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Infertility Impact of Cotton Seeds (*Ceiba pentandra*) and Eggplant Cepoka (*Solanum torvum*) on the Nfkb Expression and Seminiferous Tubules Diameter

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KEYWORDS

Solasodine

Gossypol

Infertility

NFkB

Seminiferous tubule diameter

ABSTRACT

The presence of the solasodine and gossypol compounds in Kapok seeds (*Ceiba pentandra*) and cepoka eggplant (*Solanum torvum*) suggest the use of these two as an herbal contraceptive ingredient in male animal fertility. This study aimed to determine the effect of cepoka eggplant extract (*S. torvum*) and kapok seed (*C. pentandra*) on infertility in rats (*Rattus norvegicus*) through NFkB expression and seminiferous tubule diameter. The study was carried out on 75 to 90 days old male Wistar rats having 150-200 grams of body weight. The experimental design used in this study was a completely randomized design (CRD) with three treatment groups i.e. C (control), KP1, and KP2 having six rats in each group. Here the rats of group C were not treated with gossypol or solasodine while the rats of group KP1 were treated with solasodine compound at a dose of 1g/kg BW and rats of group KP2 were treated with gossypol induction at a dose of 0.1 g/kg BW. Kapok seed extract and eggplant cepoka extract were extracted by the maceration method with 70% ethanol solvent. NFkB expression was examined using the immunohistochemical (IHK) method, which was analyzed with immunoratio software. Histopathological preparations (HE) of seminiferous tubule diameter were analyzed using raster image software 3. All the obtained data were analyzed by the One Way ANOVA test and Tukey's follow-up test with a 95% confidence level ($\alpha = 0.05$). The results showed that the administration of cepoka eggplant extract could significantly increase the expression of NFkB ($p<0.05$) with an average amount of 87.22 ± 6.89 with a dose of 1 g/kg BW. Treatment with Kapok seed extract can reduce the diameter of the seminiferous tubules (STs) by an average of 0.31 ± 0.016 at a dose of 0.1 g/kg BW. The results of the study can be concluded that the extract of cepoka eggplant and kapok seeds can be used as candidates for herbal contraceptives.

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1 Introduction

The explosion of the dog population in areas prone to zoonotic diseases can cause unrest in the community because it can trigger the occurrence of rabies and other diseases. The population of stray dogs in big cities such as East Denpasar, Indonesia has a ratio of 13.2: 1, with 96 dogs on a human population of 1272, and this has highly increased from 2008-2009 (Puja et al. 2012). The wild stray dog population can cause the risk of disease transmission in fellow animals or from animals to humans. The methods that have been frequently used for controlling the stray dog population include surgery, ovariohysterectomy, and castration. These methods are ineffective due to the time-consuming, expensive, and limited specialties. Elimination of wild animals no longer follows the principle of animal welfare, so it is necessary to develop a population control method using herbal compounds that are more effective.

Previous literature suggested that eggplant cepoka (*S. torvum*) and kapok seed (*C. pentandra*) can affect fertility and especially have a notable effect on male fertility. Fruit extract of eggplant cepoka contains alkaloid solasodine and steroids which are thought to have contraceptive potential and can be used as a male contraceptive as it decreased the number and motility of spermatozoa. Further, it has been reported that solasodine can suppress the functioning of the anterior pituitary and can reduce the secretion of FSH and LH. The decrease in LH causes a decrease in the production of testosterone in Leydig cells (Astrid 2012; Rafiqa et al. 2013).

Gossypol is obtained from kapok extract and can cause disturbances in the reproductive system; besides it can interfere with transcription factors (p53, p50, and p65) also, thereby it inhibiting the formation of hormones needed for reproduction (Moon et al. 2011). Cunha et al. (2012) reported that giving kapok extract in sheep feed can reduce the number of Leydig cells and make the testes smaller. Further, gossypol compounds found in the blood caused damage to the testes tissue. Damage to the testes tissue triggers an inflammatory mechanism and increases cell apoptosis which is characterized by the expression of Nuclear Factor kappa-B (NFκB) found in Sertoli cells. NFκB (Nuclear Factor kappa B) has an essential role in activating the inflammatory response and cell proliferation. Inflammation occurs in Sertoli cells and Leydig cells activate the expression of NFκB, which increases the cell apoptosis, and reduces the rate of spermatogenesis in Sertoli cells which results in infertility in males. Based on the background above, a study was conducted on the effect of kapok seed extract and eggplant cepoka as contraceptive candidates in male rats (*Rattus norvegicus*) through NFκB expression and seminiferous tubule diameter.

