

## Effect of Curcumin on the Hypothalamus Levels of the Potent Inhibitory Neurotransmitter, Gamma Aminobutyric Acid

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### Abstract

**Background:** There are some reports in the literature showing that hypothalamus synthesizes and secretes amino acid neurotransmitters. According to several studies, elevated serum levels of gamma-amino butyric acid (GABA), a potent inhibitory neurotransmitter, have recently been implicated in the pathogenesis of neural diseases. The purpose of this research was to estimate the effects of curcumin on GABA's level in rat's hypothalamus.

**Materials and Methods:** We used a standard animal model of rats (n=18) with mean weight 190-210 g, to determine the effects of administration of curcumin at the end of the experimental period, one week, two weeks, four weeks and eight weeks, at doses of 250 mg/kg and 625 mg/kg on GABA level in hypothalamus. On the day of experiment, hypothalamus was extracted and homogenized through a 10  $\mu$ m filter, rinsed with PBS, re-filtered, and centrifuged at 1200 rpm for 15 min. Then rat hypothalamus was weighed, and homogenized (10% w/v) in 0.1 M PBS with poltron homogenizer at pH 7.4. Homogenates were used immediately for determination of GABA level. Quantifications of GABA in all samples were performed by enzymatic method.

**Results:** Our results indicate that curcumin has a potential to increase GABA content in the rats' hypothalamus. These results suggest that curcumin holds promise as a natural agent to control or decrease the signs of lack of GABA level.

**Conclusion:** Curcumin may be used clinically as a neuro-protective drug for treatment of patients suffering from neuron damage.

**Keywords:** Curcumin; gamma-aminobutyric acid; hypothalamus

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### Introduction

Gamma-amino butyric acid (GABA) is identified as a normal constituent of brain tissue and the main inhibitory transmitter in the central nervous system which has a significant depressant effect on nerve transmission (1). As reported by many other investigators GABA is one of the most important regulators of neurotransmission and essential functions of the body, additionally it is an anti-epileptic and natural relaxation agent in the brain. Many studies showed that GABA attaches to another cell (2). It is important to emphasize that GABA blocks the

transmission of nerve impulses between neurons, bringing a calming or relaxing influence (3). Previous studies established that, with a deficiency in GABA, nerve cells can fire easily and far too often. Previous studies demonstrated that due to loss of GABA, various anxiety disorders are triggered such as seizure, panic attacks, headaches, and Parkinson's disease (4). Many studies showed that drugs such as Valium, Baclofen, Valproate and Neurontin cause an increase of GABA production in the brain (5). As reported by many other investigators, natural GABA

is much safer and incredibly effective with minimal side effects (6). There are some reports in the literature showing GABA is synthesized from glutamate using the enzyme L-glutamic acid decarboxylase and pyridoxal phosphate. It is now clear that GABA is converted back to glutamate by a metabolic pathway called the GABA shunt (7). Different studies have shown that curcumin is useful for treatment of different disease (8). In general, curcumin has effects similar to other polyphenols (9). As described previously it is a polyphonic non-flavon compound in the rhizome of *Curcuma longa*, and induces the expression of various molecular targets, such as transcription factors, enzymes, cytokines, cell cycle proteins, receptors and adhesion molecules (10). Some studies have reported on the effects of curcumin in the treatment of psychiatric disorders (11). This study was conducted based on a hypothesis on the possible roles of two different concentrations of curcumin on increasing GABA production in the rats' hypothalamus.

#### Materials and Methods

Gamma-amino butyric acid; ID code: G 012, Alpha-keto glutaric acid; ID code: K 1128, GABase enzyme; ID code: G 7509, NADP<sup>+</sup>; ID code: N 5755, Glycerol; ID code: G 9012, were purchased from Sigma-Aldrich Co., Germany. Potassium Phosphate; ID code: P 5655, BioXtra, Germany.

#### Preparation of curcumin solution

Curcumin (2.5 g) was dissolved in 5 ml distilled water in the test tube and was mixed well by a glass agitator. It was then put in Bin-Marie bathroom in 60 °C, for 30 minutes. After wards, it was shaken with vortex mixer to dissolve curcumin compounds well. Then 2 ml of supernatant was separated by scaled pipette and transferred to other tubes, and centrifuged at 3000 rpm for 15 minutes.

