Effect of ethanolic Neem (Azadirachta indica) leaf extract as an herb contraceptive on Hepato-somatic Index of the male mice (Mus musculus)

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Abstract. Neem has been known as herb contraceptive plant which shows an antifertility effect both in male and female rats. The anti-fertility compound of Neem has the same potencies to interfere with or affect the function of several organs. The Hepato-somatic index (HSI) reflects the value of toxic compounds that enter the animal body also. HSI values can also be used to assess animal health levels. A study to examine the effect of ethanolic extract of Neem as an herb contraceptive to the hepato-somatic index of male mice has been done. Neem leaf was collected from the campus area, dried, mashed then extracted with ethanol 70%. Mature male Swiss Webster mice with 25-30 grams in weight were used as laboratory animals. Mice were divided into 4 groups: P0 (given distilled water), P1, P2, and P3 were given Neem leaf extract with 8.4, 11.2 and 14 mg/KgBW/day respectively. Each treatment group had six replications.

Treatment was given orally for 21 days. The body weight was measured every week until the end of treatment. The mice were anesthetized with chloroform at the end of treatment, continued by dissecting and isolating liver isolation. The isolated liver is then weighed to determine the HSI value. Data were analyzed with ANOVA followed by DMRT test. The results showed that the body weight of control group showed a significant difference (p<0.05) to the treatment group. The hepatic weights and HSI values of the control group showed non-significant differences (p>0.05) with the P1 and P2 treatment groups but showed a significant difference (p<0.05) with the P3 treatment group. It can be concluded that the exposure of ethanolic Neem leaves extract as herb contraceptive affects liver function which causes the increase of hepatic weight and HSI value.

Keywords: Neem, herb contraceptive, HSI

1. Background
Herb contraceptive is a method of controlling the fertility of animals and/or humans by providing plant-based contraceptives. Some plant compounds have been shown to inhibit male and female fertility and can be developed into contraceptives, but only a few plants have been tested for their anti-fertility effects. Joshi et al. [7] state that many natural plant substances have mild estrogenic properties as well as strong anti-estrogenic properties so it is likely to be an effective source of unconventional contraceptives with low side effects.

Azadirachta indica is a plant known locally as Imba, Nimba or Mimba. This plant originating from India spread to Indonesia with the main planting area is on the Java island. The use of Neem as a medicinal plant has been proven in curing bacterial infections as well as worms, anti-inflammatory, and anti-diabetic. Neem extract is also proven as the cancer cells growth suppressor [15]. The various
parts of Neem trees extracts have been shown to have antimalarial, spermicidal, anti-tuberculosis, antipyretic, antiviral, anti-seborrheic, anti-allergic, anti-enzymatic and anti-fungal effects. The \textit{A. indica} extract is also proven to be an anti-fertility effect [2].

Suryawanshi [17] states that Neem contains anti-fertility compounds which potentially active in male and female animals. The results of Bansal \textit{et al.} [3] proves that the Neem oil dosage of 0.2mL/kg BW can be applied to adults. Neem has been used as plants that affect human fertility and proven to induce temporary sterility [18]. The effects of some parts of Neem plant extracts are also known to affect reproductive function in males [1]. Sharma [15] states that the ethanol extract of Neem leaf has an anti-fertility capability, but the mechanism of the compound action is not known with certainty. Sitasiwi \textit{et al.} [16] proved that exposure of ethanolic Neem leaves extracts for 21 days to female mice resulted in a change in estradiol 17-\(\beta\) hormone levels.

Joshi \textit{et al.} [7] state that the standard method of testing an anti-fertility compound against animals should be done appropriately because the same compound has the same potencies to interfere with or affect the function of several organs in the body also. The use of Neem extract as herbal contraceptive is thought to cause disruption to the body, especially when given for long periods and high doses. Neem side effects are also thought to cause damage to the liver and kidney structures [9]. Umadevi [18] states that testing the potential for local anti-fertility and toxicity of crops can provide great confidence so widely accepted by herbal contraceptives users.

Neem plants not only contain steroid group compounds such as campesterol, beta-sitosterol, stigmasterol but also contain some compounds that are toxic. Some compounds that are toxic are Azadirachtin, Nimbin, Nimbidin, and Salannin [17]. Pankaj \textit{et al.} [12] state that Nimbidin is the main compound contained in the neem tree extract. The compound exhibits toxic properties up to a certain concentration. Other compounds suspected of having high toxic activity are Azadirachtin ([12]; [5]; [8]). Hepato-somatic Index (HSI) is the ratio between hepatic weight and body weight in animals [6]. Nunes \textit{et al.} [10] state that HSI is an indicator of energy availability in the liver. The Hepato-somatic index reflects the value of toxic compounds that enter the animal body also. HSI values can also be used to assess animal health levels.

