The Effect of Channastriata Extract on Total Amino Acid, Arginine, and Leucine Concentration in Serum of Streptozotocin Induced Diabetic Rat

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Abstract. Protein damage and decreased amino acid levels in the blood, is a phenomenon that occurs in diabetics. Several studies have shown that Channa striata is a freshwater fish has a complete amino acid content. The objective of this study was to explore the effect of Pasuruan Channa striata in an extract form, whether could increase amino acid level in Streptozotocin induced rat. Male Rattus norvegicus strain Wistar was used in this experiment, assigned into 10 groups, 5 groups were sacrificed after 4 days and the 5 similar group were sacrificed after 8 days. Channa striata extract significantly increases total amino acid, arginine and leucine serum both in groups of 4 days and 8 days treatments (P<0.01). The increasing of the CSE dosage given will likely further increased total amino acid, arginine and leucine serum. Channa striata extract has a potential food that can increase levels of total amino acid, Arginine and leucine serum in diabetic rats.

Key-Words: Channa striata extract, diabetic rat, amino acid concentration

1. Introduction

Channa striata is a freshwater fish, and has been proven to contain monounsaturated, polyunsaturated fatty acid and good quality of protein [1]. The results of the study conducted by [2] show that the Pasuruan Channa striata extract (CSE) was found contained at least 17 amino acids. The major amino acids are glutamat, lysine, leucine, asparagin, alanin, arginine and valin makes up 1.494± 0.004 g/100g, 1.152±0.0006 g/100g, 0.956±0.0002 g/100g, 0.911±0.010 g/100g, 0.725±0.0005 g/100g, 0.624±0.0002 g/100g and 0.606±0.0009 g/100g extract, respectively. It is concluded that the CSE is a potential food containing complete and balanced of essential and non-essential amino acids.

The community has utilized this fish as a commodity to support the family income and as a source of animal protein and amino acids [3]. Extract of Channa striata is one of the refined products that considered to be a potential food, because it is known to be used as the treatment of wounds, reduce pain, as additional energy during illness for the elderly as well as to accelerate the recovery of postpartum mothers either in normal or sectio caesarea delivery [4]. The mechanism how CSE stimulates wound healing is still need to be explored. This is presumably because the fish is rich in arginine. Arginine is an essential amino acid in the wound healing process because it has dual roles, first by stimulating the release of growth hormone from the pituitary and insulin secretion from the pancreas [5].

A Diabetic condition characterized by a state of chronic hyperglycemia [6], which will stimulate hyperproduction of Oxygen Reactive Species (ROS). The interaction of superoxide (•O2-) and nitric oxide (NO•) to form peroxynitrite (ONOO•-) causes oxidative stress and will result in the occurrence of DNA damage and apoptosis in pancreatic β cells and Nitric Oxide (NO) in endothelial can not be fermmored [7][8]. This situation will damage the mitochondria and impair insulin secretion by the pancreas [9], can cause blood glucose levels to remain high which will increase the contribution to vascular disease [10] such as atherosclerosis [11]; nephropathy [12][13], neuropathy [13] and retinopathy [14]. Supplementation of amino acids proven to increase amino acid in the blood and contribute to the growth and proliferation of cells [15].

One of European research using six types-2 diabetes patients were split into two groups. Both groups consumed a normal diabetic diet. One of the groups was administered a placebo to be taken three times daily for one month. The other group was administered arginine over the same time period. The insulin sensitivity levels in the patients were regularly tested. Results were shown that arginine improved these than 30 percent when compared to the placebo group. Arginine furthermore led to a considerable expansion of the blood vessels, allowing for the blood pressure in the patients to be reduced significantly. This research showed that amino acid arginine is of great importance for the sensitivity of the body’s cells towards insulin. Arginine is a precursor of nitrogen oxide, a transmitting substance which has a direct influence on insulin sensitivity [16]. Another study showed that arginine was also important for curing diabetes-related foot illnesses [17].

This study aims to determine whether there is an effect of the use of CSE with total amino acid, arginine, and leucine concentration in diabetic rat serum.
2. Methods

Research Design

This study examined the effect of CSE on total amino acid, Arginine and leucine concentration in serum. In vivo experiments using Streptozotocin (STZ) induced diabetic wounded rats were analyzed the total of amino acid, Arginine and leucine content.

2.1. Experimental Animal

This study used 40 male Rattus norvegicus strain Wistar (Prepared by Gajahmada University-Yogyakarta), aged 10-14 weeks, have shiny and thick fluffy white fur (no fur loss), weight 150-250 grams, active and desire to eat. The rats were assigned into 10 groups: the group I the healthy rat without CSE, group II the diabetic rat without CSE, group III diabetic rat with CSE dose of 3.3g, group IV diabetic rat with CSE 6.7g and group V diabetic rat with CSE 10.0g/kg body weight/day, that were sacrificed after 4 days and. The 5 similar group that was conducted the same treatment that were sacrificed after 8 days. Use of experimental animals was approved by ethical clearance from the Ethics Committee for Health Research, Polytechnic of Health Malang No: 007/2013.

