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Vicia variabilis a Nutritive Feed for Domesticated Animals: A Survey about Cytotoxic and Antioxidant Activity

Research Article

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ABSTRACT

Safety and beneficence of farm animal nutrition plays a great role in their healthy life which in turn have impact on human nutrition via diary or meat products. Assessing safety and beneficence of *Vicia varibilis* Freyn and Sint. Which is chosen intrinsically by domesticated animals of Kohgiluyeh and Boyerahmad Iran is the aim of the present work. Our results not only show no cytotoxic activity in 3-(4,5-dimethylthiazol-2-yl)-2,4-diphenyltetrazolium bromide (MTT) assay but also admit the antioxidant activity of the species through scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals confirmed in DPPH assay.

KEY WORDS antioxidant activity, cytotoxicity, domesticated animals, MTT assay, *Vicia variabilis*.

INTRODUCTION

In the mountainous regions of south-west of Iran, Kohgiluyeh and Boyerahmad is located with 634299 people living in this province (Statistical Centre of Iran, 2006). Some of these people have chosen urbanism or tribalism while others have been occupied by farming or raising domesticated animals in order to provide the need of their families as well as a way to earn their livings. Alone or along with temporary agriculture, animal husbandry is a way of subsistence for Iranian nomads. It provides them dairy, meat and even wool to prepare thread for weaving carpets for their own needs or even as an income source. In regard to the difference of temperature in different seasons in Iran, in warm seasons, the nomads move from warm to temperate regions, while they migrate to warm areas before the cold season begins in order that their herds have access to natural sources and grassland all over the year. In spring and early summer, when animals are led for grazing, they act according to their instinct. Relying on their olfactory sense and following their instinct, they pick up the food that is not toxic; local people experience for collecting fodder usually counts upon animal instincts and they will make a stock of what the herds is inclined to. The weather starts changing during March and the herd which had spent the winter in stable is led to the grassland. During the first half of the spring the newly born will accompany their parents to the lea. This will continue till the weather gets cool in autumn. To provide the food supply for the herd, dried and ground fodder along with grains such as barely will be stocked. Passing the cold months, the animals will be fed twice (morning and evening) with fodder a part of which is *Vicia variabilis*. Herdsmen usually lead their herd to places of its growth during warm seasons and they also collect and

store the species in spring to provide food for their animals during cold seasons. *Vicia variabilis* (Figure 1) is an annual, biennial or perennial, glabrous species (Fabaceae) with blue-violet elongate racemes that are longer than its leaves (Pakravan *et al*. 2000).

Figure 1 *Vicia variabilis*: photographed by H. Moazzeni, May 2010, Kohgiluyeh and Boyerahmad; Iran

The flowers begin blooming during May-July, presenting a beautiful shrub. In order to evaluate the cytotoxic and antioxidant activity of *Vicia variabilis* the methanol extract of the aerial parts has been evaluated against five cell lines and in DPPH assay.

MATERIALS AND METHODS

Plant material

The aerial parts of *Vicia variabilis* were collected from Kohgiluyeh and Boyerahmad province, Iran (May 2010) and authenticated by Traditional Medicine and Materia Medica Research Center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran. A voucher specimen is deposited at TMRC Herbarium for future reference.

Extraction

Dried powdered plant (10 g) was extracted with methanol at room temperature for 24 h. The mixture was filtered and the concentrated filtrate was subjected to MTT and DPPH assays.

Preparing the extract for MTT assay

The methanol extract was dissolved in Dimethyl sulfoxide (DMSO): 40 mg/mL to make the stock solution. Serial dilutions were prepared accordingly from the above stock solution to get the final concentrations (400 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL and 12.5 µg/mL) with DMSO not exceeding 1%.

