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Effect of *Moringa oleifera* Leaf Extract on Liver and Kidney Histopathology of Sodium Nitrite-Induced Male Rats (*Rattus norvegicus*)

Niken Ayu Prachelia Putri[1], Lucya Rahayu Putri[1*], Elsa Yuniarti[1], Moralita Chatri[1], Siska Alicia Farma[1], Yuni Ahda[1], Yusni Atifah[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang, Padang, Indonesia

*Corresponding author.
E-mail address: lucyaputri147@gmail.com

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Abstract

Background: Sodium nitrite ($NaNO_2$) is a widely used meat preservative that, despite its regulatory approval, can generate reactive oxygen species (ROS) and induce oxidative damage to the liver and kidneys when consumed in excess. This study aimed to determine the effect of *Moringa oleifera* leaf extract on the liver and kidney histopathology of sodium nitrite-induced male rats (*Rattus norvegicus*) and to identify the extract dose with the greatest effect.
Methods: This experimental study used a completely randomized design with 25 male rats allocated to five groups of five: a negative control (no induction), a positive control induced with sodium nitrite (37.5 mg/kgBW), and three treatment groups given Moringa leaf extract at 200, 400, and 600 mg/kgBW after nitrite induction. Histopathological slides of the liver and kidney were prepared by the paraffin method, stained with hematoxylin-eosin, and examined under a light microscope at 400× magnification.
Results: *Moringa oleifera* leaf extract had a significant effect on the liver and kidney histopathology of the sodium nitrite-induced male rats, and the extent of histological recovery differed across doses.
Conclusion: Among the doses tested, 600 mg/kgBW (P3) produced the greatest improvement in the liver and kidney histopathology of the sodium nitrite-induced male rats.

Keywords: *moringa leaf extract, sodium nitrite, liver, kidney, histopathology*

Introduction

Rapid lifestyle changes associated with globalization have increased consumer demand for instant food, including ready-to-eat meat products such as corned beef, sausages, and canned meat.[1] Because meat is highly perishable and susceptible to microbial spoilage, these products are commonly formulated with food additives (FA), that is, substances added during processing to influence the properties, texture, or shelf life of food.[2,3] Sodium nitrite is one of the preservatives most frequently used in processed meat.[4-6]

Sodium nitrite, with the molecular formula $NaNO_2$, is an odorless, white chemical compound that is soluble in water.[7] Its use as a food preservative is permitted, but its application requires careful monitoring: prolonged use can cause tissue damage and elevated lipid peroxide levels, as sodium nitrite can act both as a carcinogen and as a free radical that increases oxidative stress.[8-10] Excessive consumption of sodium nitrite can damage hepatic cells because $NaNO_2$ is toxic to the body; its nitrite content increases the production of reactive oxygen species (ROS), and long-term consumption can impair organ function, including that of the kidneys.[11,12] When sodium nitrite reacts with oxygen in the body, it forms nitric oxide, which can disrupt kidney function.[13,14] Consuming antioxidant-rich foods such as fruits and vegetables is one strategy to mitigate liver and kidney damage, and Moringa leaf is one plant known to be rich in antioxidants.[15]

Moringa (*Moringa oleifera*) is a tropical plant found throughout Indonesia, and every part of the plant can be used as food or medicine. Moringa leaves contain tannins, terpenoids, alkaloids, and saponins, as well as bioactive compounds such as isothiocyanates, phenolics, and glucosinolates, several of which have been associated with anti-inflammatory, antidiabetic, antimicrobial, and anticancer effects.[16] Through their antioxidant activity, flavonoids such as the quercetin present in Moringa leaves scavenge and neutralize free radicals, thereby helping to repair damaged tissue and suppress the inflammatory process.

Given this background, the present study was conducted to determine the effect of *Moringa oleifera* leaf extract on the liver and kidney histopathology of sodium nitrite-induced male rats (*Rattus norvegicus*) and to identify the extract dose with the greatest effect on liver and kidney histopathology under this induction model.

Methods

Materials and Equipment

The equipment used comprised rat cages, wire mesh, a fan or air conditioner, feeding and drinking containers, and a balance for animal husbandry; an oven, blender, sieve, water bath, hot plate, rotary evaporator, measuring cylinder, Erlenmeyer flask, beaker glass, stirring rod, and gastric sonde for extract preparation; and a dissecting set, sample bottles, incubator, refrigerator, glass slides, cover slips, microscope, brush, microtome, specimen box, and standard stationery for histological processing.

