

RESEARCH ARTICLE

The Influence of Administering Propolis CMCE Extract on FSH, LH, and Testosterone Levels in MSG-Induced Male Wistar Rat

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ABSTRACT

Introduction: *Monosodium Glutamate (MSG) increases free radicals and affects male reproductive hormone balance. Continuous Multi-Stage Countercurrent Extraction (CMCE) propolis extract is often used as supplement to prevent cellular damage induced by free radical. Objective:* Discover the effect of administering CMCE propolis extract to increase follicle stimulating hormone (FSH), luteinizing hormone (LH) and Testosterone (Te) levels in MSG-induced male Wistar rats.

Methods: *This research is an experimental research with post-test only control group design using 18 male Wistar rats as its sample, divided into 3 groups. The control group (C-G) was given MSG 140mg/day and received no propolis CMCE extract. Propolis groups 8.3 (CP-83) and 10.8 (CP-108) also given MSG 140mg/day, and each received propolis CMCE extract 8.3 mg and 10.8 mg/200grBW/day. All treatments were provided orally for 21 days. On the 22nd day, their bloods were taken through orbital sinus to have their FSH, LH and Te levels measured using ELISA. The obtained data were analyzed using ANOVA test and followed with Post Hoc LSD test.*

Results: *The ANOVA analysis indicated that significant differences occurred in FSH, LH, and Te levels among the groups, $p < 0.001$. The Post hoc analysis showed that the FSH, LH and Te levels in CP-83 and CP-108 groups were significantly higher than group C-G, $p < 0.001$. The FSH, LH and Te levels in CP-108 group was significantly higher than group CP-83, $p < 0.001$.*

Conclusion: *Administering propolis CMCE extract at 8.3 and 10.8 mg/day could increase the FSH, LH and Testosterone levels in MSG-induced male Wistar rats.*

Keywords : *CMCE propolis extract, FSH, LH, Testosteron, Monosodium glutamate*

ABSTRAK

Pendahuluan: *Pemberian Monosodium glutamate (MSG) telah terbukti dapat meningkatkan radikal bebas dan berpengaruh terhadap keseimbangan hormon reproduksi pria. Ekstrak Continuous Multi-Stage Countercurrent Extraction (CMCE) propolis sering digunakan untuk suplemen dalam menangkal radikal bebas dari luar. Tujuan:* Mengetahui pengaruh pemberian Ekstrak CMCE propolis terhadap peningkatan kadar *Follicle stimulating hormone (FSH), Luteinizing hormone (LH) dan Testosteron (Te)* pada tikus Wistar jantan yang diinduksi MSG.

Metode: *Penelitian eksperimental dengan post test only control group design menggunakan sampel 18 ekor tikus Wistar jantan, dibagi menjadi 3 kelompok. Kelompok Kontrol (C-G) diberi MSG 140mg/hari dan tidak mendapatkan ekstrak CMCE propolis. Kelompok propolis 8.3 (CP-83) dan 10.8 (CP-108) selain diberi MSG 140mg/hari, masing masing juga mendapatkan ekstrak CMCE propolis 8.3mg dan 10.8mg/200grBB/hari. semua perlakuan diberikan secara oral selama 21 hari. Hari ke-22 dilakukan pengambilan darah melalui sinus orbitalis kemudian diukur kadar FSH, LH dan Te dengan metode ELISA. Data yang diperoleh dianalisis menggunakan uji ANOVA dan dilanjutkan uji Post Hoc LSD.*

Hasil: *Uji analisis anova menunjukkan terjadi perbedaan bermakna kadar FSH, LH, dan Te di antara kelompok, $p < 0,001$. Analisis Post hoc menunjukkan bahwa kadar FSH, LH dan Te pada kelompok CP-83 dan CP-108 lebih tinggi bermakna dibanding kelompok C-G, $p < 0,001$. Kadar FSH, LH dan Te pada kelompok CP-108 lebih tinggi secara signifikan dibandingkan kelompok CP-83, $p < 0,001$.*

