

The Effect of Marigold Leaf Ethanol Extract on Inhibin B Mice Exposed to Cigarette Smoke

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Article History	Abstract
<p>Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 01 Oct 2023</p>	<p>Cigarette smoke can cause oxidative damage because the amount of free radicals (oxidants) generated is far greater than the endogenous antioxidant capacity of cells. Inhibin B is used as an indicator of reproductive function in men. The content of antioxidants present in marigold leaves (<i>Tagetes erecta</i>), can maintain reproductive function. This study aims to analyze differences in levels of inhibin B resulting from the administration of marigold leaf ethanol extract (EDM) to mice (<i>Mus musculus</i>) exposed to cigarette smoke. This investigation is a laboratory experimental study with a randomized posttest-only control group design. There were 40 mice divided into 5 groups (8 mice/group). K- was only given a placebo, K+ was given a placebo and exposed to cigarette smoke 1 cigarette per day, P1 was exposed to cigarette smoke 1 cigarette per day + EDM 0.25g/kg-BW, P2 was exposed to cigarette smoke 1 cigarette per day + EDM 0.5 g/kg-BW, P3 was exposed to cigarette smoke 1 cigarette per day + EDM 1.0 g/kg-BW. The results showed that there was a significant difference between groups in the inhibin B variable with a p-value of 0.000. The results of this study showed that there was a significant difference in inhibin B levels between the K+ group with P1, P2, and P3, and the K- group with P1, P2, and P3. The average levels of inhibin B K- 0.672 ng/L; K+ 0.615 ng/L; P1 0.340ng/L; P2 0.299 ng/L; P3 0.284ng/L. The findings of this research indicate that administering an ethanol extract derived from marigold leaves to mice subjected to cigarette smoke leads to a decrease in inhibin B levels in spermatogenesis.</p>
<p>CC License CC-BY-NC-SA 4.0</p>	<p>Keywords: Marigold leaf ethanol extract, Cigarette smoke, Inhibin B, Seminiferous tubules</p>

1. Introduction

Cigarette smoke contains various chemical compounds such as carbon dioxide (CO₂), hydrogen peroxide (H₂O₂), sulfur monoxide (SO), carbon monoxide (CO), and nitrogen oxides (NO) which are free radicals. Indonesia is the third country with the largest number of smokers in the world after China and India. The number of smokers in Indonesia is approximately 48 million people, with each smoker smoking 1-3 cigarettes per day (Maheyasa, 2017). It is known and proven from previous studies that cigarette smoke can cause infertility. There are approximately 60-80 million infertile couples spread all over the world (Anggi, 2016). Cigarette smoke can cause oxidative damage because the amount of free radicals (oxidants) generated is far greater than the endogenous antioxidant capacity of cells. Oxidative stress is defined as an imbalance between the high production of reactive oxygen species (ROS) and the ability of the organism's natural protective mechanisms to remove or repair damaged cells and prevent adverse effects (Maheyasa, 2017).

Oxidative stress can interfere with the hypothalamus-pituitary gonadal axis pathway so that hormone secretion becomes abnormal (Adelati, et al. 2016). Oxidative stress in the testes can affect the hypothalamus so that the secretion of the hormones LH and FSH is disrupted, which can cause the Leydig cells and Sertoli cells to decrease. This mechanism causes a decrease in the number of Leydig cells and Sertoli cells that will disrupt the process of spermatogenesis (Malini, 2020). Sertoli cell dysfunction can inhibit spermatogenesis and inhibin B cannot be produced (Rasyid, et al. 2018). If the hormones in the testes are disrupted, the stages of spermatogenesis will be disrupted, eventually leading to infertility problems (Adelati, et al. 2016). Inhibin B is used as an indicator of reproductive function in men. Inhibin B is considered a good marker for Sertoli cell mass and spermatogenic function in men (Clavijo, 2018).

Antioxidants are compounds that can prevent oxidative stress, one of the natural antioxidants is flavonoids. Flavonoids, acting as antioxidants, possess the ability to obstruct the generation of ROS by impeding redox reactions that generate fresh oxidants. Marigold leaves (*Tagetes erecta*) represent a plant rich in these flavonoids. The *Tagetes erecta* plant is a plant that can be used as a living fence and as an ornamental plant by the general public. The flowers of *Tagetes erecta* have yellow and orange colors with an unpleasant odor (Edy & Parwanto, 2019). The marigold plant, scientifically known as *Tagetes erecta*, and commonly referred to as the kotok flower, is an additional variety of healing herb that also possesses polyphenols and two categories of carotenoids, specifically carotenes and xanthophylls (Ariana, et al., 2011). *Tagetes erecta* is spread in several countries, namely India, China, Myanmar, Indonesia, and other countries with tropical climates. Aside from being an antioxidant, this plant can also be used as an antibacterial, anti-inflammatory, hepatoprotective, anticancer, antiepileptic, antidepressant, antifungal, etc (Sing, 2020). It is hoped that the antioxidant content in marigold leaves (*Tagetes erecta*) can maintain levels of inhibin B. In previous studies, no one has explained the effect of ethanol extract of marigold leaves on reproductive health, especially in male infertility. Based on the description above, the authors wanted to conduct research on the effect of giving ethanol extract of marigold leaves (*Tagetes erecta*) at a dose of 0.25 g/kg-BW, 0.50 g/kg-BW, 1.0 g/kg-BW to inhibin B mice (*Mus musculus*) exposed to cigarette smoke.

