

The *postcoital* anti-implantation research of ethanol extract of Black cumin seeds (*Nigella sativa* L.) against white female rats

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ABSTRACT

The *postcoital* anti-implantation research of ethanol extract of black cumin seeds (*Nigella sativa* L.) against white female rats with a dose variation has been done. This study uses 4 groups: 1 negative control group who were given regular drinking water, and the other 3 treatment groups were given the orally sample at a dose of 100 mg / KgBW, 200 mg / KgBW, 400 mg / KgBW for 18 days, on the day 19, rats were dissected, the tested parameters is anti-implantation, fertility index and anti-fertility. The results showed that the ethanol extract of black cumin seeds can provide anti-implantation effect which significantly different ($p < 0.05$) in the dose of 400 mg / kg body weight compared with the control group. Fertility index showed the impairment of fertility with the increase in dose, anti-fertility showed significantly different effects ($p < 0.05$) at a dose of 200 mg / kgBW and 400 mg / kgBW in the control group and the test group at a dose of 100 mg / kgBW.

KEY WORDS: Anti-implantation activity, *Nigella sativa* L., Fertility.

1. INTRODUCTION

The increase of population and natural resources were inadequate, causing birth control becomes a necessity in building a quality population. Based on data from the year 2014 the population of Indonesia as many as 252. 370. 792 inhabitants. The average population growth rate for 2010-2014 of about 1.40% percent per year. To address the issue of population, the fertility control efforts are needed to establish the Family Planning (KB) (BKKBN, 2014).

Contraception is a way to prevent pregnancy after sexual intercourse by blocking the sperm that will be in contact with the mature egg cells, so this will prevent the fertilized ovum implanted successfully in endometrium. The way contraceptives works may be temporary or permanent. The modern contraceptives method are oral and transdermal, injectable estrogen and progestin, poscoital contraceptives, implants progestin, condoms, spermicides, tubal ligation and vasectomy. The traditional contraceptives are coitus interruptus, periodic abstinence, massage and herbal medicine (Fauziah, 2012).

Poscoital contraceptive are used to prevent pregnancy if given soon after unprotected coitus (intercourse), which means it can block implantation in the endometrium (Anwar, 2011; Dipiro, 2005; Supriati, 2009). According to Rajsekhar (2011), research, the black cumin is a small herbal plant that widely cultivated in Punjab, Himachal Pradesh, Bihar and Assam. Medical experts said the seeds of this plant has been used for abortion / substances that stimulate or increase menstrual flow like in Brazil, Ethiopia, India, Nepal and several other countries (Kesri, 1995; Rajsekhar, 2011).

Black cumin seeds contain chemical compounds such as alkaloids (nigelin), flavonoids, saponins, amino acids, dithymoquinon and timoquinon. Recent research from saponins and flavonoids from other plants have antiestrogen properties and can be synthesized by the body that cause the ovaries inactive, disruption of follicular growth and endogenous estrogen secretion so that ovulation can also be interrupted. Another influence can be seen on cervical glands which slightly more viscous so there is no fertilization despite intercourse. The other effects of antiestrogen is endometrial atrophy though fertilization is happening the implantation process will be disrupted. Alkaloid compounds also have anti-implantation effect, which can dissolve or break down the proteins that play a role in the attachment of the embryo to the wall of the endometrium (Akbar, 2010; Ezzat, 1994; Sirat, 2001).

By the fact that the side effects from the use of modern contraception lead us to study the the anti-implantation effect of ethanol extract of black cumin seeds against white females rats, in which this extract contains secondary metabolite such as alkaloids (Nigelon), flavonoids, saponins and cumin that later can be used as an alternative of a safe contraception which given after the copulation.

