Fructose Level of Male White Rats’ Semen after the treatment of *Pluchea indica* within various observations

Rr. Eko Susetyarini * and M. Ariesandy *

* Biology Education Dept., University of Muhammadiyah Malang Indonesia  
  * Laboratory of Chemistry, University of Muhammadiyah Malang, Indonesia

Abstract

Traditional medicine as man antifertility has not been much researched, especially as antifertility per oral. One of the plants traditionally used as antifertility per oral is *Pluchea indica*. The purposes of this study were to prove: 1) the treatment of tannin in *Pluchea indica* that might affect fructose level of male rats’ cement, 2) the effective treatment time to give tannin from *Pluchea indica* in converting fructose level of male white rats’ cement. This research was an experimental research. Steps of the study included: Group I was the control group without any treatment. Group II was given five time treatments; they were given the tannin treatment of *Pluchea indica*. The oral treatment was delivered as much as 0.8 ml (which was assumed to be the most effective level of tannin to obstruct fertilization taken from previous study) once in a day for 98 days consecutively. The observation was delivered in the timeframe of 49+3 days, 49+16 days, 49+26 days, 49+36 days, and 49+49 days (from the previous research) to examine cement’s fructose level. HPLC analysis was implemented with the data analysis of two-way ANOVA, whereas to examine the differences, the researcher applied BNT further testing. The results showed that the levels of fructose within various observations decreased after given *Pluchea indica*’s tannin for (p <0.05), and the most effective treatment time was on day 49 + 3 days.

**Keywords:** Fructose, *Pluchea indica*, semen, tannin, male white rats

INTRODUCTION

Traditional medicine as man antifertility has not been much researched, especially as antifertility per oral. A number of phytomedicines have positive effects on
spermatogenesis and sperm parameter (sperm motility, count, viability, abnormal sperm). One of the plants used as antifertility per oral is *Pluchea indica*. Some active compounds found in the leaves of *Pluchea indica* are tannins, alkaloids, and flavonoids. From the previous study, it was found that these active compounds influenced the process of spermatogenesis, testosterone level of male rats, and the decrease of female rats tiller number [1, 2, 3, 18, 21]. Tannin is able to reduce fertilization potential of rat’s spermatozoa [4, 18]. There are some differences in Tannins level of fresh and dry *Pluchea indica* leaves: 0.61% in fresh leaf, and 1.885% in dry version [5]. Tannin is classified into active compounds existed in botanical phenol which has a sense of astringent taste and the ability to tan leather. Tannin is capable of inhibiting protein synthesis as well [6, 19]. Tannin affects male reproductive organs so that it will affect the secretion of the glands. If a biochemical secretion from the glands of the male sex is disrupted, it will affect its biochemical semen substances (fluid from accessory gland and spermatozoa).

Based on several previous researches, Tannin of *Pluchea indica* leaf and *Azadinachta indica* can reduce the potential fertilization of male white rats (*Rattus norvegicus*) [4, 20] and the amino acid content of semen [7]. However, it has not been revealed more about the capabilities and mechanisms of tannin in influencing fructose level of male white rats’ cement that may result in the observation of spermatozoa quality.

The result of this study is expected to reveal the adequate level of fructose semen in male white rats. Spermatozoa of male rats are produced from the process of spermatogenesis in the seminiferous tubules of the testes. The quality of spermatozoa determines the fertilization success. Fertilization potential reduction occurs as the result of sperm quality decline. One of the glands that plays a significant role in the life of spermatozoa is epididymis plasma. Epididymis is an organ that is used for the process of maturation of spermatozoa. One of the secretion products from epididymis gland is fructose. Fructose is a monosaccharide. Fructose serves as a source of energy for spermatozoa. Fructose generates ATP for fibrils contraction in the tail of the sperm, so that spermatozoa may have some movements [8].

Tannin may interfere metabolic processes occurred in epididymis plasma. Epithelial cells of epididymis plasma that actively secrete fluid required for spermatozoa while in epididymis [9] become inactive. Semen fructose levels in white rats with observation according to spermatogenesis process has not been studied.

In the long run, after a clinical test, the result of this study is projected to be used more to help the family planning program, as male antifertility drugs. This study aims to prove the interaction between the treatment of tannin from *Pluchea indica* leaves with a wide range of observation time (49 + 3 days, 49 + 16 days, 49 + 26 days, 49 + 36 days, 49 + 49 days), which affect the levels of fructose in male white rats’ semen. The study was also expected to prove which fructose level is the most effective one to be administered after the treatment of *Pluchea indica*’s tannin within the observation times of (49 + 3 day, 49+ 16 day, 49 + 26 day, 49 + 36 day, 49 + 49 days).
Table 1. Result of Two-Way ANOVA Analysis on Fructose Level of Male White Rats’ Semen treated by Tannin of Pluchea indica Leaves