Based on the above problems this research was conducted to examine whether the use of ethanolic Neem leaf extracts as herbal contraceptive affecting the hepato-somatic index of male mice.

2. Methodology
2.1. Laboratory Animals
The laboratory animal was twenty-eight males mice (\textit{M. musculus}) with 2.5 months old, weighing 25-30 grams. The mice were acclimated to laboratory conditions for 2 weeks. During the acclimation, the mice were kept in a cage with 5 mice per cage in densities. Maintenance is done by temperature and humidity control. Feed and drinking water are given in \textit{ad libitum}.

2.2. Method of making ethanol extract of \textit{A. indica} leaf
The Neem leaves were collected by picking leaves from one Neem plant tree planted around the FSM campus. The leaves that were obtained, rinsed with running water then dried in the oven at 40 °C for 10 days. Leaves that have been dried, made into flour by crushing with a blender, then sifted. The leaves were extracted with ethanol 70%. The Neem leaf ethanol extract powder was made of the preparation material according to the determined concentration as proposed by Sitasiwi \textit{et al.} [16] ie. 8.4; 11.2 and 14 mg/KgBW/day.

2.3. Treatment
Male mice with the means body weight were placed in the cage by the draw, 3 mice in every cage. The mice were divided into 4 treatment groups as follows: P0, the control group, the aquadest treated group; Treatment group P1, P2 and P3, treated with ethanolic extract of Neem leaf with dose 8.4; 11.2; 14 mg/animal/day respectively. The treatment was administrated orally with a 0.3 mL/animal in dosage respectively, every 15:00 to 16:00 pm, for 21 consecutive days. The body weight of mice is measured
every 1 week, while the feed and drink consumption of was measured daily. At the end of treatment, the mice have fasted for 12 hours. The weight of the mice was measured before the mice were sacrificed by anesthesia using chloroform. Hepar was isolated by dissecting the abdomen of the mice, starting from the lower part of the abdomen to the thorax. After the liver was isolated, it is cleaned from the attached tissue, then washed with saline solution and dried with a tissue. The liver weight was measured using the analytical balance. The effect of ethanolic Neem leaf extract exposure to the mice HSI was determined by comparing HSI values of mice in control and treated groups. The Hepatosomatic index was determined using the formula expressed by Nunes et al. (2011) as follows: 

\[(\text{Liver weight/Body weight}) \times 100\]

2.4. Data analysis

HSI data were analyzed with ANOVA. The result of ANOVA showed significant difference then followed by DMRT test at 5% significance level.

3. Result and Discussion

Exposure of Neem leaf ethanol extracts orally for 21 consecutive days resulted in the death of some test animals. The mortality of test animals during the study occurred in the experimental group, 1 mouse in the P2 group and 2 mice in the P3 group. It is suspected that the death caused by toxic effects of Neem extract being tested.

The ANOVA results continued with Duncan test on body weight, hepatic weights and HSI values presented in Table 1. The lower body weight of the treated group showed significantly different (p<0.05) compared to the control group (P0). The body weight between treatment groups (P1, P2, and P3) showed no significant difference (p>0.05).

The hepatic weights and HSI values of control group (P0) showed no significant differences (p>0.05) compared to the P1 (dose 8.4 mg/KgBW/day) and P2 (dose 11.2 mg/KgBW/day) but showed significant differences (P<0.05) with P3 (dose of 14 mg/KgBW/day) group. The histogram in Figure 1. shows that the change in hepatic weight goes along with changes in HSI value, the greater the hepatic weight, the HSI value the greater the value as well.

Table 1. Mean of Body Weight, Hepatic Weight and HSI of male mice after treated with ethanolic Neem leaf extracts for 21 days orally

| Group | Body Weight X ± SD | Hepatic Weight X ± SD | HSI X ± SD |
|-------|--------------------|-----------------------|------------|
| P0 (n=6) | 36.62 ± 3.52 | 1.79 ± 0.93 | 4.91 ± 0.47 |
| P1 (n=6) | 33.02 ± 1.69 | 1.93 ± 0.37 | 5.89 ± 1.28 |
| P2 (n=5) | 32.40 ± 2.89 | 1.94 ± 0.26 | 6.11 ± 1.37 |
| P3 (n=4) | 32.93 ± 1.79 | 2.28 ± 0.30 | 6.91 ± 0.66 |