Kesimpulan: *Pemberian ekstrak CMCE propolis dengan dosis 8.3 dan 10.8mg/hari dapat meningkatkan kadar FSH, LH dan Testosteron pada tikus Wistar jantan yang diinduksi MSG.*

Kata Kunci: *ekstrak CMCE propolis, FSH, LH, Testosteron, Monosodium glutamate*

INTRODUCTION

Infertility can be defined as the inability to achieve pregnancy after sexual intercourse for 12 months with no protection (Practice committee of American Society for Reproductive Medicine, 2013). Infertility can be caused by various factors, neither from the male nor

the female partners. The factors from male partners contribute to forty percent of infertile couples (World Health Organization (WHO), 2010). One of the causes of infertility in men is oxidative stress resulting from increased Reactive Oxygen Species (ROS) level (Ko, Sabanegh and Agarwal, 2014). The evidence shows that

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30%-80% infertile men are correlated with increased ROS (Tremellen, 2008). Furthermore, in infertile men, inclining testosterone hormone level is also frequently found (Nasihun, 2009). Under such a circumstance, exogenous antioxidant is needed to fix the oxidant and antioxidant balance, and to prevent oxidative stress (Agarwal and Sekhon, 2010). Propolis Continuous Multi-Stage Countercurrent Extraction (CMCE) extract can be used as an alternative in suppressing the number of free radicals within the body and improve men's infertility, yet further research on this topic needs to be conducted.

The use of exogenous antioxidant is important, considering the fairly high infertility incidence rate. For example, in developed countries in the last 50 years the rate is reportedly around 5%-8%, and in developing countries, in the last 20 years, it is reportedly about 30% (Andriani, 2017). WHO predicts that around 8%-10% or around 50-80 million couples throughout the world have infertility for various factors (World Health Organization (WHO), 2010). In addition, infertility also often gives raise to medical, psychosocial, and economic issues to both men and women in the world (Kefer, Agarwal and Sabanegh, 2009).

One of the compounds which trigger ROS increase is Monosodium glutamate (MSG). Monosodium glutamate is glutamic acid frequently used as a flavoring ingredient for cooking to stimulate appetite (Rangkuli R, 2012). Glutamic acid is an excitotoxic neurotransmitter, once accumulated in the body, leading to damage to arcuate nucleus in the hypothalamus (Uke Y, 2008). This affects hypothalamus in producing Gonadotropin-Releasing hormone (GnRH) which can then inhibit anterior pituitary to produce Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) (Nasihun, 2012). Follicle Stimulating Hormone (FSH) serves the spermatogenesis process, and the main function of LH is to stimulate leydig cells to produce testosterone hormone which plays an important role in sperm maturation (Megawati, 2008; Nasihun, 2012; Andriani, 2017). The glutamic acid accumulated within the body can also increase the ROS level and oxidative stress (Edward, 2010). This glutamic acid makes the brain cells particularly active, leading to their death as a result of fatigue (excitotoxic) (Uke Y, 2008). In addition to affecting the hypothalamic-pituitary-testicular axis, the negative effect of glutamic acid can also take form of direct disruption in damaging various cells inside the testicle (Guyton and Hall, 2011). Moreover, giving MSG 4g/kgBW has been found to cause decreased number of leydig and Sertoli cells in rat's testicle (Susianingsih, 2018).

The results of medication using low molecular weight antioxidant in infertile men remain inconsistent, thus an alternative antioxidant such as propolis is needed. Some studies on propolis extract have reported that propolis extract can reduce MDA level, inhibit the decrease in number of spermatid, and improve the sperm quality affected by free radicals from cigarette smoke exposure (Hoesada, Nasihun and Isradji, 2016). According to Khaled et al., (2016), propolis can protect reproductive system in MSG-induced rats with increased testosterone hormone, body weight, and testicle relative weight. (Khaled, Yousef and Kamel, 2016). Yousef & Salama's research result indicates that propolis has been found capable of fixing infertility by increasing the sperm production, motility, quality and improved production of testosterone (Yousef and Salama, 2009). Another study finds that propolis extract of minimum doses at 3mg, 6mg and 10mg/kgBW/day for 36 days can increase spermatozoa production and epithelial thickness (Legowo, 2008).

