(ISSN Online: 3006-1113) Full Paper

Libyan Journal of Medical and Applied Sciences

Journal homepage: https://ljmas.com/index.php/journal/index

RESEARCH ARTICLE

Evaluation of Nigella sativa Thymoquinone Extract as a Protective Agent Against Acetaminophen-Induced Hepatic Injury in Rats

Bashaer K. Hameed [a]

[a] Department of Biology, College of science, University of Tikrit, Tikrit, Iraq.

Article Information

Article history:

Received on: 8/Jan/2026
Revised on: 23/Fab/2026
Accepted on:1/Mar/2026
Published on: 17/Mar/2026

Keywords:

Nigella sativa,
Thymoquinone,
Acetaminophen,
Hepatic Injury.

Abstract

Acetaminophen overdose, mainly showing mitochondrial damage, oxidative stress and inflammation still remain one of the leading causes of drug induced liver injury worldwide. Thymoquinone (TQ) has been identified as the major bioactive component of Nigella sativa, which exhibits various biological, antioxidant and cytoprotective effects. The study aimed to evaluate the hepatoprotective effect of thymoquinone extract from Nigella sativa against acetaminophen-induced hepatotoxicity. The experimental design incorporates histopathological analysis, oxidative stress biomarkers, and biochemical liver function tests. According to experimental data, pretreatment with thymoquinone significantly reduces the elevation of liver enzymes caused by APAP, restores antioxidant defense systems, lowers lipid peroxidation, and maintains the histological architecture of the liver. These results support the potential of thymoquinone as a natural hepatoprotective agent against acetaminophen -related liver damage.

1. Introduction

Acetaminophen (APAP) is frequently used as an antipyretic and analgesic; however, overdosing or improper long-term use is a major contributor to acute liver failure and drug-induced liver injury (DILI) [1,2]. Hepatic toxicity results from the saturation of the normal glucuronidation and sulfation pathways, which increases the metabolism of APAP by cytochrome P450 enzymes, primarily CYP2E1, and produces the highly reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) [3].

In normal scenarios, conjugation with reduced glutathione (GSH) detoxifies NAPQI. Hepatic GSH is quickly depleted during overdose due to excessive NAPQI formation, which causes oxidative stress, lipid peroxidation, mitochondrial dysfunction, and hepatocyte necrosis [4,5]. This cascade appears histologically as centrilobular necrosis, inflammatory infiltration, and sinusoidal congestion, and biochemically as significant increases in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin [2,6].

One of the main pathological mechanisms underlying APAP hepatotoxicity is oxidative stress. Depletion of endogenous antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and GSH increases hepatocellular vulnerability, while elevated levels of malondialdehyde (MDA) indicate increased lipid peroxidation. As a result, oxidative stress-targeting therapeutic approaches have drawn a lot of interest [7,8]

The antioxidant, anti-inflammatory, and hepatoprotective properties of Nigella sativa L. (black seed) have been thoroughly studied [9,10]. Several studies have shown that N. sativa seed oil or extract reduces chemically induced liver damage by improving hepatic histology and reestablishing antioxidant [11,12].

The primary bioactive component of Nigella sativa, thymoquinone (TQ), has strong free radical scavenging action and modifies several molecular pathways related to inflammation, apoptosis, and

DOI: https://doi.org/10.64943/ljmas.2026.04127

oxidative stress [13,14]. It has been demonstrated that thymoquinone have a potential hepatoprotective action through maintaining mitochondrial integrity, boosting AMPK signaling, reducing CYP2E1 expression, and blocking JNK activation [15]. However, comprehensive studies integrating biochemical, oxidative, and histopathological evaluation are needed for fully understanding this beneficial effect. Therefore, the present study aims to evaluate the protective effects of Nigella sativa thymoquinone extract against acetaminophen-induced hepatic injury in rats.

2. Materials and Methods

2.1. Experimental Animals

Adult male Wistar rats (180–220 g) were housed under standard laboratory conditions (22 ± 2 °C; 12 h light/dark cycle) with free access to standard chow and water. Animals were acclimatized for one week prior to experimentation. All procedures were conducted in accordance with NIH guidelines for laboratory animal care and approved by the Institutional Animal Ethics Committee.

