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Vicia ervilia L. seeds newly explored biological activities

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Abstract: Within the global context of increasing poverty in the developing countries, natural products are important in devising new drugs. *Vicia ervilia* L. Willd., unlike several fabaceae seeds, is not used for human consumption till now. We aim to discover any possible medicinal use of the seed. Analgesic, anti-inflammatory, anti-ulcerogenic and antihyperglycemic activities were evaluated using hot plate, carrageenan-induced rat paw edema, ethanol-induced ulcer model and alloxan-induced diabetes methods, respectively. Antiviral activity was evaluated using Methylthiazol Tetrazolium assay. *V. ervilia* seeds ethanol (70%) extract had significant anti-inflammatory, analgesic, antiulcerogenic, antihyperglycemic and antiviral activities. It is of excellent choice for treatment of several illnesses in developing countries due to its diverse resource, easy accessibility, affordability and its newly proved significant wide range of biological activities.

Subjects: Environment & Agriculture; Food Science & Technology; Economics, Finance, Business & Industry; Health and Social Care

Keywords: antiviral; antihyperglycemic; antiulcerogenic; anti-inflammatory

1. Introduction

The use of herbal medicine is an age-old tradition worldwide. The recent progress in modern therapeutics has stimulated the use of natural products not only for its effectiveness but also for its



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ABOUT THE AUTHORS

Dr Mona M. Okba, is a lecturer in the department of Pharmacognosy. Okba completed bachelor's degree at the Faculty of Pharmacy – Cairo University (FOPCU) in 2004. Okba completed master's and PhD in 2004–2014. Okba's research interests include Pharmacognosy and Phytochemistry. Okba's publications include (1) *Bull. Fac. Pharm. Cairo Univ.*, Vol. 47. No. 1, 87–96, (2009), (2) *Bull. Fac. Pharm. Cairo Univ.*, Vol. 45, No. 2, 157–179, (2007), (3) *Z. Naturforsch.*, 64 c, 611–614 (2009), (4) *Egypt. J. Biomed. Sci.*, Vol. 23, 121–134, (2007), (5) *Int. J. Pharm. Pharm. Sci.*, Vol 5, Suppl 3, 311–329, (2013), (6) *Int. J. Pharm. Pharm. Sci.*, Vol 6, Issue 1, 246–253, (2014), and (7) *Journal of Natural Sciences Research*, Vol. 6, No. 22, (2016). Okba's future is to explore other pharmacological activities and phytochemical content especially of *V. ervilia* L. to be incorporated in large-scale production of a dosage form that can be used for human consumption.

PUBLIC INTEREST STATEMENT

Within the global context of increasing poverty in the developing countries, natural products are important in devising new drugs. *Vicia ervilia* seeds are not used for human consumption till now although it is well known as an excellent sheep and cattle feed concentrate. This perspective article discovers some possible medicinal use of the seed. Analgesic, anti-inflammatory, antiulcerogenic, antihyperglycemic, and antiviral activities were evaluated. These treasure seeds are of excellent choice for treatment of several illnesses for low-income people in developing countries due to its diverse resource, easy accessibility, affordability and its newly explored wide range of biological activities.

relatively low prices and availability. Cultivation of high valued medicinal plants should be creating new dimension in the field of agriculture in developing countries (Amujoyegbe, Agbedahunsi, & Amujoyegbe, 2012). Egypt is a country with a developed economy according to the International Monetary Fund's World Economic Outlook report (2015).

Vicia ervilia L. Willd. (syn. *Ervium ervilia* L., Karsanah, kursene كرسنه), Family Fabaceae, is an annual herb distributed in the Western Mediterranean coastal region (Täckholm, 1974). The seeds are extensively used as stock feed in several countries (Haddad, 2006; Sadeghi, Pourreza, Samei, & Rahmani, 2009). However, to the best of the authors' knowledge, only one report concerning the medicinal importance of *V. ervilia* for human beings was traced (Fornstedt & Porath, 1975). The use of such cheap and easily cultivated seeds in treating several illnesses in developing country, like Egypt, will be of excellent value especially in the current state of increasing poverty. Antiulcerogenic and antihyperglycemic activities are among activities to be screened in this study due to high incidence of gastric ulcers (Hussein, 2010) and diabetes (National Center of Health & Population, 2004; Shaw, Sicree, & Zimmet, 2010) in Egypt. The seeds' anti-inflammatory and analgesic potentials are to be explored due to their major role in relieving hepatitis, cancer and rheumatic musculoskeletal disorders of high incidence in the country (Abdel-Tawab, Abdel-Nasser, & Darmawan, 2004; Ibrahim, Khaled, Mikhail, Baraka, & Kamel, 2014; Lotfi, Abdel-Nasser, & Hamdy, 2007; World Health Organization, 2011).

