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Acute Toxicity Studies of Nutmeg Pulp Ethanol Extract (*Myristica fragrans* Houtt.) in Balb/c Mice

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ABSTRACT

Myristicin is a chemical frequently present in nutmeg (*Myristica fragrans* Houtt.). Excessive consumption might result in Toxicity and hallucinogenic effects. The compound is found throughout the nutmeg fruit, with the most significant quantity found in the pulp, measuring 36.05%. This research seeks to ascertain the acute toxicity of nutmeg pulp ethanol extract (*Myristica fragrans* Houtt.) in Balb-C mice (*Mus musculus*). A total of 20 female mice were categorized into four groups: the control group, NPE 750 mg, NPE 1500 mg, and NPE 2500 mg. The test preparation was administered orally with a single dose at the study's commencement, and toxicological symptoms were observed over 14 days. The findings indicated that the ethanol extract of nutmeg pulp (*Myristica fragrans* Houtt.) at doses of 750 mg/kgBW, 1500 mg/kgBW, and 2500 mg/kgBW exhibited signs of toxicity, including grooming, tremors, anorexia, ptosis, and fluctuations in body weight, with an LD_{50} value of 2964.83 mg/kgBW. Histopathological examination of the liver tissue revealed increased hepatocyte necrosis at the highest dose of 2500 mg/kgBW. In conclusion, the ethanol extract of nutmeg pulp exhibits toxic potential when administered at doses exceeding 750 mg/kgBW. Moreover, it affects the liver when consumed at doses over 2500 mg/kgBW

Keywords: *Acute, LD_{50}, Myristica-fragrans, Nutmeg-Pulp, Toxicity. Liver*

Introduction

The healthcare industry has undergone significant development due to the advancements in science and technology, particularly herbal medicine.[1] Herbal medicines originating from plants have several merits, such as low cost, availability, and relatively few side effects.[2,3] Nutmeg (*Myristica fragrans* Houtt.), which is one of the spices used commonly in Indonesia is widely consumed by the community, and it is produced in greater amounts in Maluku and North Maluku islands. Numerous studies have demonstrated the benefits of nutmeg, including promoting digestion, controlling appetite, relieving pain, decreasing gastric acidity.[4] Other benefits include improved blood circulation, stimulation of the cardio system, and the relief of respiratory disorders such as colds and cough.[5,6] The bioactive compounds that exert benefits of nutmeg include, among others, alkaloids, flavonoids, saponins, tannins, and myristicin.[5,7,8] Myristicin is the primary metabolite in nutmeg. The pulp of nutmeg contains the highest concentration of myristicin, at 36.05%, compared to the mace at 17.54% and the seeds at 16.50%.[9] Myristicin exhibits various pharmacological activities, but when consumed in excess, it can lead to toxic effects.[10,11] Between 1996 and 1998, there were reports of myristicin poisoning in humans whoingested 14-80 g of ground nutmeg.

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Additionally, studies on nutmeg toxicity in animals have shown that administering doses of 500mg and 1000mg/kgBW of nutmeg orally for 42 days can result in organ degeneration and atrophy.[11] Toxic effects commonly target the liver, one of the body's largest and heaviest organs, which plays a key role in metabolic processes.[12] As myristicin is metabolized in the liver, it can lead to liver degradation and produce toxic compounds.[10] Myristicin is also known to cause hepatic steatosis and hepatic necrosis, with the borderline toxic dose ranging from 1-2 mg. Exceeding this dose can result in systemic disturbances, affect the central nervous system, and potentially lead to death.[13] However, no research has yet been conducted to determine the toxicity threshold for consuming nutmeg pulp. Preclinical testing, including acute toxicity studies, is necessary to establish the toxicity limits of this compound.

Materials and Methods

Animals Preparation

A number of 20 female Balb/c mice aged around 2 months and weighing between 20-30 g were acclimatized for 2 weeks at a 27°C lab facility, and during acclimatization, the mice were fed 20% of their body weight as pellets and 45 ml of water daily. The mice were housed in four square plastic boxes with wire lids and husk bedding. The cages were cleaned every two weeks, the bedding was replaced, and the enclosures were scrubbed down with disinfectant.

