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Short Communication

BIOLOGICAL ACTIVITIES OF BROAD BEAN (*VICIA FABA* L.) EXTRACTS CULTIVATED IN SOUTH ANATOLIA IN FAVISM SENSITIVE SUBJECTS*

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SUMMARY

Aqueous extracts of a different variety of fresh broad bean seeds obtained from a favism endemic area in Turkey, were incubated with blood from sensitive and non-sensitive (control) subjects. Red blood cells were characterized by a whole blood glutathione (GSH) and a deficiency of Glucose-6-phosphate dehydrogenase (G-6-PD) activity. As the decrease in GSH percent is taken as an index of haemolytic activity, the test results were as following: Sakiz, Milas-Region, French broad bean extracts reduced the blood GSH levels 48%, 70%, 46% and 53%, respectively, in favism sensitive subjects.

Active principles which are responsible for the haemolysis (Vicine and Convicine) were isolated from broad beans and their effects on GSH levels of blood were 99% and 81%, respectively, in favism sensitive subjects and 33.3% and 19% in normal subjects.

Key words: Favism; Glucose 6-phosphate dehydrogenase deficiency; Glutathione; *Vicia faba*; Vicine-Convicine; South Anatolia

Favism, a remarkable type of acute haemolytic anemia developing as a result of ingestion of broad bean (*Vicia faba*) seeds and in some instances as a result of inhalation of *Vicia faba* pollen, is associated with glucose-6-

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phosphate dehydrogenase (G-6-PD) deficiency and instability of glutathione. The incidence of this enzymatic defect is significantly higher in the Mediterranean people, particularly among Greeks, Jews, Sicilians, Sardinians and Arabs [1,2]

Some investigators [3,4] suggested that pyrimidine derivatives iso-uramil (2,4,5-trihydroxy-6-amino pyrimidine) and divicine (2,6-diamino 4,5-dihydroxy pyrimidine) occurring in the fava beans, as the aglycone moieties or their corresponding β -glycosides (convicine and vicine, respectively), may be responsible for the induction of the haemolytic crisis known as favism [3,4]. The haemolytic activity was determined by the reducing effect of the extracts and toxic constituents of *Vicia faba* on G-6-PD deficient blood GSH levels [5,6]. Some investigations have been done on the incidence of G-6-PD deficiency and favism in the Turkish population, [7,8] but no research has been carried out to determine the relationship of broad bean to favism although consumption and cultivation is fairly high in Turkey.

This present communication reports the investigation on the in vitro haemolytic activity of some varieties of *Vicia faba* extracts and pyrimidine glycosides isolated from broad beans cultivated in the South part of Turkey.

MATERIALS AND METHODS

Fresh broad bean varieties (Grex minor Milas 114, Grex major Sakiz 115, Grex equina Region 5108 and Grex equina French 116) cultivated in Turkey were obtained from the Mediterranean Institute of Agricultural Research, Antalya, Turkey.

Blood specimens from 30 favism subjects and 20 normal (control) persons were collected in an acid-citrate-dextrose (ACD) solution and kept in a refrigerator. Favism subjects were traced by the help of Hematology Research Laboratories of the Childrens Hospital, Hacettepe University.

GSH tests were performed, as described by Beutler, Duron and Kelly [9], and were based upon the development of a relatively stable colour when 5,5'-dithiobis (2-nitrobenzoic acid) was added to sulfhydryl compounds. G-6-PD was determined by specific procedures according to Beutler [10]. Before the quantitative determination, G-6-PD deficiency was screened by a rapid spot test. This method depends upon the fluorescence of reduced NADP (NADPH) as an indicator of G-6-PD activity.

Extraction of broad bean samples

A different variety of broad bean was extracted as described by Jamalian [11]. The aqueous extracts, obtained using 250 g of fresh seeds, were evaporated and the final residues were dissolved and taken up in 10.0 ml phosphate-buffered physiological saline (pH 7.4). They were stored frozen under N₂ in screw-cap sample bottles.

Haemolytic activity of extracts

Hematocrit (Hct) values of blood samples were measured by the micro-

haematocrit procedure, described by Dacie and Lewis [12], and adjusted to have a Hct value of 30% ACD solution before incubation. One millilitre of blood sample was incubated (at 37°C for 3 h) with 0.5 ml extract solution in buffered physiological saline (20% total extract) in 5-ml rubber stoppered glass tubes. One millilitre of blood incubated with buffered physiological saline was used as an internal control for each subject. Blanks were prepared by mixing 0.5 ml of each extract solution with 1 ml of buffered physiological saline and were incubated simultaneously. At the end of 3 h GSH was determined in duplicate [9].

Isolation of toxic principles

Vicine was isolated from dry seeds [13] and convicine was isolated from fresh seeds of the Milas variety [14]. Physical and chemical properties, UV, IR and NMR spectras and elementary analysis of isolated substances were determined and compared with standards according to Bien [14].

