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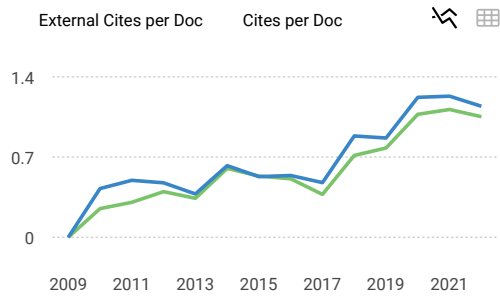
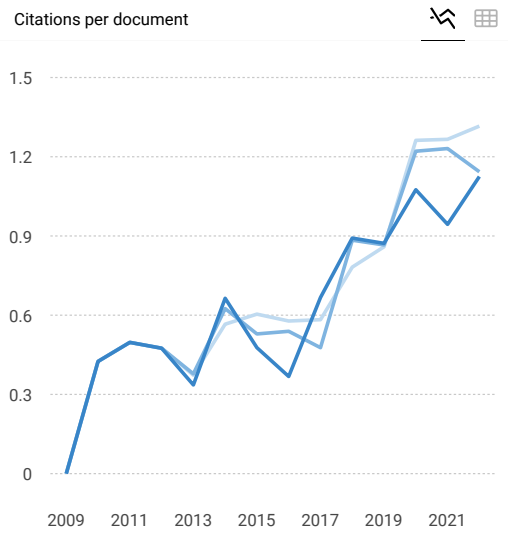
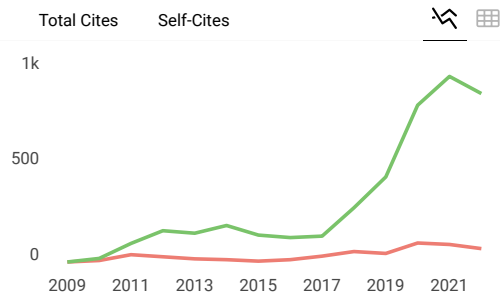
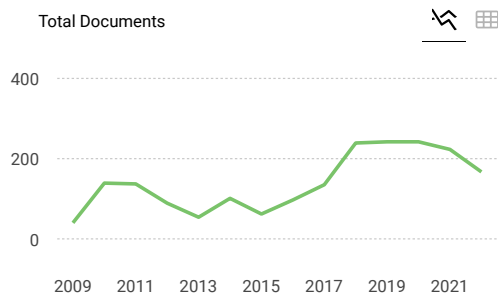
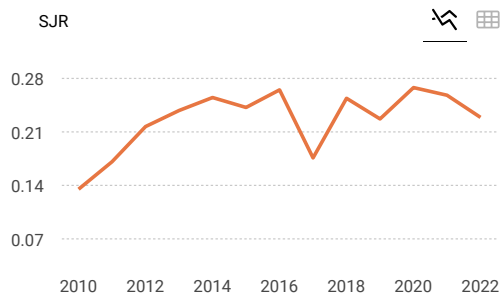
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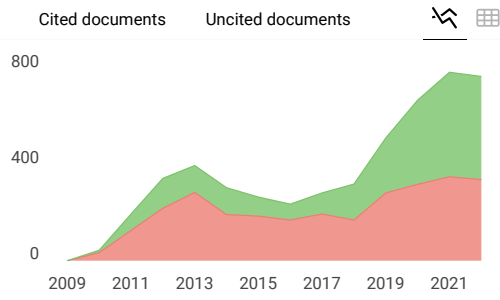
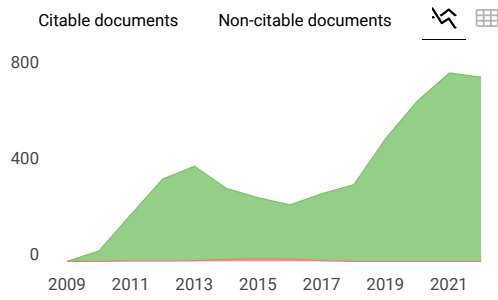
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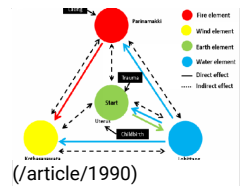
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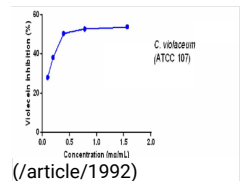
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### The Effect of Preconception Moringa Leaf Extract Supplementation on Cotinine Serum Levels in Pregnant Women Exposed to Tobacco Smoke (/article/1995)