Extract preparation

Nutmeg pulp (*Myristica fragrans* Houtt.) was sourced from Ternate City, North Maluku, Indonesia, in January 2024. The classification of the nutmeg plants was conducted by a taxonomist at the Pharmaceutical Biology Laboratory, Department of Pharmacy, Medical Faculty, Khairun University, under reference number 020/UN44.C9/PF/DT/2024. Following collection, the nutmeg pulp underwent a series of preparatory steps, including peeling, washing, and drying. A total of 500 g of the dried pulp was then subjected to

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maceration with a 70% ethanol solvent at a ratio of 1:4 for 24 hours. The resulting liquid extract was subsequently concentrated using a rotary vacuum evaporator and air-dried to eliminate residual solvent.

Acute Toxocity Studies
The mice were categorized into four experimental groups: a control group, NPE 750 mg/kgBW, NPE 1500 mg/kgBW, and NPE 2500 mg/kgBW. Each group received a single oral dose of the extract or carboxymethyl cellulose (negative control) on the first day. Observations persisted for 14 days post-administration of the extract, concentrating on behavioural symptoms, including Straub reaction, piloerection, pineal reflex, corneal reflex, grooming behaviour, and seizures. Body weight was recorded, and the LD_{50} was established by quantifying the number of mortalities within 24 hours after administering the test substance.[14]

Preparation of Liver Histopathology
On the 15th day, the animal was euthanized, and the liver was excised for histopathological examination. After the samples were fixed in a 10% buffered formalin solution, the tissue underwent processing with graded alcohol concentrations (80%, 90%, and 95%) for dehydration, xylene clearing, and paraffin embadding. After the fixation of the tissue in a paraffin embadding, it was micro-sliced to a thickness of 4µm utilizing a microtome. Each slide was stained with hematoxylin-eosin (HE).[15] All experimental techniques have obtained ethical approval from the Animal Ethics Committee of the Medical Faculty and Health Sciences, Khairun University (number 015/KEPH/PH/2024).

Preparation of Liver Histopathology
Liver histopathology slides were examined under a light microscope (Olympus CX31, Japan) at 400x magnification.

Data Analysis
The research data consists of both quantitative and qualitative components. The quantitative data, including the count of mice mortalities to ascertain the LD_{50} value and the average body weight of the mice, were analyzed using one-way ANOVA, followed by post hoc Tukey's test to identify significant differences at $p<0.05$ compared to the negative control group. The qualitative data encompasses behavioural observations and liver histological examinations, also compared to the negative control group

Results and Discussion

The test material utilized in this study was nutmeg pulp (*Myristica fragrans* Houtt.). Nutmeg is a spice plant with multifaceted utility since every portion of the plan can be utilized in various industries.[16] The nutmeg pulp was processed into a concentrated extract using the maceration method in this study. According to the observations of murine behaviours (refer to Table 1). The grooming behaviour of mice involves cleaning themselves by licking their bodies, a practice commonly observed in animals. A decrease in grooming frequency signifies stress on the central nervous system. An increase in grooming frequency signifies stimulation of the central nervous system. The grooming in the control group exhibited minimal variation; the treatment group also did not undergo significant alterations (consistent).[7,17]

In the Straub behaviour test, no groups displayed mice tails perpendicular to the floor and appeared rigid. This indicates the absence of central nervous system stimulation, particularly in the spinal cord.[18-20] The control group had negative symptoms in piloerection and tremor behaviour, while all test groups demonstrated positive symptoms. Piloerexia is characterized by hair standing on the bodies of mice, resulting from a sensitive reaction to tactile stimuli, spontaneous activity occurs due to central nervous system (CNS) activation.[18] Simultaneously, tremor symptoms are characterized by the shaking of the mice body. This occurs when stationary or during action due to the stimulating effect of the central nervous system (CNS) from the test material. Sixteen and seventeen test groups exhibited positive behaviour in ptosis behaviour. [21,22] Ptosis was noted in the eyelids of the mice, which were partially closed or appeared lethargic. The effects of Myristicin, which has a calming influence, induce patient symptoms.[20,23,24]

