CLINICAL STUDY OF ETHANOL EXTRACT OF
Justicia gendarussa Burm. f. LEAVES IN MALE FERTILITY

Uji klinik ekstrak etanol daun Justicia gendarussa Burm. f. terhadap kesuburan pria

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ABSTRACT

In the efforts to develop male contraceptive method, research using traditional ressources from Indonesia has been kept going. According to ethnomedicinal study, Justicia gendarussa Burm. f. has been used by Papuan male for contraceptive purposes. From chemotaxonomy and pharmacognocy study, it was found that this plant has flavonoid components and, phamacologically, has spermatozoa hialuronidase inhibitor activity. In this study, 70% ethanol extract of alkaloid-free J. gendarussa leaves was used. Two kinds of dosage were used, 284.5 mg/capsule and 213 mg/capsule, given one capsule orally after meal a day for 108 days. In phase 1, 36 volunteers were recruited, with the main requirement to be fertile-normospermia male. The single blind method was used, and it aimed to find the safe dosage for human being. Complete blood test and sperm test were conducted and showed that subjects are in healthy conditions and sperms are normal. The hialuronic binding assay (HBA) examination shows that there is a decrease in the percentage of hialuronat bond, compared to the placebo. Therefore, it is concluded that phase 1 shows that the subjects are healthy and having normal sperm condition. However, there is a decrease in the activity of hialuronidase spermatozoa enzym which might prevent sperm penetration and fertilization in subjects taken the 284.5 mg capsules.

Key words: ethanol extract, Justicia gendarusa, male fertility
INTRODUCTION

Contraception that used for reproduction controlling must be considered its safety and security aspects, effectivity (the results can be trusted and its working power can be adjusted as needed), acceptability (easy to use, not interfere during intercourse and the partner can receive it well), reversibility and avoid surgery act. Other than that, the ideal contraception must not be expensive, easy to obtain, and can be received well by religions, ethics, and user's culture, so contraceptive use doesn't need to be encouraged all the time (Albar, 1991; Lissner, 1994; Liu, 1998).

There are three kinds of contraception that used by men. They are interrupted coitus (or withdrawal), condom, and vasectomy (Hatcher et al., 1990). But those methods have weakness, for example in interrupted coitus that considered less effective, condom that often caused psychology resistance and has failure rate 3-15%. Vasectomy method has low reversibility rate and allegedly connected with prostat cancer emergence (Comhaire, 1994; Engelmann et al., 1990; Hargreave, 1992). There are many kinds of contraception for women, including diaphragma; sponge; IUD; cervical caps; pill; morning after pill; Norplant; Provera Depot; natural methods; woman condom; cream/gel, foam tablet, suppositoria (three of them are spermicides); sterilization, and many others (Albar, 1991; Hatcher et al., 1990). It's needed to be developed new contraception for man that safe yet effective so they can more responsible for heredity control in family.

Hormonal and non-hormonal contraceptions have been developed and been learned deeper to realize male pill (Lissner, 1994). One of non-hormonal contraception is concoction that has been used by specific nation, for example is gossypol in Chinese concoction. Further study shows toxical side effect and its reversibility rate still doubted, so it can be concluded that gossypol is not an ideal choice for contraception (Comhaire, 1994; Jones III, Wentz and Burnett, 1988).

There have been conducted many preclinical test for Justicia gendarussa to make it as a phytopharma dosage form, including sub-acute toxicity study, mutagenic, bioavailability, blood chemistry in non-rodent, and biochemistry study of human spermatozoa hyaluronidase inhibitor in vitro. The result shows positive effect that support of potential male contraceptive (Prajogo, 2007). Preliminary research proves inhibitory effect for spermatozoa function in fertilization process. Ovum cell fertilization by spermatozoa is a penetration process of spermatozoa into ovum then stimulates growth of ovum become embryo. In order to approach inside ovum, spermatozoa must penetrate protective thin-layer follicular cell (cumulus oophorus). This penetration process need hyaluronidase enzyme that will loosen follicular cell layer (Keeton, 1980). Disturbance of quality and quantity of hyaluronidase enzyme will cause disturbance of spermatozoo ability in fertilization process. The other research proves that there acrosin activity and β-glucosidase decrease in rabbit (Prajogo et al., 1997), and qualitatively there are hyaluronidase inhibitor characteristic in cow's testis (Prajogo, 1997). Presence of active compounds in gendarussa
from flavonoid group are called gendarusin A and B, that will prevent spermatozoa penetration into human ovum in vivo (IVF, In Vivo Fertilization) and show hyaluronidase activity decrease with micro plate method (Prajogo, 2002).

