treated with the standard drug (glibenclamide, 0.5 mg/kg, orally) [19]. The third to seventh groups treated with the TME and different fractions in a single dose (300 mg/kg, orally) [17]. The blood samples were taken and measured at zero, 1, 2, 3 and 4 h following drug treatment.

Wound healing activity

The wound healing activity of the TME of *F. tenacissima* L. aerial parts was evaluated using the excision wound model [20]. Rats were divided into three groups, each group of five animals. The first group treated with simple ointment base served as control. The second group treated with gentamycin ointment used as reference standard for comparison with tested ointments. The third group treated with the TME which incorporated into simple ointment base in concentrations of 5% w/w [21]. Excision wound model is the wound type used in this experiment in which the rats in each group were anesthetized by administering thiopental sodium (50 mg /kg, i.p.) [22]. A full thickness of the excision wound by a surgical blade (size 12) of circular area (approx. 200 mm^2) and 2 mm depth [23] was inflicted on the shaved back of the rats 30 min later, the administration of thiopental sodium injection. The wounding day was considered as day zero. The wounds were treated with topical application of the ointment preparations mentioned above (once daily), till the wounds were completely healed. The wounds were monitored and the area of the wound was measured on 3^{rd}, 6^{th}, 9^{th}, 12^{th} and 15^{th} post-wounding days. Wound healing rate was expressed as (%) of wound healing from the following equation:

$$\%\ \textbf{of Wound closure} = \frac{\textbf{Wound area on day 0} - \textbf{Wound area on day n}}{\textbf{Wound area on day 0}} \boldsymbol{X}\ \mathbf{100}$$

Where n = number of days 3^{rd}, 6^{th}, 9^{th}, 12^{th} and 15^{th} [24].
The percentages of wound closure were summarized in **(Table 2)**.

Statistical Analysis

Experimental results are expressed as mean ± S.E.M. (standard error of the mean). SPSS 20 version was used for the statistical analysis. Results were statistically analyzed using analysis of variance (one-way ANOVA) followed by Tukey's test for comparison between different groups. Probability values (*P*) less than 0.05, 0.01 and 0.001 indicated statistical significance (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$).

3. Results and discussion

LD_{50} determination

The result of acute toxicity study was observed that LD_{50} was 7 g/kg body weight of the TME of *F. tenacissima* L. aerial parts during the first 24 h after oral administration. Therefore, the TME is considered as a safe herbal drug due to its high LD_{50}, which was more than ten times of the therapeutic dose (300 mg/kg).

Table 1: Effect of the TME and different fractions on blood glucose level compared with standard and negative control groups.

No.	Tested samples	Blood glucose level (mg/dl)/h (%) Percentage of decrease in blood glucose levels				
		0 h	**1 h**	**2 h**	**3 h**	**4 h**
1	**Negative control**	260 ± 1.84 0 %	258 ± 1.70 0.77 %	255 ± 3.40 1.92 %	254 ± 2.00 2.31 %	253 ± 2.72 2.69 %
2	**Glibenclamide**	285 ± 2.72 0 %	230 ± 3.41*** 19.30 %	205 ± 3.16*** 28.07 %	190 ± 4.74*** 33.33 %	182 ± 2.61*** 36.14 %
3	**TME**	282 ± 3.84 0 %	235 ± 3.07* 16.67 %	225 ± 3.67* 20.22 %	199 ± 2.96* 29.43 %	184 ± 3.63* 34.75 %
4	***n*-Hexane fr.**	267 ± 3.53 0 %	250 ± 2.12 6.37 %	234 ± 2.50 12.36 %	226 ± 2.31 15.36 %	222 ± 1.98 16.85 %
5	**DCM fr.**	277 ± 2.34 0 %	242 ± 3.35 12.64 %	230 ± 1.70 16.97 %	226 ± 3.37 18.41 %	211 ± 2.29 23.83 %
6	**EtOAc fr.**	275 ± 2.02 0 %	247 ± 2.30*** 10.18 %	216 ± 1.58*** 21.46 %	193 ± 2.35*** 29.82 %	176 ± 2.70*** 36.00 %
7	***n*-BuOH fr.**	270 ± 3.42 0 %	243 ± 3.60** 10.00 %	211 ± 2.51** 21.85 %	203 ± 2.92** 24.82 %	187 ± 1.63** 30.74 %

Values are expressed as mean±SE.M; Number of animals in each group= 5, Significant difference (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$).
All doses were 300 mg/kg except glibenclamide 0.5 mg/kg.

Table 2: Effect of the TME group on the wound area in mm^2 and (%) percentage of wound healing (closure) compared with gentamycin standard and negative control groups.

No.	Time	Wound area in mm^2 (%) Percentage of wound healing (Closure)		
		Negative control	**Standard control (Gentamycin)**	**TME**
1	**Day (0)**	197.6 ± 0.93 0 %	196.6 ± 1.21 0 %	198.0 ± 0.71 0 %
2	**Day (3)**	186.4 ± 1.36 5.7 ± 0.49 %	185.2 ± 1.39*** 5.8 ± 1.19 %	184.2 ± 1.56*** 6.9 ± 1.08 %
3	**Day (6)**	171.0 ± 0.71 13.4 ± 0.74 %	157.2 ± 2.61*** 19.9 ± 1.45 %	152.2 ± 3.40*** 23.1 ± 1.53 %
4	**Day (9)**	129.4 ± 3.37 35.0 ± 1.60 %	90.0 ± 1.70*** 54.2 ± 0.93 %	82.4 ± 1.91*** 58.4 ± 0.95 %
5	**Day (12)**	95.8 ± 2.10 51.5 ± 0.97 %	40.0 ± 3.53*** 79.7 ± 1.83 %	30.4 ± 3.55*** 84.6 ± 1.82%
6	**Day (15)**	63.2 ± 2.60 68.0 ± 1.18 %	6.2 ± 1.01*** 96.8 ± 0.55 %	4.0 ± 0.71*** 97.9 ± 0.34 %

Values are expressed as mean±SE.M; Number of animals in each group= 5, Significant difference (***$P < 0.001$).

