Nigella sativa's protective effect in acetaminophen induced liver toxicity in mice

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Abstract

Acetaminophen has contributed to acute liver failure disease in more than half of the USA and Britain but as an analgesic and antipyretic it is very effective. For many decades in Europe, Middle East and Africa, Nigella sativa has been used for various medical purposes, it is part of the botanical family Ranunculaceae of Gently sloping plants, and is called black cumin seed., Nigella sativa conjugated sterols could be used as precursors to many hydrosoluble steroids for hemisynthesis. The aim of the Study is to examine the promising hepatoprotective effects of Nigella sativa against Acetaminopheninduce hepatotoxicity in mice in this experiment Forty adult male albino mice, incorporated in the experiment and Acetaminophenwas used to induce hepatotoxicity in a dose of 1 gm /kg by the oral route.

A number of biochemical and histopathological tests have been used to evaluate liver damage and Nigella sativa protective effects. The result showed a significant protective effect of Nigella sativa against acetaminophenhepatotoxic effect as Nigella sativa in this study tended to normalize the serum levels of liver enzymes, and the protective effects observed clearly by the histopathological evaluation confirming that it effectively protected mouse livers against severe damage caused by acetaminophen. Conclusion in our study it shows that Nigella sativa have a very significant protective effects against acetaminophen induced liver toxicity which is recommended to be fully investigation on human especially to people on risk of acetaminophen liver toxicity

Key words: Nigella sativa, Acetaminophen, hepatoprotective, mice, liver enzymes.

التأثير الوقائي للحبه السوداء ضد التاثير السمي للاسيتومينوفين على الكبد في الفئران

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الخلاصة:

ساهم عقار الاسيتامينوفين في الإصابة بأمراض الفشل الكبدي الحاد في أكثر من نصف الولايات المتحدة الأمريكية وبريطانيا ، ولكنه مسكن وخافض للحرارة فعال للغاية. لعدة عقود في أوروبا والشرق الأوسط وأفريقيا ، تم استخدام حبة البركة للأغراض الطبية المختلفة وتعتبر الحبه السوداء جزاء من النباتات المنحدره من الفصيله الحَوْذَانِيَّة وتسمى بذور الكمون الأسود. تم استخدام عدد من الاختبارات البيوكيميائية والنسيجية لتقييم تلف الكبد وآثار الحبة السوداء. أظهرت النتائج تأثيرًا وقائيًا كبيرًا للنيجيلا ساتيفا ضد التأثير السام للأسيتامينوفين على الكبد وقد لوحظ ان الحبه السوداء ساعدت على تقليل ارتفاع إنزيمات الكبد الى المستوى الاقرب الى الطبيعي ، والتأثيرات الواقية التي لوحظت بوضوح من خلال التقييم النسيجي تؤكد أنها تحمي بشكل فعال كبد الفأر ضد الأضرار الشديدة التي تسببها الاسيتامينوفينُ . الخلاصة في

دراستنا تظهر أن الحبة السوداء لها تأثيرات وقائية كبيرة جدًا ضد سمية الكبد و نوصى بإجراء تحقيق كامل عليها على الإنسان خاصة للأشخاص المعرضين لخطر سمية الكبد الناتج عن استخدام عقار للأسيتامينوفين
الكلمات المفتاحية: الحبه السوداء, الاسيتامينوفين, الوقايه الكبديه, فئران, انزيمات الكبد

Introduction

Acetaminophen is an analgesic and antipyretic that is effective and efficient. It is generally available as a single-component medication and has been approved for more than half of the cases of acute liver failure in the US and Great Britain as part of the excess of combination over - the-counter and prescription medicines. In fact, in the US, the medication has an estimated direct death risk of about US 87 million. [1]

Acetaminophen toxicity's precise mechanism of action is unclear [2]. Apparently understood by the glutathione depletion hypothesis the toxicit y role of Acetaminophenis. [3]

regardless of great advances in modern medicine,there are nearly no trust worthy medicines which protect

the liver against damage or help hepatic cel l regeneration. The Conventional medi-cines used to treat liver diseases are sometimes inadequate and ma have serious adverse effects [4].