Propolis extracted with CMCE method contains Caffeic acid phenethyl ester (CAPE) and flavonoid which is effective as an antioxidant. CAPE is the strongest antioxidant substance and it can obstruct lipid peroxide at around 97% (Gocer and Gulcin, 2011). This is because CAPE belongs to hydroxycinnamic acid phenols that contain CH₂=CH-COOH cluster, thus it has the capacity of high-electron donor, leading to its extremely high activity as an antioxidant (Farooqui and Farooqui, 2010). Propolis CMCE extract has Oxygen Radical Absorbance Capacity (ORAC) value of up to 21.921, i.e. twice the standard of other propolis, making it a rational choice as a natural antioxidant in repelling ROS and fix the FSH, LH, and testosterone hormone levels. Therefore, this research aims to discover the influence of propolis CMCE extract on the increased FSH, LH and testosterone hormone levels in monosodium glutamate-induced Wistar rats.

METHODS

This was experimental research with post-test only control group design. The number of its samples was 18 male Wistar rats, aged 8 weeks, weight 200-250 grams and grouped randomly into 3 groups of 6 rats. In the control group (C-G), the rats were induced with MSG 140mg/day orally and did not receive propolis CMCE extract. In the CMCE propolis-83 (CP-83) group, the rats were induced with MSG 140mg/day orally and received propolis CMCE extract 8.3 mg/200 gram BW/day. In the CMCE propolis-108 (CP-108) group, the rats were induced with MSG 140mg/day orally and received propolis CMCE extract 10.8 mg/200 gram

BW/day. The treatment is administered orally each day for 21 days. During the research term, the foods and water were given ad libitum. This research was conducted after obtaining credential from the Medical Research Bioethics Committee No. 57/II/2019/Komisi Bioetik.

Propolis CMCE Extract

Propolis CMCE extract was a superblend of propolis Poplar and propolis Baccharis extracted using Continuous Multi-Stage Countercurrent Extraction (CMCE) method by combining 3 extraction methods, namely Solvent Extraction, Repercolation with dynamic and Countercurrent Extraction. CMCE method could maintain the flavonoid and phenolic content, and the Caffeic Acid Phenethyl Ester (CAPE) increased twice as much as the standard propolis. The result of analysis using Oxygen Radical Absorbance Capacity (ORAC) method showed that CMCE propolis had higher antioxidant content up to 21.921.

Propolis CMCE Extract Doses

The propolis CMCE extract dose was converted from the adult dose which was recommended at 3 x 200 mg, meaning 600mg/day. This dose was converted into rat dose using the formula $0.0018 \times 600 \text{ mg} = 10.8 \text{ mg}/200 \text{ gr BW}$.

Measurement of FSH, LH, and Testosterone Hormones

After the treatment was given for 21 days, on the 22nd day, their blood was taken from their eye orbital sinus to have their FSH, LH and Testosterone levels examined using ELISA method at 450 nm wavelength.

Statistical Analysis

The statistical analysis used ANOVA parametric test, and was followed with post Hoc LSD. The research results were deemed significant if $p \leq 0.05$.

RESULTS

After being induced with MSG 140mg/day and receiving propolis CMCE extract for 21 days, the mean FSH, LH, and Testosterone hormone levels were as seen in Table 1.

From Table 1, it could be seen that the highest LH, FSH, and Testosterone hormone levels was found in group CP-108, followed by group CP-83, and the lowest was in group C-G. The normality test using Shapiro wilk and the homogeneity test using Levene test indicated that the data were distributed normally and homogenous ($p > 0.05$). Taking this result into consideration, ANOVA test could then be performed and it was found that there were significant differences between the groups, $p < 0.05$. To figure out which group had the significant differences, a Post hoc LSD test was carried out and explained below:

Luteinizing Hormone (LH) Level

The Post Hoc LSD test result showed that the mean LH mean in CP-83 and CP-108 groups was significantly higher than C-G group, $p < 0.05$. Meanwhile, the mean LH level in CP-108 group was significantly higher than CP-83 group, $p < 0.05$ (figure 1). From this analysis result, it could be concluded that administering propolis CMCE extract at 10.8 mg dose had better effect than propolis CMCE extract at 8.3mg dose.

