The North African Journal of Scientific Publishing (NAJSP)
مجلة شمال إفريقيا للنشر العلمي (NAJSP)
E-ISSN: 2959-4820
Volume 3, Issue 3, 2025
Page No: 259-264
Website: https://najsp.com/index.php/home/index
SJIFactor 2024: 5.49 **معامل التأثير العربي (AIF) 0.71 :2024** **ISI 2024: 0.696**

Hepatorenal Protective Effect of *Lepidium sativum* Seed Extract Against Sodium Nitrite Toxicity in Rabbits

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التأثير الوقائي الكبدي الكلوي لمستخلص بذور الرشاد *Lepidium sativum* ضد سمية نتريت الصوديوم لدى الأرانب

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Received: July 15, 2025	Accepted: August 21, 2025	Published: August 25, 2025

Abstract:
This study investigated the hepatorenal protective effects of a cold aqueous extract of *Lepidium sativum* (garden cress) seeds against toxicity induced by the food preservative sodium nitrite ($NaNO_2$) in rabbits. Over a 30-day period, sixteen animals were divided into four groups: a control group (G1), a group administered *L. sativum* extract alone (G2, 50 mg/kg), a group given sodium nitrite alone (G3, 30 mg/kg), and a group pre-treated with the extract before sodium nitrite (G4).The results demonstrated that sodium nitrite administration (G3) caused significant hepatorenal damage, evidenced by a marked increase in serum liver enzymes (ALP, AST, ALT) and elevated levels of urea and creatinine, alongside a decrease in total protein, albumin, and globulin. Macroscopic examination revealed hepatic and renal atrophy and bladder enlargement. Furthermore, G3 exhibited significant hematological alterations and weight loss.In contrast, co-administration of the *L. sativum* extract (G4) significantly ameliorated these toxic effects, normalizing the biochemical, physiological, and hematological parameters. The group receiving the extract alone (G2) showed no adverse effects, with results comparable to the control group. It is concluded that the cold aqueous extract of *L. sativum* seeds possesses significant efficacy in protecting against sodium nitrite-induced hepatorenal toxicity. This protective role is likely attributable to the antioxidant properties of the phytoconstituents present in the extract

Keywords: Sodium nitrite, *Lepidium sativum*, Liver function, Kidney function, Antioxidants, Rabbits.

الملخص
هدفت هذه الدراسة إلى تقصي التأثيرات الوقائية للمستخلص المائي البارد لبذور الرشاد *(Lepidium sativum)* ضد السمية التي يسببها مادة النتريت الصوديوم *($NaNO_2$)* في الأرانب. على مدى فترة تجريبية استمرت 30 يومًا، تم تقسيم ستة عشر حيوانًا إلى أربع مجموعات: مجموعة التحكم*(G1)* ، ومجموعة عولجت بمستخلص الرشاد فقط*(G2)* ، 50 ملغ/كغ)، ومجموعة عولجت بالنتريت الصوديوم فقط*(G3)* ، 30 ملغ/كغ)، ومجموعة عولجت بالمستخلص قبل إعطاء النتريت الصوديوم.*(G4)* أظهرت النتائج أن معالجة مجموعة *(G3)* بالنتريت الصوديوم تسببت في ضرر كبدي وكلوي كبير، يتجلى في زيادة ملحوظة في مستويات إنزيمات الكبد *(ALP, AST, ALT)* وارتفاع في مستويات اليوريا والكرياتينين في المصل، إلى جانب انخفاض في البروتين الكلي، الألبيومين، والجلوبيولين. كما كشف الفحص التشريحي عن وجود ضمور في الكلى والكبد وتضخم في المثانة البولية. علاوة على ذلك، أظهرت مجموعة *(G3)* تغيرات دموية معنوية وفقدانًا في الوزن وعلى النقيض من ذلك، أدى المعالجة المشتركة بمستخلص الرشاد *(G4)* إلى تخفيف كبير لهذه التأثيرات السامة، حيث عادت المعايير الكيميائية الحيوية والفيزيولوجية والدموية إلى مستوياتها الطبيعية تقريبًا. ولم تُظهر المجموعة التي تلقت المستخلص وحده *(G2)* أي آثار ضارة، وكانت نتائجها مماثلة لمجموعة التحكم. يُستنتج من هذه الدراسة أن المستخلص المائي البارد لبذور الرشاد يمتلك فعالية وقائية كبيرة ضد السمية الكبدية والكلوية التي*induces* ها

