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Effects of Aqueous Extract of Saffron on Gamma-Amino Butyric Acid Content in Rat Hypothalami

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Abstract

Background: Preliminary studies revealed that 4-aminobutyric acid (GABA) is a key and major inhibitory neurotransmitter in the brain. Evidence from many animal studies and even some clinical studies indicate that GABA is responsible for regulating behavior and also plays an important role in brain functions. Previous studies presented Glutamic acid decarboxylase as a catalyst for the conversion of glutamic acid to GABA.
Objectives: The aim of this study was to evaluate the effects of saffron on GABA content in the hypothalamus of rats.
Materials and Methods: Male rats weighing 190 - 210 g were used. They were maintained in a temperature-controlled room with a 12-hour light/dark illumination cycle. The rats were fed standard pellet feed and had access to water ad libitum. The animals were divided into three groups: The first group received a 250 μL intraperitoneal injection of 0.05 g/mL saffron (Group I). The second group received a 250 μL intraperitoneal injection of 0.1 g/mL saffron (Group II). The third group acted as the control and received only water (Group III). The time intervals chosen for this experiment were 1, 2, 4, and 8 weeks following the administration of saffron. At least six animals were assigned to each experimental group. At each time interval, the animals were anaesthetized and brain tissue extracted, hypothalami separated and homogenized in PBS solution, rinsed with PBS, re-filtered, and centrifuged at 1200 g for 10 minutes.
Results: In this study, both doses of saffron (0.05 g/mL [Group I]; and 0.1 g/mL [Group II]) caused significantly increased GABA content in each hypothalamus. GABA in Group I increased significantly compared to the control group (1.00 $\pm$ 0.05 [mean $\pm$ SD, n = 8] vs. 0.29 $\pm$ 0.05, mM). GABA in Group II also increased significantly compared to the control group (1.45 $\pm$ 0.07 [mean $\pm$ SD, n = 8] vs. 0.29 $\pm$ 0.05, mM). The effect of saffron on GABA was also dose dependent; the only exception occurring during the final time interval for the 0.1 g/mL saffron concentration.
Conclusions: The results of this study demonstrated a significant increase in hypothalamus GABA content from saffron administration. One explanation for this observation could be the stimulation of glutamic acid decarboxylase the primary enzyme responsible for the production of GABA. Saffron may be a potential therapeutic agent for improving neurotransmitter levels.

Keywords: 4-Amino Butyric Acid (GABA), Saffron, Neurotransmitter

1. Background

As previously demonstrated, 4-amino butyric acid (GABA) is produced by glutamate decarboxylase through the decarboxylation of L-glutamate or catalysis from L-glutamine by glutaminase enzymes (1, 2). Multiple studies have shown that glutamate decarboxylase enzymes have a sufficient effect on GABA's synthesis quantity. Earlier studies have suggested that GABA is metabolized into succinic acid, tri-carboxylic acid cycle intermediate (3, 4). It is clear that the GABA-aminotransferase catalysis converts GABA to succinic semi-aldehyde, which then converts to succinic acid via succinic semi-aldehyde dehydrogenase (5, 6). Also, succinic semi-aldehyde can reduce to 4-hydroxy butyric acid via another pathway (7, 8). Some researchers have reported that GABA is an important inhibitory neurotransmitter in the central nervous system (9, 10). As reported by many other researchers, it plays a role in regulating neuronal excitability throughout the nervous system (11, 12). Previous studies presented that a lack of GABA in the elderly can cause trouble in brain superior activity, such as video diagnosis and verbal perceptions (13, 14). Previous studies established that herbal remedies have been important in healthcare and disease prevention (15, 16). According to recent works, some of the potential uses of saffron in

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the treatment of brain disorder include: anti-depression, anti-anxiety, sedation, improved cognition and memory, and cough suppression (17, 18). Researchers have reported that saffron is able to treat depressed patients (19, 20). Many researchers have reported that saffron extract was used as a novel drug in the treatment of disorders like Alzheimer's disease (21, 22). Several researchers also report that saffron can reduce blood pressure (23).

2. Objectives

The aim of this study was to evaluate the effects of saffron on GABA content in the hypothalamus of rats.

3. Materials and Methods

3.1. Materials

Gamma-amino butyric acid, ID code: G 012; Alpha-keto glutaric acid, ID code: K 1128; GABase enzyme, ID code: G 7509; $NADP^+$, ID code: N 5755; and Glycerol, ID code: G 9012 were all purchased from Sigma-Aldrich company, Germany. Potassium phosphate, ID code: P 5655 was purchased from BioXtra company, Germany.