2. Materials And Methods

This study used a type of laboratory experimental research with a randomized posttest-only control group design, namely by comparing the results of observations of the control group and the treatment after being given treatment. The sampling technique in this study used simple random. Eight samples of male mice aged 8-12 weeks with a body weight of 20-25 grams for each group were exposed to cigarette smoke and ethanol extract of marigold leaves with graded doses. There were 5 groups, namely K- was given a placebo, K+ was only exposed to cigarette smoke, P1 was exposed to cigarette smoke + EDM 0.25 g/kg-BW, P2 was exposed to cigarette smoke + EDM 0.5 g/kg-BW, P3 was exposed to cigarette smoke + EDM 1.0 g/kg-BW. Inhibin B hormone examination using the ELISA method.

Approval for conducting this study has been authorized by the Animal Ethics Commission at Airlangga University's Faculty of Veterinary Medicine. The study is covered by ethical permit number 2.KEH.092.08.2022.

3. Results and Discussion

Inhibin B

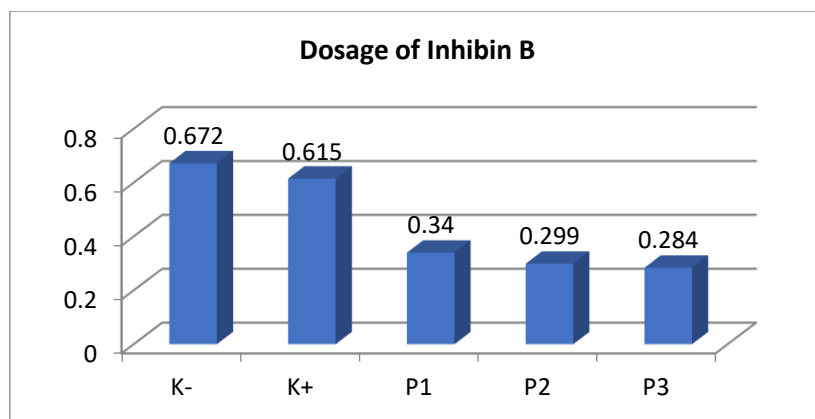


Figure 1 Distribution of Inhibin B hormone levels based on treatment groups

Figure 1 exhibit that the levels of the hormone inhibin B in the K+ group decreased compared to the K- group. Inhibin B hormone levels in the P3, P2, and P1 groups decreased compared to the K+ group. Group P1 had the highest levels of the hormone inhibin B when compared to groups P2 and P3 which were both given marigold leaf extract.

Figure 1 shows that giving cigarette smoke as much as one cigarette per day, from day 1 to day 35 can reduce levels of the hormone inhibin B. Giving cigarette smoke as much as one cigarette per day and followed by administration of marigold leaf ethanol extract at a dose of 0.25g/kg-BW (P1), a dose of 0.5g/kg-BW (P2), and a dose of 1.0g/kg-BW (P3) on day 1 to day 35 reduces levels of the hormone inhibin B compared to the group that only gets smoke cigarettes without marigold leaf ethanol extract (K+). This can explain why the ethanol extract of marigold leaves at doses of 0.25g/kg-BW, 0.50g/kg-BW, and 1.0g/kg-BW have not been able to improve/increase inhibin B hormone levels. The normality test for inhibin B hormone levels using Shapiro-Wilk can be seen in Table 1.

Table 1. The normality test results for inhibin B hormone level

Treatment Group	Mean± St.dev	"p-value"
K-	0.672 ± 0.157	0.082*
K+	0.615± 0.129	0.584*
P ₁	0.340 ± 0.056	0.507*
P ₂	0.299 ± 0.115	0.131*
P ₃	0.284 ± 0.079	0.633*

*Normal Distribution (significance if $p > 0,05$)

Table 1 exhibit that the parameter inhibin B levels are normally distributed because all groups show a $p\text{-value} > 0.05$. The homogeneity test for the inhibin B hormone level variable can be seen in Table 2.

