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Histopathological changes in pregnant mice's hepar and kidney following exposure to kelor (*Moringa oleifera*) leaf extract

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ABSTRACT

Background: Moringa provides extra dietary benefits. The immune system is significantly influenced by the nine necessary amino acids, calcium, iron, potassium, magnesium, zinc, and vitamins A, C, E, and B that are included in this food. Previous studies state that moringa causes liver and kidney damage. Another study found that moringa has an abortive effect.

Objectives: This study aimed to determine how exposure to kelor (moringa oleifera) leaf extract affected the hepar and kidney in pregnant mice and found the secondary metabolite of the moringa leaves.

Materials and Methods: An experimental laboratory design was conducted in 24 pregnant female Balb/c mice. They were randomized into four groups. Group K was not given anything. Group P1-P3 were given moringa leaf extract during pregnancy at days with a dose dose of 10, 20, and 30 mg in 10, 20, and 18 days, and termination was done to take the organs and make the tissue processing.

Results: We found no dead mice and no aggression during the experiment. Significant statistics differed between the experimental group in the microscopic appearance of the kidney and hepar. This study found degeneration and inflammation in the hepar and kidney in the treatment group. Qualitative phytochemical tests on leaves contained alkaloids, flavonoids, phenolics, tannins, and steroids.

Conclusion: The ethanol extract of Moringa leaf has shown a mild heparin and kidney effect compared to the control.

Keywords: *hepar; kidney; Moringa oleifera; phytochemical; pregnant*

BACKGROUND

Various regional names for *Moringa Oleifera* include benzolive, drumstick tree, kelor, marango, mlonge, mulangay, nébéday, saijhan, and sajn. [1] Every part of this tree is edible, from the leaves, trunks, and stems down to its root. [1]

Moringa trees have been used in developing tropical countries to combat malnutrition, especially among infants and nursing mothers.[1] Previous research states that one of the efforts to address malnutrition is the use of Moringa as a source of additional dietary because moringa leaves contain complete protein (contains nine essential amino acids), calcium, iron, potassium, magnesium, zinc, and vitamins A, C, E, and B which have a major role in the immune system. A study on the provision of Moringa leaf powder was randomly assigned to two groups of breastfeeding mothers with babies 3-4 months, each given moringa leaf powder and tablets of iron / folic acid (control). After three months of therapy, the average levels of hemoglobin concentration increased significantly in treatment groups, although plasma ferritin levels were not significant in the group receiving.[2] Moringa leaves dry powder contains 25 times more iron than spinach, one of the therapeutic agents for anemia.[3] Moringa leaves powder in the nutritional recovery from severe acute malnutrition, consumption of Moringa leaves powder should be encouraged to improve the nutritional status of children and prevent some micronutrient deficiencies such as iron and Vitamin A because of the nutritional value of its leaves commonly used in Burkina Faso. [4]

A large number of additional biological activities, such as antioxidants, tissue protection (for the liver, kidneys, heart, testes, and lungs), analgesics, anti-ulcers, antihypertensives, radioprotective, and immunomodulatory actions, have been demonstrated by a large number of studies that are being published at a rapid rate. [5]

The qualitative methods of phytochemical analysis using dry extracts of Moringa leaves found flavonoids, alkaloids, steroids and triterpenoids, saponins, Tannins, and Phenols.[6] Other research found that the root extract possesses contraceptive potentials and is teratogenic and abortifacient in rats. The root has an

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equally induced post-coital antifertility effect in rats and has been demonstrated to induce fetal resorption in pregnant rats. [7]

Paul and Didia (2012) investigated the impact of methanol extract of M. oleifera root on the histoarchitecture of the liver and kidney in 24 guinea pigs, which were/were examined. Three weeks of daily intraperitoneal injections of the root extract at dosages of 3.6, 4.6, and 7.0 mg/kg were used as the experimental protocol. All treated groups' histological sections showed bloating liver deterioration, which is more consistent with a time-dependent toxicity than a dose-dependent response. In the 4.6 mg/kg group, examination of the kidneys revealed minor tubular damage and interstitial inflammation. In contrast, in the 7.0 mg/kg group, the interstitium had been invaded by inflammatory cells and amorphous eosinophilic debris.[8] Although some literature claimed that Moringa oleifera extract has abortive properties, this presented a problem for users.[9]