Follicle Stimulating Hormone (FSH) Level

The Post Hoc LSD test result indicated that mean FSH level in CP-83 and CP-108 groups was significantly higher than C-G group, $p < 0.05$. The mean FSH level in CP-108 group was significantly higher than CP-83 group, $p < 0.05$ (figure 1). Considering this analysis result, it could be inferred that propolis CMCE extract at 10.8 mg dose had better effect than propolis CMCE extract at 8.3mg dose.

Testosterone Hormone Level

Table 1. Result of Analysis of Mean FSH, LH, and Te Hormone Levels

Variables	C-G	CP-83	CP-108	P value (ANOVA)
	N=6 Mean (+SD)	N=6 Mean (+SD)	N=6 Mean (+SD)	
LH level (mIU/ml)	25.90±0.52	29.06±0.41	33.00±0.40	0.000
Shapiro wilk	0.366	0.421	0.879	
Levene test		0.411		
FSH level (mIU/ml)	22.21±0.31	33.88±0.59	42.65±0.49	0.000
Shapiro wilk	0.484	0.638	0.076	
Levene test		0.411		
Testosterone Level (ng/ml)	2.79±0.67	4.60±0.57	6.13±0.96	0.000
Shapiro wilk	0.395	0.450	0.242	
Levene test		0.082		

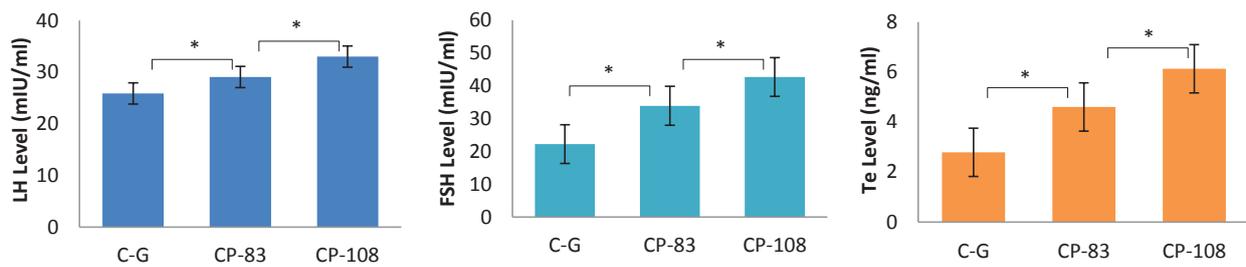


Figure 1. FSH, LH, and Te Level in C-G, CP-83, and CP-108. * Post Hoc Test of $P < 0.05$

The Post Hoc LSD test result indicated that the mean Testosterone hormone level in CP-108 and CP-83 groups was significantly higher than C-G group, $p < 0.05$. The mean Testosterone level in CP-108 group was significantly higher than CP-83, $p < 0.05$ (figure 1). Based on this result analysis, it could be inferred that propolis CMCE extract at 10.8 mg dose had better effect than propolis CMCE extract at 8.3mg dose.

DISCUSSION

This research showed that administering propolis CMCE extract resulted in increased FSH, LH and testosterone hormones in MSG-induced male Wistar rats. The induction of MSG at 140mg/BW dose in control group showed the lower FSH, LH and testosterone levels than CP-83 and CP-108 groups. This results also indicated that inducing MSG had been proven capable of lowering FSH, LH, and Testosterone levels. This research finding was consistent with Edward, stated that administering MSG orally to rats had been found to be able to decrease FSH and LH levels (Edward, 2011). Furthermore, LH decrease followed by testosterone level decrease (Nasihun, 2012). MSG, as reported by previous studies, could cause neuron changes to the cerebral, cerebellum, brain stem, and hypothalamus which were associated with ROS (Iremonger et al., 2010). In addition, MSG could also lead to increase MDA level in liver, kidney and brain (Nasihun, 2012).

