

A substance in broad beans (*Vicia faba*) is protective against experimentally induced convulsions in mice

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Abstract

Vicia faba (VF, broad beans) constitutes a major food item for the River Nile populations. Contrary to tropical Africa, the prevalence rate of epilepsy (0.9–1 per 1000) among schoolchildren of Khartoum Province, Sudan, is lower compared with the rates in Europe and North America. To explore whether broad beans contain any anticonvulsant that can explain this observation, Balb/c mice were either treated with VF extract (0.01 mL/g) or kept as a control. Various doses of strychnine and picrotoxin were used to explore the effect of VF extract on strychnine-sensitive glycine receptors and GABA_A receptors. Diazepam (DIZ) was used as anticonvulsant. Thin-layer chromatography was run for the extract against phenobarbital, DIZ, and/or glycine. VF extract demonstrated a clear protective effect against strychnine-induced convulsions and death in Balb/c mice. Diazepam (20 mg/kg, intraperitoneally), administered 20 minutes prior to strychnine (0.112 mg/kg, intraperitoneally), increased the survival rate to 66.7% and, when given with VF extract (0.01 mL/g), to 100%. Various doses of DIZ protected against picrotoxin-induced convulsions (40 mg/kg, intraperitoneally) and deaths. Pretreatment of mice with VF extract was not protective. On chromatography, VF extract separated in a manner similar to the glycine spot, and revealed one peak coinciding with, but not identical to that of glycine. The extract of broad beans (VF) protects against convulsions probably through the inhibitory glycine receptors, and may contain a substance that is intimately related to glycine. Further research is needed to substantiate this by directly assessing the binding of the VF extract to the glycine receptors or a change in receptor physiology.

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1. Introduction

The epilepsies constitute a heterogeneous group of neurological conditions characterized by recurrent, unprovoked, paroxysmal seizure activity. Data on the prevalence of epilepsy indicate that it is two to three times more prevalent in tropical Africa than in industrialized countries in nontropical areas [1]. Infections, in particular cysticercosis in its endemic areas, are thought to be causative in most cases [1], and a relationship between epilepsy

and malnutrition has recently been suggested [2]. In contrast, a survey of epilepsy among the entire school population of Khartoum Province, Sudan [3], documented a prevalence rate of epilepsy of 0.9/1000. Another survey [4], which included 21,127 children in elementary and intermediate schools of Khartoum, reported a prevalence of 1/1000. These rates are lower than rates for European and American schoolchildren. For comparison, a relatively recent study on epilepsy in primary schoolchildren in Buenos Aires, Argentina, reported an active prevalence of 2.6/1000, similar to the rates for developed countries [5]. The prevalence of epilepsy among schoolchildren in Izmir, Turkey, was considerably higher at 11.2/1000 [6].

The contrast between the prevalence of epilepsy in Khartoum and that reported from other neighboring or developing countries may be influenced by the different

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types of food consumed by these populations. Cysticercosis is a rarity in northern regions of Sudan and in Turkey, as pork is excluded from the diet. However, a legume, *Vicia faba* (VF) or broad beans, constitutes the major food consistently eaten for breakfast by populations living along the River Nile and its tributaries. It is also frequently eaten at dinner. In boarding schools in Sudan, broad beans are a major food source. During the “breakfast break,” between 9:00 and 10:00 AM, following the first two classes, most schoolchildren in Sudan will have broad beans for their breakfast. It has anecdotally been reported that the children are “sleepy” in the third class, which follows the “breakfast break.”

As a food source, the neuropharmacological and other potential therapeutic benefits of VF have been documented. Ingestion of VF (which is known to be rich in easily absorbable L-DOPA) produces a substantial increase in plasma L-DOPA levels, which has been correlated with substantial improvement in motor performance in Parkinson's disease [7]. Dopamine (DA) is also known to increase diuresis and natriuresis through its action on renal dopaminergic receptors. In this respect, ingestion of VF is followed by a significant increase in plasma L-DOPA and urinary excretion of sodium and DA [8]. Hence, it has been suggested that VF might be of value in treating conditions such as heart failure, liver cirrhosis, renal failure, and hypertension in which natriuresis and diuresis are medically beneficial [8].