The materials used consisted of standard pelleted feed, drinking water, and rice husk bedding for the rats; Moringa leaves, distilled water, sodium nitrite, Bouin's solution, graded ethanol (70%, 80%, 90%, and 95%), absolute ethanol (I, II, and III), xylol (I, II, and III), paraffin, hematoxylin-eosin stain, egg white, tap water, gloves, and filter paper.

Experimental Design and Animal Grouping

This study used a completely randomized design (CRD) comprising five groups: two control groups and three treatment groups. K1 (negative control) received neither Moringa leaf extract nor sodium nitrite; K2 (positive control) was induced with sodium nitrite at 37.5 mg/kgBW; P1 received Moringa leaf ethanol extract at 200 mg/kgBW after induction with sodium nitrite at 37.5 mg/kgBW; P2 received Moringa leaf ethanol extract at 400 mg/kgBW after induction with sodium nitrite at 37.5 mg/kgBW; and P3 received Moringa leaf extract at 600 mg/kgBW after induction with sodium nitrite at 37.5 mg/kgBW.

Preparation of Moringa Leaf Extract

Moringa leaves were separated from their stems and dried in an oven at 60°C for 5 hours until brittle enough to be crushed by hand. The dried leaves were then ground into powder using a blender and sieved to obtain a fine, homogeneous sample.

The powdered Moringa leaves were extracted by maceration using ethanol as the solvent. A 300 g portion of the leaf powder was placed in a soaking vessel and immersed in 96% ethanol at a ratio of 1:10 to facilitate extraction of the compounds. The vessel was then sealed and left to stand for three days in a location protected from direct sunlight, with periodic stirring to enhance diffusion of the dissolved compounds. After three days, the macerate was filtered through filter paper to obtain the first extract. The residual leaf powder was then re-macerated in fresh 96% ethanol for a further three days to obtain a second macerate. The first and second macerates were combined and concentrated using a rotary evaporator at 60°C to yield a more concentrated ethanol extract.

Organ Sampling

Before organ collection, the rats were euthanized by cervical dislocation and positioned on a dissection board with pins. The abdomen was then opened using dissection scissors, and the liver and kidneys were removed, separated, and cleaned of any adherent fat.[17]

Histological Slide Preparation

Histopathological slides were prepared as follows. Liver and kidney tissues were fixed in Bouin's solution for 2–10 hours, depending on the tissue being fixed. The tissues were then dehydrated using a reagent compatible with the fixative, namely graded ethanol (70%, 80%, 90%, and 95%), followed by absolute ethanol I, II, and III. Clearing was then carried out with xylol I, II, and III, followed by infiltration. The tissues were subsequently embedded: after infiltration, each tissue was placed in a mold, which was filled with liquid paraffin, left at room temperature until the paraffin hardened, and then transferred to a refrigerator. Once hardened, the paraffin block was trimmed and mounted. Sections were cut at a thickness of 4 µm and stained with hematoxylin-eosin.

Staining began with deparaffinization by immersion in xylol I, II, and III for three minutes. The second stage was rehydration in absolute ethanol I, II, and III for three minutes each, followed by 70%, 80%, 90%, and 95% ethanol for three minutes each. The slides were then rinsed twice with tap water for 5–10 minutes. This was followed by staining with hematoxylin for five minutes, rinsing with tap water, immersion in eosin for five minutes, and a further rinse with tap water. Dehydration was then performed to remove water from the tissue, using 70%, 80%, and 90% ethanol for thirty seconds each, absolute ethanol I for thirty seconds, absolute ethanol II for one minute, and absolute ethanol III for three minutes, followed by xylol I, II, and III for three minutes each. The finished slides were mounted and examined under a light microscope.

Data Analysis

Data were analyzed descriptively based on the liver and kidney histopathology of male rats (*Rattus norvegicus*) after administration of Moringa leaf extract over the 35-day study period. The liver parameters observed were the hepatocytes, central vein, and sinusoids, whereas the kidney parameters observed were the glomeruli, Bowman's capsule, and proximal tubules. The results were presented as photomicrographs with accompanying descriptions.