The study suggests that the lipid-rich seed extracts of Moringa seeds may be harmful during pregnancy, causing liver and kidney damage. On the other hand, the lipid-free Moringa does not have inherent toxicity, as the hydrophobic component has been found to be toxic, as observed in the study. This toxicity can have an impact on the development of female offspring in rodents. [10]

Several research have reported contradictory results about the impact of Moringa on pregnancy. While some individuals have claimed that it had abortifacient characteristics, others have seen its potential benefits for pregnancy. The conflicting information has resulted in perplexity among end users. [9] This study aimed to determine how exposure to kelor (moringa oleifera) leaf extract affected the hepar and kidney in pregnant mice and found the secondary metabolite of the moringa leaves.

MATERIAL AND METHODS

Research design

This was an experimental research conducted in the Department of Histology-Faculty of Medicine Diponegoro University and Animal Laboratory Faculty of Medicine Diponegoro University within six months from March to August 2017.

Our experimental work utilized an in vivo post-test-only control group design with pregnant Balb/c mice. The assignment of animals to each group was conducted randomly by a straightforward randomization technique to mitigate selection bias. All medications were provided orally during a designated gestational time. Upon conclusion of the treatment period, the animals were euthanized, and the liver and kidney tissues were harvested for histological examination.

Subjects and treatment of the study

Twenty female Balb/C strain mice that were 8–10 weeks old, 25–35 grams in weight, healthy, and anatomically typical served as the participants. For 12 hours, from 18:00 to 6:00 am, each cage of three female mice was housed with one male mouse. The presence of a vaginal plug and sperm in the vaginal smear was used to identify the day of conception. [11] Four groups formed from all the pregnant mice. Six mice in each group were randomly selected, acclimated for seven days, and then divided into groups. The P1-P3 treatment groups received moringa leaf extracts at doses of 10, 20, and 30 mg/kgBB, while the control group received aquades. Since the start of the therapy, the aggressiveness of the mice has been observed, as well as their daily behaviors and combative behavior with other mice in one cage up until the 18th day of pregnancy. [12] The extract using the maceration method.

Histopathological examination

On the 19th day, pregnant mice were terminated for microscopic preparations. The mouse hepar and kidney were treated microscopically using hematoxylin and eosin (HE). Every preparation was examined under a microscope to determine the histopathological index from five different fields of view. A grading system was used to assess the level of tissue injury: 0 for no damage, 1 for 10% damage, 2 for 11% to 25% damage, 3 for 26% to 45% damage, 4 for 46% to 75% damage, and 5 for more than 76% damage. An Olympus EP50 microscope equipped with a digital camera and image processing software was used to measure the field. This study's ethics were approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Diponegoro Number. 05/EC/ H/ FK-RSDK/2017.

