The Administration Effect Of Tryptophan On Testosterone And Estradiol Hormone Levels In White Male Rats

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Abstract: Objective to assess the influence of administering the tryptophan in the white male rats Rattus novergicus's testosterone and estradiol hormone levels. This research is experimental with the design of the posttest only control group design. The samples used as many as 28 rats were divided into 4 treatment groups. Each group is given an intraperitoneal injection of tryptophan with a dose level of 40mg, 50mg and 60mg/kg of bady weight. While one group was separated and made as control group. The treatments are given for 14 days and measured hormone levels using RIA. Based on assessment results, it was observed that there was a decrease in the average levels of testosterone (13,78nmol/L) and also observed an increase in the average level of the estradiol (8,65pg/dl) in the male white rat Rattus norvegicus when compared to the control group. The doses of tryptophan administered in the treatment group 1 is 40mg obtained the average testosterone levels 12,95nmol/L and average estradiol level of 9,87pg/dl. The doses of tryptophan administered in the treatment group 2 is 50mg obtained an average testosterone level of 11.03nmol/L and average estradiol level of 10,17pg/dl. The administration of tryptophan caused a decrease in the average testosterone levels in the control group and an increase in the average levels of estradiol compared to the control group even though the increase and decrease in the mean was not significant. It is likely that intraperitoneal administration of tryptophan has inhibitory effect of estradiol on testosterone.

Keywords: tryptophan, testosterone, estradiol, RIA

I. INTRODUCTION

Tryptophan is one of the essential amino acids needed in the process of protein biosynthesis. Tryptophan cannot be synthesized in the body; its needs can be obtained from food(1). Tryptophan can be found in several types of food, such as egg whites, cod, soybeans, beef, salmon, turkey, chicken, sesame seeds, and several other types of food(2, 3). After consumption, tryptophan is metabolized into bioactive metabolites, including serotonin, melatonin, and kynurenine(4).

Tryptophan functions as a supporter of the serotonin neurotransmitter in the brain generated by the hypothalamus(5, 6). Furthermore, serotonin will affect the hypothalamus' function as a central regulator of behavior, regulating body temperature, the osmolality of body fluids, the urge to eat, drink, regulate body weight, and regulate hormonal functions including reproductive hormones(7, 8). Tryptophan in the blood is mostly converted to serotonin in the intestine, and a small portion enters the brain. Then it will be changed to 5-Hydroxytriptopan with the help of the tryptophan hydroxylase enzyme. Finally, it is converted to serotonin with

the help of the enzyme Dopa Decarboxylases[4, 9]. Previous research has shown that the administration of tryptophan with different intensities can increase serotonin levels in the brain, depending on the brain's area being evaluated and the time of administration[10].
Serotonin will influence the hypothalamus' central regulation, one of which is reproductive hormones such as testosterone and estrogen. Research has been conducted that these two hormones exert direct and indirect effects on the serotonin transporter protein (5 HTT)[11]. These two hormones are secreted by different cells. Begins with the secretion of Gonadotropin-Releasing Hormone (GnRH) in the hypothalamus. Later this hormone will stimulate the anterior pituitary to secrete two hormones that play a role in spermatogenesis. The first is LH; it will stimulate the interstitial Leydig cells to produce testosterone. Testosterone plays a role in the maturation of spermatozoa [12]. Many studies have been conducted on the effects of giving this tryptophan to female rat sex hormones, but are still rare in male rats. The second is the FSH hormone that works on the Sertoli cells [13]. FSH will stimulate the Sertoli cells to produce estrogen [12]. Estrogen functions at the stage of changes of spermatid to spermatozoa. Elevated estrogen levels in male rats have proved that spermatogonia and spermatid also suffer damage[14].

II. SUBJECTS AND METHODS

Animals
Research is experimental with design. The post-test only control group design is the design used to measure the influence of treatment in the experiment group by comparing it with the control group [16]. The animals used are white male rats (Rattus norvegicus) found in the pharmaceutical laboratory of the faculty of pharmacy Andalas University with the consideration of rats, which is bred and kept for scientific research and often referred to as laboratory animals. The laboratory animal was used as a model for research before being treated to humans with the intrinsic criteria of rat weight is 200 – 250 grams, age 12 weeks (3 months). While the exclusivity criteria are sick rats and dead rats. Samples in research based on qualifying criteria with intrinsic criteria are male white rat; samples were taken from the population using a simple random sampling technique. The number of treatment groups, as many as four and samples are set using the formula: $(T-1)(n-1) \geq 15$, where T = number of treatment groups and n = number of samples per group. From the above formula obtained samples as $(4-1)(n-1) \geq 15$, $n \geq 6$. So the number of samples according to the above formula is six, and to anticipate the dropout, the final sample added one rat each group. So the total sample count is 7 x 4 = 28 rats.