<table>
<thead>
<tr>
<th>SK</th>
<th>F Calculation</th>
<th>F Table</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>288.68</td>
<td>3.02</td>
<td>p &lt; 0.05 (significant)</td>
</tr>
<tr>
<td>Tannin Treatment</td>
<td>2370.22</td>
<td>4.96</td>
<td>P &lt; 0.05 (significant)</td>
</tr>
<tr>
<td>Time of Treatment</td>
<td>0.38</td>
<td>3.48</td>
<td>P &gt; 0.05 (not significant)</td>
</tr>
<tr>
<td>Treatment and Time</td>
<td>56.59</td>
<td>3.48</td>
<td>P &lt; 0.05 (significant)</td>
</tr>
</tbody>
</table>

METHOD
This experimental research was basically conducted by using the "Post test control group design". The population in this study was the strain wistar rat population, that was the population with mature age (3-4 months) derived from the inbreed rats treated from birth until the sampling period. The sample consisted of 15 male rats with random sampling technique. The researcher selected male rats that had body weight between 200-300 g.

Definition of operational variables in this study, namely: the treatment of Pluchea indica leave’s tannin in 0.8 mL dose was given once in a day for 98 days. Observations of semen were conducted on day 49 + 3, 49 + 16, 49 + 26, 49 + 36 and 49 + 49. Fructose level was derived from the total of semen’s fructose level from male white rats by using HPLC.

Stages of the research were as follows: Phase I: Tannin isolation from Pluchea indica’s leaves 1). Simplification of characterization by using WHO or Meteria Medica Indonesia, 2). Phytochemical screening, 3). Extract making: a. maceration-percolation with ethanol; b. soxletIsolasi; c. extracts fractionation; d. components separation in fraction [10] of Pluchea indica leaves to generate active substances such as tannin. Tannin extracting was executed by using Lowenthal-Procter. Fractionation component of Pluchea indica leaves’ active substance was in the form of tannin, by using fingerprint thin layer chromatogram (TLC) test [11]. Step II: 1) Observations of fructose level in semen. They were conducted on day 49 + 3, 49 + 16, 49 + 26, 49 + 36, and 49 + 49; during the treatment stage, tannin was prescribed during the whole 98 days. The treatment was given as much as 0.8 mL·day⁻¹. The research plans were: (1) to prove that the treatment of tannin should be able to reduce fructose level in male white rats’ semen on day 49 + 3 of the treatment, (2) to prove that tannin treatment might also influence the level of fructose in semen on day 49 + 16, (3) to assert that tannin could also influence the level of fructose in semen on day 49 + 26, (4) to make sure that tannin would also give contribution in fructose level reduction on day 49 + 36 of the treatment, as well as (5) to find out the level of fructose reduction post treatment of tannin on day 49 + 49. When the rats had been given enough time to adapt to the laboratory, which took two weeks, the treatments were conducted in accordance with the designated groups as planned.
After 98 days, the first surgery was conducted for the samples of day 49 + 3, 49 + 16, 49 + 26, 49 + 36 and 49 + 49 of the treatments. The observation was elaborated by putting three rats per classification to death by using isofluorine and decapitation techniques [12]. Immediately after the death, surgery was administered to open their abdomens in order to take their vas deferent organs. Fructose level test was done by applying HPLC. Data analysis was administered through quantitative design. The statistical calculation was executed by two-way ANOVA, further clarified and justified by LSD test to find out the differences by its level of significance \( (a) = 0.05 \), if there is no difference, LSD test would not be proceeded.

RESULTS

The results of the observation in administering tannin towards fructose level in rats’ semen are displayed as follows, 1) data on fructose level in semen after tannin’s treatment with the observation time frame (day 49 + 3, 49 + 16, 49 + 26, 49 + 36 dan 49 + 49). The observation results were as follows. Figure 1 and Table 1 displayed the fructose level of rats’ semen of the given time frame that was extracted from vas deferent of the male white rats.

Figure 1 showed that the average male white rats’ fructose levels in the group without tannin treatment within observation time of 49 + 3 days reached to \( (303.64 \text{ mg} \cdot (100 \text{ mL})^{-1} \); the observation of 49 + 16 days up to \( (307.57 \text{ mg} \cdot (100 \text{ mL})^{-1} \); the observation of 49 + 26 days hit \( (311.02 \text{ mg} \cdot (100 \text{ mL})^{-1} \); the observation of 49 + 36 days amounted to \( (317.74 \text{ mg} \cdot (100 \text{ mL})^{-1} \); the observation of 49 + 49 days was equal to \( (329.05 \text{ mg} \cdot (100 \text{ mL})^{-1} \).

Figure 1 confirmed that the mean score for non-treatment of male white rates was \( (303.64 \text{ mg} \cdot (100 \text{ mL})^{-1} \) on the 49 + 3 day; \( (307.57 \text{ mg} \cdot (100 \text{ mL})^{-1} \) on the 49 + 16 day; \( (311.02 \text{ mg} \cdot (100 \text{ mL})^{-1} \) in the 49 + 26 day treatment; \( (317.74 \text{ mg} \cdot (100 \text{ mL})^{-1} \) in day 49 + 36; and lastly, as much as \( (329.05 \text{ mg} \cdot (100 \text{ mL})^{-1} \) on day 49 + 49. Whereas the mean score for the experimental groups with tannin treatment everyday for 49+3 day was \( (265.12 \text{ mg} \cdot (100 \text{ mL})^{-1} \), for 49 + 16 day was \( (262.12 \text{ mg} \cdot (100 \text{ mL})^{-1} \), for 49 + 26 day was \( (254.95 \text{ mg} \cdot (100 \text{ mL})^{-1} \), for 49 + 36 day was \( (248.07 \text{ mg} \cdot (100 \text{ mL})^{-1} \); and for 49 + 49 day was as much as \( (238.39 \text{ mg} \cdot (100 \text{ mL})^{-1} \).