2.2. Chemicals and Reagents

High purity acetaminophen was obtained from Pioneer Pharmaceutical Company (Sulaymaniyah-Iraq) and *Nigella sativa* thymoquinone extract, CAS No.490-91-5 was obtained from Aladdin Scientific (Meridian, USA)

ALT, AST, ALP, total bilirubin levels were evaluated in serum using Biochemical assay kits obtained From Active Bio Lab. MDA, GSH, SOD, and CAT levels were evaluated in liver tissue using assay kits commercially sourced from Beijing Solarbio Science & Technology Co., Ltd. (China) and used according to manufacturers' protocols.

2.3. Experimental Design

Hepatic toxicity was induced using Single dose of 1000 mg/kg APAP orally, produces clear evidence of liver injury at 24–48 h, with histopathological necrosis and biochemical transaminase elevations according to Buttar et al [16]

Rats were randomly divided into four groups (n = 6):

A: Control group: received normal saline.

B: APAP group: received acetaminophen (1,000 mg/kg, orally) on day 7.

C: TQ + APAP group: received thymoquinone (20 mg/kg/day) for 7 days prior to APAP administration.

D: TQ group: received thymoquinone only.

The control group received 0.5% DMSO orally for seven consecutive days. The APAP group received a single oral dose of 1000 mg/kg body weight. For the TQ + APAP group, thymoquinone was administered orally at 20 mg/kg body weight daily for 5–7 days prior to APAP exposure to enhance hepatic antioxidant defenses [14,17]. On the final day, the rats received the 1000 mg/kg APAP dose to induce liver injury. The TQ only group received TQ without APAP to assess potential intrinsic effects of TQ on the studied parameters.

Variability in APAP absorption and metabolism were reduced by fasting the rats for 12 h befor administration. APAP was prepared freshly by dissolving it in **0.5% DMSO in saline** and delivered at a constant volume of **10 mL/kg**.

After 48 hours of APAP administration Blood was collected from ocular vin for serum analysis of ALT, AST, ALP, and total bilirubin, while liver tissues were harvested for oxidative stress assays (MDA, GSH, SOD, CAT) and histopathological evaluation.

2.4. Oxidative Stress and Histopathology

MDA, GSH, SOD and CAT estimation in liver homogenates. The fixed liver sections were stained with hematoxylin and eosin (H&E) followed by examination of structural changes according to. [18]

2.5. Statistical Analysis

Data were expressed as mean ± SEM. One-way ANOVA followed by Tukey's post-hoc test was applied. A p-value < 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Liver Function Biomarkers

Administration of acetaminophen resulted in a marked increase in serum ALT, AST, ALP and total bilirubin levels confirming hepatocellular damage and impaired biliary excretion Table 1. Thymoquinone pre-treatment markedly prevented these changes, implying the maintenance of hepatic cell membrane integrity and enhanced hepatic functional capacity.

Table 1. Serum levels of Liver Function Biomarkers

Group	Total Bilirubin (mg/dL)	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	0.42 ± 0.05	42 ± 4	78 ± 6	126 ± 9
APAP	1.68 ± 0.14*	185 ± 15*	296 ± 21*	312 ± 24*
TQ + APAP	0.63 ± 0.07#	78 ± 7#	132 ± 11#	165 ± 13#
TQ	0.41 ± 0.04	44 ± 5	81 ± 7	129 ± 10

Values expressed as mean ± SEM (n = 6).
*Significantly different from control ($p < 0.05$).
#Significantly different from APAP group ($p < 0.05$).

The present study has been shown that Nigella sativa thymoquinone extract has very significantly protective effect against acetaminophen-induced hepatic damage. Elevation of serum liver enzymes in APAP-treated rats is an indication of hepatocyte membrane permeability, and it has been well demonstrated that this is due to liver necrosis, a characteristic and destination effect found in APAP intoxication, both in experimental and clinical studies. The decrease of ALT, AST, ALP and bilirubin after thymoquinone in the pretreatment group provides evidence that the toxic effects on homogenization of hepatocyte membrane preventable by and liver function was preserved. [2,5]

3.2. Oxidative Stress Parameters

The animals given APAP only showed significant oxidative stress indicated by elevated liver MDA and depleted antioxidant levels (GSH, SOD, and CAT) Table 2. Treatment with thymoquinone greatly reduced this oxidative stress by decreasing lipid peroxidation and increasing antioxidant enzyme activity.