#### Animal

Male rats (n=18), 5-7 months old weighting 190-210 g were purchased from animal center affiliated to Babol University of Medical Sciences. They were housed in individual cages in an environmentally controlled room, with a 12-hour light /dark cycle. The rats had free access to water and were fed standard rat chow. The animals were divided into three groups, each of which contained 6 rats. Group I: Rats treated with 500µL water and served as control group. Group II: Received 500 µL of 250mg/kg curcumin intraperitoneally. Group III: Received 500 µL of 625mg/kg curcumin intraperitoneally. At the end of the experimental period, one week, two weeks, four weeks and eight weeks the animals were anesthetized with solution including 500 µL of an aqueous mixture

(made of 300 µl Ketamine 10% and 200 µl Xylazine 2%). After 20 minutes, injected rats were fully anesthetized. At each time point same procedure was performed and brains tissue were extracted and hypothalamus were separated and homogenized in PBS and filtered through a 10 µm filter, and were centrifuged at 1200 rpm at 5° C for 15 min, then rinsed with PBS solution, re-filtered and re-centrifuged at 1200 rpm for 15 min. Then the supernatants were collected and finally filtered through Millipore.

#### Preparing GABA determination solutions

This study was conducted according to enzymatic method (12, 13) for measurement of GABA content that includes GABA-transaminase (GABA-T) and succinate semi aldehyde dehydrogenase (SSADH) enzymes. In this method, one unit GABase, inverts one micromole GABA to succinic semi aldehyde and finally to succinate, per minute, which equivalent one micromole NADP<sup>+</sup> reduction in pH= 8.6 and 25°C temperature. Capability of measuring in this method is 98%, as micrograms. GABase was added to solution including 10 ml standard gama amino-butyric acid, 20 ml pyrophosphate buffer, 5 ml NADP<sup>+</sup>, 10 ml alpha-keto glutarate solution, 20 ml GABase solution, concentration: 5 units in 0.15 ml solution, 0.5 ml homogenate solution. To prepare GABase solution, we dissolved 5 units of GABase powder in 5 ml pyrophosphate buffer 0.075 mole in pH= 7.4 and added 1 ml glycerol 25%. Homogenate solution (0.5 ml) was added to each tube which contained hypothalamus tissue, and mixed by glass mixing tube. Then shacked them with vortex mixer to dissolve hypothalamus components well. Afterwards, 2.5 ml pyrophosphate buffer, 0.15 ml NADP<sup>+</sup>, and 0.15 ml GABase enzyme solution were added, and 0.1 ml of hypothalamus homogenate solution supernatant was removed. Then 2.5 ml phosphate buffer, 0.15 ml NADP<sup>+</sup>, and 0.15 ml GABase enzyme solution were added to each tube.

The measurement of GABA level was performed with spectrophotometer method, in 340 nm wavelength. Then, primary absorption of sample tubes was read. Then, 0.15 ml alpha-ketoglutarate was added to sample tubes and after 30 minutes, the secondary absorption was read. Based on average absorption of 1 mM standard, GABA solution in different concentrations 0, 0.2, 0.4, 0.6, 0.8, 1, and 1.2 mM as a standard GABA were prepared and standard GABA absorption diagram was drawn. Using this diagram, GABA's concentrations were determined in each sample.