Cell lines

MCF-7 (human breast adenocarcinoma), HepG-2 (hepatocellular carcinoma), MDBK (bovine kidney cells), A-549 (non-small cell lung carcinoma) and HT-29 (human colon adenocarcinoma) cells were obtained from Pasteur Institute, Tehran, Iran. MCF7 cells were cultured in Dulbecco's Modified Eagle Medium **(**DMEM: Gibco) with 5% Fetal Bovine Surum (FBS: Gibco), HT-29 cells were cultured DMEM medium (Gibco) with 20% FBS (Gibco), while the other three cell lines were cultured in RPMI 1640 medium (Sigma) with 10% FBS (Gibco) to maintain the desired growth. All cell lines were treated with 1% penicillin-streptomycin (Sigma), in a humidified incubator at 37 ˚C in an atmosphere of 5% CO_2. The growth curve of each cell line was assessed.

MTT assay

Assessing cell viability was carried out in a micro culture tetrazolium / formazan assay (MTT assay), according to the method of Alley *et al.* (1988) with some modifications (Mosaddegh *et al.* 2010; Rahimifard *et al*. 2010).

The cells were seeded in 96-well plates at 9 x 10^3 for MCF-7, 15 x10^3 for HepG-2, 11 x 10^3 for MDBK , 8 x 10^3 for A-549 and 5 x 10^3 for HT-29 cells. The plates were then incubated at 37 ˚C in an atmosphere of 5% CO_2. After 24 h the medium was replaced with fresh medium containing different concentrations of the extract to be tested-(triplicate). After 72 h exposure of cells at 37 ˚C to the methanol extract, the medium was replaced with fresh medium contaning MTT [3-(4,5-dimethylthiazol-2-yl)-2,4-diphenyltetrazolium bromide]; Sigma, with a final concentration of 0.5 mg/mL.

The cells were incubated for another 4 h in a humidified atmosphere at 37 ˚C, after which the medium containing MTT was removed and the remaining formazan crystals were dissolved in DMSO. The absorbance was recorded at 570 nm with an (Enzyme Linked Immunosorbent Assay) ELISA reader (TECAN). Tamoxifen was used as positive control.

The relative cell viability (%) related to control wells was calculated by $[A]_{samples}$ / $[A]_{control}$ x 100. Where $[A]_{samples}$ is the absorbance of test sample and $[A]_{control}$ is the absorbance of wells containing cells, cell culture medium and DMSO 1%.

DPPH radical scavenging assay

DPPH assay is based on scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals by antioxidant agents, which produces a decrease in absorbance at about 520 nm. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by loss of color.

This delocalization is also responsible for the deep violet color, characterized by an absorption band at about 520 nm (Molyneux, 2004; Mohammadi Motamed and Naghibi, 2010).

In order to determine DPPH radical scavenging activity of *Vicia variabilis* methanol extract, 2 ml of a 100 µM DPPH methanol solution was added to 2 mL of various concentrations of the extract. The mixture was shaken vigorously and left to stand at room temperature for 30 min. The absorbance of the solutions was measured at 517 nm and antioxidant activity calculated using the following equation:

Scavenging capacity %= 100 - [(ABS of sample-ABS of blank)×100/ABS of control].

Methanol (2 mL) plus plant extract solution (2 mL) was used as blank, while DPPH solution (2 mL) plus Methanol (2 mL) as negative control and Vitamin C as positive control.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against extract concentration. The tests were performed in triplicate (Sharif Ali *et al*. 2005; Vundać *et al*. 2007).

RESULTS AND DISCUSSION

MTT assay

In vitro cytotoxic evaluation of the methanol extract of *Vicia variabilis* against MCF-7 (human breast adenocarcinoma), HepG-2 (hepatocellular carcinoma), MDBK (bovine kidney cells), A-549 (non-small cell lung carcinoma) and HT29 (human colon adenocarcinoma) cells exhibited no dose dependent cytotoxic activity.

The extract was assessed with serial twofold dilutions starting with 400 µg/mL and ending with 12.5 µg/mL of the extract. In each cell line the viability versus concentration was graphed by Microsoft Excel; however the viability was much more than 50% for every concentrations tested and a dose dependant decrease in the viability was not observed thus the IC_{50} could not be calculated in any of the above cell lines. Moreover one in the five cell lines was originally a normal cell line (MDBK: bovine kidney cells). The extract not only was not cytotoxic to the tumor cell lines but also it did not decrease the viability in the cell line with a normal origin (Table 1).