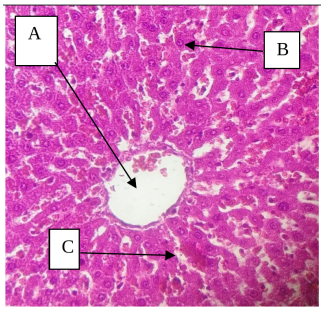
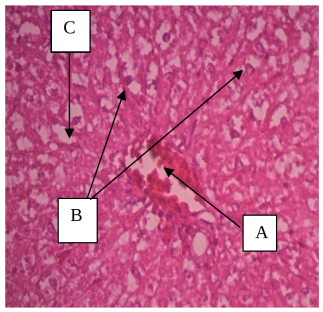
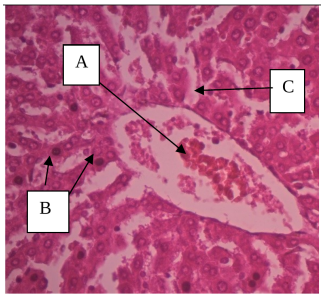
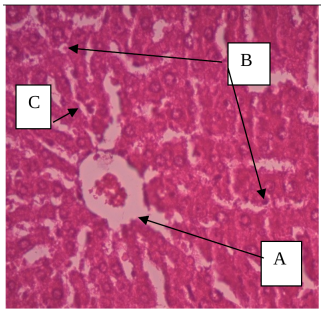
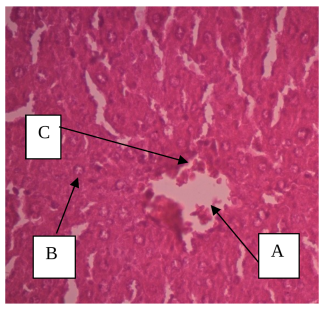
Results

In this study, the animals were first induced with sodium nitrite at a dose of 37.5 mg/kgBW for 14 days. The rats were then given Moringa ethanol leaf extract at three doses: 200 mg/kgBW (P1), 400 mg/kgBW (P2), and 600 mg/kgBW (P3). The findings showed a significant effect of Moringa leaf extract on the liver and kidney histopathology of the male rats, as detailed below and illustrated in Tables 1 and 2.

Liver histopathology was examined under a light microscope at 400× magnification, with the central vein, hepatocytes, and sinusoids assessed as the damage parameters. A normal liver is characterized by a round, empty-looking central vein, polygonal hepatocytes with centrally located nuclei, and sinusoids radiating toward the central vein, as observed in the K1 (negative control) group. In the K2 (positive control) group, the central vein showed congestion, the hepatocytes displayed fatty degeneration with necrosis and pyknotic or karyolytic nuclei, and the sinusoids were dilated.

In P1 (Moringa extract 200 mg/kgBW), the central vein remained dilated and congested, although to a lesser extent than in K2; the hepatocytes still showed necrosis with pyknotic and karyolytic nuclei; and the sinusoids remained dilated. In P2 (400 mg/kgBW), the central vein appeared smaller than in P1, with reduced congestion; the hepatocytes showed karyolysis and less pyknosis than in K2; and the sinusoids began to approach a normal, less dilated appearance. In P3 (600 mg/kgBW), the liver structure approached normal, with a near-normal central vein, reduced congestion, hepatocytes with fewer pyknotic and karyolytic nuclei, and sinusoids that appeared largely normal. Overall, dilation, necrosis (pyknotic and karyolytic nuclei), and fatty degeneration were observed at all three treatment doses, but were consistently less severe than in the K2 control.

Table 1. Liver histopathology of male rats by treatment group.

