

## Risk assessment for subjects with G6PD-deficiency of new fava bean cultivars with low content of vicine and convicine (FEVITA Beans)

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SPG Contributions	Project Status	Duration/Timeline of Project (Year to Year)	Total Project Cost
\$34,000.00	Completed	October 2008 – April 2009	\$34,000.00

### Project Description

Favism is an acute hemolytic syndrome occurring in severely G6PD-deficient individuals after consumption of *Vicia faba* beans.

Faba beans contain high levels of the pyrimidine glycosides, vicine, and convicine. Consumption of faba beans can lead to hemolytic anemia in some glucose-6 phosphatedehydrogenase (G6PD)-deficient individuals. The factors in the bean implicated as the causative agents are divicine and isouramil, the aglycones of vicine and convicine, respectively, produced through the action of beta-glucosidase. The aglycones are released in the large intestine and caecum when faba beans are consumed, and are transported to the blood where, in the presence of oxygen, they form products that can bring about oxidative destruction of red blood cells (RBC) in sensitive individuals.

The enzyme G6PD is the main producer of NADPH in the human RBC. NADPH is required by the reduced glutathione-oxidized glutathione- reduced glutathione (GSH-GSSG-GSH) cycle that RBCs utilize to protect themselves from oxidative stress. G6PD-deficient individuals have insufficient G6PD enzyme activity and cannot cope with oxidative stress, which ultimately leads to RBC damage and destruction.

G6PD-deficiency is a sex-linked trait with the majority of favism cases occurring in males hemizygous for the deficiency, with a male/female ratio of VL| WR RQH. Females are heterozygous for the G6PD-deficiency and phenotypically express varying activity. On average, females heterozygous for the G6PD deficiency have approximately 50% of the normal activity, while some show very low G6PD activity.

Favism is prevalent in the Mediterranean area, Middle East, Southern China and Taiwan. Severe favism generally affects children between two and five years of age; however, older adults who used to eat faba beans without harm may suddenly experience severe hemolysis.

Several cultivars of faba bean with low and very low levels of vicine and convicine (FEVITA beans) have been developed. Studies are needed to provide clear evidence of lack of toxicity (lack of hemolytic potential) of FEVITA beans in sensitive individuals.

Hypothesis: That FEVITA beans are safe to consume in G6PD deficient individuals and excess amounts of fresh FEVITA beans will cause no noxious effects even in highly sensitive individuals.

To develop protocols suitable to assess toxicity, or lack of toxicity, of faba bean cultivars low in V/C. The design entailed 2 studies:

Study No. 1:

- a) Study 1a: In vitro study: Analyze intact RBC isolated from G6PD-deficient and normal individuals with the RBC being treated with pure divicine or faba bean extracts treated with beta-glucosidase to release free active aglycones
- b) Study 1b: In vivo study: Where RBC parameters modified by divicine performed in intact RBC isolated from G6PD deficient and normal individuals fed with FEVITA beans (normal and G6PD-deficient subjects) and normal beans (normal subjects only)

Study No. 2:

The clinical trial (Study 2) was performed on healthy normal (7 & 9 volunteers) and healthy G6PD-deficient volunteers (7 female; 21 to 42 years). There were three trial arms:

- 1) Normal volunteers ingested faba beans with low tannin, high V/C content
- 2) Normal volunteers ingested faba beans with low tannin, low V/C content
- 3) G6PD-deficient volunteers only ingested beans with low tannin, low V/C content

Each group was fed 450g/70kg fresh, raw de-hulled beans. This amount exceeds faba bean quantities normally consumed in human nutrition (usually 100-150g/70kg body weight).

Blood was drawn before ingestion, and then 8-10, 24, and 48 hours after ingestion.