Table 2. Inhibin B hormone level homogeneity test results

Variable	"p-value"
Inhibin B	0.472*

*Homogeneous Data (Significance if $p > 0,05$)

Table 2 gives the result that the homogeneous inhibin B hormone level variable is indicated by a $p\text{-value} > 0.05$. Data on inhibin B hormone levels were homogeneous, so the analytical test used was the One-Way Anova test as an analysis of variance test. The results of the One-Way Anova test can be seen in Table 3.

Table 3. Result of One Way Anova

Variable	"p-value"
Inhibin B	0.000*

* Significantly different (significance if $p < 0,05$)

According to Table 3, the p-value for inhibin B hormone levels is <0.05 , indicating noteworthy distinctions in inhibin B hormone levels among the groups (K-, K+, P₁, P₂, and P₃).

Following the One-Way Anova test, the BNT Post hoc analysis was performed to assess the variations in hormone inhibin B levels across distinct groups. The outcomes of the BNT Post hoc examination are presented in Table 4.

Table 4. Post hoc BNT test results for levels of the hormone inhibin B

Treatment Group	K+	K-	P1	P2	P3
K-	-	-	-	-	-
K+	0.352	-	-	-	-
P1	0.000*	0.000*	-	-	-
P2	0.000*	0.000*	0.505	-	-
P3	0.000*	0.000*	0.363	0.806	-

* Significantly different (significance if $p < 0.05$)

The BNT post hoc test results for the variable levels of inhibin B hormone in Table 4 showed that the levels of inhibin B hormone that were significantly different ($P < 0.05$) were in the K+ group with P₁, P₂, and P₃, then the K- group with P₁, P₂, and P₃.

Inhibin B

The findings from this research suggest that giving male mice (*Mus musculus*) exposed to cigarette smoke an ethanol extract of marigold leaves (*Tagetes erecta*) did not lead to any improvement or increase in inhibin B levels. There was a decrease in inhibin B levels in the K+ group exposed to cigarette smoke when compared to K-.

This study shows that cigarette smoke has a significant effect on inhibin B levels. This is following several research results. A study conducted by Akbar (2021) said that cigarette smoke contains more than 4,000 compounds that can cause oxidative stress on the male reproductive organs and sperm which has the potential to cause infertility in men. The increased risk of infertility is directly proportional to the number of cigarettes consumed and the duration of smoking (Akbar. 2021). Tobacco smoking is considered one of the main factors causing male infertility (Bundhun, et al. 2019). The nicotine levels contained in cigarette smoke can damage Sertoli cells, which are Sertoli cells that produce the hormone inhibin B. When Sertoli cells are disrupted, the production of inhibin B is also disrupted (Kurniati & Nugraheni, 2020).

Among the essential serum markers of spermatogenesis, inhibin B holds great significance. Several clinical studies also show that serum levels of inhibin B reflect the functional state of spermatogenesis because it is one of the elements of the hypothalamus-pituitary-testicular axis inverse relationship. In a study conducted by Demyashkin in 2018, it was shown that increased serum FSH levels reflect an inverse relationship with decreased serum levels of inhibin B.

The findings from this study demonstrate that administering ethanol extract derived from marigold leaves (*Tagetes erecta*) at dosages of 0.25 g/kg-BW, 0.50 g/kg-BW, and 1.0 g/kg-BW was carried out has not been able to increase inhibin B levels. According to Semercioz (2017) suppression of inhibin B levels in testicular tissue may be a marker of oxidative stress. The substantial decrease found in inhibin B levels paralleled the increase in oxidative stress. Analysis of inhibin B can serve as a marker of oxidative stress. Sertoli cells are the main producer of inhibin B in the human body.

Therefore, the function of Sertoli cells and the quality of spermatogenesis are both closely related to the concentration of inhibin B. Marigold leaf ethanol extract contains antioxidants in the form of flavonoids. Flavonoids are antioxidants that are used as chain-breaking antioxidants (Kurniati & Nugraheni, 2019). Flavonoids will capture free radicals by releasing hydrogen atoms from their hydroxyl groups and breaking the chain reaction of free radicals (Sumiarsih & Kuslestari. 2018). In this study, administration of marigold leaf ethanol extract (EDM) to mice exposed to cigarette smoke reduced levels of inhibin B. Marigold leaf extract had no curative effect on inhibin B levels of mice exposed to cigarette smoke for 35 days.

4. Conclusion

In this study, administration of marigold leaf ethanol extract (EDM) reduced inhibin B levels. Marigold leaf extract did not have a curative effect on inhibin B levels in mice that had been exposed to cigarette smoke. Giving marigold leaf ethanol extract (EDM) can affect on inhibin B levels in spermatogenesis. However, to see the benefits of EDM on inhibin B levels, further examination is needed, because the normal value for inhibin B levels can vary.

This research requires further research with different methods. The method that can be given is in the form of protective research. Further research can also replace exposure to free radicals other than exposure to cigarette smoke.

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Conflict of interest:

The authors declare no conflict of interest.

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