IOP Conf. Series: Earth and Environmental Science **116** (2018) 012028 doi:10.1088/1755-1315/116/1/012028

Clinical Outcome And Arginine Serum of Acute Ischemic Stroke Patients Supplemented by Snakehead Fish Extract

Dwi Pudjonarko[1,2], **Retnaningsih**[1], **Zainal Abidin**[1]
1. Department of Neurology Faculty of Medicine Diponegoro University / Kariadi Central Hospital. Semarang – Indonesia.
2. Corresponding Author, E-mail: dr.onang@fk.undip.ac.id

Abstract:
Background: Levels of arginine associated with clinical outcome in acute ischemic stroke (AIS). Arginine is a protein needed to synthesis nitric oxide (NO), a potential vasodilator and antioxidant. Snakehead fish is a source of protein which has antioxidant activity. Snakehead fish contains mineral, vitamin, and amino acids. One of the amino acids that were found quite high in snakehead fish extract is arginine. The aim of this study was done to determine the effect of snakehead fish extracts (SFE) on serum arginin levels and clinical outcome of AIS patients.
Methods: It was double-blind randomized pretest-posttest control group design, , with. AIS patients were divided into two groups i.e. snakehead fish extracts (SFE) and control. SFE group were administered 15 grams SFE for 7 days . Arginine serum levels and clinical outcome (measured by National Institute of Health Stroke Scale= NIHSS) were measured before and after treatment, other related factors were also analyzed in Logistic regression.
Results: A total of 42 subjects who were performed random allocation as SFE or control group. There was no differences in subject characteristics between the two groups. There was a differences Δ arginine serum levels between SFE and control (33.6±19.95 μmol/L*;* 0.3±2.51 μmol/L*;* p<0.001). Change in NIHSS score in SFE improved significantly compared to the control group (4.14 ± 2.03; 2.52 ± 1.81;p=0.009). Logistic regression analysis showed only female gender factor that affected on improvement of NIHSS (OR=7; p=0,01).
Conclusion: There is Clinical outcome improvement and enhancement of arginine serum levels in AIS patient with snakehead fish extract supplementation.

Keywords: snakehead fish extract, arginine serum, acute ischemic stroke, NIHSS

1. Introduction

Stroke if not handled properly can have a negative impact on quality of life, social life, psychology, and patient's economy. Therapy for ischemic stroke in general is to overcome the emergency problem that occurs, treatment of underlying disease such as hypertension, handling complications and special therapies such as prevention of reperfusion and protecting brain tissue.

Previous studies by Cherubinni showed that antioxidant levels of patients with acute ischemic stroke were associated with the output of ischemic stroke. Low levels of antioxidants associated with the incidence of death or severe neurological dysfunction.[1] Based on this, antioxidant treatment are put into consideration as an additional therapy in ischemic stroke. The benefits of antioxidants in ischemic stroke are through the mechanism of inhibiting the production of free radical, elimination of free radicals and increasing free radical degradation.[2]

Study done by Jurcau showed that administration of antioxidants are improving neurological functionalities, assessed by the *National Institutes of Health Stroke Scale* (NIHSS)

score.[3] Metaanalysis result by Schurks M, et al showed vitamin E supplementation may reduce the risk of ischemic stroke but, on the other hand, may increase the risk of hemorrhagic stroke, which concluded that administration of Vitamin E should be done carefully.[4]

Another alternative in the administration of antioxidants in ischemic stroke is to use Nitric Oxide (NO). NO is an antioxidant that can effectively break the chain reaction of lipid oxidation caused by free radicals. NO will react with the peroxyl radical ($OH^{\bullet}$).[5] Clinical trials by Jara using a combination of L-Arginine (NO precursor), Lamotrigine (inhibitor of glutamate and aspartate release) and Tianeptine (inhibitor of plasma serotonin) in patients with ischemic stroke may decrease the NIHSS score compared with the control group.[6]

Snakehead fish extract is a source of nutrient that has potential therapeutic effect. Snakehead fish extract rich with amino acids, vitamins and minerals as antioxidant. One of amino acids in snakehead fish extract is Arginine (10.74±0.15/ 100 grams snakehead fish protein).[7] Arginine used to NO Synthesis that has potential effecs. NO has several benefits in ischemic stroke therapy. NO can potentially used as vasodilator, can inhibits platelet aggregation and adhesion, inhibits leucocyte adhesion and chemotaxis, and blocks *N-Methyl-D-aspartate* (NMDA)receptors. One of NO's source is NO donor and L-arginine. Administration of NO donor and L-arginine was also reported to reduce the extent of infarction in ischemic stroke.[8] On the other hand, there is currently insufficient evidence to recommend the use of NO donors, L-arginine or NOS-I in acute stroke, and only transdermal glyceryl trinitrate (GTN), an NO donor has been assessed. In people with acute stroke, GTN reduces blood pressure, increases heart rate and headache, but does not alter clinical outcome.[9]

Objective

This study was aimed to determine the effect of snakehead fish extracts (SFE) on serum arginin levels and clinical outcome of acute ischaemic stroke (AIS) patients.