النتريت الصوديوم. ويعزى هذا الدور الوقائي على الأرجح إلى الخصائص المضادة للأكسدة للمكونات الفعالة النباتية الموجودة في المستخلص.

الكلمات المفتاحية: نتريت الصوديوم، *Lepidium sativum* ، وظائف الكبد، وظائف الكلى، مضادات الأكسدة، الأرانب.

Introduction

Recent rapid development in the food industry necessitates stringent scientific measures to ensure the production of safe and high-quality food products [1,2]. Food additives are commonly used to enhance taste, color, and aroma [3-5] and to extend shelf life, ensuring year-round availability [6]. Nitrite, used in the form of salts, is a common preservative added to meat products at precise concentrations [7]. However, sodium nitrite can react with amines in the stomach to form nitrosamines [8], which generate free radicals contributing to oxidative stress and subsequent damage to organs such as the liver and kidneys [9]. Furthermore, sodium nitrite induces methemoglobinemia, impairing oxygen transport [10] and its conversion to nitric oxide can affect renal function by relaxing vascular smooth muscles [11-13].

Plants represent a vital source of bioactive compounds that mitigate oxidative stress with minimal side effects compared to synthetic drugs [14-18,]. Garden cress (Lepidium sativum) is an annual herb whose seeds are rich in medicinal components, including antioxidants and vitamins, and are known to aid in treating anemia, joint pain, and boosting immunity [19,20]. This research highlights the vital role of medicinal plants, particularly their antioxidant content, in counteracting the adverse effects of synthetic preservatives like sodium nitrite on crucial internal organs such as the liver and kidneys.

Research Problem

This study addresses the toxicity of long-term exposure to food additives, specifically sodium nitrite, used in canned and preserved meats, and its detrimental impact on human health through oxidative stress. It explores the potential of medicinal plants to mitigate this damage.

Aim of the Study

This research aims to demonstrate the protective role of a cold aqueous extract of *L. sativum* seeds on liver and kidney function in rabbits exposed to sodium nitrite, by evaluating liver enzymes, renal function parameters, and antioxidant status.

Material and methods

Experimental Animals:

The study was conducted over 30 days in May 2024. Sixteen adult rabbits (3-4 months old, 800-900 g) were housed in suitable cages under controlled conditions of lighting, temperature, and ventilation.

Preparation of *L. sativum* Extract:

Red garden cress seeds were cleaned, dried, ground, and stored. A cold aqueous extract was prepared by adding 5 g of powder to 100 ml of distilled water. The solution was filtered and centrifuged at 5000 rpm for 10 minutes [21-23]. The final concentration administered was 50 mg/kg body weight, given orally via gavage.

Preparation of Sodium Nitrite Dose:

Sodium nitrite ($NaNO_2$, E250) was dissolved in distilled water to achieve a concentration of 30 mg/kg body weight for oral administration.

Experimental Design:

Rabbits were divided into four groups (n=4):

- G1 (Control): Received 1 ml distilled water daily.
- G2 (*L. sativum*): Received *L. sativum* extract (50 mg/kg) daily.
- G3 ($NaNO_2$): Received sodium nitrite (30 mg/kg) daily.
- G4 (*L. sativum* + $NaNO_2$): Received *L. sativum* extract (50 mg/kg) two hours prior to sodium nitrite (30 mg/kg) daily.