The objective of this study was to explore whether VF has any anticonvulsant effects that might partially explain the reported low prevalence of epilepsy in schoolchildren of Khartoum Province.

2. Materials and methods

2.1. Animals

Balb/c mice (25–30 g) were used throughout the study. Animals were deprived of solid food for 18 hours prior to the experiment. They were kept in wire-mesh cages to prevent cropophagia. They were allowed water ad libitum. Research on experimental animals was conducted in accordance with internationally accepted principles for laboratory animal use and care.

2.2. Preparation of *Vicia faba* extract

Three hundred grams of brown broad beans was soaked in 1.0 L of distilled water overnight, and boiled in a water bath until the extract was reduced to 300 mL (about 3 hours). The extract was homogenized and filtered. The filtrate was concentrated by cold evaporation to a volume of 50 mL. This treatment resembles the way in which broad beans are cooked as a meal in Sudan.

2.3. Strychnine-induced convulsions

Animals were pretreated with oral VF extract (0.01 mL/g per mouse) and divided into two groups (6 mice each). Then, after 30 minutes, the two groups were injected intraperitoneally (IP) with strychnine 0.112 and 0.225 mg/kg, respectively. The numbers of animals that died after

treatment with extract and strychnine were recorded and compared with the numbers of control mice (two groups, 6 mice each) that had received the same doses of strychnine only.

2.4. Potentiation of diazepam action by VF

Two groups of animals were used. One ($n = 12$) received diazepam (DIZ, 20 mg/kg, IP) alone. The second group ($n = 6$) received VF extract (0.01 mL/g, orally) together with DIZ (20 mg/kg, IP). Both groups of animals were injected with strychnine (0.112 mg/kg, IP) 20 minutes later. The number of deaths was recorded.

2.5. DIZ protection against picrotoxin-induced convulsions

Three groups of animals (10 mice each) received 2.5, 5, and 10 mg/kg DIZ, IP, respectively. After 15 minutes, each animal was injected with picrotoxin (40 mg/kg, IP). Time to death was recorded for each animal.

2.6. Effect of VF extract on picrotoxin-induced convulsions

Animals were divided into three groups. Groups A ($n = 16$), B ($n = 16$), and C ($n = 20$) received 10, 20, and 40 mg/kg picrotoxin, IP, respectively. Half of the animals in each group had been pretreated with VF extract (0.01 mL/g) given orally 30 minutes earlier. Control animals for the three groups were given 0.05 mL/g 0.9% saline, orally. Time to death was recorded for each animal.

2.7. Chromatographic methods

Thin-layer chromatography consisted of a stationary phase immobilized on a glass and an organic solvent (55% phosphate buffer and 45% methanol). The constituents of a sample could be identified by simultaneously running standards with the unknown. The bottom edge of the plate was placed in a solvent reservoir, and the solvent moved up the plate by capillary action. When the solvent front reached the other edge of the stationary phase, the plate was removed from the solvent reservoir. The separated spots were visualized with ultraviolet light or by placing the plate in iodine vapor. The different components in the mixture move up the plate at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

2.8. Statistical analysis

Statistics were analyzed using SPSS (Version 12.0). Paired t test (two-tailed), Fisher's exact test, ANOVA, and multiple regression analysis were used to compare data among the different groups. A P value < 0.05 was considered to indicate significance.

3. Results

The animals started to become “sleepy” about 20 minutes, and at least four animals were asleep, 30 minutes after administration of VF extract (as shown in the video clip—see Appendix A. Supplementary video).

3.1. Strychnine-induced convulsions

VF extract protected against death after injection of two different doses of strychnine (Fig. 1). All 12 animals in the two control groups (6/group) died following administration of 0.221 and 0.225 mg/kg, IP. The mortality of animals pretreated with VF extract (6 mice/group) was significantly lower (respectively 16.6 and 33.3%, $P = 0.008$ and 0.03).

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