Antidiabetic activity

The results of antidiabetic activity of the TME and different fractions of *F. tenacissima* L. aerial parts were represented in **(Table 1)**. EtOAc fraction showed significant decrease in blood glucose level (36.00 %, $P < 0.001$) in comparison with glibenclamide (36.14 %, $P < 0.001$) as standard followed by the TME (34.75 %, $P < 0.001$) then *n*-BuOH (30.74 %, $P < 0.001$) after 4 h of administration of single dose (300 mg/kg) of the TME and different fractions in the hyperglycemic rats. The hypoglycemic activity of the TME and different fractions may be attributed to the presence of sterols, triterpenes, polyphenols and flavonoids [25-27]. Many previously reported triterpenes have proved their ability to inhibit glucose absorption, glucose uptake, insulin secretion and enzymes which are involved in glucose metabolism. They also prevented the development of insulin resistance and inhibited the formation of advanced glycation end products. Moreover, they had strong antioxidant activity and could inhibit the expression of several genes which are responsible for diabetes and progression of diabetic complications [28, 29]. Furthermore, polyphenols and flavonoids were reported to promote regeneration of β-cells of Islets of Langerhans and exhibited significantly radical scavenging activity and thus antioxidant activity confirms the possibility on the protection of vital tissues including the pancreas, thereby reducing the causation of diabetes in these animals [30].

Wound healing activity

The results of wound healing activity of the TME of *F. tenacissima* L. aerial parts were represented in **(Figure 1**) and **(Tables 2)**. The TME group showed a marked increase in wound healing activity (97.9 %, $P < 0.001$) compared with standard group (96.8 %, $P < 0.001$) and negative control group (68.0 %, $P < 0.001$) after 15 days of treatment. The higher wound healing activity may be attributed to the presence of different classes of constituents which possess different activities in the wound healing process as sterols (e.g β-sitosterol), which is one of the active compounds which may be responsible for the epithelization activity [31], anti-inflammatory and anti-ulcer activities [24]. Triterpenes showed anti-inflammatory and antimicrobial activities, which are also supporting the wound healing process. Moreover, polyphenolic compounds acted as free radical scavenger from the site of injury, which may cause more tissue damage and delay wound healing process [24]. Moreover, ceramides had anti-inflammation activity which suppressed the inflammatory mediators such as chemokines and cytokines in the tissue injury involved the coordinated delivery of blood components to the

site of injury [32]. Also, ceramides played a role in damaged skin which facilitate the repair process [33, 34].

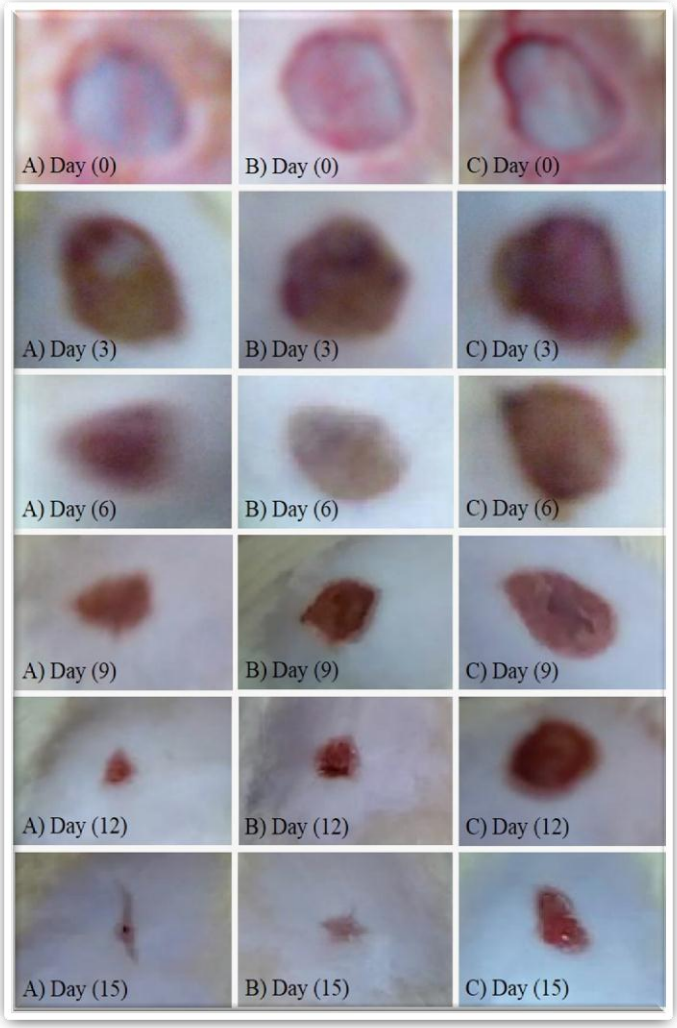

Figure 1: Progress of wound healing (during 15 days) of the TME group **(A)** compared with standard **(B)** and negative control groups **(C).**

Conclusion

The assessment of biological activities of *F. tenacissima* L. aerial parts revealed that the EtOAc fraction had a potent antidiabetic activity as well as the TME showed a powerful wound healing activity. In addition, the TME is safe due to its high value of LD_{50}. Therefore, this plant could be considered as an outstanding source for discovering new bioactive natural products.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding these studies.

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