Tryptophan
Tryptophan is an essential amino acid in the form of powder dissolved with aqua pro injection administered to the mouse by injection intraperitoneal (IP) using a scale measuring ratio with the measured results in mg/kg of body weight. Dosages are administered in each group, i.e., 40, 50, and 60 mg administered for 14 days [15]. Before being injected, tryptophan in powder form will be dissolved with Aqua Pro injection with a degree of solubility 10 g/L.

Testosterone and estradiol
Data on free testosterone and estradiol levels were obtained from rat blood serum obtained from the tail vein taken in the morning after 14 days of treatment using microhematocrit capillaries. The collected blood is then put into a measuring cup and placed on a tube test rack, then let stand for about 10 minutes. The blood serum is obtained after centrifuging 3000 RPM for 20 minutes, then separated into a new measuring cup. Subsequently, the hormone

levels are measured using the Radio Immuno Assay (RIA) method in the Biochemistry laboratorium of Andalas University.

Results:

Research on the effect of tryptophan on testosterone and estradiol levels in male white rat Rattus norvegicus has been conducted. First, the normality test is done with Kolmogorov-Smirnov. Then proceed with the ANOVA statistical test with a 95% confidence level than the Multiple Comparisons statistical test (Post Hoc Test Benferroni type).

Testosterone and estradiol hormone levels male white Rat (Rattus norvegicus)

Table 1. The test result of ANOVA hormone levels of testosterone (nmol/L) and estradiol (pg/dl) male white rat (Rattus norvegicus) in the control group and treatment group after tryptophan administration

Hormone	**Mean**				
	Control group	Tryptophan Doses			
		40 mg	50 mg	60 mg	*p**
Testosterone	13.78	12.95	11.03	13.57	0.041
Estradiol	8.65	9.87	10.17	8.08	0.001

**p*: Probability

From the table of the ANOVA test obtained the p-value of < 0.05, which means there is a significant difference between the control group and the treatment group in the male white Mouse (Rattus norvegicus). Thus it can be continued with Post HOC Test Bonferroni test to see more details of the average significant difference in testosterone and estradiol hormone levels in the following table 2:

Table 2. Test Results of Benferroni Multiple Comparations on hormone testosterone and estradiol male white Rat (Rattus norvegicus) in the control group and treatment group after tryptophan administration

Group		Testosterone		Estradiol	
		Mean difference	***p***	**Mean difference**	***p***
Control	D1*	0.83	1.00	-1.22	0.09
	D2 †	2.75	0.06	-1.52	0.02
	D3 ‡	0.22	1.00	0.57	1.00
D1	D2	1.92	0.37	-0.30	1.00
	D3	0.62	1.00	1.78	0.01
D2	D3	2.53	0.10	2.08	0.00

*D1: Dose 1; †D2: Dose 2; ‡D3: Dose 3

Table 2 It is known that among the control groups with groups D1, D2, and D3, there are no meaningful differences evidenced by the value of $p > 0.05$. Likewise, the Inter-group treatment of D1, D2, and D3, There are also no meaningful differences ($p > 0.05$). The differences showed estradiol levels between the treatment groups is that between the group D1 and the D3 group, between the D2 group and the D3 group indicates a meaningful difference ($p < 0.05$). This shows that administrations of tryptophan significantly affect estradiol level but not testosterone. For more details, the difference in testosterone hormone levels can be seen in the figure below:

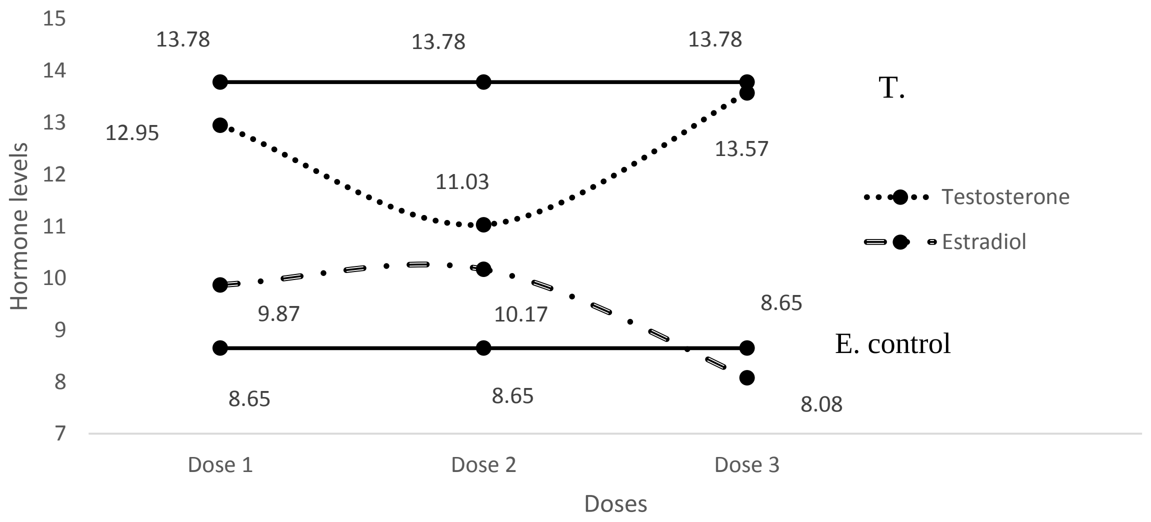

Figure 1. Means plot of testosteron and estradiol hormone levels male white Rat (Rattus norvegicus) in control group and treatment group after tryptophan administration

The figure above shows that there is a decrease in testosterone levels. At D1 and D2, levels of testosterone hormone decreased from control. However, at D3, hormone levels are re-increased. Despite its improvement, the hormone levels in D3 remain below the control levels. It appears that there are differences in the levels of estradiol hormones between the controls with the treatment group. In D1 and D2, there was an increase in estradiol hormone levels rather than control. At the same time, D3 hormone levels of estradiol are re-increased. This increase can occur due to the reduction of estradiol inhibition against Tryptophan 2,3-Dioxygenase (TDO) in the liver, which causes the reduction of tryptophan to the brain, thereby reducing the synthesis of serotonin (5-hydroxytryptamine). It happens when both hormone levels are at the closest level, which is administrating dose 2.

III. DISCUSSION

Rat blood samples are taken on the 15th day after 14 days of administering tryptophan injection. Based on the assessment results, the experiment showed a decrease in the average levels of testosterone hormone in the male white rat Rattus norvegicus compared to the control group (13.78 nmol/L). In the treatment group 1, given a tryptophan does 40mg obtained average testosterone hormone levels of 12, 95nmol/l, and average estradiol 9, 87pg/dl. In the treatment group, two doses of tryptophan administered 50mg obtained an average testosterone hormone level 11, 03nmol/L followed by estradiol increase by an average of 10, 17pg/dl. This suggests both doses may increase the amount of Tryptophan to the brain by inhibition of the activity of TDO in the liver, so that serotonin levels in the brain are increased, resulting in testosterone levels decreased [19]. Bethea et al. ever conducted previous research., discovering that estrogen is shown to increase brain serotonin [20]. Esteban also led to similar results, but he intervened with oral tryptophan administration, and the results turned out to increase serotonin [21]. In its way in the blood, testosterone binds to the sex hormone-binding globulin (SHBG) and albumin, where the amount that binds to the SHBG is less in males with females. Only a small fraction of this binds the testosterone is passed to the brain. At the same time, the majority are free [17, 18].

However, when the dose was increased to 60mg in the third group, acquired testosterone hormone levels jumped up by an average of 13, 56nmol/L, and followed a drastic reduction in the average estradiol levels of 10, 17pg/dl. It is estimated that the levels of free testosterone have begun to decline, followed by the decline of estradiol, which is the result of conversion from testosterone, so that estradiol that was played in the inhibition of TDO in the liver will reduce the amount of tryptophan to the brain, so that serotonin levels regain a sustained decline to increase back testosterone levels. The automatic balance regulation that occurs in this hormonal system is by what has been mentioned in the Qur'an surah Al-Infithar verse 7, which reads ٱلَّذِى خَلَقَكَ فَسَوَّىٰكَ فَعَدَلَكَ means "Who has created you then perfected your events and made your (body structure) balanced."

Acknowledgement:
We would like to gratefully acknowledge Prof. Eti Yerizel as head of Biochemistry Laboratory and staff, Pharmaceutical Laboratory Andalas University, and Yayasan Griya Husada Batam University.