2. Experimental

2.1. Plant material

Samples of *V. ervilia* seeds were imported from Jordan in July 2012. They were cultivated in the Experimental Station of Medicinal Plants, Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Giza. Photos of the cultivated plant were sent to Kew Garden, England to confirm their identity. Identification was studied by same authors in a previous publication (Okba, Yousif, El Deeb, & Soliman, 2014). Voucher samples were deposited at the Museum of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University (herbarium No. 14.4.2013.2).

2.2. Extract preparation

Seeds were powdered and extracted with ethanol (70%) by percolation at room temperature for three consecutive days. The solvent was evaporated under vacuum to yield seeds ethanol (70%) extract (SEE).

2.3. Biological activities

Animals: were obtained from the animal house of the Laboratory National Research Center, Giza, Egypt. They were kept on standard laboratory diet. This study was conducted in accordance with ethical procedures and policies approved by Animal Care and Use Committee of Faculty of Pharmacy, Cairo University which follows the World Medical Association Declaration of Helsinki (WMA General Assembly 1964).

Determination of median lethal dose (LD50): was estimated according to (Kerber, 1931).

Acute anti-inflammatory activity: carrageenan-induced rat paw edema method was followed (Winter, Risley, & Nuss, 1962). Hind paw volume was measured by water displacement method using 7410, Ugo Basile, plythesmometer, Comerio, Italy (Chattopadhyay et al., 2002; Li, Hyun, Jeong, Kim, & Lee, 2003). Indomethacin (Epico, Egyptian Int. Pharmaceutical Industries Co.) was used as a positive control.

Analgesic activity: Hot plate method was carried out using (7280 Ugo Basile Biological Research Apparatus Company, Comerio, Italy) according to (Laviola & Alleva, 1990; Woolfe & McDonald, 1944). Indomethacin was used as a positive control.

Antiulcerogenic activity: ethanol-induced ulcer model was adopted (Morimoto, Shimohara, Oshima, & Sukamoto, 1991). Ranitidine was used as a reference drug. Lesions were examined under an illuminated magnifier (Adami, Marazzi-Uberti, & Turba, 1964).

Antihyperglycemic activity: The technique used by (Zhang, Huang, Hou, & Wang, 2006) was followed. Hyperglycemia was induced by alloxan monohydrate (Sigma Co., USA). BioMerieux kits were used for the assessment of blood glucose, triglycerides and cholesterol levels.

Antiviral activity: VERO cells (kidney epithelial cells of African green monkey) incubated into culture bottle were checked using inverted microscope. Healthy cells propagation, determination of extract cytotoxicity and MTT assay protocol were done according to (Alley, Scudiero, & Monks, 1988; Van de Loosdrecht, 1994).

3. Results and discussion

The LD_{50} of seed ethanol extract (SEE) was up to 5 g/kg b.wt. It is thus of high safety margin at the tested dose level (Buck, Osweiler, & Van Gelder, 1976). These reflect the possibility of the plant use for nutritional or medicinal purposes after elimination of its anti-nutritional factor canavanine (Berger, Robertson, & Cocks, 2003; Sadeghi, Samie, Pourreza, & Rahmani, 2004).

SEE showed a significant *anti-inflammatory* activity ($p < 0.05$). When indomethacin was administered the edema volume after 4 h of carrageenan injection was 24.5% of its original volume. The edema volume was 46.51% of its original volume when SEE was tested (500 mg/kg). The extract did not cause gastric ulcers, during the course of study, as usually happens with anti-inflammatory drugs. SEE showed a significant *analgesic activity*. SEE (500 mg/kg) analgesic activity was 80.21% of indomethacin (Table 1).

SEE showed a pronounced *antiulcerogenic* activity (Table 2). SEE (250 mg/kg) reduced ulcers number and severity by 80.6 and 82.32%, respectively. The antiulcerogenic activity at 500 mg/kg approached that of ranitidine.

SEE caused significant ($p < 0.05$) reduction in glucose, triglycerides, and cholesterol levels of *hyperglycemic* rats (Table 3). At 500 mg/kg b.wt SEE cause reduction in glucose level by 79.06%, a

Table 1. Anti-inflammatory and analgesic activities of *V. ervilia* seeds

		Control	Indomethacin	SEE	
			Dose (mg/kg)		
			10	250	500
Edema volume ^a	1 h	100.33 ± 7.5	59.71 ± 2.7*	60.32 ± 3.3*	55.53 ± 4*
	2 h	142.88 ± 7.7	67.22 ± 3.1*	75.52 ± 3*	65.69 ± 4.1*
	3 h	156.52 ± 9.5	40.46 ± 4.6*	68.80 ± 5.2**,**	54.16 ± 4.2*
	4 h	160.29 ± 10.8	24.50 ± 4.7*	50.70 ± 4.7**,**	46.53 ± 3.1*
Reaction time (min)	B.l.	6.84 ± 0.19	6.78 ± 0.23	6.02 ± 0.17	5.88 ± 0.13
	30	6.52 ± 0.18	7.94 ± 0.22*	7.02 ± 0.16	6.82 ± 0.16
	60	6.44 ± .09	9.46 ± 0.22*	8.48 ± 0.29*	7.92 ± 0.16*
	90	6.16 ± 0.07	11.22 ± 0.26*	9.62 ± 0.3*	9 ± 0.19*

Notes: Data represent the mean value ± S.E. of six rat per group and the percent changes vs. basal (zero min) values and 1, 2, 3 and 4 h post-carrageenan injection. Statistical analysis was done using one-way ANOVA Followed by LSD and Tukey for multiple comparisons.