Nigella sativa (N. sativa) is belonging to the botanical family of Ranunculaceae of herbaceous plants and known as black cumin seed.

The height of the plant exceeds 60 cm and its

flowers are blue and the foliage is finely di vided. [5.]

Nigella sativa seed has, for decades, been used for therapeutic purposes in Europe, the Middle East and Africa. A variety of diseases have been traditionally used it as a natural cure, such as diarrhea, hypertension,lung disorder , fatigue, diabetes, fever, bronchitis, coug and dizziness. [6]

In pharmaceutical propose, Nigella sativa conjugated sterols could be used as precursors for the hemisynthesis of many hydrosoluble steroids. and aroma compounds [7].

aimed of the study is to highlight the promising of hepatoprotective effects of *Nigella sativa* seed against Acetaminopheninduce hepatotoxicity in mice.

Materials & methods

Animals

Forty adult male albino mice, weighing (25-36 gm) were obtained from AL-Nahrien biotechnology research center, the mice were kept in a controlled temperat ure setting of (23 ± 2) C° and humidity of (50 ± 5) % with a 12– 12 h light–dark cycle. Food and water were available ad libitum. All experimental protocols employed here in were approved by the Committee on the Care of Laboratory Animal Resources

Nigella sativa seeds extraction

Nigella sativa Seeds treated with filtered water to remove foreign substances. Then dried and grounded to powder form. *Nigella sativa* powder soaked with distal water at 1:10 ratio for 48 h, then it positioned in a water bath at 50°C for 6 h before it filtered with filter papers. It was placed in a rotary evaporator at 37 ° C (60 rpm) to remove excess solvents during the extraction process and obtained a black extract of Nigella sativa seed.

Induction of acetaminophenliver toxicity

Acetaminophenwas used to induce hepatotoxicity in a dose of 1 gm /kg by the oral route[8]

Experimental design

Animals were divided into five groups containing eight animals for each group.

Group1 Serve as a default command, providing with onl y distilled water. no hepatotoxic induction (negative control group)

Groups 2
dose of paracetamol (1 gm / kg) was given to the mice. without using any drug (positi ve control group)
Group 3 oraly treated group (Nigella sativa **5gm/kg/day**) for 8 days followed by induction of hepatotoxicity on day 8.
Group 4 oraly treated group (Silymarin 200 mg/kg) for 8 days followed by induction of hepatotoxicity on day 8.

Collection of blood samples
After the experimental time, fasted blood samples from the mouse are collected from the medial retronorbital venous plexus under ether anesthesia immediately with capillary tubes (microhematocrit capillary, Mucaps). Blood was then centrifuged at 3000 r / min for 15 minutes and several biochemical analysis sera were collected.
Assessment of liver damage
Biochemical and histopathological tests have been used to determine liver damage.
Biochemical investigations
The following biochemical parameters were used: Serum (S.) alanine aminotransferase (ALT) (serum glutamic-pyruvic transaminase [SGPT]), Sr. aspartate aminotransferase (serum glutamic-oxaloacetic transaminase [SGOT]), Sr. alkaline phosphatase and Sr. bilirubin.

Histopathological examination of liver
Histopathological assessment of liver damage was carried out by using the National Health Services Meryland, U.S. method for measuring structural changes.
Upon extracting the liver tissue from the animals, the histological analysis for the tissues began. The tissues were softly rinsed in order to remove blood and adhering debris with a Saline Physiological (0,9% NaCl). They are set in 5% formalin for24 hours and washed off with hot tap water over the day. The tissues are purged in methyl benzoate and coated in paraffin after the oxidation of a graded sequence of alcohols. Sections were cut by a microtome with a thickness of 6 mm and stained with Hematoxylines stained in 95 percent ethanol hematoxyline and eosin stains) as described byGabe and counter stained with eosin.
After dehydration and clearing, sections were mounted with digital picture exchange and observed under a microscope.