Solutions of vicine and convicine crystals were prepared in buffered physiological saline (100 mg/10 ml) and 0.5 ml of the solution was used for the incubation.

RESULTS AND DISCUSSION

1. The effects of different varieties of fresh broad beans on the blood GSH levels is shown in Table I. All the favism subjects tested have shown G-6-PD deficiency (0 units/g Hb). It was noted that each of the extracts decreased the level of GSH in favism subjects to a greater extent than in the normal control. Using mean values of Table I, the following can be calculated; Sakiz and Milas broad bean extracts decreased the blood GSH levels in normal subjects by 15% and 18.5% and in favism subjects by 48% and 70%, respectively. It was observed that the Milas variety has the greatest effect (Table I).

TABLE I

EFFECTS OF *VICIA FABA* EXTRACTS ON BLOOD GSH LEVELS OF SENSITIVE AND NON-SENSITIVE SUBJECTS

GSH in mg/100 ml red blood cells $\pm$ standard deviation.

Groups	No. of subjects	Treatment	Mean GSH	
			Before increase	After increase
Non-sensitive	20	Milas extract	108.535 $\pm$ 19.404	88.315 $\pm$ 19.675
Non-sensitive	20	Sakiz extract	108.535 $\pm$ 19.404	92.275 $\pm$ 20.645
Sensitive	30	Milas extract	58.913 $\pm$ 17.792	17.757 $\pm$ 12.602
Sensitive	30	Sakiz extract	58.913 $\pm$ 17.792	30.620 $\pm$ 13.566
Sensitive	12	Bölge extract	53.583 $\pm$ 16.232	24.817 $\pm$ 7.391
Sensitive	12	Fransiz extract	53.583 $\pm$ 16.232	28.533 $\pm$ 8.824

TABLE II

EFFECTS OF VICINE AND CONVICINE PYRIMIDINE GLUCOSIDES ON GSH LEVELS OF SENSITIVE AND NON-SENSITIVE SUBJECTS

GSH in mg/100 ml red blood cells $\pm$ standard deviation.

Groups	No. of subjects	Treatment	Mean GSH	
			Before increase	After increase
Non-sensitive	10	Vicine	81.250 $\pm$ 24.848	54.310 $\pm$ 23.248
Non-sensitive	10	Convicine	81.250 $\pm$ 24.848	65.930 $\pm$ 25.481
Sensitive	10	Vicine	46.970 $\pm$ 21.858	2.090 $\pm$ 3.497
Sensitive	10	Convicine	46.970 $\pm$ 21.858	8.730 $\pm$ 4.819

The extracts other than the Milas variety had no statistically significant difference on the fall of the GSH level (Table III).

Some investigators such as Walker and Bowman [5], Bottini et al. [6] and Jamalian [14] reported that aqueous extracts of broad bean reduced the blood GSH levels between 27% and 91% (average 60%) in favism sensitive subjects.

2. The effect of isolated pyrimidine glycosides vicine and convicine on blood GSH levels of sensitive and non-sensitive subjects is shown in Table II. Vicine and convicine reduced the GSH levels in normal subjects by 33% and 19% and in favism subjects by 99% and 81%, respectively. The yield was 9.6 vicine from 1 kg dry seed Milas variety and 0.6 g convicine from 1 kg Milas variety fresh Faba beans.

Statistical results are shown in Table III. The haemolytic effect of vicine has been found more potent than convicine ($P < 0.01$).

Vicine content of some varieties of broad bean was reported earlier by

TABLE III

EFFECTS OF VARIOUS BROAD BEAN EXTRACTS, VICINE AND CONVICINE GLYCOSIDES ON BLOOD GLUTATHIONE LEVELS (Chi-square test, No. 12)

No statistically significant difference ($P > 0.05$) was observed, $P < 0.001$ was found to be significant.

Comparison of various extract types	X	t	P
Bölge-Milas	24.817	14.750	5.508
Bölge-Sakiz	24.817	26.375	0.590
Bölge-Fransiz	24.817	28.535	1.119
Milas-Sakiz	14.750	26.375	7.752
Milas-Fransiz	14.750	28.533	6.527
Sakiz-Fransiz	26.375	28.533	0.955
Convicine-Vicine	8.730	2.090	3.527

Lin and Ling [3] and Jamalian [13]. Jamalian reported that vicine concentration of *Vicia faba* can change due to the variety, culture area, different parts of pod and pod development [15]. There are some investigations on the isolation methods, characterisation and effect of reduction on GSH levels [4,14,16] dealing with convicine but we could not find any research on its comparative haemolytic activity.

The toxic constituents vicine and convicine of *Vicia faba* seem mainly to be the responsible active principles in the haemolytic activity although some other factors such as L-DOPA may also be effective [16].

Our results, until now, confirm and amplify previous reports and serve to indicate the degree of variability in toxicity that occur in broad beans.

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