3.2. Preparation of Saffron Solution

For preparation, 1 g of saffron was dissolved in 5 mL of distilled water in a test tube and was mixed thoroughly by a glass agitator. The total solution was then placed in a Bin-Marie bathroom at 60°C for 30 minutes. After 30 minutes passed, the solution was shacked with a vortex mixer to better dissolve the saffron compound. It was then centrifuged at 3000 rpm for 10 minutes. Afterwards, 2 mL of supernatant was separated by scaled pipette and transferred to other tubes. Saffron-treated groups received intraperitoneal 250 μL suspensions of saffron.

3.3. Animals

Male rats (n = 24), 5 - 7 months old and weighing between 190 - 210 g were purchased from the animal center in Babol University of Medical Sciences. They were allowed water ad libitum. The animals were divided into three groups, each of which contained 8 rats. Group I received 250 μL of a 0.05 g/mL saffron concentration intraperitoneally. Group II received 250 μL of a 0.1 g/mL saffron concentration intraperitoneally. Group III rats were treated with 250 μL water and served as the control group. At the end of the experiment, the animals were anesthetized with a solution containing 500 μL of an aqueous mixture (made of 300 μL Ketamine 10% and 200 μL Xylazine 2%) at the following set intervals (two rats per interval): one week, two weeks, four weeks, and eight weeks. After 15 minutes, injected rats were fully anesthetized. At each interval, the two animals were anaesthetized and their brain tissues extracted; each hypothalamus (150 mg samples) was separated and homogenized in PBS and filtered through a 100 μm filter, centrifuged at 1200 g at 5°C for 10 minutes, rinsed with a PBS solution, and then re-filtered and re-centrifuged at 1200 g for 10 minutes. The supernatants were then collected and finally filtered through 0.22 μm Millipore. These experiments were also conducted for the control group. Each test was repeated 6 times for each sample obtained from animals. In total, laboratory tests were performed for each group 12 times.

3.4. Preparing GABA Determination Solutions

The GABA content was measured according to the enzymatic method as described previously (24, 25). It is worth mentioning that for the measurement of GABA content, the experiment method included GABA-transaminase (GABA-T) and succinate semi-aldehyde dehydrogenase (SSADH) enzymes. One unit of GABase enzyme inverts one micromole GABA to succinic semi-aldehyde, and finally to succinate (equivalent to one micromole $NADP^+$ reduction in pH = 8.6 and 25°C temperature per minute).

GABase enzyme was added to the solution including 10 mL standard gamma amino-butyric acid, 20 mL pyrophosphate buffer, 5 mL $NADP^+$, 10 mL alpha-keto glutarate solution, 20 mL GABase solution; with concentrations of 5 units in 0.15 mL solution, and 0.5 mL homogenate solution. To prepare the GABase solution, we dissolved 5 units of GABase powder in 5 mL pyrophosphate buffer 0.075 mol/L in pH = 7.2 and followed this with a 1 mL glycerol 25% addition. We added 0.5 mL homogenate solution to each tube containing hypothalamus tissue and mixed them via a glass mixing tube. We then shacked them with a vortex mixer to thoroughly dissolve the hypothalamus components. We followed this with adding a 2.5 mL pyrophosphate buffer, 0.15 mL $NADP^+$, and a 0.15 mL GABase enzyme solution. We then removed 0.1 mL of the hypothalamus homogenate solution supernatant. For the brain samples, the same steps mentioned above were carried out, and then we ultimately removed 0.1 mL of brain homogenate supernatant. For preparation of the GABA standard solution, 0.0, 0.5, 1, and 2 mM of content were prepared. Next, a 2.5 mL phosphate buffer, 0.15 mL $NADP^+$, and a 0.15 mL GABase enzyme solution were added to each tube. The measurement of GABA levels was performed via the spectrophotometer method in 340 nm wavelengths. To prepare the spectrophotometer, distilled water was used for initialization; setting the 0 absorbance level on the device. This was followed by reading the primary absorptions of the sample tubes. Then, 0.15 mL of alpha-keto glutarate was added to

the sample tubes; after 30 minutes, the secondary absorption level was read and recorded. Based on the average absorptions of GABA solutions in different concentrations (0, 0.5, 1, and 2 mM), a standard GABA absorption curve was drawn. By using this curve, the GABA concentrations were determined for each sample.

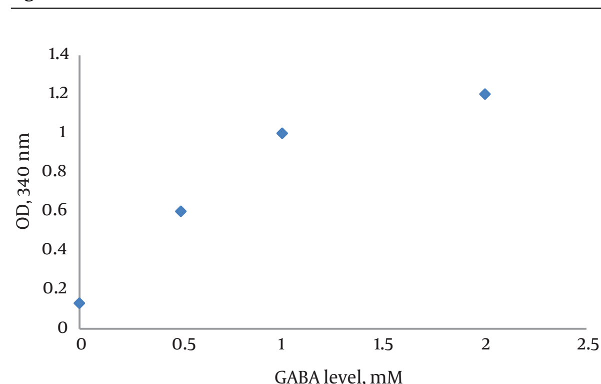
3.5. Statistical Analysis

Data were presented as mean ± SD. Statistical significance was defined as $P < 0.05$. Analysis was performed using the SPSS software package version 20. Comparisons between groups were performed using the two-tailed Mann-Whitney test.