Blood Sample Collection:

Blood was drawn from the auricular vein into gel-free tubes as illustrated in Figure 1. Serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at -20°C for analysis of liver enzymes (AST, ALT, ALP), renal parameters (urea, creatinine), and protein profiles (total protein, albumin, globulin). A complete blood count (CBC) was also performed.

Figure (1): Blood collection from the auricular vein.

Results

Biochemical analysis revealed a significant increase ($P < 0.05$) in liver enzymes (ALP, AST, ALT) in G3 compared to G1. No significant differences were observed in G2. G4 showed a significant decrease ($P < 0.05$) in these enzymes compared to G3.

A significant decrease ($P < 0.05$) in total protein, globulin, and albumin was observed in G3. G2 showed a significant increase ($P < 0.05$) in total protein and globulin. G4 exhibited significant improvement in these parameters compared to G3.

Urea and creatinine levels were significantly elevated ($P < 0.05$) in G3. No significant changes were noted in G2, while G4 showed a significant decrease compared to G3. Weight monitoring showed a substantial decrease in G3 (to 600g from 800g), while G1, G2, and G4 maintained or increased their weight.

CBC analysis indicated significant hematological disturbances ($P < 0.05$) in G3, which were not present in G2 and G4.

Table (1): Effect of aqueous extract of *L. sativum* seeds on liver enzyme activities (ALP, AST, ALT) in rabbit serum (Mean ± S.D.).

Group	Treatment	ALP (U/L)	AST (U/L)	ALT (U/L)
G1	Control (Distilled water)	350.42 ± 0.19	115.33 ± 0.61	48.10 ± 0.89
G2	*L. sativum* extract (50 mg/kg)	350.71 ± 0.72	112.16 ± 0.33	47.00 ± 0.27
G3	Sodium nitrite (30 mg/kg)	397.10 ± 0.77*	150.33 ± 0.55*	56.33 ± 0.06*
G4	*L. sativum* (50 mg/kg) + $NaNO_2$	375.01 ± 0.11 **	142.14 ± 0.79**	53.35 ± 0.28**

**Significantly different from control ($P < 0.05$)*
***Significantly different from G3 ($P < 0.05$)*

Table (2): Effect on serum total protein, globulin, and albumin levels (Mean ± S.D.).

Group	Treatment	Total Protein (g/dl)	Globulin (g/dl)	Albumin (g/dl)
G1	Control	5.32 ± 0.08	1.30 ± 0.10	4.01 ± 0.03
G2	*L. sativum* extract	5.40 ± 0.07*	1.40 ± 0.09*	4.00 ± 0.02
G3	Sodium nitrite	3.43 ± 0.10*	0.69 ± 0.42*	2.74 ± 0.30*
G4	*L. sativum* + $NaNO_2$	5.06 ± 0.64**	1.18 ± 0.13**	3.88 ± 0.16**

**Significantly different from control ($P < 0.05$)*
***Significantly different from G3 ($P < 0.05$)*

Table (3). Effect on serum urea and creatinine levels (Mean ± S.D.).

Group	Treatment	Urea (mg/dl)	Creatinine (mg/dl)
G1	Control	32.66 ± 0.81	0.81 ± 0.11
G2	*L. sativum* extract	30.83 ± 0.45	0.80 ± 0.03
G3	Sodium nitrite	75.33 ± 0.99*	1.99 ± 0.05*
G4	*L. sativum* + $NaNO_2$	62.00 ± 0.89**	1.20 ± 0.03**

**Significantly different from control ($P < 0.05$)*
***Significantly different from G3 ($P < 0.05$)*

Table (4): Changes in body weight.

Group	Treatment	Initial Weight	Final Weight
G1	Control	800 g	1000 g
G2	*L. sativum* extract	800 g	900 g
G3	Sodium nitrite	800 g	600 g
G4	*L. sativum* + $NaNO_2$	800 g	900 g

Table (5): Effect on complete blood count (CBC) parameters (Mean ± S.D.).