2 Materials and Methods

The study was carried out on 75 to 90 days old male Wistar rats having 150-200 grams of body weight. Other used materials are rat food and drink, 95% albumin vaseline, alcohol (70%, 80%, 90%, 95%, 100%), physiological NaCl 0.9%, PBS pH 7.4, H_2O_2 3%, object-glass, formalin buffer 70%, NFκB antibody, extract of kapok seed, extract of eggplant cepoka, Hematoxylin Eosin (HE) dye, distilled water, paraffin wax, ether 70%, xylol, and chloroform.

This is a laboratory study that was carried out in Completely Randomized Design (CRD). The experimental rats used in this study were first acclimatized for seven days with regular feeding and drinking water ad libitum. A total of eighteen male rats were randomly divided into three groups, and each group consisted of 6 rats. The treatment groups in this study are (i) C (control) - rats of this group were given standard feed and drinking water ad libitum, and not induced by gossypol and solasodine extract with ethanol as solvent, (ii) KP1 - rats of this group were given standard feed and drinking water ad libitum and orally given solasodine extract (eggplant cepoka extract) at a dose of 1 g/kg BW and (iii) KP2 - rats of this group were given standard feed and drinking water ad libitum and orally induced by gossypol extract (kapok extract) at a dose of 0.1 g/kg BW. Predefined doses of the kapok seed extract (0.1 g/kg body weight) and eggplant cepoka extract (1 g/kg body weight) were given orally once a day in the morning before the mice were fed. Euthanization of rats was carried out on the 11th day of rearing using the chloroform inhalation method. After euthanization, surgery was performed with an incision in the linea abdominis, then a pair of testes were collected. The 0.9% physiological NaCl was selected to wash the organ before putting them into a 10% formalin buffer. The diameter of the seminiferous tubules was calculated by observing the stained histology preparations under a microscope in five fields of view with a magnification of 100x. The measurement of the diameter of the seminiferous tubules was carried out using image raster three macros software with units of millimeters (mm).

Quantitative data were analyzed by one-way ANOVA with an accuracy of 95%, provided that the data were homogeneous and normally distributed. Tukey's test was also used to identify the difference between treatment groups (α = 5%).

3 Results

The comparative results of giving cepoka eggplant and kapok seed extract on male rats' infertility through NFκB expression and seminiferous tubule diameter were observed using the immunohistochemical method (IHK). The results of the testicular CPI were indicated by the expression of NFκB in male rats.

Table 1 Treatments result on NFκB expression

Groups	The average expression of NFκB(%) ± SD
Control	64.38 ± 13.84[a]
KP 1 (1 g/kg BW)	87.22 ± 6.89[b]
KP 2 (0.1 g/kg BW)	85.52 ± 6.94[b]

Value given are the average of four replicates; mean ± SE value followed by the different letters in the same vertical column are significantly different (p <0.05)

Table 2 The diameter of the STs in rats

Groups	Average diameter of the STs± SD
Control	0.44 ± 0.016[a]
KP 1 (1 g/kg BW)	0.40 ± 0.033[b]
KP 2 (0.1 g/kg BW)	0.31 ± 0.016[c]

Value given are the average of four replicates; mean ± SE value followed by the different letters in the same vertical column are significantly different (p <0.05)

Observations were made using a 400x magnification microscope with five times the field of view in the seminiferous tubule area. A brown color indicated nFκB expression in the tissue due to the binding between the primary anti-rat NFκB antibody and a secondary antibody labeled biotin, anti-Rabbit IgG biotin-labeled with the addition of DAB substrate (Diaminobenziddine). NFκB observations were carried out in all treatment groups, namely the control group (C), KP1, and KP2.

The accumulation of NFκB expression was statistically analyzed using SPSS software with the one-way ANOVA test. The results of the study showed a significant difference (P<0.05) between the control and the treatment group. Among the treatment group, group KP1 had a higher average NFκB expression than the negative control and KP2.