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RESULTS AND DISCUSSION

Microscopic of Hepar

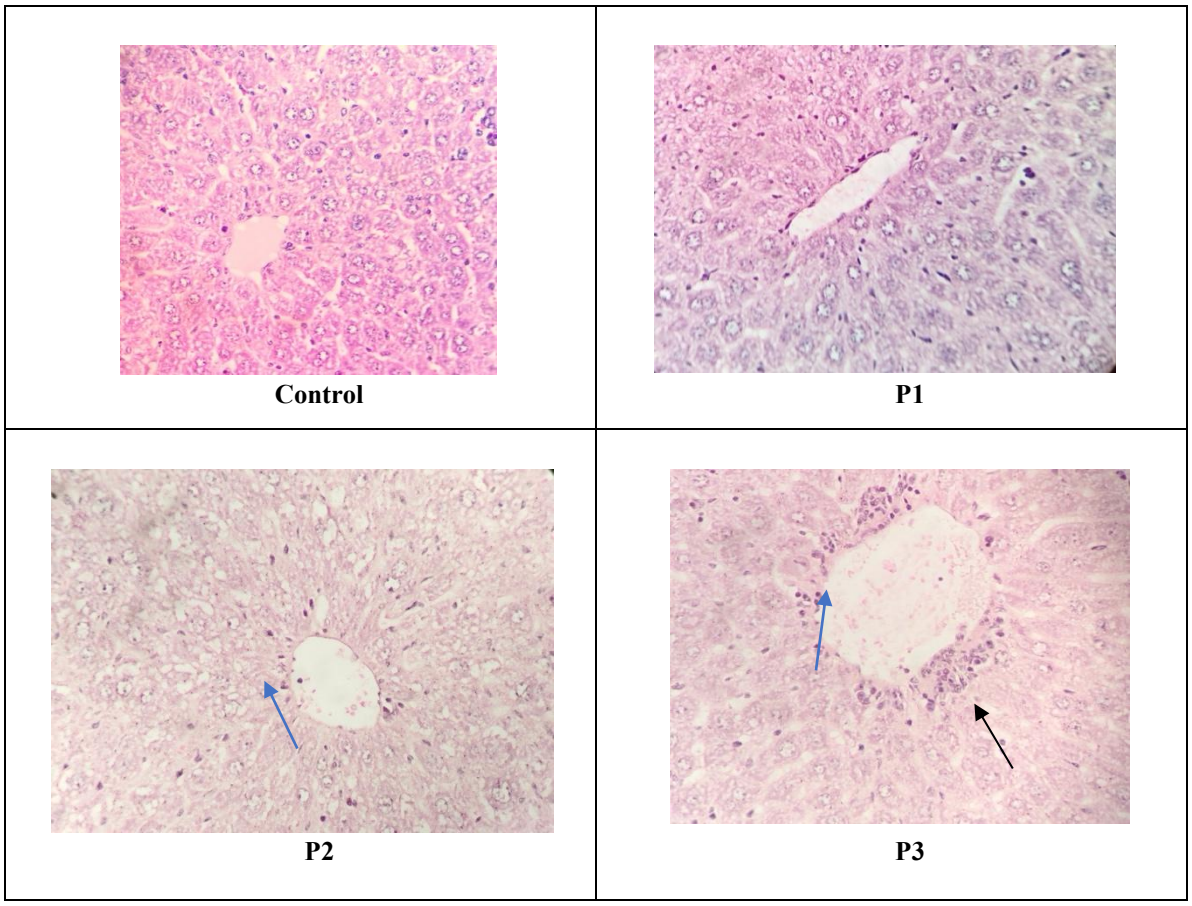

Figure 1. Microscopic appearance of mice hepar
Blue arrow: degeneration, black arrow: inflammation (leucocyte infiltration), yellow arrow (erythrocyte infiltration)

According to Figure 1, there was a significant difference in hepar damage compared to the control group. Significant damage was observed in the histopathological appearance, especially lobular degeneration and inflammatory cell infiltration in P2 and P3. (Table 1).

Table 1. P values of Hepar Damage with Kelor Leave Extract Test Results by Mann Whitney

	K	P1	P2	P3
K	-	0.359	**0.008***	**0.003***
P1		-	0.085	**0.037***
P2			-	0.718
P3				-

The comparisons between K and P2, K and P3, and P1 and P3 were significantly compared, whereas those between K and P1, P1 and P2, and P2 and P3 were not compared considerably.