2. Methods

This study was a *double-blind randomized pretest-posttest control group design*, performed on 42 patients with acute ischemic stroke. The subjects were differentiated into treatment groups with snakehead fish extract (SFE) therapy and control group that received placebo within 48 hours after the onset of stroke for 1 week. The snakehead fish extract dosage was 15 grams per day, divided to 5 grams every 8 hours. Variable of snakehead fish extract were considered independent variable, Serum arginine sample as intermediate variable was analyzed with *Microplate reader*, Neurological clinical outcome as dependent variable was measured with *National Institutes of Health Stroke Scale* (NIHSS) score. All data were measured on day 1 and day 8. This study has received approval from the Commission of Ethics Faculty of Medicine UNDIP / RSUP dr. Kariadi with the number 944/EC/FK-RSDK/IX/2016. Data were analyzed using statistics software. The p value was considered significant if $p<0.05$

3. Results

A total of 42 subjects were included and then classified randomly to SFE/treatment group or control group. The characteristic feature of the subjects by age showed no significant mean age difference between the two groups ($p=0.5$). Similarly, other characteristics include sex ($p = 0.06$), hypertension ($p = 0.3$), diabetes mellitus ($p = 0.2$) and smoking ($p = 0.6$). (Table 1)

Serum arginine levels before treatment were significantly different ($p=0.002$), whereas in the snakehead fish extract group, the majority of subjects had low serum arginine levels/11 people (52.4%) and subjects with normal serum arginine levels/10 people (47.6%). In control group, serum arginine level before treatment was extreemly low in all subjects.

Serum arginine level after treatment was significantly different ($p <0.001$), seen in snakehead fish extract group, most subjects had normal serum arginine levels (90.5%) and the rest of it had low serum arginine levels (9.5%). In the control group, all of subject's serum arginine levels after treatment remain low (100%)

Table 1. Characteristics of study subject in groups

Characteristics	**Group**		**p**
	Treatment (n=21)	**Control (n=21)**	
Gender			
- Male	7 (33.3%)	13 (61.9%)	0.06*
- Female	14 (66.7%)	8 (38.1%)	
Age (years old)	58.3 ± 9.39	56.7 ±6.17	0.5¶
Age Category(years old)			
- < 55	6 (28.6%)	8 (38.1%)	0.4¶
- 55-65	10 (47.6%)	11 (52.4%)	
- > 65	5 (23.8%)	2 (9.5%)	
BMI	24.3±4.04	21.4 ±2.40	0.01¶
BMI Index			
- ≥ 25	10 (47.6%)	2 (9.5%)	0.06*
- < 25	11 (52.4%)	19 (90.5%)	
Hypertension			
- Present	20 (95.2%)	17 (81.0%)	0.3§
- Not present	1 (4.8%)	4 (19.0%)	
Diabetes Mellitus			
- Present	9 (42.9%)	5 (23.8%)	0.2*
- Not present	12 (57.1%)	16 (76.2%)	
Smoking Habit			
- Present	3 (14.3%)	1 (4.8%)	0.6§
- Not present	18 (85.7%)	20 (95.2%)	

¶Independent t-test; *χ^2test; § Fisher-Exact test
Value in table are : mean (value±Standard Deviation); n(%) percentage are calculated in the column

There was no correlation between change in serum arginine level with change in NIHSS score, with value in snakehead fish extract group (*r = -0.03* and *p = 0.90*) and value in the control group (*r = - 0.12, p = 0.60*).(Fig.1) But the change of serum Arginine in the snakehead fish extract group are higher than the control group (33.6±19.95 μmol/L*;* 0.3±2.51 μmol/L*;* p<0.001) (Table 2), while the change in NIHSS score in snakehead fish extract group improved significantly compared to the control group (4.14 ± 2.03; 2.52 ± 1.81;p=0.009). (Table.3)

Table 2. Serum arginine level before and after treatment in SFE and control group