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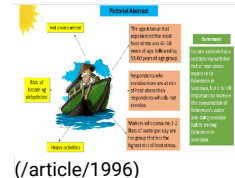
### Relationship Between Individual Characteristics and the Risk of Exposure to Heat Stress in Indonesian Fishermen (/article/1996)

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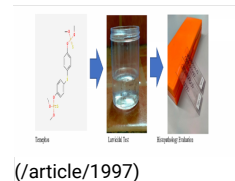
### Another Mode of Action of Temephos Against Aedes aegypti Larvae: A Stomach Poison Investigation (/article/1997)

Hebert Adrianto, Sri Subekti, Heny Arwati, Etha Rambung, Hanna Tabita Has Silitonga, Etik Ainun Rohmah

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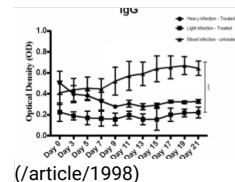
### The Aqueous Leaf Extract of Moringa Oleifera had Immunomodulatory Effects on Sheep that had Been Infected by Fasciola Gigantica, Clostridium Novyi Naturally and Impact to Fasciola gigantica Non-Embryonated (/article/1998)

Maslichah Mafruchati, Wan Irjani Wan Ismail

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### The Effect of Moringa Leaf Extract Intervention Since Preconception Period on the Prevention of Oxidative Stress in Pregnant Women and Adverse Pregnancy Outcomes (/article/1999)

, Veni Hadju, Arsunan Arsin, Aminuddin Syam, Anwar Mallongi, Abd. Farid Lewa, Haerani Harun,

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### Antihypertensive Choices during Pregnancy in Limited Setting (/article/2000)

, , Salsabila Nabilah Rifdah, Agus Sulistyono

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### The Influence of Job Satisfaction and Organizational Commitment on Nurse Performance with Work



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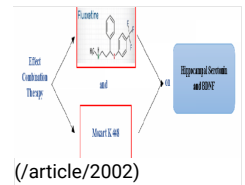
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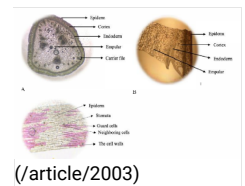
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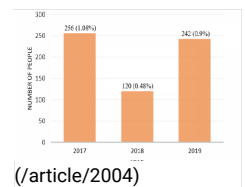
Teguh Triyono, Tsaniatul Afifah, Usi Sukorini

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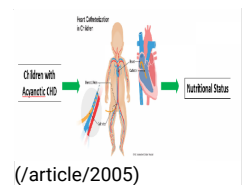
Muqiemuddin Salim, Ketut Alit Utamayasa, Roedi Irawan, Azwin Mengindra Putera, Meity Ardiana

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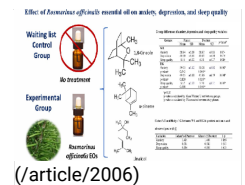
Paul Alan Arkin Alvarado-García, Marilú Roxana Soto-Vásquez, Luis Enrique Rosales-Cerquin, Santiago M. Benites, Taniht Lisseth Cubas-Romero, Demetrio Rafael Jara-Aguilar, José Gilberto Gavidia-Valencia, Iris Melina Alfaro-Beltrán

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### Phytochemical Screening, Antibacterial Activity and Fatty Acids from Heliotropium Indicum (/article/2007)

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Arifin Seweng, ,Ella Andayani,Anwar Mallongi

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(/article/2009)

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## Diuretic Potency of Belalai Gajah Plants (Clinacanthus nutans (Burm.fil.) Lindau) (/article/2010)

Ruqiah Ganda Putr Panjaitan, ,Syarifah Ditha Aprilia

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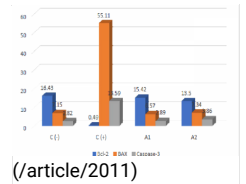
Novi Primadewi,Harijono Kariosentono,Ari Probandari,Budiyanti Wiboworini

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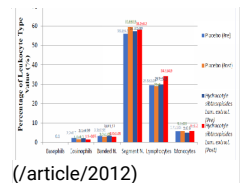
,Adrul Fauzan,Salman Umar,Yufri Aldi