Verification process to show there aren’t any toxic, mutagenic, and terratogenic characteristics in gendarussa administration, it must be conducted molecularly because mutation characteristic related with gen as herediter material that hold all information about growth, organization, and organism’s function as a live biologic system. Carcinogenic test in water fraction of *J. gendarussa* leaves doesn’t show any carcinogenic effect in testis, liver, kidney, intestine, and lung. Test conducted with PCR shows any profile difference between 802 base pairs in hyaluronidase of mice’s testis, after administration of water fraction of *J. gendarussa* leaves using Primer Forward 5’-GCT TAG CTA TCA TTG ACT GG-3’ and Primer Reverse 5’-GCA CAT TTT GGC TGC TAG GG-3’ with PCR product (Marsusianti, 2006).

Mice’s testis RNA total analysis after administration water fraction of *J. gendarussa* leaves per oral with Northern-Blot method shows there aren’t any change in RNA composition. This research used water fraction with dose 1/12 LD50; 1/17 LD50; 1/90 LD50 and 1/100 LD50 (Nidom, 2005).

Preclinical test that has been conducted significantly proved *J. gendarussa* leaves as male contraceptive. The next phase test that required for realize gendarussa leaves extract as phytopharmaka product is clinical test. This proposal created as basic implementation of first phase clinical test that conducted in normospermatozoa males to identify effect of gendarussa leaves extract to male fertility rate, specifically for parameter such as semen quality, toxicity in blood chemistry, and electrolyte fluid from blood serum, spermatozoa hyaluronidase activity, and gendarusin A pharmacokinetic’s in plasm blood, and ejaculate.

The objective of the research is to conduct first phase of clinical test with usage of ethanolic extract of *Justicia gendarussa* leaves capsule as male contraceptive. The specific objectives of the research are as follows:

1. To know effect of administration ethanolic extract of Justicia gendarussa leaves capsule as male contraceptive in subject’s blood chemistry and electrolyte fluid aspect from blood serum.
2. To know effect of administration ethanolic extract of Justicia gendarussa leaves capsule as male contraceptive in spermatozoa quality and quantity.
3. To know effect of administration ethanolic extract of Justicia gendarussa leaves capsule as male contraceptive in spermatozoa hyaluronidase activity.
4. To know pharmacokinetic of gendarusin A in blood plasma and ejaculate.

The benefit of the research are as follows:

1) Obtain contraceptive function for reproduction control.
2) As an alternative method of male contraceptive that:
3) Relatively cheap, practically, safe and comfort, effective, because it can prevent fertilization and reversible.
4) Information of health generally and reproductive health caused by J. gendarussa exposure.
5) Founding of male contraceptive development model with natural source.
6) As an early information for further research development.

MATERIALS AND METHODS
Clinical test pre and post treatment, single blind, random with three groups parallel.

Sample amount
A. Benefit Parameter
Sample amount in 3 groups \((n_1, n_2, n_3) = 12 \times 3 = 36\) research subjects
Break end 10 % = 3,64 research subjects.
Amount of research subjects that will be included is 40 people, to get minimum amount of research subject is 36 people that can be evaluated as benefit analysis.

B. Safety Parameter
Sample amount in 3 groups \((n_1, n_2, n_3) = 7 \times 3 = 21\) research subjects
Break end 10 % = 2,13 research subjects.
Amount of research subjects that will be included is 24 people, to get minimum amount of research subject is 21 people that can be evaluated as safety analysis.

C. Randomization
Using lottery where each subjects pick a number which represent treatment first dose, second dose, or placebo (lottery/follow the order).

Research subject
It’s needed 36 research subjects healthy male, already married and normospermia. Then they divided into 3 (three) groups with balance amount on each each group just like this:

1. Group I get treatment gendarussa dosage form with dose 284,5 mg, once a day per oral half hour after meal for 108 days.
2. Group II get treatment gendarussa dosage form with dose 213 mg, once a day per oral half hour after meal for 108 days.
3. Group III get placebo once a day per oral half hour after meal for 108 days.

Before the research conducted, all subjects must signing in informed consent. This research conducted according to provision in Helsinki Declaration and must be approved by local research ethic committee.