Results presented in table 1 suggested that the control group (C) had the lowest mean NFκB expression compared to the other treatment groups (64.38 ± 13.84). It might be due to the rats used in the control group being only given food and drink ad-libitum and did not get exposure to toxic compounds as inflammatory mediators. Some NFκB expression was also found in normal rats and it might be because of germ cell apoptosis which occurred to eliminate defective germ cells and carry DNA mutations (Pentikainen 2002). Further, among the treatment groups, group KP1 has the highest average NFκB (87.22 ± 6.89) followed by the KP2 (85 .52 ± 6.94), and these treatments showed a 26.18 and 24.71% increase from the control group (C), respectively.

The quantitative data of the diameter of the STs were analyzed using SPSS software with the one-way ANOVA method. The results showed a significant difference (P<0.05) between the control group (C), treatment group KP1, and KP2. Results of the study suggested that giving cepoka eggplant extract at a dose of 1 g/kg BW and kapok seeds at a dose of 0.1 g/kg BW can reduce the diameter of the STs in rats (Table 2).

4 Discussion

This study proved that treatment group KP1 could inhibit the process of spermatogenesis and increase the expression of NFκB in testicular tissue. The results also showed the presence of NFκB expression in treatment group KP2 and this is marked by brown color in the testicular tissue (Figure 1). These results are in line with previous research conducted by Susilo and Akbar (2016), who found a decrease in spermatozoa motility when treated with 1 g/kg BW cepoka extract and also reduced the number of spermatozoa by 20.33 million/ml. Cepoka has solasodine as an active ingredient which is thought to be used to increase infertility in male rats. Previous studies suggested that solasodine compounds have activities that can suppress the function of the anterior pituitary and reduce the secretion of FSH and LH hormones through negative feedback to the Hypothalamus.

The results of this study revealed that treatment group KP2 had a lower NFκB mean (85.52 ± 6.94) but this is not significantly different (P>0.05) from the treatment group KP1 (Table 1). The lower NFκB mean of KP2 might be due to the lower concentration of gossypol compounds in kapok extract. The results of this study are in agreement with Gadelha et al. (2014) who suggested that gossypol could act as an antifertility, and anti steroidogenic. Further, gossypol can cause changes in the structure of the epididymis, the diameter of the seminiferous tubules of the testes, damage to the plasma membrane, and cause regression in Sertoli and Leydig cells. Gossypol compounds have a cytotoxic and reactive effect that can disrupt and damage cellular activities. Further, gossypol can bind to the protein connexin 43 (Cx-43) on Sertoli cells and inhibit the production of gonadotropin hormones by interfering with the action of FSH and LH hormones in the anterior pituitary. Gossypol also affects transcription factors (NFκB, p53, p50, and p65), and interferes in the formation of hormones and enzymes needed in reproduction (Moon et al. 2011). Yurekli et al. (2009) suggested that gossypol interferes in steroidogenesis by inhibiting the activity of the cytochrome enzymes p450, 3β–hydroxysteroid dehydrogenase (3β–HSD), 5α–reductase, and aromatase. Barriers to the mechanism of action of these enzymes cause the steroid hormone produced to decrease so that spermatogenesis is disrupted.

This study indicates that eggplant cepoka extract with a dose of 1 g/kg BW increased the expression of NFκB, and this extract has a more significant effect on seminiferous tubules by increasing apoptosis and inhibiting the process of spermatogenesis in male rats.