DPPH radical scavenging assay

The ability of the extract to scavenge the free 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was evaluated by plotting the percent of inhibition of DPPH radicals versus different concentrations of the extract and the IC_{50} (the concentration in which 50% of DPPH was inhibited) was calculated. The results showed antioxidant activity with IC_{50} of 189 µg/mL for *Vicia variabilis* methanol extract, which suggests the beneficence of the species as nourishment for farm animals (Figure 2).

Animal husbandry plays a great role in the economic life of many parts of the world as in Kohgiluyeh and Boyerahmad Iran. Both farmers who raise domesticated animals and nomads, who move to places where enough food could be found, have a challenge for supplying the animals' food. *Vicia varibilis* is a species from Fabaceae family which has been collected by stockmen to feed the farm animals in Kohgiluyeh and Boyerahmad; Iran. Also the animals would be guided to the place where the species is grown either by the nomads or in special times of the year by farmers to provide the animals needs.

Vicia variabilis has been traditionally used in Kohgiluyeh and Boyerahmad, Iran as a nutritive feed for domesticated animals. The data presented here could be a support to the non-cytotoxicity of the species and as evidence to the intrinsic selection of safe food among domesticated animals.

Antioxidants are important health-protecting factors (Milan, 2006). Scientific evidences suggest that they reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are plants. Antioxidants from plants belong to various classes of compounds with a wide variety of physical and chemical properties. The main characteristic of antioxidants is their ability to scavenge free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative diseases. Antioxidant compounds quench free radicals such as peroxide, hydroperoxide or lipid peroxide and inhibit the oxidative mechanisms that lead to degenerative diseases (Molyneux, 2004; Tripathi *et al*. 2007).

Table 1 The viability of cell lines treated with different concentrations of *Vicia variabilis* methanol extract for 72 h. Values represent the Mean±SD of 3 experiments

Concentration (µg/mL) of VM*	Viability in different cell lines (%)				
	MCF7	HepG2	MDBK	A549	HT29
12.5	96.3±0.048	99.9±0.01	93.08±03	103.9±0.07	108.6±0.03
25.0	90.3±0.037	106.5±0.05	99.47±05	92.3±0.03	106.4±0.05
50.0	97.5±0.041	109.2±0.07	100.24±01	88.3±0.04	102.1±0.01
100.0	103.6±0.02	97.8±0.08	102.09±.09	82.0±0.07	100.1±0.00
200.0	109.9±0.05	103.8±0.08	96.95±.09	96.8±0.04	112.2±0.07
400.0	104.84±0.06	119.8±0.02	109.64±.02	104.8±0.05	124.8±0.04

VM*: *Vicia variabilis* methanol extract.

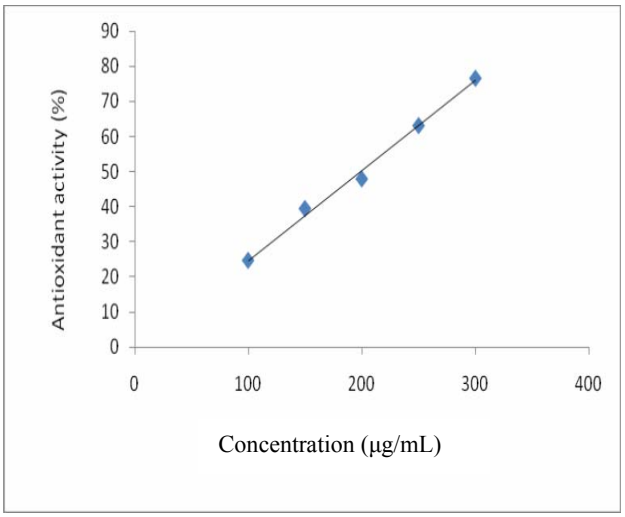

Figure 2 The antioxidant activity of *Vicia variabilis* extract evaluated in DPPH assay as described in materials and methods

Vicia variabilis methanol extract has demonstrated antioxidant activity in our study; Thus including the species in the domesticated animal diet might provide an antioxidant source to maintain their health.

CONCLUSION

Non-cytotoxicity and antioxidant activity of *Vicia variabilis* suggests it as a healthy nourishment not only to feed but also to improve farm animals health in any part of the world that the species grows.

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