Serum Arginine Level	**Group**		**t**	**p**
	SFE (n=21) **(**μmol/L)	**Control** (n=21) **(**μmol/L)		
Before	41.2 ±22.20	23.7±=8.78	3.344	0.002*
After	74.8 ±21.36	24.0±=8.10	10.178	< 0.001*
Δ	33.6±19.95	0.3±=2.51	7.594	<0.001*

Table 3. NIHSS before and after treatment in SFE and control group

NIHSS	Group		t	p
	SFE (n=21)	Control (n=21)		
Before	8.67± 4.39	7.57 ± 4.73	0.777	0.442
After	4.59± 3.23	5.05± 4.63	-0.425	0.673
Δ	4.14± 2.03	2.52 ± 1.86	-2.729	0.009*

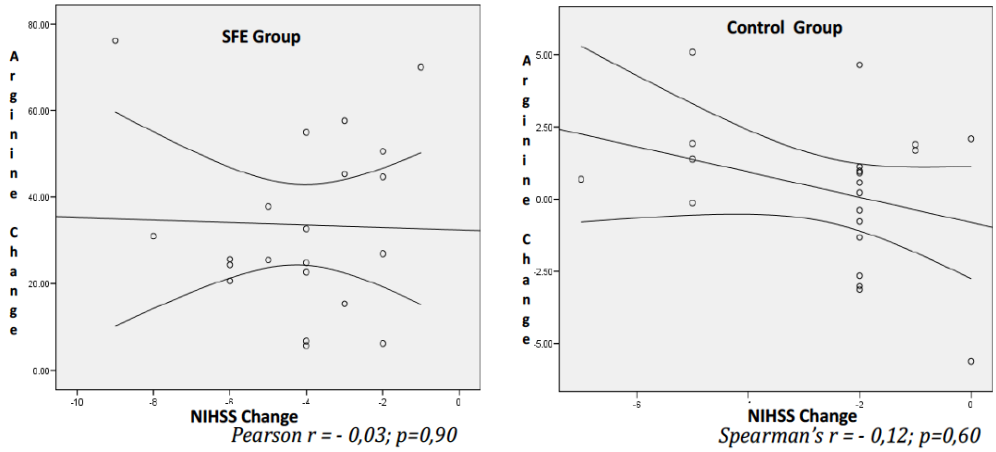

Figure 1. Correlation between change in arginine serum level with change in NIHSS score in both group

4. Discussion

The role of Arginine in ischemic stroke is as NO donor. NO synthesized by eNOS is an important endogen mediator in the regulation of cerebral blood flow and protection of brain blood vessel. NO synthesis by eNOS is through the oxidation of L-arginine to L-sitrulin. NO formed by endothelial blood vessels diffuses to adjacent cells such as capillaries, platelets and leukocytes, activates guanilate cyclase enzymes, and increases intracellular cGMP levels. NO from eNOS will cause neuroprotective effects such as vasodilation, anti thrombotic, anti-inflammatory and anti-proliferative effects.[10]

This study found that the change in NIHSS score in snakehead fish extract group improved significantly compared to the control group (*4.14 ± 2.03: 2.52 ± 1.81; p-0.009)*) and it is consistent with the previous study. The administration of NO donors such as L-arginine is reported to provide benefits in reducing the extent of infarction in ischemic stroke..[11] L-arginine may improve endothelial function through several mechanisms, namely increased intracellular transport, competitive antagonist Asymetric Dimethyl Arginine (ADMA), antioxidant effect, stimulates Histamine mast cells release, inhibition of norepinephrine activity, increased insulin secretion and change in intracellular pH. Previous study has shown that arginine supplementation is a major source of the availability of intracellular L-arginine required for the manufacture of eNOS.[12] Arginine also plays a role in inhibition of ADMA formation. ADMA is a competitive inhibitor of eNO synthase. Increased levels of ADMA inhibit NO synthesis and therefore impair vascular endothelial function.[13]

NO synthesized by eNOS is an important endogen mediator in the regulation of cerebral blood flow and brain blood vessel protection. NO synthesis by eNOS is through the oxidation of L-arginine to L-sitrulin. NO formed by endothelial blood vessels diffuses to adjacent cells such as capillaries, platelets and leukocytes, activates guanilate cyclase enzymes, and increases intracellular cGMP levels. NO from

eNOS have neuroprotective effects such as vasodilation, anti thrombotic, anti-inflammatory and anti-proliferative effects.[10]

The content of arginine in snakehead fish extract has an important role in wound healing process. Arginine stimulates the release of various chemical compounds for wound healing, such as growth hormone and insulin. Arginine is also described to cause a positive nitrogen balance. Arginine is a substrate of NOS and arginase that plays a role in the wound healing process. Arginine is metabolized in the wound through arginase. During the formation of connective tissue, arginine is used to produce hydroxy-proline which is important in the formation of collagen. The high content of arginine in snakehead fish extract [14-15] can potentially be utilized as NO donor for additive therapy of ischemic stroke.