The liver sections were graded numerically to evaluate the degree of histopathological appearance of acute hepatic injury. Hepatocyte necrosis, fatty change, and infiltration of lymphocytes were important in the histological findings and The liver pathology was numerate as described by French et al. (2000) as follows[9] :
Score 0-1 no noticeable cell damage;
Score 1-2 hepatocyte damage on less than 25% of the tissue;
Score 2-3 hepatocyte damage on 25–50% of the tissue;
Score 3-4 extensive hepatocyte lesions;
Score 4 comprehensive, hepatocyte necrosis.
The morphology of any lesions observed was classified and registered (Gray, 1964) [10].
Statistical analysis the values are presented as mean ± SD standard deviation. Differences between group means were estimated using a one-way ANOVA. significant differences among the different groups. $P <$ 0.05 was considered statistically significant difference.

Results

Effect of acetaminophen (Group 2)
The following changes have been reported after acetaminophen therapy (Groupe A-II). rise in Sr. AST, ALT Sr. alkaline phosphatase level, and Sr. bilirubin level. There have also been reported histopatholo gical changes including degeneration, necr osis, and fibrosis (graph 1). All these variable variations are statistically significant compared to control values. (Tables 1 and 2).

Effect of simultaneous administration of acetaminophenand NS oil (Group 3)

Co-administering acetaminophen with NS oil significantly prevented acetaminophen toxicity., administration of acetaminophen significantly elevated levels of Sr. AST, Sr. ALT, Sr. alkaline phosphatase and Sr. bilirubin. This increase was significantly prevented by co-administration of NS oil. All of these alterations in the parameters were statistically significant as compared to Acetaminophen treated group (Table 1). Classical changes in degeneration, necrosis and fibrosis occurred in livers of mice that have been treated with acetaminophen. Co-administration of NS oil with acetaminophen reduced degeneration, necrotic and fibrosis levels significantly (graph 2).

Table (1): comparism of the ALT , AST , bilirubin and Alkaline phosphatase between Nigella sativa treated group , Silymarin treated group to both positive control and negative control groups

	negative control	positive control Acetaminophen (1 gm/kg)	Nigella sativa treated group 5gm/kg/day +Paracetamol (1 gm/kg)	Silymarin treated group (50mg/kg) +Acetaminophe n(1 gm/kg)
ALT (Units/ml)	54.67±1. 03	92.0±3.098	82.1 ±2.04***	61 ± 6.13
AST (Units/ml)	58.80 ± 4.44	74.9±7.2	69.3±6.69*#	68.8 ± 2.81***#
Bilirubin (Mg/dl)	0.80±0.1 09	2.5 ±0.10328	1.3 ±.08619#	0.76 ± 0.02***
Alkaline phosphatas e (Units/ml)	175.83± 3.488	331.33±5.085	230.61±4.24**# #	200.4 ± 12.44***###

All values are average ± SD, data have been analyzed with unpaired t students., AST: aspartate aminotransferase, ALT: alanine aminotransferase, AP: alkaline phosphatise, NS: Nigella sativa. # In comparison with positive control group, * In comparison with negative control group #, * =p < 0.05, ##, ** = p < 0.01, ###, *** = p < 0.001

Table (2) : Effect of co-administration of NS oil on histopathology score in acetaminophentreated mice

	Negative Control group	Positive group	NS+ acetaminophen group	Silymarin + acetaminophengroup
degeneration	0	2.9± 0.43	1.8 ± 0.4	1.0 ± 0.35***
necrosis	0	2.4 ± 0.45	1.75 ± 0.35***	1.1 ± 0.25***
fibrosis	0	1.9± 0.3	1.2 ± 0.3***	0.9 ± 0.15***
Regeneration	0	0	0.24 ± 0.1**	0.4 ± 0.15 ***