Table 1: Observation results for test animal behavior

Groups	GR	SR	PR	TR	PT
Negative Control	+	-	-	-	-
NPE 750mg/kgBW	+	-	+	+	+
NPE 1500mg/kgBW	+	-	+	+	+
NPE 2500mg/kgBW	+	-	+	+	+

GR (grooming), SR (straub), PR (piloerection), TR (tremor), PT (ptosis)

Table 2: Mice body weight

Groups	Average Body Weight (gram±SD)	
	Initial Weight	Final Weight
Negative Control	26.80±1.93	25.33±14.06
NPE 750mg/kgBW	23.80±2.18	23.50±12.87
NPE 1500mg/kgBW	23.60±2.19	17.60±9.91*
NPE 2500mg/kgBW	26.20±2.16	10.40±14.34*

*significance $p<0.05$ compared to negative controls;
NPE: Nutmeg Pulp Extract

The subsequent parameter is the body weight of the mice, which was recorded during the testing phase. Variations in mice's body weight may signify toxicity from exposure to harmful substances. Alterations in body weight signify adverse effects from a pharmaceutical or chemical agent and diminish markedly if body weight is decreased by 10% of the test animal's baseline weight. Table 2 indicates that the mean body weight of mice diminished from the commencement to the conclusion of the 14-day dosing period. The NPE 1500mg/kgBW and 2500mg/kgBW groups exhibited the most substantial reduction, with a significance value of $p<0.05$ relative to the control group, leading to the conclusion that the test material influenced the body weight of the mice. The reduction in the weight of mice may result from diminished hunger, irregular eating patterns, and the condition of the test subjects (stress).[25] It may also result from elevated doses, signifying a toxic effect.

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The quantity of mice fatalities (refer to Table 3) is the foundation for calculating the LD_{50} value of the extract. On the initial day following the administration of the extract, no fatalities occurred among the test animals in any group. Nevertheless, by the 14th day, one mouse from each of the NPE 1500 mg/kgBW and 2500mg/kgBW groups perished, indicating that the administration of NPE at doses of 1500 mg/kgBW and 2500 mg/kgBW exhibited toxicity in toxicity subjects. Utilize the mortality data to conduct the LD_{50} calculation, ascertaining the toxic classification encountered by the test subjects upon administering the extract at that dose.

The results indicate that the administration of ethanol extract from nutmeg pulp falls within the mild toxic category, as it possesses an LD_{50} of 2964.83 mg/kgBW. The research findings diverged from those of Prasidya et al., 2024, who conducted an acute toxicity test on Myristicin using pure myristicin molecules at 5, 50, 300, and 2000 mg doses. The study's results indicated a 2000 mg/kgBW dose.[10] It did not result in mortality among test animals and was classified under mild toxicity.

Table 3: The number of animals that died during testing

Groups	Number of Mice	Number of Mice Deaths	
		1st day	14th day
Negative Control	5	0	0
NPE 750mg/kgBW	5	0	0
NPE 1500mg/kgBW	5	0	1
NPE 2500mg/kgBW	5	0	1

