



## Research article

## Analyzing the Potential Antioxidative Effects of Omega-369 in Preventing Acetaminophen-Induced Liver Damage

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### Abstract

**Background:** As acetaminophen (APAP) toxicity has become more common in many countries, related cases of poisoning, whether deliberate or unintentional, have been identified as a key contributor to acute liver failure. **Aime:** To discover if omega-369 fatty acids could protect the liver of male mice from the effects of acetaminophen. **Methods:** Thirty-five albino male mice were allocated to one of five groups at random. Group 1 served as the "negative control" and received a single intraperitoneal injection (10 ml/kg) of normal saline on the eleventh day of the test following ten days of receiving liquid paraffin orally at a dose of 10 ml/kg. The liquid paraffin was given to group 2 "positive control". Group 3 received Omega 369 (50 mg/kg). Group 4 received Omega 369 (100 mg/kg). Group 5 received N-acetylcysteine (100 mg/kg). The mice were given Omega-369, N-acetylcysteine, and liquid paraffin via oral gavage for 10 days. **Results:** Group 2 had significantly lower levels of glutathione peroxidase (GP-X) and superoxide dismutase (SOD) than group 1, but significantly greater levels of malondialdehyde (MDA). GP-X and SOD levels were significantly higher in mice given the doses of omega-369, and N-acetylcysteine prior to acetaminophen administration, whereas MDA levels were significantly lower in groups 3,4 and 5 when compared with group 2. **Conclusion:** Omega-369 fatty acids, when taken orally, exhibit antioxidative effects and may reduce the risk of acetaminophen-induced liver injury.

**Keywords:** Omega-369, Liver toxicity, Acetaminophen, Antioxidant activity.

تحليل الآثار المحتملة المضادة للأكسدة لأوميغا 369 في منع تلف الكبد الناجم عن الأسيتامينوفين

### الخلاصة

**الخلفية:** نظراً لأن سمية الأسيتامينوفين أصبحت أكثر شيوعاً في العديد من البلدان، فقد تم تحديد حالات التسمم ذات الصلة، سواء كانت متعمدة أو غير مقصودة، كمساهم رئيسي في الفشل الكبدي الحاد. **الهدف:** لاكتشاف ما إذا كانت أحماض أوميغا 369 الدهنية يمكن أن تحمي كبد ذكور الفئران من آثار الأسيتامينوفين. **الطريقة:** تم توزيع خمسة وثلاثين من ذكور الفئران البيضاء لواحدة من خمس مجموعات عشوائية. كانت المجموعة 1 بمثابة "عنصر تحكم سلبي" وتلقّت حقنة واحدة داخل الصفاق (10 مل/كجم) من محلول ملحي عادي في اليوم الحادي عشر من الاختبار بعد عشرة أيام من تلقي البارافين السائل عن طريق الفم بجرعة 10 مل/كجم. تم إعطاء البارافين السائل لمجموعة "التحكم الإيجابي". تلقت المجموعة 3 أوميغا 369 (50 ملغ/كجم). تلقت المجموعة 4 أوميغا 369 (100 ملغ/كجم). تلقت المجموعة 5 N-أسيتيل سيستين (100 ملغ/كجم). أعطيت الفئران أوميغا 369، وN-acetylcysteine، والبارافين السائل عن طريق الفم لمدة 10 أيام. **النتائج:** كان لدى المجموعة 2 مستويات أقل بكثير من الجلوتاثيون بيروكسيداز (GP-X) وديسموتاز الفائق الأكسيد (SOD) من المجموعة 1، ولكن مستويات أكبر بكثير من الملوندايديهيد (MDA). كانت مستويات GP-X و SOD أعلى بشكل ملحوظ في الفئران بسبب جرعات أوميغا 369، و N-acetylcysteine قبل إعطاء الأسيتامينوفين، في حين كانت مستويات MDA أقل بكثير في المجموعات 3 و4 و5. **الاستنتاج:** الأحماض الدهنية أوميغا 369، عند تناولها عن طريق الفم، تظهر تأثيرات مضادة للأكسدة وقد تقلل من خطر إصابة الكبد التي يسببها الأسيتامينوفين.