All values are mean +- SD data were analyzed by using spss 22 statistical test. * in comparison with control group * = p < 0.05, ** = p < 0.01, *** = p < 0.001. NS: Nigella satava

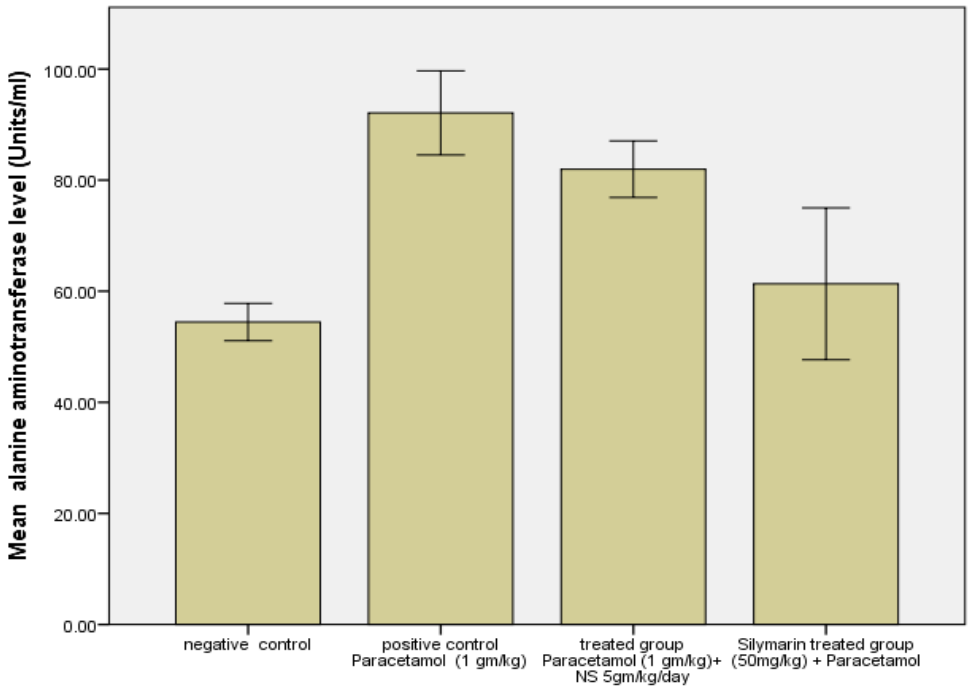


Figure (1): Difference in alanine aminotransferase between Normal control (received distilled water (negative control group) and positive control group acetaminopheninduction of hepatotoxicity (1 gm /kg) and Nigella sativa treated group (5gm/kg/day) for 8 days followed by induction of hepatotoxicity on day 8. And Silymarin treated group 200 mg/kg for 8 days followed by induction of hepatotoxicity on day 8.