NPE: Nutmeg Pulp Extract

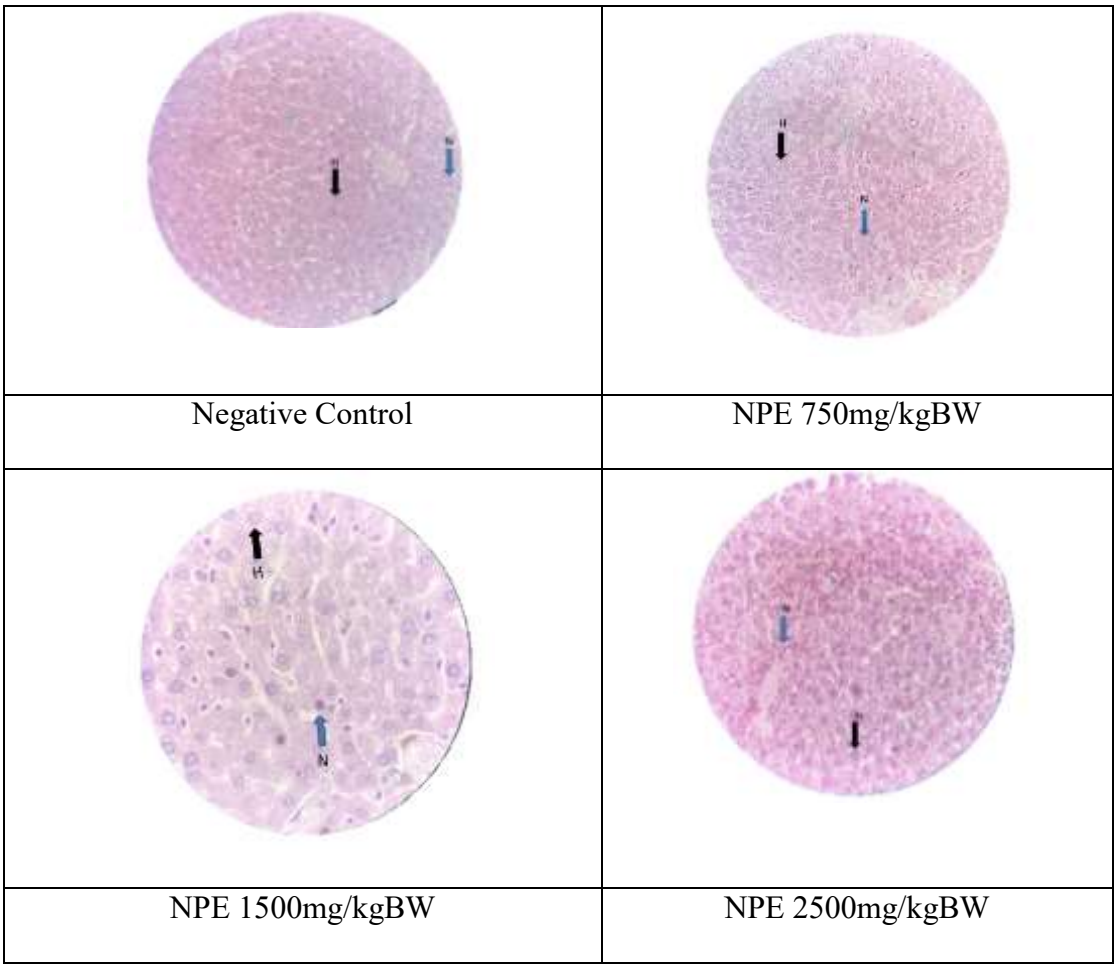

Figure 1: Histopathology of the liver of female Balb-c mice with HE staining at 400x magnification, showing hepatosis/normal cells (H) and necrosis/damaged cells (N).

The discrepancy may arise from the nature of the test material, as the extract contains residual components that require separation, thus serving as a confounding variable in the research outcomes. The final parameter is a histological examination of the liver, focusing on liver cells that have undergone necrosis (refer to Figure 1). Microscopic examinations at 400x magnification reveal variations in hepatocyte cells across different histological sections of the liver in mice. The number of healthy hepatocytes in the control group appeared normal, whereas hepatocyte cellular damage was more pronounced in the NPE 2500 mg/kgBW group. Consequently, an increased dose administered to the mice resulted in a more significant presence of necrotic cells within the hepatocytes. Cell necrosis is a process of cellular damage marked by cellular swelling, protein denaturation, and organ impairment, resulting in significant tissue malfunction.[26,27] The impact of nutmeg poisoning on the liver is contingent upon the length and dose of administration.[28]

Conclusion

The research findings suggest that the ethanol extract of nutmeg pulp exhibits toxic effects when ingested at doses surpassing 750 mg/kgBW.

Moreover, it impacts the liver when consumed at doses over 2500 mg/kgBW.

Conflict of interest
The authors declare no conflicts of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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