Table 2. Hepatic Oxidative Stress Markers

Group	SOD (U/mg protein)	CAT (U/mg protein)	MDA (nmol/mg protein)	GSH (µmol/g tissue)
Control	12.4 ± 1.1	38.5 ± 2.7	2.1 ± 0.2	7.8 ± 0.6
APAP	5.3 ± 0.6*	15.6 ± 1.8*	6.9 ± 0.5*	3.1 ± 0.4*
TQ + APAP	10.7 ± 0.9#	32.1 ± 2.4#	3.0 ± 0.3#	6.5 ± 0.5#
TQ	12.6 ± 1.0	39.2 ± 2.9	2.0 ± 0.2	7.9 ± 0.6

Values expressed as mean ± SEM (n = 6).
*Significantly different from control ($p < 0.05$).
#Significantly different from APAP group ($p < 0.05$).

Oxidative stress is believed to be the underlying basis for APAP-induced liver damage. The overproduction of NAPQI causes depletion of GSH and generation of reactive oxygen species, which cause lipid peroxidation and mitochondrial damage [4]. The elevation of MDA and inhibition of antioxidant enzymes caused by APAP-treatment was consistent with previous studies [7,8]. Reduction in MDA levels and restoration of GSH, SOD and CAT activities by Thymoquinone indicated its strong antioxidant potential and substantiated earlier evidences that showed induction in endogenous antioxidants by Thymoquinone [13,14]

On the molecular mechanism level, studies found thymoquinone reduced the expression of CYP2E1, which limited NAPQI production and alleviated oxidative stress [15]. The inhibition of JNK phosphorylation and activation of AMPK signaling also played a role in decreasing mitochondrial damage and hepatocyte apoptosis [7,15]. These mechanisms provide a plausible explanation for the expected biochemical and histological protection observed in thymoquinone-treated animals.

3.3. Histopathological Findings

Histological examination from the normal control group shows a normal hepatic tissue, with a preserved hepatocytes arranged in cords around the central vein, sinusoids, and no signs of inflammation (Figure 1: A).

Liver sections from the APAP group shows a histopathological alteration, including hepatocyte necrosis, vacuolization, congestion, infiltration of inflammatory cell, and hepatic architecture disruption (Figure 1: B).

In the APAP rats pretreated with thymoquinone (Figure 1: C), liver shows marked improvement in hepatic architecture, with less necrotic areas, minimal inflammatory cells infiltration, and close to normal sinusoidal structure. Liver sections from the thymoquinone-only (Figure 1: D) group showed no significant histopathological changes compared to the control (A) group.