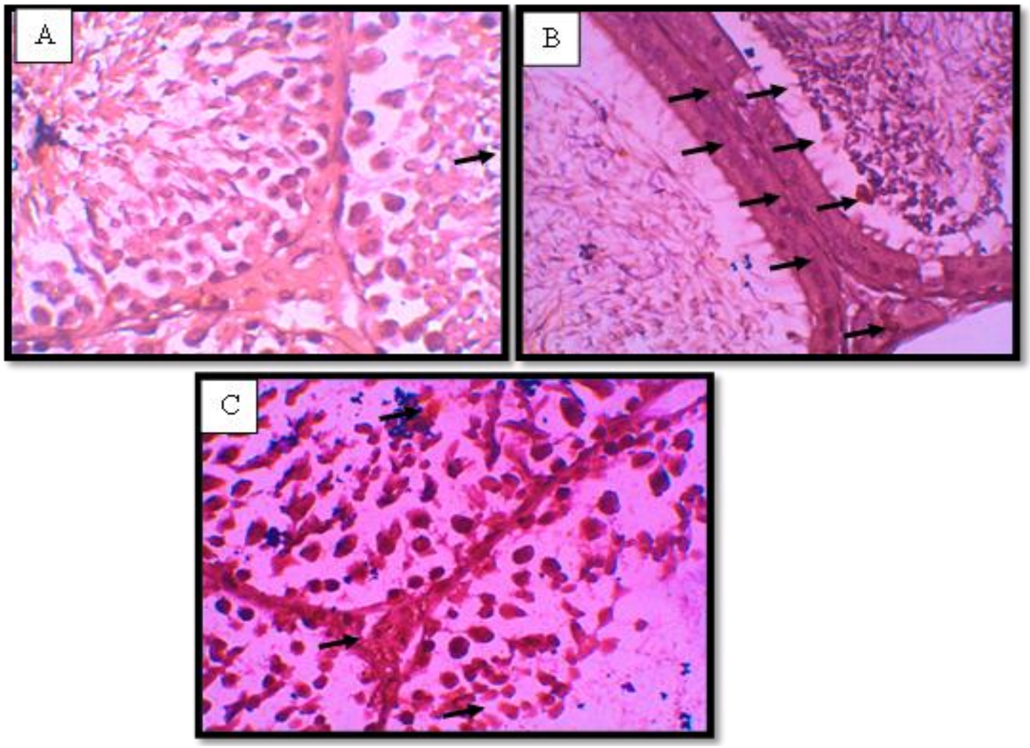

Figure 1 Immunohistochemistry of Testicular Tissue (400x magnification); Black arrows indicate NFκB expression
A = Negative Control Group; B = KP1; C = KP2

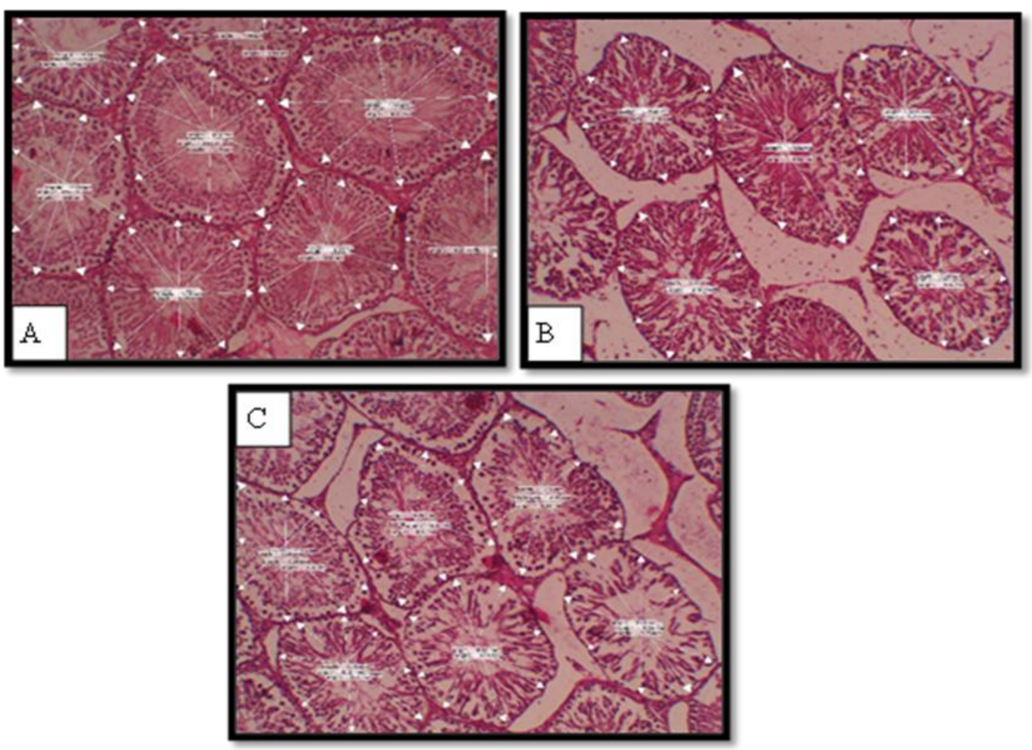

Figure 2 Histology of rat seminiferous tubules (100x magnification); A = cross section of control group testicle (C); B = cross section of KP1; C = B = cross section of KP2 here white arrows indicate the diameter of the STs