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Microscopic of Kidney

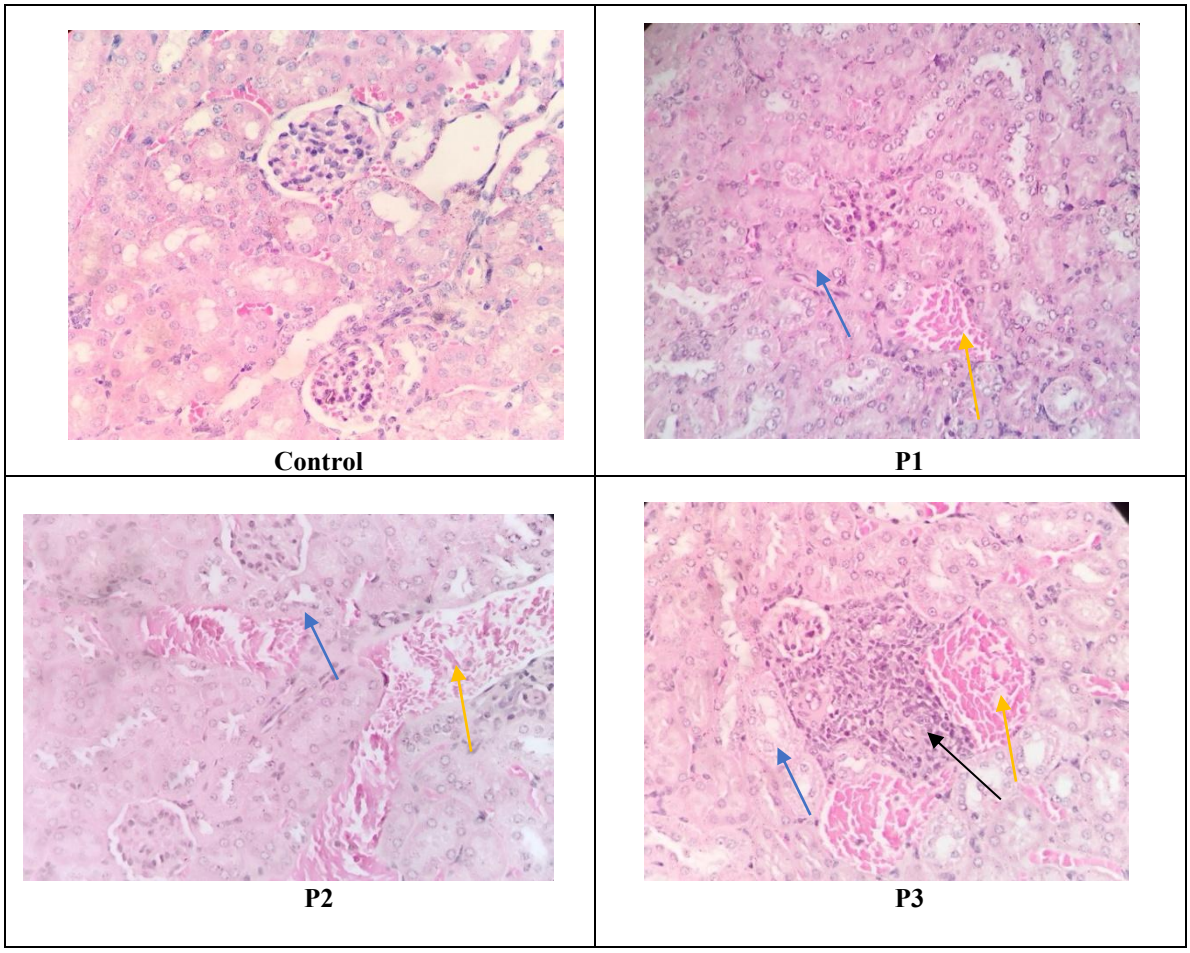

Figure 2. Microscopic appearance of mice kidney
Blue arrow: degeneration, black arrow: inflammation (leucocyte infiltration), yellow arrow (erythrocyte infiltration)

A histopathology kidney slide found degeneration, especially degeneration and infiltration in lymphocytes in all experimental groups. Higher doses showed higher damage.

Table 2. P values of Kidney damage with Kelor Leave Extract test results by Mann Whitney

	K	P1	P2	P3
K	-	**0.047***	**0.003***	**0.000***
P1		-	0.307	0.126
P2			-	0.610
P3				-

The comparisons between K and P1, K and P2, and K and P3 were significantly compared, whereas the comparisons between P1 and P2, P2 and P3, and P1 and P3 were not compared considerably.

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Table 3. Secondary Metabolite in Moringa Leave Extract.