The main mechanism of cell damages from MSG consumption was the incidence of oxidative stress. Excessive use of MSG resulted in increased glutamate level in blood serum, triggering free radical production in the body and react with unsaturated fatty acid of the cell membranes (Hoesada, Nasihun and Isradji, 2016). These free radicals resulted from excessive glutamate receptor activities, created Ca^{2+} influx, forced Ca^{2+} ion to get into the nerve cells, excitotoxicity occurred, resulted in cell death (Ruca C.B., 2016). The damage would spread to all glutamate receptors such as hypothalamus and reproductive organ. The damaged hypothalamus prompted disruption to pituitary adrenal axis area in

secreting FSH and LH (Khaled, Yousef and Kamel, 2016). The decrease in LH and FSH hormones which was then followed by another decrease in testosterone would disrupt the spermatogenesis process (Edward, 2010). In addition to affecting the hypothalamic-pituitary-testicular axis, administering MSG could also cause spermatogenesis disruption through testicular mechanism in such a way that it damaged the cells within the testicle (Guyton and Hall, 2011; Khaled, Yousef and Kamel, 2016).

The decreased FSH, LH and testosterone hormones resulting from oxidative stress would make men infertile. To protect the cells from ROS attack, the body provided endogenous antioxidant. However, when the number of ROS had been too much, the body required antioxidant from the outside to help maintain the prooxidant-antioxidant balance. Antioxidant itself was divided into 2, i.e. preventive and chain-breaking-reaction antioxidants. The preventive antioxidant was the one capable of obstructing the formation of ROS during the initiation phase such as catalase, peroxidase, and glutathione peroxidase enzymes. Nevertheless, when ROS was formed excessively, the reaction of preventive antioxidant could still left some ROS. This number of remaining ROS could accumulate when added by the ROS induced by MSG from the outside. Hence, this needed an antioxidant which was useful to break the ROS reaction chain during its propagation phase (chain breaking reaction) (Ruca C.B., 2016).

Propolis CMCE extract could also act as exogenous antioxidant by playing the chain breaking antioxidant role (Molina, 2009). Propolis CMCE extract contained Caffeic acid phenethyl ester (CAPE), terpenoid, flavonoid and phenolic acid ester which had high antioxidant activity. Caffeic acid phenethyl ester was the strongest antioxidant substance in propolis since it belonged to hydroxycinnamic acid phenols which contained $CH_2=CH-COOH$ cluster, leading to its high electron donor capacity. Meanwhile, the mechanism of CAPE antioxidant was similar to other flavonoid as an effective compound to serve the role of

scavenger species reactive which worked by transferring H+ atom, hence it could prevent cell damage which affected hormone balance (Gocer and Gulcin, 2011).

In this research, the FSH, LH and testosterone hormone increased as the dose of propolis CMCE extract to the MSG-induced male Wistar rats was increased. Administering propolis CMCE extract at 10.8 mg dose was found to have higher increase than at 8.3 mg dose. This study result was consistent with Khaled who found that administering propolis extract could increase testosterone hormone, testicle weight and testicle relative weight in MSG-induced rats (Khaled, Yousef and Kamel, 2016). The study conducted by Hoesada also found that administering propolis extract was proven capable of reducing MDA level and improve the quality of sperm which was affected by cigarette smoke exposure (Hoesada, Nasihun and Isradji, 2016). Numerous data on propolis extract administration could prove that propolis CMCE extract could be used as an alternative in protecting cells from oxidative stress due to ROS attack in which it increased the antioxidant level in the body to allow the prevention of infertility due to excessive consumption of MSG. This research has its own limitation in that it did not examine such parameters as sperm and lipid peroxide level as the indicators of oxidative stress. Therefore, on these various markers, further research is needed.

CONCLUSION

Administering propolis CMCE extract at 10.8mg/day could increase the Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and testosterone hormone levels in Monosodium glutamate (MSG)-induced male Wistar rats.

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