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The Level of Glutamic Acid in the Semen of Male White Rat (*Ratus norwegicus*) after Being Treated with Tannin of *Pluchea indica*

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Abstract

This current research aims at proving that the treatment of *Pluchea indica*'s tannin could affect the level of glutamic acid in the semen of male white rat. Experimental method was employed, under the following details: control group (without being treated) and experiment group with the treatment of *Pluchea indica*'s tannin. The observation was conducted based upon the treatment; the white rats were dissected, and vas deferens is taken to get the semen of the white rat. The amino acid of the semen was then analyzed by utilizing high-performance liquid chromatography. The data are analyzed by using ANOVA. The results show that there is an effect after the treatment of *Pluchea indica*'s tannin on the level of glutamic acid in the semen of male white rat ($p < 0.05$). The male white rats in the control group had averagely 1 547.48 mg · $(100\ g)^{-1}$ glutamic acid level; while the male white rats in the experiment group (after the treatment of *Pluchea indica*'s tannin) showed the decrease on glutamic acid level (1 494.43 mg · $(100\ g)^{-1}$ to 1 341.40 mg · $(100\ g)^{-1}$). The high and low level of glutamic acid determined the quality of spermatozoa.

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Keywords*:* glutamic acid level of semen; male white rat *(Ratus norwegicus)*; *Pluchea indica*'s tannin

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Nomenclature	
ANOVA	The data analysis employed variant analysis.
Post-test control design group	This is the research design which uses control and experiment groups; and the data are taken after the experiment is completed.
d	This letter represents 'day'.
h	This letter represents 'hour'.
min	This is the contraction of 'minute'.
rpm	revolution per minute, 1 hertz is equal to 60 RPM.

1.Introduction

Pluchea indica is one of the traditional herbs used as anti-fertility for men per oral which has not been overtly researched. Pre-clinical research proves that *Pluchea indica* contains active compounds such as tannin, alkaloid, and flavonoid[1] These active compounds, in the form of fraction, can reduce the amount of spermatogenic cells, the level of testosterone[2] and the number of female white rat breeding. Tannin is active compounds existing in plant which has phenol characteristic, with bitter taste. Tannin in *Plucea indica* may reduce the fertility potential of male rat spermatozoa[3]. The level of tannin in fresh *Plucea indica* leaf is 0.61 %, as for the dry one reaches 1.885 %[4]. This study utilizes the dry leaves since it has not been well researched. Tannin is found to be able to inhibit protein synthesis[5] that disrupts male reproduction. Disrupted protein synthesis will influence the quality of spermatozoa in the reproduction cycle. Spermatozoa are produced inside testis in two phase spermatogenesis processes; spermatositogenesis and spermiogenesis. The products of spermatogenesis comprise spermatogonia cells, primary spermatosit, secondary spermatosit, spermatid, and spermatozoa which are located in the *tubulus seminiferus testis*. It takes up to 3 d to form spermatogonia, 16 d for primary spermatosit, 26 d for secondary spermatosit, 36 d for spermatid, as well as 49 d to form spermatozoa[6]. Spermatozoa are distributed through vas efferent into epididimis for maturation process purpose. Afterwards, they are transferred to vas deferent. Spermatozoa, in this state, experience nutrition or liquid addition from various accessory glands in order to maintain their life span. The liquid derived from various glands and spermatozoa is called semen. Semen brings essential nutrition to be used in spermatozoa movement.

Tannin compounds may disturb protein metabolism process in the epididimis plasma, which should be the source of antiaglitinin. Types of protein reproduced in epididimis consist of various amino acids such as lysine, histidine, arginine, cysteine, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, tyrosine, phenylalanine, and tryptophan. Glutamate has a significant role in metabolism that is to produce ATP as an energy source for spermatozoa motility[7]. Ideal spermatozoa motility should be accompanied by quality plasmatic liquid or semen because it will result in fertility[8]. Glutamic acid is necessary for spermatozoa metabolism; the decrease of glutamic acid level results in reduction of spermatozoa motility[9]. However, further research needs to be conducted to observe the capability of tannin to influence glutamic acid level in male rat semen in fertility cycle. This study aims at proving the effect of tannin in *Pluchea indica* leaves towards glutamic acid level in male white rat.