2.2. Diabetics Rats

Normal rats with blood glucose levels < 150 mg/dL (n=32), were made diabetic with streptozotocin induces (STZ) which had been diluted in 0.5 ml of sodium citrate buffer pH 4.5 via the intraperitoneal with a single dose of 55 mg/kg body weight [18][19]. On the fifth day, the blood glucose levels are checked using chromatography Multicheck (Nesco). The rats are approved to be hyperglycemia if the blood glucose levels are > 300 mg/dL [18], and they are approved to be diabetic if the blood glucose levels remain high for 3 weeks based on preliminary studies [2].

2.3. Extraction of Channa striata

Extraction process uses a plain steaming water/distillate water, with the following procedures. Extraction results from one kg Channa striata is 230-250 ml, with an average of 240 ml, in 1 ml of CSE containing 1.114 g [2]. The extracts then keep in -20°C until used.

2.4. Administration of Channa striata Extract (CSE)

CSE was given to treated group rat every morning per oral by using a special stomach cannule according to the dosage of 3.3g, 6.7g and 10.0g/kg body weight/day. Given from the first day until day four for group I, and up to 8 days for group II. CSE dose oral administration of approximately 0.8 ml, 1.3 ml of an 1.8 ml/rat.

2.5. Statistical Analysis

Data were presented as mean±SD. Differences in the effect of treatment were analyzed by one-way analysis of variance (ANOVA) follow by the Duncan’s multiple range test. Independent t-test was used to see the differences in the mean levels of total amino acids, Arginine, leucine and albumin levels among healthy rat without CSE and the diabetic rat without CSE, as well as after administration of CSE for 4 days to 8 days. P-value < 0.05 was considered statistically significant. All statistical analysis were perform using the Statistical Package for Social Sciences (SPSS) version 20 software for Windows

3. Results and Discussion

The mean of amino acid level in rats serum according to the groups are in the figure 1. CSE administration can increase the amino acid serum level, along with the increasing dosage of the extract. The highest increase occurred in the group treated by 10.0g/kg body weight/day of CSE administration, either in 4 days or 8 days treatment. Independent t-test show that mean amino acid level in rat serum between normal (21.67±0.38) and diabetic groups without CSE administration (16.04±0.33) of 4 days period significantly different (P=0.000; α:0.01). For the 8 days period mean of normal group are (21.65±0.27) and diabetic groups...
without CSE administration (15.43±0.27) significantly different ($P=0.000; \alpha=0.01$) The One way ANOVA results show that the increasing levels of amino acid serum level on rats between diabetic groups significantly different either on 4 or 8 days period ($P=0.000; \alpha=0.01$).

**Figure 1** Description: The level of amino acid among diabetic rats decreases significantly compared with control healthy rats and ($p=0.000; \alpha=0.01$) either on 4 days or 8 days treatment. Increasing dose of CSE in diabetic rat the amino acid concentration increase in dose dependent manner, either in 4 days or 8 days treatment, and the concentration of amino acid significantly higher in the respective treatment days. Group 1 the healthy rat without ECS, group 2 the diabetic rat without CSE, group 3 diabetic rats with CSE dose of 3.3g, group 4 diabetic rat with CSE 6.7g and group 5 diabetic rat with CSE 10.0g/kg body weight/day, that were sacrificed after 4 days and 8 days treatment. Blue=4 days treatment, red=8 days treatment. The same notation indicates that there is no significant difference, while different notations indicate significant differences ($p<0.01$). $t$-test; ANOVA and Post Hoc Duncan.

Leucine and arginine are two amino acids are closely related to the stimulation of wound healing. Analysis of these two amino acids is shown in the figure 2 and 3.

Serum arginine levels seen increase along with increase doses of extract. The highest increase occurred in the group treated with 10.0g/kg body weight/day, either in the treatment 4 days or 8 days. Independent $t$-test show that the mean of arginine serum level between normal (0.60±0.06) and diabetic groups without CSE administration (0.44±0.02) on 4 days period or 8 days period mean of normal (0.59±0.02) and diabetic groups without CSE administration (0.43±0.03) differ significantly ($p=0.006; \alpha=0.01$ and $p=0.001; \alpha=0.01$) respectively.

One-way ANOVA show that elevated levels of these rats serum Arginine differed between the diabetic group both in 4 days period and in the 8 days period ($p=0.000; \alpha=0.01$).

**Figure 2** Description: The level of arginine in diabetic rats decreases significantly compared with control healthy rats ($p=0.000; \alpha=0.01$) either on 4 days or 8 days treatment. With the increasing dose of CSE in diabetic rat the arginine concentration increase in dose dependent manner, either in 4 days or 8 days treatment, and the concentration of arginine is significantly higher in the respective treatment days ($p=0.000$) Group 1 the healthy rat without CSE, group 2 the diabetic rat without CSE, group 3 diabetic rat with CSE dose of 3.3g, group 4 diabetic rat with CSE 6.7g and group 5 diabetic rat with CSE 10.0g/kg body weight/day, that were sacrificed after 4 days and 8 days treatment. Blue=4 days treatment, red=8 days treatment. The same notation indicates that there is no significant difference, while different notations indicate significant differences ($p<0.01$). $t$-test; ANOVA and Post Hoc Duncan.