2. MATERIALS AND METHODS

Plant material: The using samples are black cumin seeds (*Nigella sativa* L.) obtained from Pasar Raya Padang. Black cumin seed sample are weighing 1 kg and then being powdered. The extract was made by maceration, the sample was inserted into the dark bottle sheltered from sunlight and soaked with 70% ethanol. Strain, and repeated for several times until the maceration extract was clear and the macerate being viscoused with a rotary evaporator, to obtain a viscous extract.

Characteristics of ethanol extract:

a. Phytochemical screening: The ethanol extract of black cumin seed are weighed 0.5 g and then inserted into a test tube. Chloroform and water are added each 5 mL (1: 1) and allowed a moment to form two layers, namely water and chloroform. Layer of water used for examination of flavonoids, phenolics and saponins. Chloroform layer used for examination of terpenoids, steroids and alkaloids.

b. Determination of drying shrinkage: Weigh porcelain crucible which had previously been dried for 30 minutes in the oven at a temperature of 105 °C and cooled in the desiccator (A). Weigh 1 gram of extract and then enter it into the crucible (B). Put it in the oven, open it's lid and left in the oven. Heat for one hour, then cool back into the desiccator, weigh back in.

c. Determination of total ash: Carefully weighed 2-3 grams of test material, put in a porcelain crucible which has flamed and has being constants earlier, then it was slowly being flamed to form charcoal. Crucible was added into the furnes by 600 °C temperature for 8 hours, then it was being cooled in a desiccator and then weigh the total ash.

Preparation of Experiments Animal: The experimental animals that being used were 12 white female rats weighing of 150-200 g. The test methods used were divided into 4 groups, each group consisting of three rats. Three experimental groups for treatment with three doses variety, and one groups for the control group. Prior to the study, the mice were acclimatized beforehand for two weeks. Experimental animals declared healthy if during acclimatization showed no change in body weight more than 10% and was visually not showed any disease symptoms.

During the period of acclimatization, the determination of the estrous cycle under the microscope using the pipette method by spraying physiological saline using a pipette blunt-ended by two drops of the test animals vagina, then sucked back, flatten on the slide and given dye methylene blue 0.1% left 3 minutes and then observed the shape of epithelial cells and the leucocyte cells under a microscope. Animals that are in estrus period are grouped. Mating experimental animal were conducted by ratio of 1: 2 between male and female animals during estrus and if found vaginal plug in the following morning, it was considered to have occurred the first day of gestation (Almahdy, 1993).

Doses used: The dose used to test anti-implantation activities poscoital is 100 mg / KgBW, 200 mg / KgBW, and 400 mg / KgBW and negative controls.

Anti-Implantasi poscoital: Acclimatization of experimental animals for a week to adjust the experimentally animals to the environment. Female mice that had been pregnant, were randomly divided into four groups with each group consists of 3 animals. Test preparation administered orally as much as 1% of rats BW from day 1 to day 18 of pregnancy and weighed daily and observed for unwanted effects.

Group 1: negative control

Group 2: given the ethanol extract at a dose of 100 mg / kgBW

Group 3: given the ethanol extract at a dose of 200 mg / kgBW

Group 4: given the ethanol extract at a dose of 400 mg / kgBW

On day 19 of pregnancy, each rat dissected in the abdomen which earlier being anaesthetized using ether. Observing implantation and, the number of corpus luteum, the number of live fetuses and weight of the fetus.

Fertility index were evaluated using parameters Post Coitum (Abu, 2011):

- The percentage of female pregnancies each group (PFP)
- The average number of live fetuses per pregnancy females (ANLF)
- The average length of the fetus (ALF)
- The average number of pregnancies per female corpus luteum (ANPF)

Fertility Index (FI) of each group was calculated:

$$FI = \frac{ANLF \times ALF \times PFP}{ANPF}$$

Antiimplantation calculated as follows:

$$\frac{\text{implantation number of a control group} - \text{implantation number of a test group}}{\text{implantation number of a control group}} \times 100$$

Antifertility calculated as follows :

$$\frac{\text{unimplantation number}}{\text{number of experimental animal}} \times 100$$

Data processing: The experimental data was statistically processed by one-way ANOVA method then followed by a further test of Duncan.