Group	Treatment	RBC (10^6/µl)	WBC (10^3/µl)	Hb (g/dl)	Platelets (10^3/µl)
G1	Control	5.30 ± 0.35	8.32 ± 2.079	10.3 ± 0.51	235.9 ± 109.51
G2	*L. sativum*	5.17 ± 0.354	8.16 ± 2.066	10.6 ± 0.51	229.5 ± 100.5
G3	Sodium nitrite	3.01 ± 0.230*	12.02± 0.22*	8.1 ± 0.66*	173.0 ± 73.0*
G4	*L. sativum*+$NaNO_2$	5.17 ± 0.353**	8.80 ± 2.063**	10.3 ± 0.50**	230.5 ± 100.5**

**Significantly different from control (P < 0.05)*
***Significantly different from G3 (P < 0.05)*

Discussion

The observed elevation in liver enzymes (ALP, AST, ALT) in G3 indicates hepatocellular damage, a consequence of sodium nitrite-induced oxidative stress leading to lipid peroxidation and membrane disintegration [24, 25]. The hepatic atrophy confirmed upon dissection as shown in Figure 2, aligns with these findings. The amelioration of these effects in G2 and G4 is attributed to the antioxidant constituents of L. sativum, such as flavonoids, alkaloids, and fatty acids [20, 26, 27, 28], which neutralize free radicals, as evidenced by the healthy liver morphology in these groups as presented in Figure 3. The hypoproteinemia and hypoalbuminemia in G3 likely result from accelerated protein catabolism due to oxidative stress and potential renal protein loss [29, 30]. The extract's ability to improve protein levels suggests a protective effect on metabolic pathways.

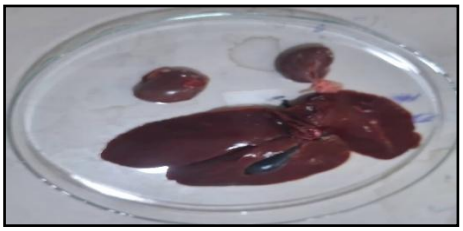

Figure (2): Liver atropy in the sodium nitrite-treated group (G3).

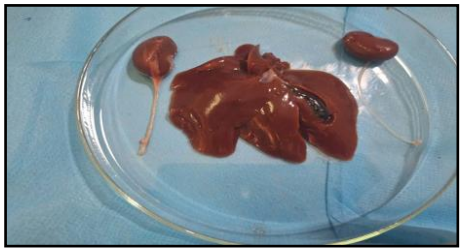

Figure (3): Normal liver morphology in the group receiving *L. sativum* extract (G2).

Figure (4): Bladder enlargement in the sodium nitrite-treated group (G3).

The significant rise in urea and creatinine in G3 indicates impaired renal function, likely due to reduced glomerular filtration rate and renal blood flow caused by nitrite toxicity [31,32,33,34]. The bladder enlargement may be a secondary effect as demonstrated in Figure 4. The normalization of these parameters in G4 demonstrates the extract's renoprotective role. The severe weight loss in G3 reflects the overall systemic toxicity and muscle wasting. The weight stability in G4 and G2 can be linked to the nutritional (high energy content) and protective properties of the cress seeds. The hematological toxicity (anemia, leukocytosis) in G3 is consistent with the oxidative damage to blood cells and bone

marrow. The prevention of these changes in G4 further underscores the extract's comprehensive protective action.

Conclusion

The present study demonstrates that sodium nitrite administration induces marked hepatorenal toxicity, oxidative stress, and hematological disturbances in rabbits. However, treatment with the cold aqueous extract of Lepidium sativum seeds effectively alleviated these adverse effects. The protective action was evident through the significant improvement in liver enzyme profiles, renal function markers, protein levels, body weight, and hematological parameters, as well as the prevention of organ atrophy. These beneficial outcomes are likely attributed to the strong antioxidant properties of L. sativum. Accordingly, L. sativum seed extract may be considered a promising natural protective agent against the toxicities induced by sodium nitrite and potentially other harmful food additives.

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