Nuclear Factor kappa B (NFκB) is the part of transcription factors that regulate the expression of gene encoding cytokines, chemokines, and several acute-phase proteins in health, which have an essential role in inducing the regulation of various genes in the inflammatory response, and cell proliferation. Generally, NFκB is in an inactive state because in the cytoplasm it has bonding with IkB inhibitors (IkB) (Riawan and Samodijanti 2006). Treatments with cepoka eggplant and kapok seed extracts act as inflammatory mediators and cause the activation of the Toll-Like Receptor (TLR), which is used to activate NFκB and trigger apoptosis in germ cells.

The result of Hematoxylin Eosin (HE) staining shows the presence of normal seminiferous tubule tissue (Figure 2). The histological examination of the seminiferous tubules revealed the presence of a basement membrane structure with a dense and tight arrangement of spermatogonia cells. Spermatogonia cells are still attached to the basement membrane of the seminiferous tubules. Further, in treatment group KP1, a decrease in thickness, separation of spermatogonia cells from the basement membrane, and widening of the lumen were observed in the seminiferous tubules (Figure 2). Treatment group KP2 also experienced similar symptoms and reported a decrease in the thickening, change in the histological shape of seminiferous tubules that were no longer reported, and also experienced atrophy (Figure 2). The atrophic testicular morphology shows the absence of spermatogenic cell development, thinning of the basement membrane epithelial layer, and a decrease in the number of interstitial cells in the testes (Epstein 2012).

The measurement of the diameter of the STs showed a 9.1% decrease in the average tubular diameter in a treatment group KP1 as compared to the control group. The control group had a mean and standard deviation of 0.44 ± 0.016, while it was reported 0.40 ± 0.033 in the treatment group KP1. The results of this tubular diameter measurement proven that the extract of cepoka eggplant and kapok seed can cause a significant decrease in the diameter of the STs and cause disturbances in the process of spermatogenesis. The histology of seminiferous tubules in the control group was thicker as compared to both treatment groups. Ismail (2018) suggested that regular feeding did not suppress intratesticular testosterone levels, which functioned to develop spermatogenic cells.

Histological description of seminiferous tubules after induction of cepoka eggplant extract in treatment group KP1 showed a decrease in the number of spermatogenic cells in the basement membrane, this might be due to the presence of solasodine compounds and steroid content in cepoka eggplant which suppressed the testosterone levels and caused detrimental effects. The development of spermatogenic cells, causes the thickness of the seminiferous tubules to become smaller (Ismail 2018). The content of solasodine compounds in cepoka eggplant results in an increase in testosterone concentration in the blood resulting in a negative feedback effect on the pituitary gland. It has an impact on inhibiting the secretion of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) (Hidayati and Nofianti 2014).

Treatment group KP2 has the highest effect in reducing the diameter of the STs with a decreasing percentage of 29.5% and an average of 0.31 ± 0.016. Histology of the tubules in the treatment group KP2 experienced a decrease in diameter, indicated by the histological shape of the seminiferous tubules that were no longer round and atrophied (Figure 2 C). Several other studies have stated that gossypol compounds disrupt the process of spermatogenesis, resulting in a decrease in sperm motility and the number of spermatozoa as a result of degeneration of testicular tissue. Disruption of the process of spermatogenesis will increase the percentage of abnormal sperm. The increase in abnormal sperm and degeneration of testicular tissue by gossypol compounds are probably caused by damage to mitochondria in the tail of the sperm and damage to the germinal epithelium in the seminiferous tubules.

Conclusion

According to the results of the study, it can be concluded that the administration of cepoka eggplant extract with kapok seeds at a dose of 1 g/kg BW and 0.1 g/kg BW orally can increase the expression of NFκB in the seminiferous tubules of white rats, with the best dose of 1 g/kg BW. Oral administration of cepoka eggplant extract with kapok seed decreased the diameter of the STs of white rats, with the best dose of kapok seed extract at 0.1 g/kg BW.

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