#### Statistical analysis

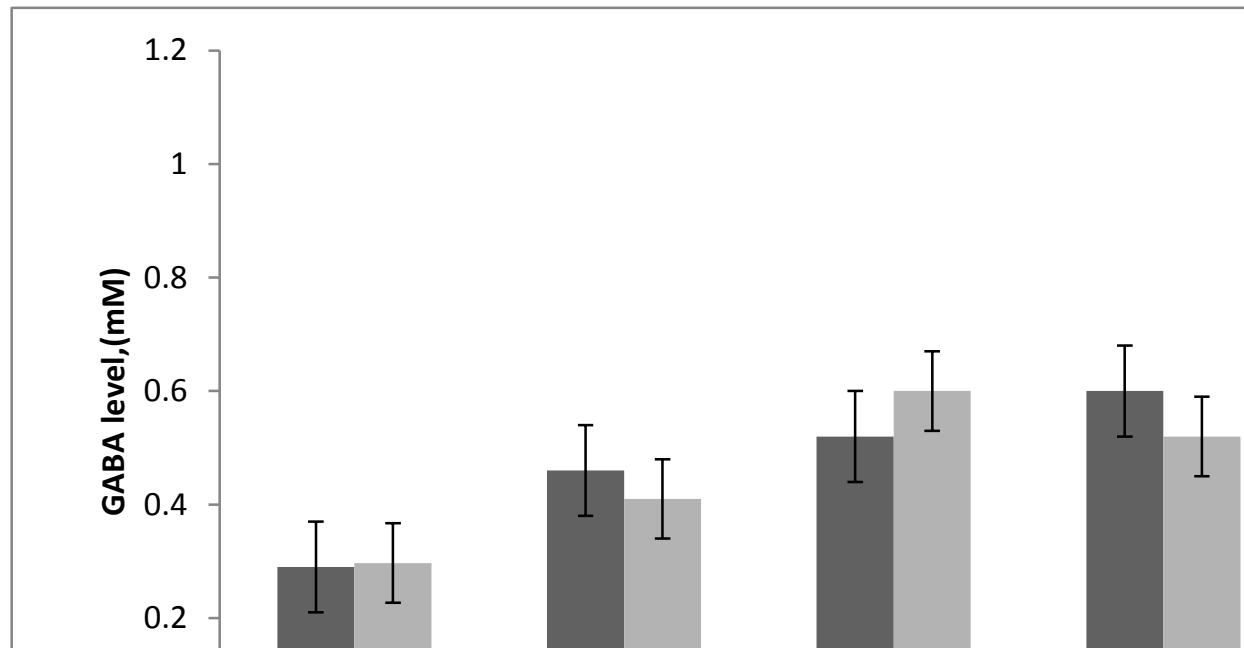
Data were presented as mean ± SD. Statistical

significance was defined as  $p < 0.05$ . Analysis was performed using SPSS version 20. Comparisons between the groups were performed using the two tailed Mann -Whitney test.

### Results

Based on standard curve, quantification of GABA content in all samples was performed using

enzymatic methods. In a comparison between the control group and the intervention group it was revealed that the lower dose of curcumin (250 mg/kg) resulted in higher GABA content compared to that of the higher dose (625mg/kg), (Figure 1). GABA level were found significantly affected by administration of curcumin.



**Figure 1.** GABA content in rat hypothalamus. (Column one: 250 mg/kg curcumin was administrated and column two: 625 mg/kg curcumin was administrated. (0; control group, 1; after one week administration of curcumin, 2; after two weeks administration of curcumin, 4; after four weeks administration of curcumin, 8; after eight weeks administration of curcumin). Values are mean  $\pm$  SD of 4-6 separate experiments.

### Discussion

In the present study, the effects of aqueous extract of curcumin were investigated on GABA content in rat's hypothalamus. We observed that aqueous extracts of curcumin with different concentrations, caused a marked increase in the GABA level in the rat's hypothalamus. After the treatment the mean of GABA's content in hypothalamus of all treated groups at each time point was higher compared to that of the control group. Data demonstrated that at 250 mg/kg dose increase in GABA content occurred from first week until the eighth week maximum effect was seen. These increases showed significant differences in treated groups of all times compared to the control group ( $P < 0.05$ ). But, in 625mg/kg dose, GABA level elevated slightly in the rat hypothalamus.

Interestingly, in all times GABA's level in group II (treated by 250 mg/kg curcumin) was higher than GABA's content in group III (treated by 625mg/kg curcumin), except after 2 weeks. This may express that the increasing GABA content by administration of curcumin is not dose dependent.

According to these results, we suggest that the effect of curcumin may be a stimulatory influence on the enzymes involved in production of GABA, or the possible mechanism of curcumin action in hypothalamus may be by inhibition of enzyme related to GABA catabolism. Curcumin may stimulate the glutamic acid decarboxylase that catalyzes the conversion of glutamic acid to GABA. Glutamic acid decarboxylase is a critical enzyme involved in the metabolism of GABA. On the other hand, it is possible that Curcumin could inhibit the GABA transaminase or succinic semialdehyde dehydrogenase. Both of which are main enzymes in catabolism of GABA. Further studies are needed to understand the main mechanism action of curcumin.