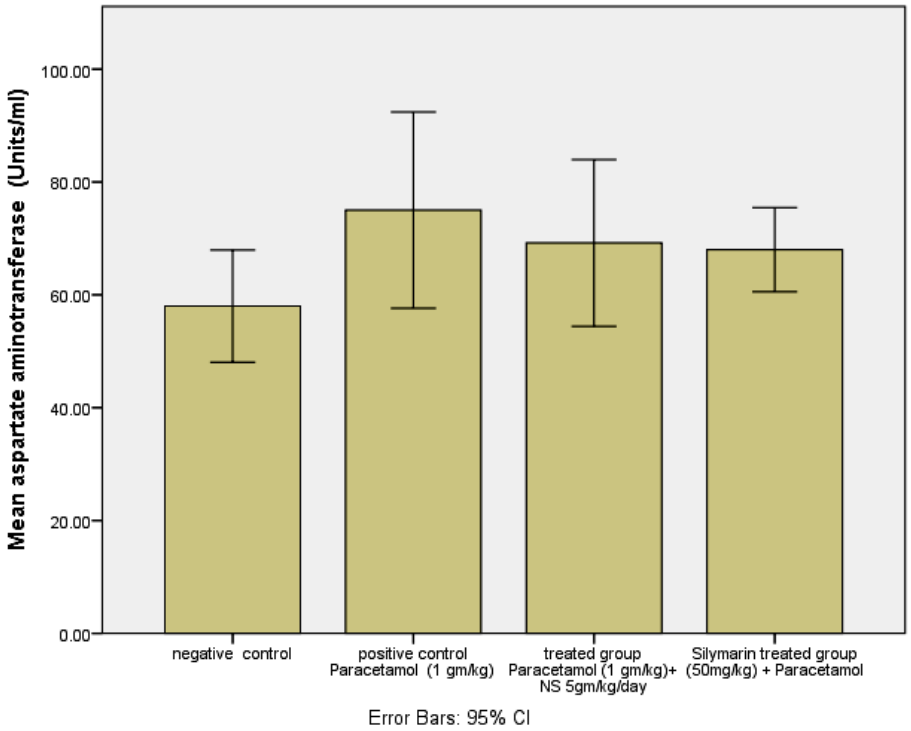
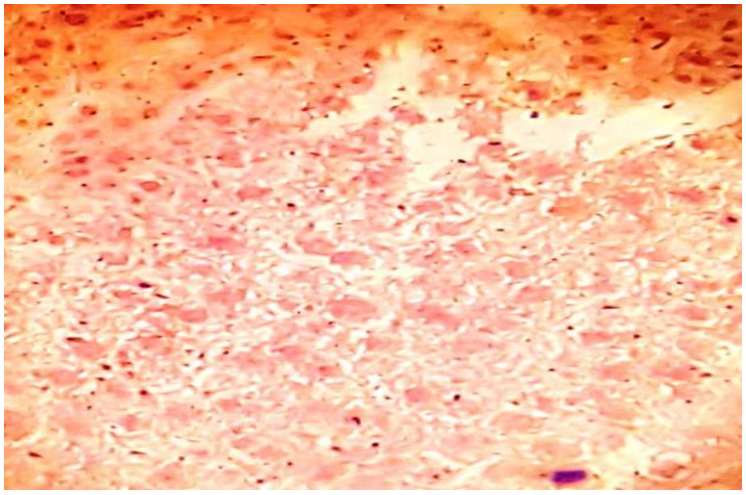
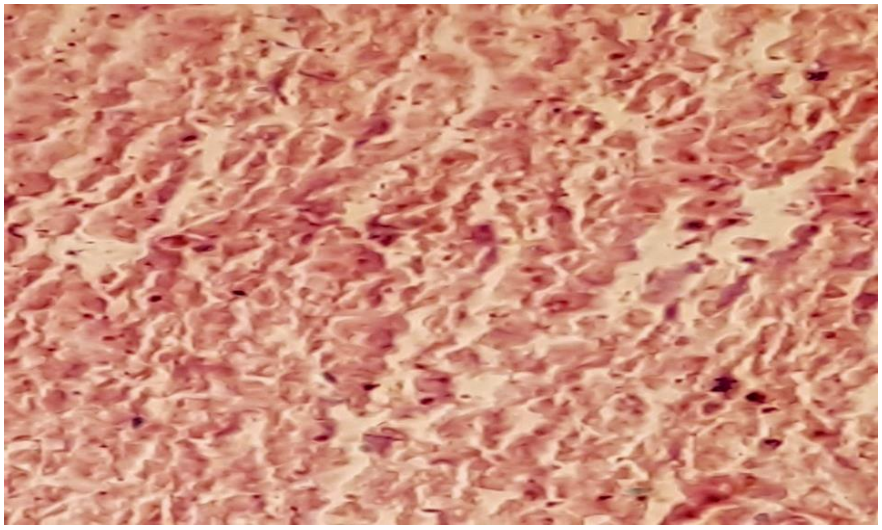


Figure (2): difference in aspartate aminotransferase Normal control (received distilled water negative control group) and positive control group acetaminopheninduction of hepatotoxicity (1 gm /kg) and Nigella sativa treated group (5gm/kg/day) for 8 days followed by induction of hepatotoxicity on day 8. And Silymarin treated group 200 mg/kg for 8 days followed by induction of hepatotoxicity on day 8.



