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Effect of Habbatussauda (*Nigella sativa*) Extract on Rat Sperm Count (*Rattus norvegicus*)

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Abstract

Sperm sample from epididimal source can be determined its motility using minimal amount of equipment. These methods will aid researcher and practitioner in sperm motility analysis to determined sperm quality rapidly and practically.

***Keywords**: Sperm quality, Sperm motility, Hemocytometer.*

Introduction

Habbatussauda (*Nigella sativa*) belong to the family Ranunculaceae and are widely grown in the Middle East and West Asia. Habbatussauda used as medicinal plant to improve health and treat various diseases including high blood pressure, diabetes, cancer, infection, and infertility. However, previous scientific studies that supporting the traditional use of habbatussauda are still lack. There are only a few scientific studies that show the pharmacological effect of habbatussauda (Najmi et al., 2008; Yar et al., 2008).

Infertility in men is a serious problem (Turner 2003; Clouatre 2005). An estimated 10% of men experienced fertility disorders and 50% of infertile couples are due to male factors (Pei et al. 2005). Infertility can cause serious psychological problems. Family happiness can be disrupted, that will reduce work productivity in an individual or in a family.

Medical treatment for male fertility disorders is still not entirely successful (Hamadeh et al. 2001; Hannan, 2008; Kohn 2001). Existing methods of treatment such as assisted reproductive technology (ART) require high costs, while the percentage of success for getting a child is low (Orgebin-Crist 1998), even causing side effects (Lefie'vre et al. 2007).

Previous study by Tohamy et al. (2010) and Al-Sa'aidi et al. (2009) showed an increase in fertility of male animals treated with habatussauda. This study aims to determine the effect of habbatusauda one of the important factors on male fertility parameter, namely sperm count.

Method

Material

Habatussauda seeds (*Nigella sativa*) obtained from pharmacy distributors in Yogyakarta.

Chemical material

All chemicals used in this study are analytical unless stated otherwise. The material used were NaCl, KCl, KH2PO4, MgSO4.7H20, NaHCO3, bovine serum albumin, glucose, and methanol obtained from Merck, Germany. $CaCl_2$ obtained from Scharlau, Spain. Penicillin-Streptomycin Glutamine from Life Technology, USA. Sodium pyruvate was obtained from Gibco, USA, while sodium lactate and glycerin were obtained from Sigma, USA. Giemsa dyes are obtained from GCC, UK.

Test organisms

The animals used in this study were mature male Sprague-Dawley rats. 14 mature male rats were obtained from the Experimental Animal Research Unit, Faculty of Veterinary, Gadjah Mada University, Yogyakarta. The rats are given pellets as food and drink water *ad libitum*.

Tool

The tools used in this study are:

-. Cage
-. Hemocytometer
-. Micropipette
-. Incubator
-. Microtome
-. Oven

-. Syringe -. Glassware
-. Microscope

Steps

1. Sperm Samples Preparation
   Caudal epididymis is separated by epididymal division method determined by Hamilton (1975), then cut into pieces and incubated in 15 mL of Biggers, Whitten & Whittingham (BWW) media solution (Biggers et al. 1971) for 30 minutes at 37 ° C in incubator 5 % CO2 to let sperm swim in BWW media (swimp up technique).
2. Sperm Count Determination
   Sperm count was calculated using improved Neubauer Haemocytometer following the Prasad et al method (1972) and Kvist & Bjorndahl (2002) with modifications. A total of 10 µL of sperm suspension from sperm sample preparation was taken using a micropipette, then put into the space between the glass inserts on the hemocytometer, and left for 5 minutes so that the sperm was scattered on the calculation path. Sperm in 25 boxes of each chamber in the hemocytometer is calculated through examination under a microscope at 100x magnification.

Discussion

From the results of the study, the results of the calculation of the sperm count of the treatment group and the control group were as follows:

Table 1. Average sperm count of habatussauda-treated rats compared to control rats.

Treatment	Sperm Count (x 10^6)
Control	39.7000 ± 10.63686
Habatussauda	52.6000 ± 2.69037

Based on the results of the t-test in the group of mice with habatussauda and the control group that has been done, it shows a significant value of 0.008. This shows that the value is less than 0.05 and proves that the two groups have significant differences. These results are in line with the results of the calculation of the average score which shows that the treatment group has a higher average value than the control group.

The results show that the treatment with habatussaudah has beneficial effect on rat sperm count. This is in line with the research conducted by Al-Sa'aidi et al. (2009) that the effect of Black Seed alcohol extract showed an increase in sexual behavior of male rats. This study also shows the positive effects of Black Seed on spermatogenesis. In the transverse preparations of testicular histology slices of treated rats, there was an increase in seminiferous tubule diameter and thickness, number of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and free spermatozoa.

This is confirmed by the study of El-Tohamy et al. (2010) on "The Beneficial Effects of *Nigella sativa*, *Raphanus sativus*, and *Eruca sativa* Seed Cake to Improve Male Rabbit Fertility, Immunity and Production". This study showed that a mixture of *Nigella sativa*, *Raphanus sativus*, and *Eruca sativa* extracts increased sperm motility and immunity of male rabbits.

Conclusion

From the results of the study it is concluded that the treatment of habatussauda has a positive effect on sperm count.

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