

# A hemolysis trigger in glucose-6-phosphate dehydrogenase enzyme deficiency

*Vicia sativa (Vetch)*

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## ABSTRACT

يعتبر أنزيم محلل الماء جلوكوز 6 فوسفات (G6PD) أنزيمًا ضروريًا، حيث يلعب دورًا مهمًا في إعادة التركيب الحيوي لجميع الخلايا التي تتنفس هوائيًا. وقد تم التوصل إلى أن النقص في أنزيم محلل الماء جلوكوز 6 فوسفات (G6PD) عند الأشخاص الذين يتناولون أدوية معينة، فول، أو الذين لديهم أمراض يعتبر النقص فيه دلالة وإشارة على وجود فقر دم من نوع انحلال كريات الدم الحمراء. نستعرض في هذا التقرير حالة طفل يبلغ من العمر 8 سنوات، تم تنويمه في المستشفى حيث كان يعاني من وجود دم في البول، صداع، دوار في الرأس، خمول، فقدان للشهية، واصفرار في بياض العين، وكان ذلك بعد 24 ساعة من تناوله كمية كبيرة من حبات نبات (البيقة). تبين من خلال التحاليل الطبية التي تم إجراؤها وجود فقر دم من نوع انحلال كريات الدم الحمراء، ارتفاع في نسبة البليروين، ونقص في أنزيم محلل الماء جلوكوز 6 فوسفات (G6PD). يوجد في بذور البقوليات نسبة ما يقارب 0.5% من مادة الفيسين ومادة الكونفيسين، والذي يعتبر المكون الأساسي لهما عدد 2 من بيريميدين بيتا جليكوزيد، ويوجد في نبات (البيقة) أيضًا ما يقارب 0.731% من مادة الفيسين، و 0.081% من مادة الكونفيسين، و 0.530% من مادة سيانو الانين جليكوزيد. الهدف من هذا التقرير، أنه يجب الأخذ بعين الاعتبار ما يسببه استهلاك نبات (البيقة) من حدوث فقر دم من نوع انحلال كرات الدم الحمراء، حيث انه يتم استهلاك ما يقارب مليون طن من (البيقة) في بلادنا، وخاصة المناطق التي يتم زراعتها فيها.

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme, playing an important role in the redox metabolism of all aerobic cells. It was reported that certain medications, fava beans, and infections can trigger acute hemolytic anemia in patients with G6PD deficiency. An 8-year-old male patient was admitted to the hospital with blood in the urine, headache, dizziness, fatigue, loss of appetite, and jaundice in the eyes, 24 hours after eating large amounts of fresh, vetch grains. Laboratory investigation revealed hemolytic anemia, hyperbilirubinemia, and

G6PD deficiency. Approximately 0.5% of fava bean seeds have 2 pyrimidine beta-glycosides called, vicine and convicine. Vetch has 0.731% vicine, 0.081% convicine, and 0.530% beta cyanoalanine glycosides. The aim of this case report is to emphasize the importance of vetch seeds as a cause for hemolytic crisis in our country, where approximately one million tons of vetch is produced per year, especially in the agricultural regions.

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An important enzyme in the redox metabolism of all aerobic cells is glucose-6-phosphate dehydrogenase (G6PD). It was reported that certain medications, fava bean, and infections are triggers of acute hemolytic anemia in patients with G6PD deficiency. The acute form of hemolytic anemia caused by consumption of fava bean (*Vicia faba*) is called "favism".<sup>1</sup> The scientific classification of the vetch seed (*Vicia sativa*) is shown in Table 1. It is an important livestock fodder.<sup>2</sup> Fava bean and vetch seeds are from the same genus, but of different species. Fava beans contain high levels of vicine, divicine, convicine, and isouramil, all of which are oxidants. Vetch has 2 pyrimidine beta-glycosides: 0.73% vicine, 0.08% convicine, and 0.53% beta cyanoalanine glycosides.<sup>3</sup> The vicine and convicine amount in fava bean seeds is 0.5%.<sup>4</sup> Vetch is also consumed by humans in the form of seeds. It is consumed all over Turkey.