IV. REFERENCES

[1] Friedman, Mendel. Analysis, Nutrition, and Health Benefits of Tryptophan. Int J Tryptophan Res. 2018; 11:1178646918802282.
[2] Holden, Joanne. USDA National Nutrient Database for Standard Reference, Release 22. Nutrient Data Laboratory, Agricultural Research Service, United States Department of Agriculture. 2009.
[3] Rambali B, Van Andel I, Schenk E, Wolterink G, van de Werken G, Stevenson H., et al., The contribution of cocoa additive to cigarette smoking addiction. RIVM. The National. 2005.
[4] Badawy, Abdulla A-B. Modulation of Tryptophan and Serotonin Metabolism as a Biochemical Basis of the Behavioral Effects of Use and Withdrawal of Androgenic-Anabolic Steroids and Other Image Performance-Enhancing Agents. Int J Tryptophan Res. 2018; 11:1178646917753422
[5] Wang L EH, Haavik J, Knappskog PM, Stevens RC. Three-Dimensional Structure of Human Tryptophan Hydroxylase and Its Implications for the Biosynthesis of the Neurotransmitters Serotonin and Melatonin. Biochemistry. 2002; 41:12569-12574.
[6] Wang W, Cui G, Jin B, Wang K, Chen X, Sun Y. Estradiol Valerate and Remifemin ameliorate ovariectomy-induced decrease in a dorsal serotonin raphe–preoptic hypothalamus pathway in rats. Ann Anat. 2016; 208:31-39.
[7] Alexander N, Klucken T, Koppe G, Osinsky R, Walter B, Vait D. et al., Interaction of the Serotonin Transporter-Linked Polymorphic Region and Environmental Adversity: Increased Amygdala-Hypothalamus Connectivity as a Potential Mechanism Linking Neural and Endocrine Hyperreactivity. Biol Psychiatry. 2012; 72:49-56
[8] Flores OG, Arati PG, Juárez MG, Martínez AM, Villegas AA, Arroyo IC. Progesterone receptor isoforms differentially regulate the expression of tryptophan and tyrosine hydroxylase and glutamic acid decarboxylase in the rat hypothalamus. Neurochem Int. 2011; 59:671-676.
[9] Mohammad M, Siddiqui MR. A Literature Review on the Effect of Changing Blood Tryptophan Concentration on Mood, in Particular Depression and How Concentration of Tryptophan can be Altered Through Diet and Supplements. WebmedCentral PSYCHIATRY 2011;2(4):WMC001844.

[10] Carneiro IBC, Toscano AE, Lacerda DC, Cunha MB, Castro RM. et al., L-tryptophan administration and increase in cerebral serotonin levels: Systematic review. Eur J Pharmacol. 2018; 836:129-135.
[11] Jovanovic H, Maras LK, Rådestad AF, Halldin C, Borg J, Hirschberg AL. et al., Effects of estrogen and testosterone treatment on serotonin transporter binding in surgically postmenopausal women's brains – a PET study. Neuroimage. 2015, 106:47-54.
[12] Scanlon, VC, Sanders T. Essentials of Anatomy and Physiology. 3rd ed. F.A. Davis Company. 2007.
[13] Guyton and Hall. Buku Ajar Fisiologi Kedokteran. ed 9. EGC. 2002
[14] Verderame M, Migliaccio V, Scudiero R. Role of estrogen receptors, P450 aromatase, PCNA and p53 in high-fat-induced impairment of spermatogenesis in rats. C R Biol. 2018, 341:371-379.
[15] Hansen F, Oliveira DL, Amaral FUIA, Guedes FS, Schneider TJ, Tumelero AC. et al., Effects of chronic administration of Tryptophan with or without concomitant fluoxetine in depression-related and anxiety-like behaviors on adult rat Neurosci Lett. 2011; 499:59-63.
[16] Liu HT, Lu CL. Effect of silencing Bcl-2 expression by small interfering RNA on radiosensitivity of gastric cancer BGC823 cells. Asian Pac J Trop Med. 2013; 6:49-52.
[17] Hammond GL. Sex Hormone-Binding Globulin and Metabolic Syndrome. Humana Press, Cham. 2017, pp. 305-324.
[18] Czub MP, Venkataramany BS, Majorek KA, Handing KB, Porebski PJ, Beeram SR., et al., testosterone meets albumin – the molecular mechanism of sex hormone transport by serum albumins. Chem Sci. 2018; 10:1607–1618.
[19] Badawy, Abdulla A-B. Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects. Int J Tryptophan Res. 2017
[20] Bethea, CL, Smith, AW, Centeno, M, Reddy, AP. Long-term ovariectomy decreases serotonin neuron number and gene expression in free-ranging macaques. Neuroscience. 2011; 192:675–688.
[21] Esteban, Susana; Nicolaus, Cristina; Garmundi, Antonio; Rial, Rubén Victor; Rodríguez, Ana Beatriz. et al., Effect of orally administered-tryptophan on serotonin melatonin, and the innate immune response in the rat. Mol Cell Biochem. 2002; 267: 39-46