### Table 1. Capsule Formulas

<table>
<thead>
<tr>
<th>Ingredient name</th>
<th>Weight per capsule (mg) [1st Dosage]</th>
<th>Weight per capsule (mg) [2nd Dosage]</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 % Ethanol extract Justicia gendarussa Burm. f.</td>
<td>284,5</td>
<td>213,4</td>
</tr>
<tr>
<td>Lactose 27,8 %</td>
<td>194,72</td>
<td>265,82</td>
</tr>
<tr>
<td>Primogel 3 %</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Mg Stearate 1 %</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Cab-O-sil 10,2 %</td>
<td>71,4</td>
<td>71,4</td>
</tr>
<tr>
<td>Avicel 2,04 %</td>
<td>14,28</td>
<td>14,28</td>
</tr>
<tr>
<td>Amylum 15,3 %</td>
<td>107,1</td>
<td>107,1</td>
</tr>
</tbody>
</table>
A. Population
36 research subjects healthy male, already married, normospermia, and fulfill inclusion criteria.

B. Inclusion Criteria
2. Healthy male, already married, 20 – 40 years old, has minimum 2 children.
3. Spermogram:
   - spermatozoa concentration > 20 millions/ml
   - normal morphology > 50%
   - very good motility > 25%
4. Have done laboratory check.

C. Exclusion criteria
1. Male that wants to have child in research period.
2. Leukospermia patient.
3. Males that received steroidal drugs and its derivates, including herbal drugs that can increase or decrease sexual potenti-ality.

RESULTS AND DISCUSSION

Table 2.

<table>
<thead>
<tr>
<th>NO</th>
<th>PARAMETER</th>
<th>SUMMARY</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Whole hematology</td>
<td>There isn't any significant change</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Complete urine</td>
<td>There isn't any significant change</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Liver function (SGOT, SGPT)</td>
<td>There isn't any significant change</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Fat profile (total cholesterol, triglyceride, HDL)</td>
<td>There isn't any significant change</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Kidney function (BUN, creatinine, uric acid)</td>
<td>There isn't any significant change</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Blood glucose</td>
<td>There isn't any significant change</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Electrolyte (Na, K, Cl, Ca)</td>
<td>Decrease</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Testosterone concentration</td>
<td>Normal, some subjects experience bradicardia, but subjects don’t feel any symptoms</td>
<td>T</td>
</tr>
<tr>
<td>9</td>
<td>ECG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Physical check</td>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Blood pressure</td>
<td>Increase</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Weight</td>
<td>Increase</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Abdominal circumference</td>
<td>Increase</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>Increase of appetite and libido</td>
<td>T</td>
</tr>
<tr>
<td>11</td>
<td>Semen analysis</td>
<td>There isn't any significant change</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Sperm-Hyaluronan Binding Assay</td>
<td>Decrease of spermatozoa bound-mobile spermatozoa percentage, shows decrease of spermatozoa fertility</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>DNA Analysis</td>
<td>DNA sample treatment can be amplified, shows mutation don’t occured</td>
<td>-</td>
</tr>
</tbody>
</table>
In this experimental research, the plants has obtained from Jolopeto village Pacet, Mojokerto. Which has been done to determination and making of Herbarium and stored in Pharmakognosi and Phytochemistry Department, Faculty Of Pharmacy, Airlangga University. In first step is making simplisia from the crop, according to the good way of making with phase: wet sorting, washing, draining, and dry sorting, then dried in the oven with temperature 50°C during 72 hours. The average results obtained that the plants decreased into 10% than normal condition. The drying temperature was set in 50°C, in order that the compound which contained in Justicia gendarussa leaf can be stable.

In extraction processes, the ethanol solvent recommended to use because only intended for human. The solvent have substance ethanol 70%, based on research this condition known getting more concentrate of glycosides flavonoid compound. As it is known that the main content is gandarusin A that has characteristic inhibitor hialuronidase and also as mark of candidate preparation phytopharmaca male contraception. Releasing of the alkaloid is absolutely necessary because indicated the cause of poisoning is from dried leaf powder which will be extracted. In result that J. gendarussa can't be used in fresh condition. The standart of simplisia and extract which must free from pesticide, is the main qualify of raw material preparation phytopharmaca than the other parameters.

The ethanol extract which can be dried and formulated into capsul with the addition of Cab-o-sil beside other filters. Problems were encountered is the extraction was hard to be formed into granulation tablets. Different in small scale or laboratory with small amount can be handled. So it was decided that provision of test materials in capsule.