Divicine and isouramil, which are formed as a result of hydrolysis of vicine and convicine by the intestinal flora, are responsible for favism.<sup>1,4-6</sup> Favism is a disorder characterized by a hemolytic reaction with the consumption of the fava beans. The superoxide ( $O_2^-$ ) anion is produced in the redox cycle, of which divicine and isouramil each is a pyrimidine aglycan. Then, hydrogen peroxide ( $H_2O_2$ ) is produced from  $O_2^-$  by the superoxide dismutase pathway. Glutathione (GSH) is oxidized by  $O_2^-$  or GSH peroxidase.<sup>7</sup>

Normal erythrocytes overcome the problem by the reduction of GSSG (oxidized glutathione) utilizing the GSH reductase pathway, mediated by nicotinamide adenine dinucleotide phosphate (NADPH), and increasing the G6PD activity to compensate the increased need of NADPH. On the other hand, in cells which are deficient for G6PD, the NADPH supply is quite limited as the only source is the pentose phosphate pathway. Thus, when G6PD deficient cells are exposed to divicine and isouramil, GSH, which appears to be essential for maintaining enzymatic activities and other membrane and cellular functions, will be rapidly depleted. This consumption may cause irreversible cellular changes, and may lead to cell destruction by the reticuloendothelial system.<sup>4</sup>

No case have been reported in literature declaring vetch as causing hemolysis in patients with G6PD deficiency. We hereby present a case with the clinical manifestation of intravascular hemolysis, and found to have G6PD deficiency and hemolysis that was linked to vetch consumption.

**Case Report.** An 8-year-old male patient presented to the hospital with complaints of blood in the urine, headache, dizziness, fatigue, loss of appetite, and jaundice in the eyes that had appeared 24 hours after eating large amounts of fresh, vetch grains (Figure 1). There was no history of infection or medication use. Medical history and family history were insignificant. Physical examination revealed fatigue, pallor in the skin, conjunctivae, and jaundice in the sclerae. He was hospitalized in order to undergo a work-up for the etiology of the anemia and jaundice. Laboratory results are presented in Table 2. The patient had severe anemia, hyperbilirubinemia, normal mean corpuscular volume, and no red blood cells in the urine sediment, but a positive blood reaction measured with the urinalysis stick. These results showed that the blood reaction in the urine was related to hemoglobinuria, and not hematuria.

In his detailed medical history, it was learnt that he had consumed large amounts of vetch planted as livestock fodder in the field, and that his symptoms commenced 24 hours after ingesting the vetch.

**Table 1 -** The scientific classification of the vetch (*Vicia sativa*), and the faba bean (*Vicia faba*) seeds.

Classification	Vetch	Faba beans
Kingdom	Plantae	Plantae
Division	Magnoliophyta	Magnoliophyta
Class	Magnoliopsida	Magnoliopsida
Order	Fabales	Fabales
Family	Fabaceae	Fabaceae
Subfamily	Faboideae	Faboideae
Tribe	Vicieae	Vicieae
Genus	<i>Vicia</i>	<i>Vicia</i>
Species	<i>V. sativa</i>	<i>V. faba</i>

**Table 2 -** Laboratory results.

Laboratory	Normal values	Results obtained from patient
<i>Complete blood count</i>		
Hemoglobin (g/dl)	(12-16)	5.4
Hematocrit (%)	(37-47)	16.2
MCV (fl)	(80)	88.7
MCH (pg)	(26.5-33.5)	29.5
MCHC (g/dL)	(31.5-35)	33.3
RDW (%)	(10-15)	14.3
Leukocyte ( $\times 10^9/L$ )	(4.1-10.9 $\times 10^9$ )	12.6
Thrombocyte ( $\times 10^9/L$ )	(440 $\times 9 \times 10^9$ )	185
<i>Urine analysis</i>		
Blood reaction		+++
Bilirubin		-
Urobilinogen		Normal
Cells in the urine sediment		None
<i>Blood analysis</i>		
Total bilirubin (mg/dL)		4.2
Direct bilirubin (mg/dL)		0.25
*Glucose 6 phosphate dehydrogenase (U/g Hb)		1.6
MCV - mean corpuscular volume, MCH - mean corpuscular hemoglobin, MCHC - mean corpuscular hemoglobin concentration, RDW - red cell distribution width, *normal range: 4.6-13.5 U/g Hb		