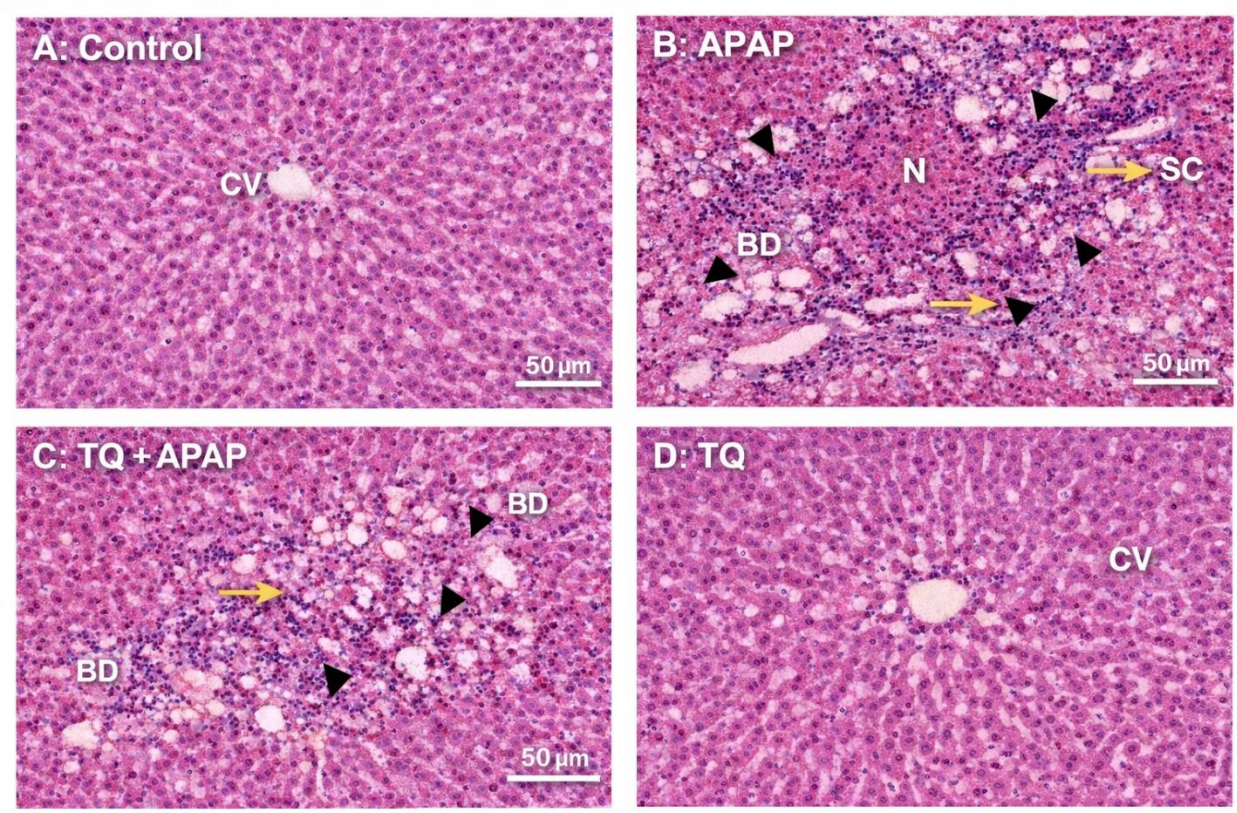

Figure 1. Representative Histopathological Changes in Rat Liver Tissue (H&E Staining) ×200.

Photomicrographs illustrate the histological architecture of liver sections from different experimental groups following acetaminophen-induced hepatotoxicity and thymoquinone treatment. (N = Centrilobular necrosis, BD = Ballooning degeneration of hepatocytes, SC = Sinusoidal congestion, I = Inflammatory cell infiltration, CV = Central vein, H = Hepatocyte, S = Sinusoid)

Histopathological preservation of hepatic architecture remains a critical endpoint in evaluating hepatoprotection. The lesions that have been noticed in the APAP group are characteristic of acetaminophen-induced liver injury and correlate with the observed biochemical disturbances [2,6]. The reduction in centrilobular necrosis, inflammatory infiltration, and sinusoidal congestion corroborates previous reports demonstrating *Nigella sativa*-mediated structural protection in chemically induced liver injury models [11,19]. The close correlation between biochemical normalization and histological improvement reinforces the integrative protective role of thymoquinone. These protective histological effects are consistent with earlier reports demonstrating thymoquinone-mediated suppression of oxidative damage and inflammatory signaling pathways [15,20].

Furthermore, thymoquinone's anti-inflammatory properties may contribute significantly to its hepatoprotective effects. APAP-induced liver injury is accompanied by activation of inflammatory mediators such as TNF-α, IL-1β, and neutrophil infiltration, which exacerbate tissue damage [2,21]. Thymoquinone has been shown to suppress pro-inflammatory cytokine release and inflammasome activation, further limiting secondary hepatic injury and inflammation [22].

4. Conclusion

Based on strong experimental evidence, *Nigella sativa* thymoquinone extract significantly protect against acetaminophen-induced hepatic injury in rats. Its hepatoprotective action is mediated through attenuation of oxidative stress, restoration of antioxidant defenses, suppression of inflammatory signaling, and preservation of hepatic histoarchitecture. Thymoquinone may therefore represent a promising natural therapeutic candidate for the prevention and management of drug-induced liver injury.

Acknowledgments

to university of Tikrit /collage of science/ biology department for their collaboration and to university of Mosul for helping me in housing the experimental animal and treatment.

Conflict of Interest

There is no Conflict of Interest regarding the current study subject.