The researchers monitored minor changes in the redox balance of the RBC to determine effect of faba bean ingestion. This included:

- a) RBC levels of reduce glutathione (GSH) levels (which always decrease in favism)
- b) Markers indicating modification of RBC membranes (indicate oxidative stress) which are modified in favism and include:
  - a. Membrane deposition of hemichromes
  - b. Formation of membrane adducts with 4-hydroxynonenal (4 HNE)- a sensitive indicator of lipid peroxidation that accompanies oxidant insult
  - c. Membrane deposition of opsonins (autologous IgG and complement fragments ) which are formed as the RBC dies for ultimate removal by phagocytes
  - d. Measurement of RBC filterability, sensitive parameter of RBC deformability that is found during favism (an indicator of G6PD activity)

In addition, the following analysis was conducted:

- a) Hematological parameters considered to be indicators of hemolysis (RBC count, RBC volume, hemoglobin, hematocrit, haptoglobin levels etc)
- b) Comprehensive clinical chemistry (vitamin status, liver status, thyroid status, diabetes, and iron) to ensure deficient subject had no associated pathologies

### Outcome

Study No. 1: Studies using extracts from low V/C and high V/C faba beans to challenge GSH solutions and RBC isolates

Study No. 1a)

1. Low V/C and high V/C extracts not treated with beta glucosidase were unable to oxidize GSH
2. Only extracts prepared from the high V/C faba beans and pre-treated with betaglusosidase were able to oxidize GSH. Extracts prepared from low V/C + betaglusosidase were almost completely inactive
3. Low V/C beans did not contain other potentially oxidant components such as L-DOPA which could possibly lower the GSH levels

Study No. 1b)

1. In the second study, RBCs from normal G6PD individuals were exposed to the same extracts from low V/C and high V/C either treated or not with beta-glucosidase
2. Only extracts prepared from high V/C faba beans and pre-treated with betaglusosidase were able to temporarily decrease the intracellular level of GSH. Oxidation was reversible

Study No. 2: Clinical Trial: Oral challenge test with faba bean ingestion

Total No. trial participants: 23; 12 females, 11 males. The seven G6PD-deficient subjects were all heterozygote female subjects with low or very low G6PD activity and had close relatives who had experienced favism.

Trial Arms:

A. G6PD-deficient subjects fed with low V/C faba beans

1. Based upon the steady-state level of GSH in RBC from the G6PD-deficient females, none showed significant variation in the GSH level from time 0 to time 8 hours after ingestion of the low V/C faba beans
2. All other study parameters were stable over time (4-HNE adducts, RBC deformability)
3. In three subjects the membrane-bound hemichromes were occasionally higher than the 10% mark, but this was attributed to the technical limitations of the test
4. All subjects, except one, had normal indicators of RBC health and clinical chemistry

B. G6PD-normal subjects fed with low tannin, high V/C faba beans

1. Nine subjects fed with low tannin, high V/C faba beans
2. Due to the high anti-oxidant potency of normal RBC, no relevant changes were expected to occur. However, the following changes were noted:
  - Short term changes in 4-HNE in all nine subjects with increases greater than 150% in 7/9 subjects
  - Increases in complement fragment C3c as well as increases in autologous IgG and RBC deformability—all indicators of senescent or damaged RBC
3. No changes in clinical chemistry parameters were noted

C. G6PD-normal subjects fed with low tannin, low V/C faba beans

1. No changes were expected due to the high anti-oxidant potency of normal RBC and this was confirmed by the test parameters:
  - Minimal fluctuations in GSH level
  - Stable levels for 4-HNE, membrane bound opsonins complement C3c fragment and autologous IgG
  - Some fluctuations in hemichrome level attributed to methods
  - No changes in clinical chemistry parameters

In a sample of seven female, G6PD-deficient subjects none of the parameters indicating hemolysis or oxidative RBD damage were changed short term (8-10 hrs) or long term (48 hrs) after ingestion of approximately ten-fold the normal amount of faba bean ingestion of low tannin, low V/C faba beans.

Tentative conclusion: low tannin, low vicine/convicine faba beans are safe to consume by females heterozygous for the G6PD-deficiency.

Value to Producers: Provides evidence that low tannin, low vicine/convicine faba beans are safe for consumption by G6PD-deficient individuals.

## Research Objective

### OBJECTIVE 1

To show that faba beans with low levels of Vicine (V) and Convicine (C) (low V/C beans) are not toxic to highly susceptible glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals, and can be ingested in large amounts by deficient subjects without any harmful effect.