2. Materials and methods

This is mainly an experimental research with "Posttest control design group" design. Eighteen male white rats were utilized as the samples by using random sampling technique. Research variables consisted of free variable that was a group of rats which received tannin treatment, and controlled variable that was the glutamic acid level in semen. The definition of operational variable was as follow: Male white rats were given *Pluchea indica*'s tannin treatment for 0.8 mL $\cdot$ d^{-1} during 98 d period. Semen observations were conducted on day 49 + 3 (W1), 49 + 16 (W2), 49 + 26 (W3), 49 + 36 (W4), and 49 + 49 (W5).

Tannin extract was made by the use of condensed method. Extraction and condensation of tannins were as follows: The leaf of *Pluchea indica* was dried and then crushed, and the tannins was isolated. The powder of *Pluchea indica* was extracted with water-saturated butanol (for 200 g of grain, 600 mL of water-saturated butanol

overnight followed by 3 times extractions with 400 mL of water-saturated butanol each), air dried, and grounded through a 40-mesh screen on a Willey mill. The 200 g of grounded grain was subject to five extractions, each with 400 mL of methanol; the first extraction is overnight, and each subsequent extraction was for 3 h to 5 h. The methanol solutions were combined, filtered (Whatman 1 paper), and vacuum concentrated to approximately 200 mL. The solutions became cloudy during concentration and centrifuged (1 500 rpm for 15 min) to remove an oily fraction containing a small amount of finely divided particulate matter. The centrifugation was repeated until the final volume was reached to 200 mL; then the small amount of similar precipitation was removed[10].

The methanol extract was placed on a column containing Sephadex LH 20 (15 × 4.5) cm that had been equilibrated in 95 % ethanol[11]. The column was eluted with 95 % ethanol until UV absorbance (280 nm) indicated that no material was eluted (approximately 2 000 mL). The ethanol fraction that contained other polyphenolics was determined to have essentially no enzyme inhibiting ability and discarding. The column was then eluted with 50 % aqueous acetone (1 : 1) until absorbance at 420 nm (420 nm avoiding the absorbance of the acetone solvent) of the effluent is nil (approximately 2 000 mL). The eluted material was exposed to a warm air current to remove the acetone to gain tannin fraction[10].

In the 98th day of the treatment, the rats were dissected for the observation on day 49 + 3, 49 + 16, 49 + 26, 49 + 36, and 49 + 49. Glutamic acid level was measured based on the observation on three male white rats. These rats were anesthetized with isoflurane and were decapitated immediately after the death[12]. Vas deferens was taken by opening their abdomens; it was massaged to excrete the semen. The level of glutamic acid in the semen was analyzed using high-performance liquid chromatography and processed with ANOVA.

3. Results and discussion

The results of the study on glutamic acid in male white rat after being treated by tannin from *Pluchea indica* are presented in the following Table.

Table 1. Average level of glutamic acid (mg · (100 g)$^{-1}$) in male white rat post tannin treatment of *Pluchea indica*

Treatment	Average (mg · (100 g)$^{-1}$)
Control (without tannin treatment)	1 547.48e
W1 (tannin treatment for 49 + 3 d)	1 494.93d
W2 (tannin treatment for 49 + 16 d)	1 486.72d
W3 (tannin treatment for 49 + 26 d)	1 457.92c
W4 (tannin treatment for 49 + 36 d)	1 432.20b
W5 (tannin treatment for 49 + 49 d)	1 341.39a

Note: The same notations show insignificant difference, different notations point significant difference.