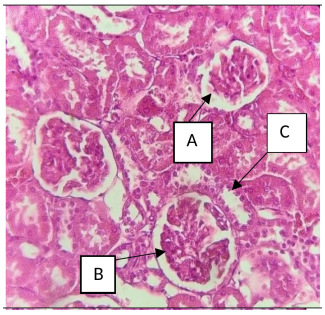
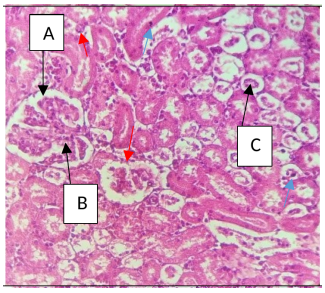
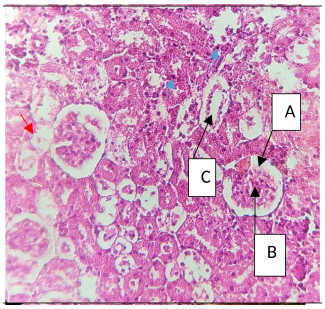
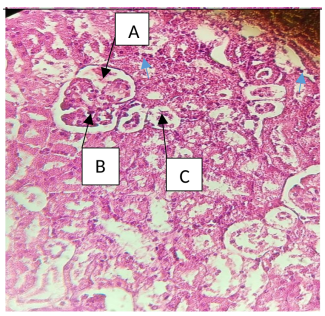
Group	Photomicrograph (400×)	Description
K1 – Negative control		*A: Central vein B: Hepatocyte C: Sinusoid* The central vein, hepatocytes, and sinusoids appeared normal, as indicated by a round, empty central vein; centrally located, polygonal hepatocytes; and sinusoids oriented toward the central vein.
K2 – Positive control		*A: Central vein B: Hepatocyte C: Sinusoid* The central vein showed congestion; the hepatocytes showed fatty degeneration, necrosis, and pyknotic or karyolytic nuclei; and the sinusoids were dilated.
P1 – 200 mg/kgBW		*A: Central vein B: Hepatocyte C: Sinusoid* The central vein showed dilation and congestion; the hepatocytes showed necrosis with pyknotic and karyolytic nuclei; and the sinusoids were dilated.
P2 – 400 mg/kgBW		*A: Central vein B: Hepatocyte C: Sinusoid* The central vein appeared smaller, with reduced congestion; the hepatocytes showed karyolysis and slight pyknosis; and the sinusoids were largely normal and less dilated.
P3 – 600 mg/kgBW		*A: Central vein B: Hepatocyte C: Sinusoid* The central vein appeared nearly normal, with reduced congestion; the hepatocytes appeared nearly normal, with fewer pyknotic and karyolytic nuclei; and the sinusoids were no longer dilated.

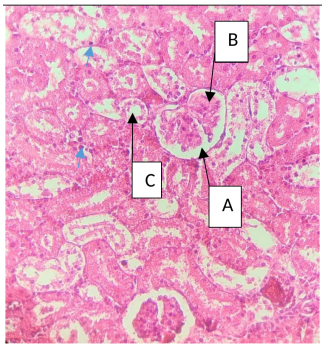
Kidney histopathology was likewise examined at 400× magnification, with Bowman's capsule, the glomerulus, and the proximal tubule assessed as the damage parameters. A normal kidney shows a nephron in which the glomerulus is surrounded by Bowman's capsule and the glomerular nucleus is round and located within the cell,

a distal tubule lined by taller, thicker cuboidal epithelium with visible septa, and a proximal tubule without septa, as seen in the K1 group. Compared with K1, the K2 group showed clear differences: glomerular narrowing, dilation of Bowman's capsule, and necrosis with degeneration.

In P1 (200 mg/kgBW), Bowman's capsule showed dilation and necrosis was present, although less severe than in K2. In P2 (400 mg/kgBW), the kidney structure approached normal but still showed necrosis and dilation of Bowman's space. In P3 (600 mg/kgBW), necrosis and dilation of Bowman's space were still present but were less severe than in P2, indicating that, although histological recovery of the kidney increased with the Moringa extract dose, it was less complete than that observed in the liver.

Table 2. Kidney histopathology of male rats by treatment group.

Group	Photomicrograph (400×)	Description
K1 – Negative control		*A: Bowman's capsule B: Glomerulus C: Distal tubule* The glomerulus appeared normal, as indicated by nuclei retained within the cell; the proximal tubule appeared normal, with a round nucleus and cytoplasm adjacent to the nucleus.
K2 – Positive control		*A: Bowman's capsule B: Glomerulus C: Distal tubule* The tubule showed necrosis (blue arrow) and cellular degeneration (red arrow); the glomerulus was narrowed; and the tubular lumen was narrowed.
P1 – 200 mg/kgBW		*A: Bowman's capsule B: Glomerulus C: Distal tubule* The cells showed necrosis (blue arrow) and degeneration (red arrow); the glomerulus was narrowed; and the tubular lumen was narrowed.
P2 – 400 mg/kgBW		*A: Bowman's capsule B: Glomerulus C: Distal tubule* The cells showed necrosis (blue arrow); the glomerulus was narrowed; and the renal tubule showed early signs of repair.

