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Effect of subchronic administration of nutmeg (*Myristica fragrans* Houtt) ethanolic extract to hematological parameters in rat

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Abstract. Nutmeg is dried kernel of broadly ovoid seed of *Myristica fragrans* Houtt. It has been mentioned in ethnomedical literature as aphrodisiac, stomachic, carminative, tonic, and nervous stimulant. In order to establish the safety of nutmeg, the effect of the repeated administration of nutmeg is needed. The study was aimed to determine the toxic effect of subchronic administration of nutmeg ethanolic extract to hematological parameters in rat. A total of 28 male adult Wistar rats divided into 4 groups. Group I as control was given by 0.5% CMC-suspension, group II, III, and IV were given by 50, 100, and 200 mg/kg bw, respectively, of nutmeg ethanolic extract. The treatments were administered daily for 31 days. On day 31 bloods were taken from orbital sinus. The hematological parameter consisted of the numbers of erythrocyte and leukocyte as well as hemoglobin and total protein levels were measured. The data were statistically analyzed by one way Anova followed by LSD test. All of observed hematological parameters in rats showed that there were no significant difference between the nutmeg ethanolic extract treated groups and control group. The result indicated that the subchronic administration of 50, 100, and 200 mg/kg bw of nutmeg ethanolic extract did not cause the change of hematological parameters in rat.

Keywords: *Myristica fragrans* Houtt, subchronic, hematological parameters

1. Introduction

Nutmeg is dried kernel of widely ovoid seed of *Myristica fragrans* Houtt. Nutmeg is an originally Indonesian plant come from the Banda and Maluku islands. Since ancient times in Indonesia, nutmeg has been used as a spice. Several medicinal and pharmacological properties of nutmeg have been reported. Nutmeg has aromatic, stimulant, narcotic, astringent, aphrodisiac, hypolipidaemic, anti-platelet aggregation, sedative and analgesic activities. Nutmeg seeds can also overcome sleep disorders, oxidative stress and be used as a remedy for stomach ache, rheumatism, and vomiting during pregnancy [1–3]. Eventhough nutmeg seed has been used for various symptoms, nutmeg can cause intoxication. Nutmeg essential oil contains pine, sabincene, camphene, myristin, elemicin, isoelemicin, eugenol, isoeugenol, methoxyeugenol, safrole, diametric phenylpropanoids, lignans and



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neolignas [4, 5]. Those compounds have been reported causing to paralysis, poisoning, drunkenness, anger, dizziness, falling, headaches, convulsions, hallucination and death [6].

Thus, the safety of nutmeg consumption has become important to be investigated. The toxic effects of the extract of nutmeg might occur with the administration of repeated doses in the animal system. The aim of this study was to investigate the toxic effect of sub chronic administration of nutmeg ethanol extract to the hematological parameters in male Wistar rat.

2. Methods

A total of 28 adult male Wistar rats (150-200 g), were randomly divided into 4 groups consisting of 7 rats per group. Rats were acclimatized for a week, and treated orally for 31 days. The treatment of each group as follows: Group I, as control, the rats were treated with a solution of 0.5% NaCl; Group II, the rats were treated with 50 mg/kg bw of nutmeg ethanolic extract; Group III, the rats were treated with 100 mg/kg bw of nutmeg ethanolic extract; Group IV, the rats were treated with 200 mg/kg bw of nutmeg ethanolic extract. At day 31, the blood were taken from orbital sinus for hematological parameters measurement including the numbers of erythrocyte and leucocyte as well as the levels of hemoglobin and total protein.

2.1. The measurement of erythrocyte number

The number of erythrocyte were obtained as previous method [7]. The average number of erythrocytes was calculated by the formula: the number of cells counted $\times 10$ (0.1 mm in) $\times 5$ ($\frac{1}{5}$ from 1 mm³) $\times 200$ (1: 200). The number of erythrocyte were expressed as 10⁶/mm³.

2.2. The measurement of leukocyte number

The number of leukocytes were evaluated according to the method described in the previous study [8]. The average number of leukocytes at day 31 in the all groups were calculated by the formula: the cells number counted $\times 20$ (1:20) $\times 10$ (0.1 mm in): 4 (number of squares in mm²). The number of leukocytes were expressed as 10³/mm³.

2.3. The measurement of Hb level

The level of Hb was evaluated according to the method described in the study by Guder, et al. [9] using spectrophotometer at 540 of wavelength. The formula for calculating hemoglobin (g/dL) is: the absorbance of standard/absorbance sample \times standard concentration. The Hb level was expressed as g/dL.

2.4. The measurement of total protein level

The number of total protein was evaluated according to the method described in the study by Guder, et al. [9]. The formula for calculating total protein (g/dL) was absorbance of sample/absorbance of standard \times standard concentration. The total protein level was expressed as g/dL.

3. Result and Discussion

Observation of blood parameters are very important in the diagnosis of a functional disorder of organs or tissues. Hematological examination may describe the function of body organs and physiological status. The data of the numbers of erythrocyte and leucocyte as well as the levels of hemoglobin and total protein in rat are presented in the Table 1.

Table 1. The number of erythrocyte and leukocyte as well as the level of hemoglobin and total protein (mean \pm SD) of blood in rat after administration of nutmeg ethanol extract for 31 days.