The recruitment of subjects with reference clinical trials implementation first phase need about 20-80 subject, and basic of statistic calculation with 21 subject by considering the risk of failure, then the amount raised to 36 subject by considering teh risk of failure then increased to 36 subjects. The main criteria is a healthy male, married, normospermia, and fulfilled the inclusion criteria. At the start of recruitment is difficult to find the desired number of subjects so that in fact there are 60 potential subjects who were then followed skrening appropriate inclusion and exclusion criteria to 36 subjects.

In this study subjects treated with divided randomly into 3 groups, each comprising: Group I was given a dose preparations gendarusa 284.5 mg (1/ 486 LD50), once daily orally half an hour after eating for 108 days; gendarusa dosage group II were given a dose of 213 mg (1/ 648 LD50) orally once a day and a half hours after eating for 108 days, group III was given a placebo orally once a day and a half hours after eating for 108 days. Before the study all subjects the test must sign a statement of willingness sheet (informed consent). This research was conducted in accordance with existing provisions in the Declaration of Helsinki and was approved by the ethics committee of the Faculty of Medicine Airlangga University.

The pattern uses a single-blind study design, of which only the researchers who know the type of drugs given to the subject, so that psycho-
logical factors can be avoided. While implementation is 11 times the visits of subjects, including: a. Stage Information (V-1); b. Screening Phase (V-2) (V1 1 week); c. Baseline phase (V-3) (V2 1 week); d. Treatment Phase (V-4) (V3 2 weeks); e. Phase Examination 1 (V-5) (V4 2 weeks); f. Examination Stage 2 (V-6) (V5 2 weeks); g. Examination Stage 3 (V-7) (V6 2 weeks); h. Examination Stage 4 (V-8) (V7 2 weeks); i. Examination Stage 5 (V-9) (V8 2 weeks); j. Examination Stage 6 (V-10) (V9 2 weeks); k. Follow Up Phase (V-11) (V10 3 weeks). This situation is intended for monitoring the condition of the development the subject of the effect of exposure to ethanol extract gendarusa every week in addition to seeing the subject compliance in taking the test drug.

The assessment of laboratory data including blood pressure, pulse, weight, ECG, hematology, LDL, TG, total cholesterol, blood sugar, creatinine serum, BUN, SGOT, SGPT, K+, Na+, Cl-, Ca++, total bilirubin, microbilirubin, uric acid, and platelet aggregation showed a normal state until the reversible condition. In case of a single subject rythme impaired heart and until now under the supervision of cardiologists. Alleged temporary situation due to congenital abnormality and not a result of exposure gendarusa.

Furthermore, sperm analysis results both qualitatively and quantitatively, including motility state of viability, morphology and concentration of spermatozoa showing normal circumstances. Similarly, the analysis supporting the macroscopic sperm: viscosity, odor, color and pH also remained normal in accordance with WHO requirements.

While the scrutiny hialuronic binding assay (HBA) looks at a dose of 1 percentage decline in bond with hyaluronic low compared to placebo. Methods HBA is a new method for early detection of spermatozoa condition of patients who want children in the process of in vitro fertilization (IVF). There is an opinion that this HBA test results on the percentage above 80% can not guarantee the fertile male, so need the support of other tests such as the micro-plate test that can measure the amount of sperm hyaluronidase enzyme. From the results of statistical analysis seems to only 284.5 mg dose (a dose of at large) that provides significant reduction compared to placebo.

As it is known that the content of compounds in the leaves J. gendarussa although not indicate a compound that is suspected to be mutagenic, but in clinical trials are still carried out mutagenic investigation of spermatozoa of subjects to ensure the safety of future users. The results obtained on PCR examination of gene coding for the enzyme hyaluronidase which is a human sperm hyaluronidase has 46.000 bp did not show signs of mutation. By relying on the known PCR amplify in vitro data is less valid, so that the necessary stages of sequencing DNA.

CONCLUSIONS

According to research of ethanolic extract of Justicia gendarussa Burm. f. leaves effect in fertile males, in can be concluded that:

1. Capsule dosage form of ethanol extract Justicia gendarussa leaves don’t effect to volunteer”s health condition.
2. Subjects’ spermatozoa quality and quantity still in normal condition.
3. Subjects’ spermatozoa hyaluronidase activity decrease because of consuming gendarussa extract capsule with dose 284.5 mg.

REFERENCES