Snakehead fish extract in addition to having a high nutritional content also has medical benefits, especially because the content of albumin in snakehead. Snakehead fish extract is used for wound healing, reduce postoperative pain, as antioxidant and various other benefits for the nervous system and cardiovascular, as carrier of metabolism substances like free fatty acids and bilirubin, provides osmotic pressure in capillaries, the albumin can maintain blood plasma fluid balance for the purpose of prevention of edema, and the formation of new cell tissues [15-16].

Mean serum arginine level in SFE group before treatment was 41.2± 22.20 μmol/L, higher compared to control group 23.7± 8.78 μmol/L, statistic result showed that the difference was significant (p=0.002). Serum arginine level in SFE group after treatment is 74.8 ±21.36 μmol/L, higher compared to control group, but the statistic result showed significant difference in arginine levels between treatment group after treatment and control group *(p <0.001)*. Table. 2 also showed a delta increase in serum arginine levels in SFE group was significantly greater than in control group (33.6 ± 19.95: 0.3 ± 2.51; p<0.001).

According to normal serum arginine levels in the laboratory is 41-114 μmol/L, all subjects in control group still categorized to low serum arginine level (100%). The statistic result showed that the serum arginine level differences after treatment in hospital between the SFE group and the control group have a significant value *(p<0.001).* Previous study by Worthmann on 67 patients showed findings of arginine deficiency characterized by increased Asymmetric Dimethyl Arginin (ADMA) and its analog counterpart, Symmetric Dimethyl Arginin (SDMA) in stroke patients.. Both an increase of ADMA known as a marker of cardiovascular risk and atherosclerosis and an increase of SDMA plasma levels after acute ischemic stroke predict poor functional outcome.[17] Similar results were found in this study of 42 patients with acute ischemic stroke in which 32 of them had low serum arginine levels (<41 μmol/L).

The statistic result showed that serum arginine level differences in the hospital admission between the SFE group and the control group have a significant value *(p=0.002).* The results of the study were also supported by characteristic data of research subjects by Jara who reported on the neuroprotective effects of arginine on ischemic stroke and cerebral trauma. Of the 49 research subjects, 44 of whom had low serum arginine level (<41 μmol/L), 5 other subjects had normal serum arginine level. This study also showed that the serum arginine levels at admission to hospital between treatment group and control group was significant *(p<0.001).*[6]

In this study, logistic regression analysis showed only female gender factor that affected on improvement of NIHSS (OR=7; p=0,01) (Table.4), it means that female got more benefit than male. This study also found that the correlation of change in serum arginine with change in NIHSS score of SFE and control group was not significant, it is possible because there is a content or mechanism of snakehead fish extract outside arginine mechanism which more influential as variable between the effect of snakehead fish extract on improvement of stroke clinical output such as albumin, zinc, glutamate and BCAA (Brain Chain Amino Acid) consisted of leucine, isoleucine and valine, and daily dosage of small and peroral arginine may also be the cause of the study.

5. Conclusion

Administration of snakehead fish extract may increase serum arginine levels in AIS patients. Snakehead fish extract administration can improve the clinical outcome of AIS patients. Snakehead fish extract administration may be considered to support the recovery of stroke patients.

Acknowledgement
We would like to thanks to all research team as well as to all patients who participate in this study.