Groups	Dose (mg/kgBW)	Erythrocyte (10 ⁶ /mm ³)	Leukocyte (10 ³ /mm ³)	Hb (g/dL)	Total Protein (g/dL)
Control	-	7.848 \pm 0.409	7.86 \pm 0.801	10.640 \pm 0.551	8.252 \pm 0.595
Group I	50	6.520 \pm 0.379*	7.70 \pm 1.134	14.910 \pm 4.134*	8.726 \pm 0.426
Group II	100	6.466 \pm 0.288*	7.47 \pm 1.082	13.914 \pm 0.867*	7.732 \pm 0.500
Group III	200	5.962 \pm 0.179*	7.20 \pm 1.259	14.124 \pm 1.034*	7.852 \pm 0.368

* = p < 0.05, significantly difference compared with control group

3.1. The number of erythrocyte

Erythrocytes are the red blood cells that travel in the blood. They are typically shaped as biconcave disks and manufactured in the bone marrow. The erythrocytes carry hemoglobin in the circulation. The main function of erythrocytes is to distribute oxygen to body tissues, and carry waste carbon dioxide back to the lungs. In humans, they survive in the circulation for an average of 120 days [10].

The result showed that there were significant difference between the rats treated extract groups and control group. The reduced of erythrocytes cells number may be caused by the suppression of blood cell synthesis or red cell lysis by saponins. This finding supported the previous study that nutmeg contains saponin component which have toxic effects on the body's systems [11]. Saponin can cause hemolysis, the destruction of erythrocytes cell, resulting in the release of hemoglobin from the stromal erythrocytes (red blood grains). Hemolysis can be caused by a decrease in the surface tension of the cell membrane affected by several factors such as organic solvents, saponins, bile salts, soaps, enzymes, and other factors [12]. However, in the current study the number of erythrocytes in the extract treated groups showed still in the normal range (5.00-10.00 million/mm³). Thus it can be concluded that administration of nutmeg seed ethanol extract of did not affect the number of erythrocytes.

The results of this study was different with the previous study [13] reported that administration of 100, 250 and 500 mg/kg bw of nutmeg ethanol extract for 14 days could significantly decrease ($p < 0.05$) the number of erythrocytes. The difference of the study may be due to several factors such as the difference in concentration of ethanol solvent, the dose and duration of administration in rat.

3.2. The number of leukocytes

Leukocytes or white blood cells are the cells of the immune system involved in protecting the body against both infectious disease and foreign invaders. Immune system refers to the body's system ability to resist or eliminate abnormal cells or xenobiotic potentially damaging. Leukocytes and their derivatives can prevent pathogens invasion (disease-causing microorganisms, such as bacteria and viruses) through the process of phagocytosis, identifying and destroying cancerous cells that appear in the body and as cleansers that clean up body waste by phagocytically debris originating from dead cells or injury [14].

The result showed that there were no significant difference between the rats treated extract groups and control group. The result in all groups was within in normal range. The number of normal leukocytes of rat is 3.00-17.00 (10³/mm³) [15]. The administration of nutmeg ethanol extract for 31 days did not affect the number of leukocytes.

3.3. The level of haemoglobin (Hb)

Hemoglobin is a molecule which play an important role for storing and carrying the oxygen in the body. It contains a small amount of iron and heme protein. Hemoglobin has a strong appeal to oxygen. When red blood cells pass through the lungs, hemoglobin will bind oxygen from the air forming oxyhemoglobin [16].

The result showed that the mean of Hb level of group treated by 50 mg / kg bw of nutmeg ethanol extract was greater than the control group, however statistically there was no significant difference ($p > 0.05$). Whereas the administration 100; 200 mg / kg bw of extract and the dose of 200 mg / kg bw significantly increase the hemoglobin levels compared to the control group ($p < 0.05$). However, the increase in hemoglobin levels are still within the normal range (11.0-19.0 g/dL) [15]. Thus it can be concluded that administration ethanol extract of nutmeg seed did not change on blood hemoglobin level.

3.4. The total protein level

Plasma total proteins are a group of plasma constituents that are not simply transported. These essential components in normal state remain in the plasma, where they perform many useful functions. Because of a large plasma constituent, plasma proteins usually did not exit to the pores in capillary walls. Unlike other plasma constituents that water soluble, plasma proteins are in the form of colloidal dispersions [17].

The decreased of total protein (called hypoproteinemia) might be caused by prolonged malnutrition, starvation, low protein diet, malabsorption syndrome, gastrointestinal tract cancers, ulcerative colitis, Hodgkin's disease, severe liver disease, severe burns and water intoxication. Meanwhile the increased of total protein levels (hyperproteinemia) can be caused by dehydration (hemoconcentration), multiple myeloma, vomiting, diarrhea, respiratory distress syndrome, and sarcoidosis. Serum protein levels may return to normal if the client is adequately hydrated

The result showed that there were no significant difference between the rats treated extract groups and control group in total protein. The result in all groups were within in normal range. The normal total plasma protein level of rat was 5.9 – 8.4 g/dL [15]. The administration of ethanol extract of nutmeg seed at all doses did not affected protein total level.

4. Conclusion

The sub chronic administration of 50, 100 and 200 mg/kg bw of nutmeg ethanol extract for 31 days did not cause the change of the numbers of erythrocytes and leucocytes as well as the levels of hemoglobin and total protein in blood of rat.

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