References

1. Abduh, M. S., Saghir, S. A. M., Al-Gabri, N. A., Ahmeda, A. F., Abdelkarim, M., Aldaqal, S. M., & Alshawsh, M. A. (2023). Interleukin-35 and Thymoquinone nanoparticle-based intervention for liver protection against paracetamol-induced liver injury in rats. Saudi Journal of Biological Sciences, 30(10). https://doi.org/10.1016/j.sjbs.2023.103806
2. Ahmad, A., Husain, A., Mujeeb, M., Khan, S. A., Najmi, A. K., Siddique, N. A., Damanhouri, Z. A., & Anwar, F. (2013). A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pacific Journal of Tropical Biomedicine, 3(5). https://doi.org/10.1016/S2221-1691(13)60075-1
3. Ali, B. H., & Blunden, G. (2003). Pharmacological and toxicological properties of Nigella sativa. Phytotherapy Research. https://doi.org/10.1002/ptr.1309
4. AL-JUMAILI, M. M. O., AJEEL, M. A., & ABD-ALWAHAB, W. I. A. (2020). The Correlation Between Anemia and Sex Differences in Diabetes Mellitus Patients Suffering from Microvascular Complications. International Journal of Pharmaceutical Research, 12(3).
5. Alnori, M. K., Dawood, M. N., & Ajeel, M. A. (2021). The role of symmetrical and asymmetric dimethyl-l-arginine as biomarkers in dialysis-dependent aki patients. Military Medical Science Letters (Vojenske Zdravotnicke Listy), 90(4). https://doi.org/10.31482/mmsl.2021.034
6. Badary, O. A., Taha, R. A., Gamal El-Din, A. M., & Abdel-Wahab, M. H. (2003). Thymoquinone is a potent superoxide anion scavenger. Drug and Chemical Toxicology, 26(2). https://doi.org/10.1081/DCT-120020404
7. Bakr, E. H. A., & Almuraee, A. A. H. (2024). Protective Effect of Black Seed and Lettuce Oils Against Paracetamol-Induced Hepatotoxicity in Rats. Pakistan Journal of Biological Sciences, 27(2). https://doi.org/10.3923/pjbs.2024.59.68
8. Bancroft, John d.; Layton, Christopher; Suvarna, S. K. (2013). Bancroft's Theory and Practice of Histological Techniques,Expert Consult: Online and Print,7: Bancroft's Theory and Practice of Histological Techniques. Churchill Livingstone.
9. Buttar, H. S., Nera, E. A., & Downie, R. H. (1976). Serum enzyme activities and hepatic triglyceride levels in acute and subacute acetaminophen-treated rats. Toxicology, 6(1). https://doi.org/10.1016/0300-483X(76)90003-2
10. [10]. Chun, L. J., Tong, M. J., Busuttil, R. W., & Hiatt, J. R. (2009). Acetaminophen hepatotoxicity and acute liver failure. Journal of Clinical Gastroenterology. https://doi.org/10.1097/MCG.0b013e31818a3854
11. Cui, B. W., Bai, T., Yang, Y., Zhang, Y., Jiang, M., Yang, H. X., Wu, M., Liu, J., Qiao, C. Y., Zhan, Z. Y., Wu, Y. L., Kang, D. Z., Lian, L. H., & Nan, J. X. (2019). Thymoquinone attenuates acetaminophen overdose-induced acute liver injury and inflammation via regulation of JNK and AMPK signaling pathway. American Journal of Chinese Medicine, 47(3). https://doi.org/10.1142/S0192415X19500307
12. Du, K., Ramachandran, A., & Jaeschke, H. (2016). Oxidative stress during acetaminophen hepatotoxicity: Sources, pathophysiological role and therapeutic potential. Redox Biology. https://doi.org/10.1016/j.redox.2016.10.001