Note: The data are presented in the mean (X) ± standard of deviation (SD). The mean followed by different superscript in the same column showed significant difference (p<0.05). HSI = Hepatosomatic Index

The results showed that there are dead mice in the treatment P2 and P3 group after exposure of Neem leaf extract for 21 days. In line with the occurrence of the death of several mice in the P2 and P3 groups, the mean of body weight of the mice in the treatment group showed lower and significantly different values (p<0.05) with the mice in the control group. In this study, we are using ethanol for Neem leaf extraction. Samsudin [14] states that ethanol can dissolve substances that have low to high polarity. Ethanol dissolvent produces optimal active ingredients and is non-toxic also. Pankaj et al. [12] stated that some component of active compounds can be isolated by using ethanol in extraction, including Azadirachtin, Nimbin, Nimbidin, Meliacin. Nimbidin serves as a hypoglycaemic agent.
Nimbin and Azadirachtin is a cytotoxic compound so that both of these compounds are suspected to cause different results in this study.

The exposure of Neem dose in this study ranged from 8.4 mg/KgBW/day to 14 mg/KgBW/day. Kupradinun et al. [9] states that Neem toxicity in rats was estimated at 0.25 g/kg BW. The doses administered in this study were still under the Neem toxic dose, but it has led to a decrease in body weight and even the death of some animal treatment groups. The effects of exposure to toxic compounds are influenced by several things, such as strains of testing animal [8]. In this study, we are using Mus musculus L. Swiss Webster strain that is suspected to be more sensitive to the given test material causing a decrease in body weight and even death in some animals in the treatment group.

The results also showed that the hepatic weight mean hepatic in the treatment group was increased along with the increased dose given. The mean of hepatic weight of the P3 group showed a significant difference (p<0.05) with the control group (Table 1). This causes an HSI value running in line with an increase of hepatic weight (Table 1) with a corresponding pattern (Figure 2) also. Omotayo et al. [11] stated that the ratio of organs and body weight is an indicator of atrophy or organ hypertrophy. The hepatic weight mean of the treatment group was greater than the control group showed that there was hypertrophy in the organ.

Hypertrophy in animal liver that occurs in treated group due to exposure to toxic substances in the treatment material allegedly occurs through several mechanisms. Those mechanisms namely the occurrence of cellular swelling (cloudy swelling), water buried in the cytoplasm so that cytoplasmic organelles absorb water causes mitochondrial and endoplasmic reticulum swelling. The next mechanism is hydrophic which microscopically appears to be a vacuole cytoplasm. A further mechanism that can cause hypertrophy in the liver is the accumulation of intracellular lipids (fatty liver), microscopically seen fat-filled vacuoles [13]. In this present study, we only observed hepatic weights so still needed observation of hepatic structures to make sure the microscopic structure changes.

Hepatic disorders due to exposure to toxic compounds in the test materials are suspected not only on weights but also in hepatic function. Hepar is an organ that serves as a place of gluconeogenesis [4]. Koriem et al. [8] stated that Nimbidin has anti-hyperglycemic action so suspected test animals have decreased energy availability for metabolism. Omotayo et al. [11] stated that exposure to Nimba extracts causes an increase in ALT, AST, ALP and GGT enzymes that indicate liver damage. Liver damage is also indicated by an increase in bilirubin which is an indication of hemolysis and obstruction in the bile ducts. Both mechanisms of renal dysfunction are thought to cause test animals to experience decreased energy availability and enzymatic disturbance in metabolic processes that can lead to weight loss and ultimately lead to death in some test animals in this study.

Neem leaf extract exposure is also suspected to interfere with the biosynthesis of HDL and triglycerides in test animals. This is in accordance with the statement of Kupradinun et al. [9] and Koriem et al. [8] which states that Nimba's extracts cause serum HDL and triacylglycerol to decrease significantly. Hepar is a place of absorption and release of cholesterol, as well as lipoprotein biosynthesis, including HDL. Triacylglycerol is a major component of fatty deposits in cells that are also synthesized in the liver [4]. Differences in the mean of body weight, hepatic weight and HSI value in the study, were thought to be the result of interference of HDL biosynthesis and triacylglycerol due to exposure of ethanolic Neem extracts.

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Figure 1. The mice body weight after treated with ethanolic Neem leaf extract for 21 days

Figure 2. The Hepatic weight and HSI values of mice after treated with ethanolic Neem leaf extract for 21 days