Graph (1): Heapatotoxic effects of Acetaminophenon mice

Section of mouse liver tissue treated by 500 mg/kg Acetaminophen (positive control group). Massive centrilobular necrosis (arrows), accumulation of inflammatory cells and congestion have been seen. (H&E. X200).



Graph (2): Heapatoprotective effects of Nigella sativa against Acetaminophen on mice.
Section of mouse liver tissue treated by 1000mg/kg Acetaminophen+ 5 mg/kg Nigella sativa. Centrizonal necrosis (arrows), accumulation of inflammatory cells and congestion are less than positive control group. (H&E. X200).

Discusion

Paracetamol is among the most effective counter medications in recommended doses but can lead to massive hepatic necrosis through acute exposure or regular dosage usage. 11.

The main objective of this study was to det ermine Nigella sativa's protective effects o n toxicity from APAP.

Compared with untreated cells, the viability of the Nigella sativa cells was significantly increased, indicating that Nigella sativa may has a protective effects against hepatotoxicity induced by APAP .it has been reported in various clinical studies as the study performed by (md.Mahbuburrahman, sei-jin lee 2016) This stated that a reduction in hepatotoxicity was associated with two

weeks oral treatment with nigella sativa. 12

Another study (Reham, 2015) has shown that the administration of Nigella sativa extract into APAP-treated mice eliminates the toxicity of this compound, aimed at improving the liver's biochemistry, histology, and ultrastructures. 13

In the current study, high levels of biochemical parameters have confirm ed that APAP-induced liver damage, namely, GPT, GOT, bilirubin, and alkaline phosphate. In particular, the increase of these serum enzymes is indicative of hepatocytic damage, changes in membrane potential and increased membrane permeability, which leads in turn to the leakage of enzymes from liver cells and increased levels of serum. 14

Before initiation of acetaminophen liver toxicity, oral administration of nigella in a mouse decreased serum levels of liver enzymes, indicating that mouse liver was effectively protected from APAP injury.

As for this study we basically support the hepatoprotective effects shown by the previous studies of nigella sativa as we can observe the significant difference between GPT and GOT in nigella sativa in paracetamol treated mice (500 mg / kg) and nontreated ($p>0.05$)

As for this study we basically support the h epatoprotective effects shown by the previ ous studies of nigella sativa as we can obse rve the significant difference between GPT and GOT in nigella sativa in paracetamol t reated mice (500 mg / kg) and nontreated ($p>0.05$)

Paracetamol- induced hepatotoxicity has Hepatic congestion in humans has been rec orded to occur (Thompson et al.1972)15 and rodents (Walker et al. 1985)16

It happens early in the mouse before necrosis occurs. Studies of the morphology in mouse showed that the congestion is caused in the endocytic vacuola by accumulation of red blood cells and the sinusoidal lumens of the Disse area (walker et al. 1983)17.

In our present study, we found that in the section of mouse liver tissue treated by Acetaminophen (positive control group). Massive centrilobbular necrosis, accumulation of inflammatory cells and congestion has been seen.

While in Section of mouse liver tissue treated by Acetaminophen+ NS, necrosis, accumulation of inflammatory cells and congestion are less than positive control group

Conclusion

Nigella sativa has been documented to have a potent antioxidant effects, in our study it shows that Nigella sativa have a very significant hepatoprotective effects against acetaminophen induced liver toxicity which is recommended to be full investigated on human especially to people on risk of liver toxicity.

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