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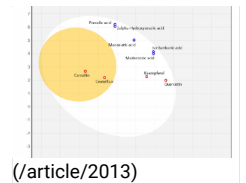
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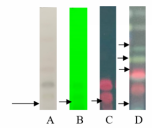
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### Analysis of the Metabolite Compound of the Ethanol Extract of *Chrysanthemum cinerariifolium* Stem and Activity for inhibition of Oral Squamous Cell Carcinoma (OSCC) in silico study (/article/2015)

Anik Listiyana, Yuanita Lely Rachmawati, Hani Susianti, Hidayat Sujuti, Roihatul Mutiah, Agustina Tri Endharti



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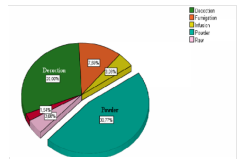
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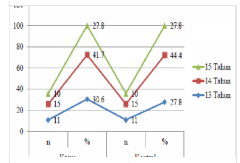
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Suriana Koro, Kameriah Gani, Ellyani Abadi, Anwar Mallongi



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# Effect of Thiamine on Serum Glutamate in Ischemic Stroke Animal Model

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## ABSTRACT

**Background:** Thiamin or vitamin b1 is a therapy that has been widely used in neurology cases such as alcohol-induced encephalopathy and neuropathy. Thiamin has the ability to penetrate the blood brain barrier so that it is expected to be used as an additional therapy in stroke cases. Stroke is currently an emergency neurology case that causes high mortality and disability. Among the causes is because damage to large areas of the brain will cause the release of neurotransmitters such as glutamate which has excitotoxicity effects to the brain when excessive. **Method:** This study used animal models of carotid artery occlusion stroke. Divided into 4 groups: control, thiamin dose of 100mg/kg, Thiamin 200mg/kg, and 400mg/kg. Serum glutamate was collected on day 7 post-action. Then evaluated by ELISA method. **Results:** There was a significant difference in the form of a decrease in the average serum glutamate level between the control and the sample that received a dose of thiamine 200mg/kg and 400mg/kg. **Conclusion:** High-dose thiamine may be considered as an additional therapy in cases of carotid artery occlusion model stroke.

**Key words:** Thiamine, Glutamate, Animal model, Ischemic, Stroke.

## INTRODUCTION

Ischemic stroke is a neurological emergency that causes high mortality and disability.<sup>1</sup> The costs incurred due to stroke in the world are considerable.<sup>2</sup> So now there is a need for better stroke management. The current management of stroke therapy is not only to save the core of damaged brain cells (core), but also to save the area around the infarct core (penumbra).<sup>3</sup> Studies have shown that there is an increased response in the form of inflammatory mediators if there is a disturbance in the nerves and brain.<sup>4</sup> In stroke, in addition to the inflammatory process, there is also a release of Glutamate neurotransmitters in the core and penumbra areas in ischemic stroke.<sup>5</sup> Research shows that glutamate levels increase in blood plasma and cerebrospinal fluid during ischemic conditions.<sup>6</sup> High glutamate levels within 24 hours have associations with neurological clinical deterioration.<sup>7</sup>

Anatomically vascular, ischemic stroke can occur due to disruption or occlusion of the carotid artery.<sup>8</sup> The carotid artery is an artery that provides extensive vascularization to the intracranial region in addition to the extracranial.<sup>9</sup> The carotid artery provides vascularization from the cortical region to the cerebral subcortex which plays a role in motor, sensory, language, memory and other functions.<sup>10</sup>

Thiamine or vitamin B1 is a vitamin that is closely related to human physiological functions such as fat, glucose and amino acid metabolism.<sup>11</sup> Thiamine administration also provides clinical improvement in cases of heart disease, which is also one of the risk factors for stroke.<sup>12</sup> However, the effect of thiamine on glutamate in ischemic stroke is still unknown.

## MATERIALS AND METHODS

### Ethics, consent and permissions

Ethics and experimental procedures were approved by the ethics committee Animal Care and Use Committee Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. (Number: 2.KEH.126.09.2022).

### Research design

This research is a True Experimental with a research design using a design in the form of a randomized posttest only control group design.

### Experimental animal

The experimental unit used in this study was male rats (*Rattus norvegicus*) Wistar strain, white in color, 4 months old weighing 140-200 grams. Then the stroke model was carried out by occluding the left carotid artery. Then the population was randomized with a total of 32 samples. Then divided into 4 groups: control, thiamine doses of 100 mg/kg/day, 200 mg/kg/day and 400 mg/kg/day.