Group	Photomicrograph (400×)	Description
P3 – 600 mg/kgBW		*A: Bowman's capsule B: Glomerulus C: Distal tubule* The cells showed necrosis (blue arrow); Bowman's capsule showed improvement; and the tubular lumen showed improvement.

Discussion

The use of chemical additives in processed foods and beverages is now widespread. Sodium nitrite ($NaNO_2$) is among the preservatives most commonly used in processed meat, where it inhibits the growth of *Clostridium botulinum*, helps retain the red color of the meat, and contributes to flavor. Excessive consumption of sodium nitrite, however, has adverse effects on the body: it can impair the oxygen-carrying capacity of red blood cells, cause anemia, and lead to the formation of carcinogenic nitrosamines. Once absorbed into the circulation, $NaNO_2$ increases ROS production and induces oxidative stress, causing erythrocytes to undergo hemolysis. Nitrite that enters the bloodstream also oxidizes the Fe^{2+} ion in hemoglobin to Fe^{3+}, forming methemoglobin, which, unlike hemoglobin, cannot bind oxygen effectively. When methemoglobin levels are too high, red blood cells undergo hemolysis, reducing oxygen delivery to the tissues and ultimately causing cellular damage and cell death.

These mechanisms are consistent with the liver findings observed in this study. The P1, P2, and P3 groups all showed some degree of dilation, necrosis (pyknotic and karyolytic nuclei), and fatty degeneration, but the severity decreased progressively as the Moringa extract dose increased and was, at every dose, less severe than in the K2 positive control, indicating a dose-related protective effect of the extract against sodium nitrite-induced hepatic injury.

A comparable pattern was observed in the kidney. The glomerular narrowing seen in the K2 group was consistent with repeated exposure of renal cells to toxic substances entering the body.[18] The proximal tubule is a segment of the kidney that is particularly vulnerable to toxic injury because it is the site of reabsorption and secretion, where toxic substances become more highly concentrated.[19] Necrosis itself is defined as cell death that occurs in a living organism as a result of pathological injury.[20]

Renal structural damage arises because ingested nitrite enters the digestive tract and is absorbed into the bloodstream, where it forms nitric oxide (NO) via reactive oxygen species (ROS) and reactive nitrogen species (RNS). Free radicals in the body can be counteracted by antioxidant intake: antioxidant molecules donate hydrogen atoms or electrons to free radicals, converting them into more stable products, interrupting the free-radical chain reaction, and inhibiting further oxidative reactions. These processes are mediated through enzymatic and non-enzymatic metabolic pathways as part of the body's defense against toxic substances, and the resulting metabolites are excreted by the kidney in the urine.[21]

The results of this study showed that, in P3, kidney histopathology exhibited significant recovery, marked by improvement relative to the other treatment groups, consistent with the antioxidant (flavonoid) activity of Moringa leaf extract described above.

Because this study relied on qualitative, descriptive histopathological assessment of a modest number of animals per group, without semi-quantitative lesion scoring or supporting biochemical markers (e.g., serum urea, creatinine, or liver enzymes), the findings should be interpreted as indicative rather than confirmatory. Future

studies incorporating quantitative histopathological scoring and biochemical analyses will help to substantiate the hepatoprotective and nephroprotective effects of *Moringa oleifera* leaf extract observed here.

Conclusion

This study demonstrated that *Moringa oleifera* leaf extract affected the liver and kidney histopathology of sodium nitrite-induced male rats (*Rattus norvegicus*). Among the doses evaluated, 600 mg/kgBW (P3) produced the greatest improvement in liver and kidney histopathology, supporting the potential of Moringa leaf extract as a protective agent against sodium nitrite-induced hepatic and renal injury.

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Declaration concerning generative AI and AI-augmented technologies in the compositional process

In the course of preparing this paper, the authors utilized ChatGPT to enhance readability and linguistic quality. Subsequent to utilizing this tool/service, the writers assessed and amended the information as necessary and assume complete accountability for the publication's content.

Declarations of competing interest

No potential competing interest was reported by the authors.

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