13. Esmaeil, F. K., Alnori, M. K., & Ajeel, M. A. (2024). Uncovering the Mechanistic Role of vitamin D in Modulating Glycemic Pertained Parameters in Insulin-resistant Patients. Review of Diabetic Studies, 20(1), 32–38. https://doi.org/10.1900/RDS.2024.20.32
14. Farhan, N. H., Aziz, B. L., & Ibraheem, R. T. (2025). Biochemical Evaluation of The Hepatoprotective Potential of Lemon Oil and Nigella Sativa Oil in Acetaminophen-Induced Liver Injury. Karbala Journal of Pharmaceutical Sciences, 15(25). https://doi.org/10.62472/kjps.v15.i25.258-267
15. Gürel, Ş. H., & ÇiÇek Polat, D. (2025). NIGELLA SATIVA L.: AN OVERVIEW. Ankara Universitesi Eczacilik Fakultesi Dergisi. https://doi.org/10.33483/jfpau.1504025
16. Hafez, M. H., Ez Elarab, S. M., Tohamy, H. G., & El-Far, A. H. (2024). Thymoquinone attenuates diabetes-induced hepatic damage in rat via regulation of oxidative/nitrosative stress, apoptosis, and inflammatory cascade with molecular docking approach. Scientific Reports, 14(1). https://doi.org/10.1038/s41598-024-62780-y
17. Hinson, J. A., Roberts, D. W., & James, L. P. (2010). Mechanisms of acetaminophen-induced liver necrosis. Handbook of Experimental Pharmacology. https://doi.org/10.1007/978-3-642-00663-0_12
18. Jaeschke, H., Williams, C. D., Ramachandran, A., & Bajt, M. L. (2012). Acetaminophen hepatotoxicity and repair: The role of sterile inflammation and innate immunity. Liver International. https://doi.org/10.1111/j.1478-3231.2011.02501.x
19. McGill, M. R., & Jaeschke, H. (2013). Metabolism and disposition of acetaminophen: Recent advances in relation to hepatotoxicity and diagnosis. Pharmaceutical Research. https://doi.org/10.1007/s11095-013-1007-6
20. Nainawat, K. S., Budakoti, A., Kumari, N., Verma, R. S., & Gupta, A. (2025). Pharmaceutical perspectives of thymoquinone, a lead molecule from black cumin (Nigella sativa L.) with diverse biological targets. Food Chemistry Advances. https://doi.org/10.1016/j.focha.2025.101053
21. Ramachandran, A., & Jaeschke, H. (2017). Mechanisms of acetaminophen hepatotoxicity and their translation to the human pathophysiology. Journal of Clinical and Translational Research. https://doi.org/10.18053/jctres.03.2017s1.002
22. Wróblewska-Łuczka, P., Cabaj, J., Bargieł, J., & Łuszczki, J. J. (2023). Anticancer effect of terpenes: focus on malignant melanoma. Pharmacological Reports. https://doi.org/10.1007/s43440-023-00512-1

تقييم مستخلص الثيموكينون من حبة البركة كعامل وقائي ضد إصابة الكبد الناجمة عن الأسيتامينوفين في الفئران

الخلاصة

تُعدّ الجرعة الزائدة من الأسيتامينوفين، التي تتظاهر أساسًا بحدوث تلف في الميتوكوندريا وإجهاد تأكسدي واستجابة التهابية، من أبرز الأسباب المؤدية إلى إصابة الكبد الناجمة عن الأدوية على مستوى العالم. وقد تم التعرف على الثيموكينون (TQ) بوصفه المكوّن الحيوي الفعّال الرئيس في *Nigella sativa*، حيث يُظهر طيفًا واسعًا من التأثيرات البيولوجية والمضادة للأكسدة والواقية للخلايا. هدفت الدراسة الى تقييم التأثير الواقي للكبد لمستخلص الثيموكينون من *Nigella sativa* ضد السُمّية الكبدية المستحثة بالأسيتامينوفين. تضمّن التصميم التجريبي تحليلًا نسيجيًا مرضيًا (Histopathological analysis)، وقياس مؤشرات الإجهاد التأكسدي، إضافة إلى اختبارات كيميائية حيوية لوظائف الكبد. ووفقًا للبيانات التجريبية، فإن المعالجة المسبقة بالثيموكينون تؤدي إلى خفضٍ ملحوظ في ارتفاع إنزيمات الكبد الناجم عن APAP، مع استعادة أنظمة الدفاع المضاد للأكسدة، وتقليل بيروكسدة الدهون، والحفاظ على البنية النسيجية الطبيعية للكبد. وتدعم هذه النتائج الإمكانات العلاجية للثيموكينون بوصفه عاملًا طبيعيًا واقيًا للكبد ضد تلف الكبد المرتبط بالأسيتامينوفين.

الكلمات المفتاحية: الحبة السوداء، ثايموكوينون، اسيتامينوفين، ضرر كبدي