4. Results

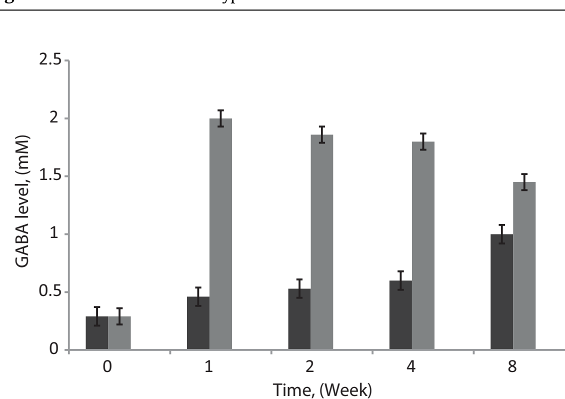
There are different techniques, such as chromatography and spectrophotometric, for measuring GABA in various regions of the brain. For this study, measurement of GABA was ascertained via enzyme systems because of the sensitivity and simplicity in these systems. Quantification of GABA in all samples was performed via the enzymatic method. The GABA standard curve determination is shown in Figure 1. When compared with the control group, the higher concentration of saffron (0.1 g/mL) resulted in higher GABA content, as shown in Figure 2. The results of this study demonstrate that GABA contents were significantly affected by saffron when compared to the control group. On week eight, GABA content was 1.45 ± 0.07 mM, ($P < 0.05$). The 0.1 g/mL treatment group was significantly higher than the control group (0.29 ± 0.07 mM). Moreover, the mean GABA level in the other treatment group (0.05 g/mL) was 1.00 ± 0.05 mM, which was also greater than that observed in the control group ($P < 0.05$). Saffron administration at the 0.1 g/mL dosage obtained the maximum GABA content ($P < 0.05$), but GABA levels first elevated and then decreased in the rat hypothalami.

Figure 1. GABA Standard Curve



Values are expressed as mean ± SD of 4 - 6 separate experiments.

Figure 2. GABA Content in rat Hypothalami



Black column, 0.05 g/mL saffron; Gray column, 0.1 g/mL saffron; 0, control group; 1, after one week of saffron administration; 2, after two weeks of saffron administration; 4, after four weeks of saffron administration; 8, after eight weeks of saffron administration. Values are expressed as mean ± SD of 4 - 6 separate experiments.

5. Discussion

It has been revealed that GABA is an inhibitory neurotransmitter. Previous studies also concluded that GABA is synthesized by glutamic decarboxylase in brain tissue (15-21). In this study, the effect of an aqueous extract of saffron on GABA content in the hypothalamus of rats was investigated. Saffron is a specific derived from the flower of the saffron Crous (Crocus Sativus), a species of crocus in the family Iridaceae (20, 22, 23). The most important findings in this research indicate that aqueous extracts of saffron in different concentrations cause a marked increase in GABA levels in a rat's hypothalamus. The mean GABA content in the treated group's hypothalamus per experiment interval was higher in comparison to the control group. Our data demonstrated that in a 0.05 g/mL dose, there is an increase from the first week till the eighth week, with the maximum readings observed in the eighth week. However, in a 0.1 g/mL dose, GABA levels first elevated and then decreased in the hypothalamus. It has been suggested that the effect of saffron may be attributed to its chemical constituents. The possible mechanism of saffron's actions in the hypothalamus may be from stimulated enzymes related to GABA metabolism. Considering that glutamic acid decarboxylase is a critical enzyme involved in the production of GABA, saffron may stimulate the glutamic acid decarboxylase that catalyzes the conversion of glutamic acid to GABA.

The injection of 0.05 g/mL saffron aqueous extract in Group I resulted GABA levels increase at each experiment interval of this study in comparison to the control group. These increases have meaningful differences in all intervals when compared with control group ($P < 0.05$). The mean of GABA levels in the first, second, fourth, and eighth weeks were observed as higher than the control group.

5.1. Limitations

Several limitations need to be considered. First, the main limitation of the present study is that we cannot describe the mechanism through which saffron increases GABA content. Second, we could not use advanced methods because we are limited financially. However, extensive experimental studies are required in large numbers of animals.

5.2. Conclusions

In conclusion, the findings of this paper suggest that the use of saffron increases GABA levels in the hypothalamus of rats. It suggested that the administration of saffron was beneficial in improving GABA metabolism in rats.

Footnotes

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Authors' Contribution: Durdi Qujeq was responsible for the study's conception, design, critical revision, and the drafting of the manuscript. Ali Asghar Rastegari Efahani acquired data. Shokoufe Nikpour Moghaddam was responsible for data analysis and interpretation.

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