Figure 1 exposed that the fructose level in rats’ semen of control group which was higher than the experimental groups for all the experiment timeframes from 49 + 3, 49 + 16, 49 + 26, 49 + 36, 49 + 49 days.

The data were further analyzed by Two-Way ANOVA test. However, before the ANOVA was conducted, homogeneity and normality tests were administered. From the fructose level of rats’ semen after the tannin treatment by *Pluchea indica* leaves, it could be seen that the data distribution was normal \( (p > 0.05) \) and homogeneous \( (p > \).
0.05); therefore, ANOVA was performed. The result of the test was displayed in Table 1.

DISCUSSION
The analysis results of diversities in tannin treatment and time frame affected the level of fructose production in male white rats’ semen shown in the following recaps; 1). Tannin treatment from *Pluchea indica* leaves for 49 + 49 days then dismissed or stopped could reduce the level of semen’s fructose compared with the treatment without tannin, 2). Tannin treatment from *Pluchea indica* leaves for 49 + 36 days then dismissed or stopped could also decrease the level of semen’s fructose compared to the treatment without tannin, 3) 49 + 26 days treatment with tannin was able to decrease levels of fructose in semen compared to the group treated with zero tannin, 4). 49 + 16 days tannin treatment was proven effective to reduce the levels of fructose in cement compared to the control group, 5). Tannin treatment for 49 + 3 days could reduce the levels of fructose in male white rats’ semen to limit the production of spermatozoa compared to the untreated group. These findings have concluded that tannin is capable of reducing the level of fructose in rats’ semen.

Spermatozoa quality influences fertilization process. Spermatozoa are produced from spermatogenesis process inside the testis. There are two processes of spermatogenesis, spermatositogenesis and spermiogenesis. Spermiogenesis is a developmental process from spermatid into spermatozoa. Spermatozoa will swim into reproductive track to experience maturation process inside epididymis. In the process of maturation in the epididymis, spermatozoa undergo structural and functional changes [13]. Components of epididymal fluid that influence the cells during maturation of spermatozoa are proteins, fructose, carnitine, gliserilfosforilkolin, masitol, sodium, potassium, and calcium. Epididymis can be divided into three regions, namely: caput (head or nucleus area, the source of ductus efferent), corpus, (middle area), and cauda (tail or end area, the meeting point with vas deferent).

Epididymis is an area of sperm maturation and storage. In the epididymis cauda, spermatozoa experience maturation by eliminating the phosphotyrosine residue from the head of spermatozoa [14]. Therefore, it has motility and fertility in accordance with spermatozoa in the ejaculating phase. Spermatozoa from the epididymis cauda have the capability of fertility equivalent to the original ejaculated spermatozoa. Vas deferent is a track that runs from the bottom of epididymis.

This is in accordance with the opinion from [15] stating that the higher tannin concentration would influence pH level because tannin contains phenol compounds carrying acid characteristics, even considered as toxic phenol compounds in plant. Semens (spermatozoa and liquids from various glands) of male white rats tend to have too high pH (alkaline) or too low (acidic) which may result in spermatozoa death. Semen’s pH level will certainly affect the survival and the life of spermatozoa [16]. The treatment of *Pluchea indica* within 49 + 49 days would likely to reduce pH level
as tannin contains phenol compounds that has acid characteristic in order to release $H^+$ ion form its hydroxyl group. The release of this ion becomes $C_6H_5O^-$ anion fenoxye that is soluble in water.

Phenol compound is classified into toxic compound in plant, which has negative influence if it is used in high level. The existence of $Ca^{++}$ ion inside plasmatic membrane of spermatozoa could be bind with poly phenol so that decomposition or ion exchange would occur [17].

![Figure 1: Diagram Mean of Fructose Level in Male White Rats’ Semen after the treatment of Tannin of *Pluchea indica*](image)

CONCLUSION
The level of fructose in male white rats’ semen with tannin treatment can be concluded as follows. The treatment of tannin extracted from *Pluchea indica* leaves within various observation timeframes was proven to be able to reduce fructose level of male white rat’s semen within 49 + 49 day treatment. The treatment of tannin from Pluchea indica within 49 + 3 days was affirmed to be the most effective time because the average level of fructose in semen was the closest to the fructose level of the control group without tannin treatment.

ACKNOWLEDGEMENT
We express our gratitude to Directorate of Research and community Service (DPPM) University of Muhammadiyah Malang for supporting the funding of this current research.
REFERENCES