3. RESULTS AND DISCUSSION

Characteristics of ethanol extract: Viscous extract obtained from black cummin seeds, macerated using 70% ethanol. Macerate evaporated using a rotary evaporator, then the viscous black cummin seeds extract can be obtained. The maceration method was being chosen for its simple implementation and it can avoid the possibility of decomposition of the active substances contained in the sample due to the effects of temperature and heating resistant compounds (thermolabile). In the process of maceration, sample were pulverized so that a larger surface area can be obtained, thus more parts of the sample in contact with the solvent so that the shrink process was more perfect. (Djamal, 2010).

In determining the chemical content of the ethanol extract of black cummin seeds, this extract have some active compounds such as phenolic, flavonoids, alkaloids and saponins. Examination of the yield obtained was 10.2%.

Drying shrinkage of 6.6% was obtained, the measurement of residual substances after drying at a temperature of 105 °C to achieve a constant weight, its purpose to provide a maximum limit on the amount of the compound is lost in the drying process. Total ash gained 3.84%, which still meets the standards of ash content (no more than 8%), ash content of interest to do this is to provide an overview mineral content obtained in the initial process to extract formation, wherein the organic compounds and derivatives was destructed and evaporated so the mineral elements and inorganic compounds stay remain (Depkes, 1979).

The preparations are given in the form of a suspension. This was done to facilitate the delivery of preparations on the body. A suspending agent used is 0.5% NaCMC. Na CMC are inert, good resistance to microbes, high clarity and at this concentration has formed good suspension (Wade, 1986). Experimental animals used were female white mice 2-3 months old with an average body weight of 150-190 grams. Selection aims to ensure the uniformity in the study. The advantage of using this animals is because its body physiologically similar to humans, its body size is relatively small, easy handling, easy to obtain and cheaper price. To avoid distortion of research results, the selected mice with strains and the same sex, age and weight are relatively equal (Thompson, 1990).

At the time of acclimatization, the determination of the estrous cycle was done under the microscope by the pipette method by spraying physiological saline using a pipette blunt-ended two drops in the vagina, sucked back, flatten on the slide by 0.1% dye methylene blue is left 3 minutes, observe the shape of epithelial cells and it's leukocytes cells. The epithelial cells were seen horned with irregular shapes and sized as well as the disappearance of leukocytes. Animals that are in estrus period are grouped. Animal mating experiments were performed with a ratio of 1: 2. The combined male and female rats at 4 pm, separated the next day, do the examination of vaginal plugs. The presence of vaginal plugs indicates mice has been declared a day of copulation and first pregnancy. Pregnant female mice that had been separated, the test preparations are given orally on days 1-18 and weigh the body weight of rats every day, day to 19 animals dissected (Akbar, 2010).

The percentage of anti-implantation test poscoital showing improvement in line with the increase in dose, the higher the dose the more variations as anti-implantation effect, can be seen in Table 1.

Table.1. The antiimplantation percentage

Group	The Implantation Number	Total implantation	% anti implantation
Control	10	30	0
	10		0
	10		0
Average	-	-	0
Doses 100mg/kgBW	10	28	66,67
	9		70
	9		70
Average	-	-	68,89
Doses 200mg/kgBW	9	25	70
	8		73
	8		73
Average	-	-	72
Doses 400mg/kgBW	7	13	76
	6		80
	0		100
Average	-	-	85,33

Results of statistical analysis using ANOVA known that ethanol extract of black cumin poscoitum has significant effect ($p < 0.05$) against anti-implantation test that showed that the group of a dose of 400mg / kg is significantly different from the control group, the dose of 100mg / kgBW. Cumin extract causes an increased of anti-implantation activity with an average value of percentage increase of 0%, 68%, 72%, 85.33%. The percentage of anti-implantation increased by administering different doses in rats after copulation other than the alleged anti-estrogens also caused by alkaloids that interfere with the process of implantation of the zygote in the uterine endometrium wall by destroying a protein that plays a role in the implantation process.