Provision of CSE increase serum levels of Leucine. The highest increase seen at a dose of 10.0g/kg body weight. The longer delivery of CSE will likely further increased serum levels of leucine. Independent $t$-test show that mean of leucine serum level on rats between normal (0.66±0.06) and diabetic groups without CSE...
administration (0.51±0.01) differ significantly either on 4 periods (p=0.010; α:0.01) or 8 days periods mean of normal (0.66±0.02) and diabetic groups without CSE administration (0.51±0.03) differ significantly (p=0.001; α:0.01).

One-way ANOVA analysis show that administration of ECS influence serum leucine levels significantly differed between groups, both in 4 days and in the 8 days period (p = 0.000; α:0.01).

**Figure 3.** Description: The level of leucine serum between control healthy rats and diabetic rats significantly different (p 0.000; α:0.01) either on 4 days or 8 days treatment. The longer delivery of CSE will likely increased serum concentration of leucine significantly in the respective treatment days (p=0.000). Group 1 the healthy rat without CSE, group 2 the diabetic rat without CSE , group 3 diabetic rat with CSE dose of 3.3g, group 4 diabetic rat with CSE 6.7gg and group 5 diabetic rat with CSE 10.0gg/kg body weight/day, that were sacrificed after 4 days and 8 days treatment. Blue=4 days treatment, red=8 days treatment. The same notation indicates that there is no significant difference, while different notations indicate significant different (p<0.01). T-test ; ANOVA and Post Hoc Duncan.

**Discussion**

The results of T-test to see the difference between the groups of healthy rat compared with diabetic rats without administration of CSE, pointing it significantly different results in the total amino acid content, arginine and leucine, both in rat of 4 days and 8 days of treatment. Glycation and protein modifications that occur in diabetic conditions resulting in oxidative damage and decrease the status of the network of proteins and amino acids in the blood [21][22]. Groups of healthy rats, still have a quite good supply of nutrients, whereas the diabetic condition, there is damage of proteins and nucleic acids as a result of an imbalance between antioxidant and pro-oxidant [23]. In this study the difference of total amino acids, arginine and leucine levels in rat serum differed significantly between normal rats and diabetic rats.

Channa striata extract has increased total amino acid serum level significantly in diabetic rats. Diabetic rats that were not given ECS had significantly lower levels of total amino acid serum compared to the normal group, the similar result was also form in the treatment group with 3.3g, 6.7g and 10.0 gg/kg body weight dosage, both the administration period of CSE during 4 days or 8 days. CSE can be absorbed properly so as to increase the concentration of amino acids in serum.

Levels of total amino acids arginine and leucine in rat serum differed significantly between the group of rat without CSE administration and normal rats, either in the treatment group during the 4 days CSE administration or 8 days period. Administration of CSE influence serum arginine and leucine levels significantly differed between groups both in 4 days period and in the 8 days period (p = 0.000; α:0.01).

Arginine and leucine is an amino acid that plays a role in neutralizing the blood glucose levels and stimulates wound healing [24]. Arginine is also known to increase insulin sensitivity [25]. Arginine is the substrate for two enzymes that play an important role in wound healing, namely nitric oxide synthase (NOS) and arginase. Arginine is a precursor of nitric oxide (NO), a substance that transmission has a direct influence on insulin sensitivity [16]. Arginine and NO are known to accelerate wound healing [26] [27]. Impaired wound healing in patients with diabetes, followed by NO reduction in the wound. Amino acid L- arginine is the only material used in the synthesis of NO. NO can affect processes in the body, both physiological and pathological conditions [28]. Leucine plays an important role in stimulating protein synthesis and for growth.

Leucine is very helpful in healing the wound of the skin and helps in regulating blood glucose levels, because it can produce energy (gluconeogenesis). In this study, Increased concentrations of arginine and leucine particular in rat serum, is a good sign to increase protein synthesis and reduce tissue oxidative damage. Supply of Arginine and leucine from CSE administration in diabetic rats could potentially hurt to accelerate the process of wound healing.

In this research note that the provision of ECS may increase serum concentrations of amino acids, arginine and leucine concentrations increase. It can be said that the CSE has the good quality and potential food for increasing amino acid level. Further research is required to confirm the role of amino acic in CSE on wound healing processes of diabetic Wistar rat.
Conclusion

In diabetic rat the concentration of amino acid, leucine and arginine were decreased due the the synthesis. CSE is one of the food sources of high quality protein. The content of essential amino acids and non-essential in the extract in balanced proportion. Treatment of diabetic rats with CSE orally significantly increase the concentration of those total amino acid, and also the concentration of leucin and arginine with increasing dose of CSE and duration of CSE treatment with the strong correlation but. The increasing amino acid especially arginine and leucine by CSE extract are possibly can be used to accelerate wound healing in diabetic.

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Conflict of Interest

None

References