**Figure 1 -** *Vicia sativa* seeds.

Considering the clinical and laboratory results, he was diagnosed as having acute hemolysis. Vetch is known to be a member of the *Fabaceae* family, and assuming that the patient may have G6PD deficiency, this would cause hemolysis and hence, the G6PD level was measured, and the patient was diagnosed as suffering from G6PD deficiency. The patient received transfusion of erythrocyte suspension. His general status improved. He and his family were advised on food and medications that he must avoid.

**Discussion.** Glucose-6-phosphate dehydrogenase deficiency is the most commonly seen erythrocyte (red blood cell) enzyme deficiency in human, estimated to affect approximately 400 million people in the world.<sup>8</sup> The deficit is most prevalent in Africa, Southern Europe, Middle East, South East Asia, and Oceania (5-25%).<sup>1,8</sup> This genetic abnormality may be asymptomatic in some people, whereas it may manifest in the form of newborn jaundice, acute hemolytic anemia, or severe non-spherocytic hemolytic anemia. There are 140 mutations of the G6PD gene, and more than 400 clinical and biochemical variants of the deficiency.<sup>8</sup> The prevalence of this enzyme deficiency in Turkey is between 0.5-20%, varying according to the geographical region and/or ethnicity.<sup>9</sup> The fava bean is one of the triggers of hemolysis in patients with G6PD deficiency, however up to the present, vetch has not been reported to be a trigger. Vetch is used as livestock fodder, especially as an alternative protein in poultry.

Hemolysis in the erythrocytes was reported in chickens fed by vetch due to vicine, convicine, and beta-cyanoalanine toxicity.<sup>3,10</sup> However, G6PD deficiency has not been mentioned. It was reported that in chickens fed by vetch, oxidant agents in vetch such as vicine, convicine, and beta-cyanoalanine, cause an increase in H<sub>2</sub>O<sub>2</sub>, and reactive oxygen radicals.<sup>10</sup> This suggests that probably the abnormal increase in H<sub>2</sub>O<sub>2</sub>, and reactive oxygen radicals in chickens are responsible for hemolysis with a similar hemolysis mechanism in humans having G6PD deficiency. Approximately 0.5% of fava bean seeds have 2 pyrimidine beta-glycosides called vicine, and convicine, and these increases the probability of acute hemolytic anemia in proportion with the consumed amount of fava beans.<sup>1</sup>

Vetch cultivation is common in our region. People in this region are used to eating vetch grains. Since vetch has a composition of vicine, convicine, and beta cyanoalanine glycosides,<sup>3</sup> it was reported that beta cyanoalanine is mainly responsible for neurotoxicity and hemolysis.<sup>3</sup> The reason for considering a relationship between consumption of vetch and hemolysis in patients with G6PD deficiency are the similar amounts of vicine and convicine in the fava bean and vetch, and vicine

and convicine in vetch causing hemolysis in chickens. The hemolytic anemia may be related to the consumed amount of vetch, because our patient had stated the consumption of large amounts of vetch 24 hours before the onset of symptoms, and small amounts do not cause acute hemolytic anemia.

The probability of raw fava beans causing favism is higher than that of cooked, frozen, or conserved fava beans.<sup>1</sup> This is due to the fact that vicine and convicine, the 2 pyrimidine glycosides found mainly in the fava bean cotyledon undergo destruction by cooking.<sup>10</sup> Turkish people usually eat the hard, raw, green bean grains after its flowers dry up. Our patient consumed large amounts of vetch. Thus, we think that he was exposed to excessive amounts of vicine, convicine, and beta cyanoalanine glycosides.

These patients should be recommended not to consume vetch. As there are no case report in the literature on hemolysis caused by vetch consumption in patients with G6PD deficiency, it has been emphasized that vetch may cause hemolysis in patients with G6PD deficiency.

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