Caused by the anti-estrogen properties that affect the ovulation process which further inhibits the maturation of ovum for fertilization, seen from the percentage of corpus luteum formed diminishing, can be seen in Table 2.

The corpus luteum is a result of the ovum follicles that have been transformed into lutein cells and enlarged doubled and charged lipid which gives the yellowish look. Corpus luteum secretes a number of hormones estrogen and progesterone, the nature of this antiestrogen will suppress the action of estrogen results in inhibition of LH and the ovum luteinizing further disrupted and it will disturbed the process of ovum formation, resulting the decreased of fertility index so that the percentage of embryos to be implanted also decreased (Supriati, 2009).



Fig.1. Ten implants in a control group



Fig.2. Nine implants in doses 100mg/kgBW group



Fig.3. Eight implants in doses 200mg/kgBW group



Fig.4. Six implants in doses 400mg/kgBW group

Table.2. Calculation Results of Fertility Index

Groups	Rats	% Pregnant	Living Embryos	footprint resorbtion	EL	Cl	FI
Control	1	Pregnant	10	-	3.29	20	173.77
	2	Pregnant	10	-	3.1	21	
	3	Pregnant	10	-	3.89	20	
Average		100%	100	-	3.43	20.33	
Doses 100 mg/kgBW	1	Pregnant	10	-	2.58	12	163.90
	2	Pregnant	9	-	2.97	20	
	3	Pregnant	9	-	2.73	15	
Average		100%	9.3	-	2.76	15.66	
Doses 200 mg/kgBW	1	Pregnant	9	2	1.96	5	143.53
	2	Pregnant	8	2	2.4	16	
	3	Pregnant	8	1	2.85	20	
Average		100%	8.33	1.67	2.40	13.66	
Doses 400 mg/kgBW	1	Pregnant	7	3	3.057	10	38.005
	2	Pregnant	6	2	2.85	13	
	3	-	-	-	-	-	
Average		66,67%	4.33	1.67	1.93	7.66	

Explanation: EL = embryo length, Cl = corpus luteum, FI = fertility Index.

Fertility and antifertility index affect anti-implantation, by the increasing dose the fertility index has decreased, and anti-fertility has increased, it can be seen in Table 3. From the statistical analysis we can observed the doses of 200mg / kgBW, 400mg / kgBW significantly different from the control dose and a dose of 100mg / kgBW, thus the higher the percentage of anti-fertility the higher effect of anti-implantation, where the process of anti-fertilization is an event inhibit the union of sperm cells and the ovum after the copulation. The increase in body weight of mice during pregnancy is allegedly because other compounds contained in extracts of black cummin that could affect the metabolism without affecting the embryo, by the fact that the content of black cummin seeds contain 30-37% carbohydrates and minerals, and 20% of protein (Wichtl. Max, 2004).

Table.3. The anti-fertility percentage counting

Groups		The Uniplant Number	% anti-fertility
control	1	0	0
	2	0	0
	3	0	0
Average		-	0
Doses 100mg/kgBW	1	0	0

	2	0	0
	3	0	0
Average	-	0	0
Doses 200mg/kgBW	1	2	66.7
	2	2	66.7
	3	1	33.3
Average	-	1.67	55.57
Doses 400mg/kgBW	1	3	100
	2	2	66.7
	3	0	0
Average	-	1.67	55.57

4. CONCLUSION

The research can be concluded that the ethanol extract of black cumin seeds (*Nigella sativa* L.) effect as antiimplantation postcoital at a dose of 100 mg/kgBW, 200 mg/kgBW, 400 mg/kgBW and most excellent effect as antiimplantation postcoital at a dose of 400 mg/kgBW. Data can be concluded from black cumin seeds be used as postcoital contraceptive in female rats.

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