Values represent the mean ± S.E. of six animals for each group ($n = 6$). B.l., base line, SEE: seed ethanol (70%) extract.
^a% change from baseline.

*Significantly different from control group at $p < 0.05$.

**Significantly different from indomethacin group at $p < 0.05$.

Table 2. Effect of *V. ervilia* seeds on the gastric ulcer number and severity

Treatment	Dose (mg/kg)	Ulcer number		Ulcer severity	
		Mean ± SE	%Reduction	Mean ± SE	%Reduction
Control		0	–	0	–
Ethanol 99.5%		13.4 ± 1.07	–	39.6 ± 2.97	–
SEE	250	2.6 ± 0.50*	80.6	7 ± 0.70*,**	82.32
	500	3.8 ± 0.22*	71.64	6.8 ± 0.31*,**	82.83
Ranitidine	50	2.4 ± 0.24*	82.09	3.8 ± 0.37*	90.4

Notes: Statistical analysis was done using K independent test followed by Kruskal-wallis for nonparametric test. SEE: seed ethanol (70%) extract.

Values are expressed as means ± SEM (n = 6).

*Significantly different from ethanol treated group at p < 0.05.

**Significantly different from standard ranitidine group at p < 0.05.

Table 3. Antihyperglycemic activity of *V. ervilia* seeds

Groups	Dose mg/kg	mg/dl		
		Glucose	Triglyceride	Cholesterol
Normal		81.26 ± 1.74	34.15 ± 1.97	31.26 ± 1.32
Hyperglycemic		361.11 ± 7.47*	220.31 ± 5.23*	228.50 ± 10.11*
Gliclazid	5	103.63 ± 1.33**	55.69 ± 2.77**	49.23 ± 2.63**
SEE	250	111.58 ± 8.43**	70.15 ± 5.36**,**	115.63 ± 6.09*,**,**
	500	75.63 ± 6.3**	57.54 ± 4.93**,**	77.57 ± 4.41*,**,**

Notes: Statistical analysis was done using one way ANOVA followed by Tukey for multiple comparisons. SEE: seed ethanol (70%) extract.

Values are expressed as means ± SEM (n = 6).

*Significantly different from control group at p < 0.05.

**Significantly different from hyperglycemic group at p < 0.05.

***Significantly different from standard gliclazid group at p < 0.05.

Table 4. Antiviral activity of *V. ervilia* seeds

Test sample	Selected dose (mg/ml)[MNTC]	Viability (%) ^a	Cytotoxicity (%)	Antiviral effect (%)
Control (VERO cell line)		100	0	0
Virus control		31	69	
SEE	0.1	57	43	26

Notes: MNTC: maximum non-toxic concentration; SEE: seed ethanol (70%) extract.

^aAverage of three determinations.

value which is higher than that caused by the standard gliclazid (71.3%). SEE (500 mg/kg b.wt) reduced triglycerides and cholesterol levels in diabetic rats by 73.89 and 66.06%.

The *Antiviral* activity was screened against *Coxsackie B4* virus. It is a serotype of *Enterovirus B* which can trigger an autoimmune reaction resulting in destruction of the pancreas insulin-producing beta cells, which is one of several different etiologies of diabetes mellitus (Ylipaasto et al., 2004). SEE had a mild activity against *Coxsackie B4* virus (Table 4). SEE proved to have significant hypoglycemic action in this study (Table 3). This suggests the use of *V. ervilia* seeds in treatment of diabetes as it may relieve the case through two different mechanisms, antiviral and hypoglycemic ones. Further studies on the ability of *V. ervilia* seeds to treat diabetes through other mechanisms as increased

insulin release from pancreatic beta cells, insulin sparing effect and controlling lipid peroxidation are recommended.

This is the first report on anti-inflammatory, analgesic, antiulcerogenic, antihyperglycemic and antiviral activities of such promising seeds. We are conducting further studies on *V. ervilia* to explore other pharmacological activities and its phytochemical content.

4. Conclusions

It was found that *V. ervilia* seeds possess a wide spectrum of biological activities. The plant can be used to relieve different illness in Egypt. The country climatic conditions are suitable for cultivation of such cheap seeds. The only restriction for the medicinal use of *V. ervilia* seed is its canavanine content which can be easily removed by soaking in boiling water before use. The seed's low price, availability, ease of cultivation, and medicinal effectiveness make it a good choice especially for low-income people in developed countries.

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Competing Interest

The authors declare no competing interests.

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