### Analysis of glutamate

Examination of serum glutamate levels was carried out on day 7 with blood serum material, using an ELISA Reader, Rat GLUD1/Glutamate ELISA Kit reagent, the method used was sandwich ELISA, unit mg/mL. Glutamate levels were measured with Rat Glutamate ELISA Kit (Wuhan Feiyue Biotechnology Co., Ltd. Catalog No: FY-ER4835).

### Data analysis

Research using *Rattus norvegicus* experimental animals was conducted at the Faculty of Veterinary

Medicine, Airlangga University in October 2022. Glutamate levels were examined at the Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia.

The mean data of Glutamate levels were presented descriptively in the form of tables. Then the data were tested for normality with the Kolmogorov-Smirnov or Shapiro Wilk test and data homogeneity with the Levene test. If the data distribution is normal and homogeneous, continue with the One-Way Anova test. If the results of the One-Way Anova test  $p < 0.05$  are followed by a post hoc test with the Tukey test.

## RESULTS

The results of serum Glutamate examination on day 7 showed Glutamate release in blood serum in the unilateral arterial occlusion ischemic stroke model. The results of the examination of serum Glutamate levels showed that the P3 group, which received a dose of Thiamine 400mg/kg, experienced a significant in serum Glutamate levels compared to the control (K), groups given Thiamine 100 mg/kg (P1), and 200 mg/day (P2) as in table 1.

Thiamine administration at a dose of 100mg/kg gave a decrease in serum Glutamate levels compared to the control, but did not show significant differences. Thiamine administration at a dose of 200/kg and 400mg/kg gave a decrease in serum glutamate levels and showed significant differences as in table 2.

## DISCUSSION

Stroke is a vascular disorder that occurs in the brain.<sup>13</sup> During the journey to the brain, disruption of blood vessels in the neck region can cause intracranial and extracranial damage.<sup>14</sup> The brain is very sensitive to conditions of insufficient oxygen and glucose supply.<sup>15</sup> When an ischemic process occurs, a condition of oxygen and glucose deprivation occurs, so that within minutes, neuronal cells and non-neuronal cells will be depolarized and calcium channel activation occurs.<sup>16</sup> The depolarization will also release excitatory neurotransmitters from the presynaptic terminal to the synapse gap. For one, the released glutamate will bind to the ionotropic receptors N-methyl-d- aspartate (NMDA) and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA).<sup>17</sup> Excitotoxicity due to glutamate is a major contributor to nerve cell damage.<sup>18</sup>

**Table 1: Mean serum glutamate levels.**

	N	Mean	Std. Deviation
K	8	12.7638	1.41785
P1	8	11.4700	1.15107
P2	8	11.0537	1.46742
P3	8	10.9750	0.69683

**Table 2: Post Hoc Tukey results of glutamate levels.**

		Mean Difference (I-J)	Sig.
K	P1	1.29375	.172
	P2	1.71000*	.043
	P3	1.78875*	.032
P1	K	-1.29375	.172
	P2	.41625	.903
	P3	-.49500	.849
P2	K	-1.71000*	.043
	P1	-.41625	.903
	P3	.07875	.999
P3	K	-1.78875*	.032
	P1	-.49500	.849
	P2	-.07875	.999

\*. The mean difference is significant at the 0.05 level

For this animal model of stroke, we used unilateral carotid artery occlusion like other studies. In this study, there was no mortality in the control and treatment groups. Research shows that glutamate levels increase in blood plasma and cerebrospinal fluid during ischemic conditions.<sup>19</sup> The increase in serum glutamate in animal stroke models indicates that the glutamate neurotransmitter is still present in the serum until day 7. This is in accordance with previous studies that glutamate in stroke will be in the serum in the acute stroke phase.<sup>20</sup> In samples that received thiamine at a dose of 400mg/kg, it showed that there was a significant difference compared to without thiamine administration on the decrease in mean serum glutamate. This is like other studies conducted on other brain disease models that thiamine reduces glutamate levels.<sup>21</sup>

## CONCLUSION

Medium dose thiamine 200mg/kg and high dose 400mg/kg can be considered as therapy in ischemic stroke unilateral carotid artery occlusion model because it is proven to significantly reduce Glutamate levels. The high dose showed a more significant difference. From statistical analysis, the use of a dose of 400mg/kg has the smallest significant value and the largest mean difference.

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