Secondary metabolites	Reagen	+/-
Alkaloid	Dragendorff	+
Flavonoid	Mg, HCl dan amil alkohol	+
Saponin	HCl	+
Fenolik	$FeCl_3$ 1%	+
Tanin	$FeCl_3$ 5%	+
Kuinon	NaOH	+
Triterpenoid	Lieberman-Burchard	-
Steroid		+
Semi Kuantitatif Steroid		++

Qualitative phytochemical tests on leaves revealed alkaloids, flavonoids, phenolics, tannins, and steroids. The examination was continued with a semi-quantitative examination of steroids. The results, namely steroid examination, obtained a greener color.

DISCUSSION

Due to their natural origins and negligible side effects, herbal medicines have experienced a significant development in popularity in both developed and developing nations. [14] This is not the case Despite the plant's supposed nontoxicity. While supplement amounts seem safe from all tested toxicity, a minimal increase (three to four times the recommended dose) can induce genotoxic damage. It may encourage cancer development, while higher doses cause overt organ damage (mainly to the liver and kidneys). Beyond that, supplementing is contraindicated (not advised) in pregnant women because it appears that even extremely low supplement dosages might cause abortions in pregnant rats. [14] Moringa is currently being utilized more frequently as a food additive, a clarifier for dirty water, and as a phytomedicine without taking into account its possible toxicity. [10] Prior studies have indicated that the liver tissues of all experimental groups exhibited infiltration of inflammatory cells.[10] Tubular necrosis, a form of kidney toxicity, was detected in all groups who were administered Moringa. Additionally, the liver of the animals under investigation exhibited pathological alterations. These data obtained from experiments conducted inside a living organism indicate that the Moringa extract, which has a high amount of lipids, could potentially cause harm to the liver and kidney. This statement contradicts the findings of various local publications that have shown the positive effects of Moringa in protecting the liver and kidneys. However, it aligns with the well-researched in vitro studies conducted by Al-Anizi et al. [15] The conflicting reports may be attributed to the specific plant component employed in the analysis, varying dosage levels, regional differences, or other relevant considerations [10]. Both male and female progeny had different levels of pathological changes in their kidney and liver tissues, indicating the presence of poisoning. The toxicity seen was eliminated when lipid-free Moringa was given at a dosage of 300 mg/kg body weight. [10]

The rat's serum sodium and potassium levels were significantly affected by the administration of 300mg and 600mg/kg body weight of Moringa aqueous leaf extract. Reduced levels of sodium in the blood might cause the body to retain water. The elevated bilirubin levels seen in the high-dose treated group indicate that the high dosage of the extract may enhance the likelihood of developing jaundice.[16]

Previous research found the extract of Moringa oleifera leaves' phytochemical studies showed flavonoids ++, alkaloids ++, saponins +, tannins +, proteins ++, carbohydrate +, reducing sugars +, steroids ++, and terpenoids +. [17] The results of the literature study show that the bioactivity content of secondary metabolite compounds from various plant families that have the potential to antifertility can be divided into two based on the principle of action of antifertility materials, namely cytotoxic (tannins and abrin) and hormonal (alkaloids, flavonoids, steroids, and triterpenoid saponins) [18] Alkaloids are one of the compounds that are believed to have toxic properties to ren that disrupt the structure and function of the cells that make up the glomerulus. [19] Tannins can induce the contraction of cell membranes, leading to the disruption of the

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transport of food substances or nutrients across these membranes. Consequently, the transportation of food substances is hindered, which in turn disrupts cell metabolism and the production of energy.[18]

According to a prior study, oral administration of Moringa oleifera dried leaf powder up to 2000 mg/kg did not affect clinical symptoms or gross pathology, and its oral toxicity (LD50) is more significant than 2,000 mg/kg. [21] This study found mild damage in the liver and kidney; this could still be due to the physiological effects of pregnancy or the effects of moringa. This study used a minimal dose, far below the toxic dose, and the daily dose was used widely in the community. However, caution is needed for pregnant women in the use of this extract so as not to exceed the toxic dose.

CONCLUSION

The ethanol extract of Moringa leaf has exhibited potential effects on the liver and kidneys. Consequently, its use during gestation should be undertaken with prudence. Additional research is required to investigate its effects on other organs and endocrine systems.

CONFLICT OF INTERESTS

There is no conflict of interest in this study.

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