References

[1] Cherubini A, Polidori M C, Bregnocchi M, Pezzuto S, Cecchetti R, Ingegni T, Di Iorio A, Senin U and Mecocci P 2000 Antioxidant profile and early outcome in stroke patients *Stroke* **31** 2295–300
[2] Shirley R, Ord E and Work L 2014 Oxidative Stress and the Use of Antioxidants in Stroke *Antioxidants* **3** 472–501
[3] Jurcau A 2007 The role of antioxidant treatment in acute ischemic stroke: a clinical study *Rom J Neurol* **6** 181–8
[4] Schurks M, Glynn R J, Rist P M, Tzourio C and Kurth T 2010 Effects of vitamin E on stroke subtypes: meta-analysis of randomised controlled trials *Bmj* **341** c5702–c5702
[5] Hummel S G, Fischer A J, Martin S M, Schafer F Q and Buettner G R 2006 Nitric Oxide as a Cellular Antioxidant: A little goes a long way *Free Radic Biol Med* **40** 501–6
[6] Jara H, García F, Torres F and Lechin F 2011 An Effective Neuroprotective Treatment in Ischemic Stroke and Cerebral Trauma with Low Doses of *Sch. Dr. Stud. (European Union) J.* 121–33
[7] Tan B H and Azhar M E 2014 Short Communication: Physicochemical properties and composition of Snakehead fish (*Channa striatus*) whole fillet powder prepared with pre-filleting freezing treatments *Int. Food Res. J.* **21** 1219–24
[8] Willmot M, Gray L, Gibson C, Murphy S and Bath P M W 2005 A systematic review of nitric oxide donors and L-arginine in experimental stroke; effects on infarct size and cerebral blood flow *Nitric Oxide - Biol. Chem.* **12** 141–9
[9] Bath P M, Krishnan K and Jason P Appleton 2017 Nitric oxide donors (nitrates), L-arginine, or nitric oxide synthase inhibitors for acute ischaemic stroke *Cochrane Database Syst Rev* **4**
[10] Sawada N and Liao J K 2009 Targeting eNOS and beyond: Emerging heterogeneity of the role of endothelial Rho proteins in stroke protection *Expert Rev Neurother* **9** 1171–86
[11] Davis M and Barer D 1999 Neuroprotection in acute ischaemic stroke. II: Clinical potential *Vasc. Med.* **4** 149–63
[12] Loscalzo J 2004 Arginine Metabolism: Enzymology, Nutrition, and Clinical Significance *J. Nutr* **134**2798S–2800S
[13] Böger R H 2004 Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the "L-arginine paradox" and acts as a novel cardiovascular risk factor *J. Nutr.* **134**2842S–2847S
[14] Mohd Shafri M A and Abdul Manan M J 2012 Therapeutic potential of the haruan (*Channa striatus*): From food to medicinal uses *Malays. J. Nutr.* **18** 125–36
[15] SH B and KA S 2000 The wound healing properties of *Channa striatus*-cetrimide cream-- tensile strength measurement *J Ethnopharmacol* **71** 93–100
[16] Mustafa A, Widodo M A and Kristianto Y 2012 Albumin And Zinc Content Of Snakehead Fish (*Channa striata*) Extract And Its Role In Health *IEESE Int. J. Sci. Technol.* **1** 1–8
[17] Worthmann H, Chen S, Martens-Lobenhoffer J, Li N, Deb M, Tryc A B, Goldbecker A, Dong Q, Kielstein J T, Bode-Böger S M and Weissenborn K 2011 High plasma dimethylarginine levels are associated with adverse clinical outcome after stroke. *J. Atheroscler. Thromb.* **18** 753–61

Table 4. Results of Logistic Regression Analysis of factors influencing the change in NIHSS score of patients with acute ischemic stroke

Variables	Bivariate Analysis						Multivariate Analysis			
	Change in NIHSS Score		p	OR	IK 95%		p	Exp(B)	IK 95%	
	Improvement	No Improvement			Min	Max			Min	Max
Change in serum arginine level										
- Increased	17 (94.4%)	17 (70.8%)	Fisher's = 0.11§	7.00	0.78	63.21	0.186	4.79	0.47	48.81
- Not increased	7 (29.2%)	1 (5.6%)								
Age (years old)										
- > 65	5 (71.4%)	2 (28.6%)	Fisher's = 0.12§	4.23	0.72	25.02	0.301	2.80	0.40	19.74
- ≤ 65	13 (37.1%)	22 (62.9%)								
Gender										
- Female	8 (36.4%)	14 (63.6%)	χ^2= 0.004*	7.00	1.73	28.34	0.01	7.00	1.73	28.34
- Male	16 (80.0%)	4 (20.0%)								
Diabetes										
- Present	6 (42.9%)	8 (57.1%)	χ^2= 1.0*	1.00	0.27	3.66				
- Not present	12 (42.9%)	16 (57.1%)								
Hypertension										
- Present	16 (43.2%)	21 (56.8%)	Fisher's = 1.0§	1.14	0.17	7.67				
- Not present	2 (40.0%)	3 (60.0%)								
Smoking Habit										
- Present	2 (50.0%)	2 (50.0%)	Fisher's = 1.0§	1.38	0.18	10.82				
- Not Present	16 (42.1%)	22 (57.9%)								
BMI Category										
- ≥ 25	6 (50.0%)	6 (50.0%)	χ^2= 0.5*	1.50	0.39	5.77				
- < 25	12 (40.0%)	18 (60.0%)								

*χ^2test
§Fisher-Exact test