### Conclusion

The findings suggest that the curcumin administration was beneficial in improving GABA metabolism in rat. Curcumin can be operant in moderating diseases that are caused by GABA deficiency and emphasized its use in monitoring the GABA content. GABA

disorder include neurodegenerative disturbances, such as Alzheimer disease, multiple sclerosis, Parkinson disease and cerebral ischemia, psychiatric disorders such as depression, schizophrenia, neuropathic pain and some other diseases in different manners such as epilepsy or brain tumors. Also, these data provide new insight into the physiological function of curcumin. It is promising that curcumin will play an important role in GABA metabolism. In contrast chemical drugs such as Valium, Baclofen, Valproate and Neurontin all have wide spreading side effects making them as a trouble instead of solution for long-term use. On the other hand, utilization of natural components, lead to stimulus GABA synthesis, are much safer and incredibly effective with minimal side effects. Beyond all these positive results and suggestions for better understanding on the mechanism of curcumin, further studies are needed to support its use in the management of neurologic diseases.

#### Limitations

Although the present study has yielded some preliminary findings, but there were some limitations that should be noted we could not describe the mechanism of how curcumin increased the content of GABA. Another limitation was that we could not use advanced method and limited finance was another issue.

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#### Author Contributions

Prof Qujeq, Study conception, design, critical revision and drafting of the manuscript. Ali Asghar Rastegari Efahani acquisition of data. Shokoufe Nikpour Moghaddam, analysis and interpretation of data.

#### Conflict of Interest

The authors declare that they have no conflict of interest in this work.

#### References

1. Lee CT, Chen J, Worden LT, Freed WJ. Cocaine causes deficits in radial migration and alters the distribution of glutamate and GABA neurons in the developing rat cerebral cortex. *Synapse*. 2011; 65(1) 21-34. PMID: 20506319.

2. Wood JD. A possible role for Gamma-aminobutyric acid in the homeostatic control of brain metabolism under conditions of hypoxia. *Exp Brain Res*. 1967; 4(1)81-84. PMID: 5598819

3. Battistin L, Varotto M, Berlese G, Roman G. Effects of some anticonvulsant drugs on brain GABA level and GAD and GABA-T activities. *Neurochem Res*. 1984; 9(2); 225-31. PMID: 6429560.

4. Nasrallah FA, Balcar VJ, Rae CD. Activity-dependent Gamma-aminobutyric acid release controls brain cortical tissue slice metabolism. *J Neurosci Res*. 2011; 89(12) 1935-1945. PMID: 21618581

5. Chen Z, Silva AC, Yang J, Shen J. Elevated endogenous GABA level correlates with decreased fMRI signals in the rat brain during acute inhibition of GABA transaminase. *J Neurosci Res*. 2005; 79(3): 383-91. PMID: 15619231

6. De Sousa A. Herbal medicines and anxiety disorders. *J Med plant Stud*. 2013; 1(6): 18-23.

7. Albrechta J, Sidoryk-Węgrzynowicza M, Zielińska M and Aschnera M. Roles of glutamine in neurotransmission. *Neuron Glia Biol*. 2010; 6(4): 263-76. PMID: 22018046

8. Mythri RB, Bharath MM. Curcumin: a potential neuroprotective agent in Parkinson's disease. *Curr Pharm Des*. 2012; 18(1):91-9. PMID: 22211691

9. John M. Ringman, Sally A. Frautschy, Gregory M. Cole, Donna L. Masterman and Jeffrey L. Cummings. A potential role of the Cury spice curcumin in alzheimer's disease. *Curr Alzheimer Res*. 2005; 2(2): 1-5. PMID: 15974909

10. Chimakurthy J, Talasila M. Effects of curcumin on pentylenetetrazole-induced anxiety-like behaviors and associated changes in cognition and monoamine levels. *Psychol Neuroscience*. 2010; 3(2):239-44.

11. Lopresti AL, Hood SD, Drummond PD. Multiple antidepressant potential modes of action of curcumin. *J Psychopharmacol*. 2012; 26(12):1512-24. PMID: 23035031

12. Yazdanparast R, Qujeq D. Significant Changes in the Activity of L-glutamic acid decarboxylase of mouse hypothalamus after peripheral injection of cholecystokinin-8 and caerulein. *Iran Biomed J*. 1999; 3 (1): 47-51.

13. Qujeq D. Determination of gama aminobutyric acid by high performance liquid chromatography. *Acta Medica Iranica*. 1998; 36 (2): 102-105.