Table 1 indicates that the average level of glutamic acid without tannin treatment was higher (1 547.48 mg · (100 g) $^{-1}$) than the level of arginine in the sample with *Pluchea indica*'s tannin treatment. The average level of glutamic acid with tannin treatment at W5,49 + 49 d, was the lowest (1 341.39 mg · (100 g) $^{-1}$) compared to other intervals. The level of glumatic acid decreased compared to control and treatment groups. The decrease of glutamic acid per 100 g was 52.6 mg at the treatment of 49 + 3 d; similarly, 60.8 mg at 49 + 16 d; 89.6 mg at 49 + 26 d; 115.3 mg at 49 + 36 d; and 206.1 mg at 49 + 49 d (Fig. 1).

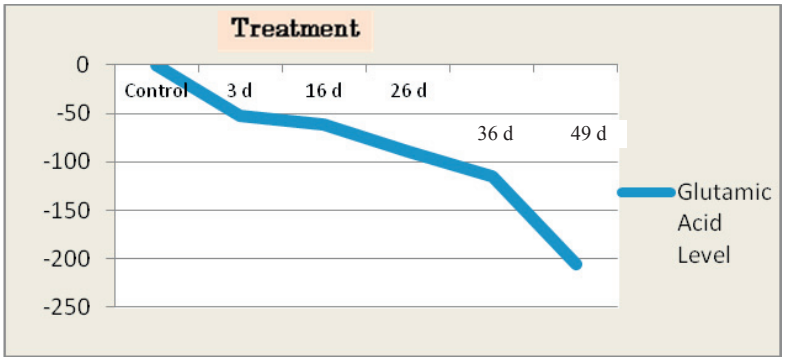

Fig. 1.Level of glutamic acid on male white rat after being treated with the tannin of *Pluchea indica*

The data were analyzed by normality (Kolmogorov-Smirnov) and normality tests. The result of normality test was presented in $p > \alpha$ (0.05) significant score, which indicated normal distribution. In addition, the result of homogeneity test (Levene test) was shown in $p > \alpha$ (0.05) significant score, which denoted the homogeneity of the data variants. The normal and homogeneous data were further analyzed with ANOVA. During the ANOVA test, $p < \alpha$ (0.05) significant score was found, which exhibited the significant effect of *Pluchea indica*'s tannin treatment towards the glutamic acid in male white rat semen.

Bitter tannin inhibits protein synthesis[5]; it binds as well asprecipitates protein[13]. These presumptions were deducted from the findings of showing a decrease in the average level of glutamic acid of the group treated by tannin from *Pluchea indica*, compared to the control group without treatment (1 547.48 mg · $(100\ mg)^{-1}$). The findings were in line with a statement[14] that the higher the tannin concentration in certain compounds was the more it influenced pH level, since tannin contained phenolic compounds with acid, poisonous characteristic in plant. Semen (spermatozoa and liquid from various glands) of male white rat with high pH (alkaline) or low pH (acid) would affect spermatozoa quality. Rapid pH change would influence the spermatozoa quality in terms of its motility and viability[15]. Spermatozoa motility may be obstructed due to the overly high tannin concentration during the treatment or due to the overtime use of tannin in long period although it is given the same amount. These circumstances occurred as tannin could bind complex proteins and/or any other proteins bound with Ca, Mg, Na and K; carbohydrate and fat[16].

Glutamic acid is required to maintain spermatozoa quality, especially to protect its plasma membrane from any damage due to lipid peroxides. This mechanism represents antioxidant mechanism to protect cells from free radicals. Nitric oxide deactivates superoxide produced by spermatozoa during the oxygen consumption process. Excessive amount of superoxide affects the peroxide of spermatozoa's phospholipids membrane that could cause functional failure. The production of lipid peroxide in spermatozoa membrane is prevented by increasing the product of Nitric oxide by glutamic acid. Glutamic acid blocks and prevents agents inhibiting the process of sugar fraction in spermatozoa. This factor supports metabolism activity and increases spermatozoa's energy availability, so the spermatogenesis inside the testis can be regulated. As the result, better quality spermatozoa are produced, especially with high quality spermatozoa morphology[9].

4. Conclusion

The treatment of *Pluchea indica*'s tannin was proven to reduce the level of glutamic acid in male white rat's semen. The findings showed that the rats without tannin treatment possess higher level of glutamic acid compared to that of the counterpart.

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