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## INTRODUCTION

The liver is regarded as the most important organ in the human body due to its function in the detoxification of several substances. At the same time, the liver may encounter many adverse or side effects of medications that can affect it. Despite the fact that some therapies have little to no hepatic metabolism, the bulk of drugs undergo some liver metabolism before being removed via the kidneys or bile [1]. Hepatotoxicity caused by drugs is a hazardous condition. It currently accounts for 20-40% of liver transplant patients in the United States and is the leading cause of hepatic dysfunction. These medications cause oxidative stress, fatty acid peroxidation, fat buildup, antibody-mediated cytotoxicity, and death in liver cells [2]. Acetaminophen (N-acetyl-para-aminophenol, or APAP), an analgesic and antipyretic, is one of the most regularly used medications. When compared to other nonsteroidal anti-inflammatory medicines, APAP is reasonably safe. An increase in APAP dose can cause a variety of liver problems, including increased liver enzymes, abrupt liver failure, and hepatic encephalopathy [3]. The primary metabolic pathways of APAP are conjugated with glucuronic acid and sulfate at the therapeutic level, and they are then removed in the urine [4]. A potentially toxic molecule, N-acetyl-p-benzoquinone imine (NAPBQI), is formed when a small quantity of APAP is oxidized by cytochrome P450 activity (CYP2E1), coupled with hepatic glutathione, and eliminated in the urine [5,6]. However, NAPBQI binds to macromolecules in the liver, causing irreversible hepatic necrosis [8,9], despite the fact that high doses of APAP cause glutathione depletion due to increased NAPBQI synthesis and saturation of its main metabolic processes (glucuronidation and sulfation) [5,7]. NAPBQI has been shown to promote the generation of reactive oxygen species (ROSS) such as superoxide, hydrogen peroxide, and hydroxyl radicals when glutathione levels are low [10]. Two enzyme defense systems, glutathione peroxidase (GPx) and superoxide dismutase (SOD), protect healthy hepatocytes from free radicals and other toxic substances. This finding is significant [11]. Previous research has found a link between oxidative stress and hepatotoxicity caused by APAP. Furthermore, excessive APAP levels can cause cell death by oxidative stress, which results in lipid peroxidation [12]. To prevent cell damage from NAPBQI, N-acetylcysteine (NAC) is utilized as a therapy for APAP toxicity. NAC therapy must enhance the clinical outcome of APAP hepatotoxicity [13]. The body requires dietary lipids since they are a fundamental component of all biological activities. All lipids can be produced by humans, with the exception of long-chain fatty acids from the Omega-3, Omega-6, and Omega-9 groups [14]. The two primary long-chain polyunsaturated fatty acid components of omega-3 are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [15]. The majority of omega-6 fatty acids are polyunsaturated, whereas the

majority of omega-9 fatty acids are monounsaturated [16]. Because mammalian cells cannot generate alpha-linolenic acid or linoleic acid, which are both Omega-3 and Omega-6 fatty acids, they are necessary fatty acids that must be acquired from the food [17]. Oleic acid (OA), a non-essential FA member of the Omega-9 family, can be generated by the body when Omega-3 and Omega-6 levels are adequate [18]. Omega-3 supplementation may increase or reduce the synthesis of pro- and/or anti-inflammatory cell signaling molecules, which has been identified as a modulator of inflammation. Omega-3 supplementation lowered the amount of proinflammatory cytokines in the blood in a recent randomized controlled experiment [19]. The omega-3 fatty acids present in cod liver oil are necessary for maintaining good health. Numerous studies have found that dietary Omega-3 PUFAs improve immune function, lipid peroxidation, and antioxidative characteristics. Anti-inflammatory effects of omega-3 PUFAs have been proven [20].

## METHODS

Thirty-five albino male mice weighing 25-33 g were utilized in this study. They were procured and housed in the animal house of College of Pharmacy, Baghdad University in conditions that ensured a constant temperature, humidity, and light/dark cycle. The animals had constant access to pellet food and an endless supply of tap water. The experimental procedure was approved by the scientific and ethical committee of the College of Pharmacy, University of Baghdad.

### *Acetaminophen (APAP) and Omega-369*

Acetaminophen ampoules (500 mg/5 mL) were purchased from Ajanta Pharma Limited® (India) and Omega-369 was purchased from Adrien Gagnon®, Canada.

### *Study design*

Thirty-five albino male mice were allocated to one of five groups at random. Group 1: The negative control received 10 ml/kg liquid paraffin via oral gavage for ten days, followed by an intraperitoneally single injection of normal saline (10 ml/kg) on day-11. Group 2: positive control, which received oral liquid paraffin (10 ml/kg) for ten days before receiving a single intraperitoneal APAP injection (IP) (400 mg/kg) on day-11 [21]. Group 3: treated with omega-369 (50 mg/kg) through oral gavage for ten days, then received intraperitoneally single injection (400 mg/kg) APAP on day-11 of the study. Group 4: treated with omega-369 (100 mg/kg) through oral gavage for ten days, then received intraperitoneally single injection (400 mg/kg) APAP on day-11 of the study. Group 5: N-acetylcysteine (100 mg/kg) was given orally for ten days, then a single intraperitoneal APAP injection was given on day-11 [21]. After 24 hours, the

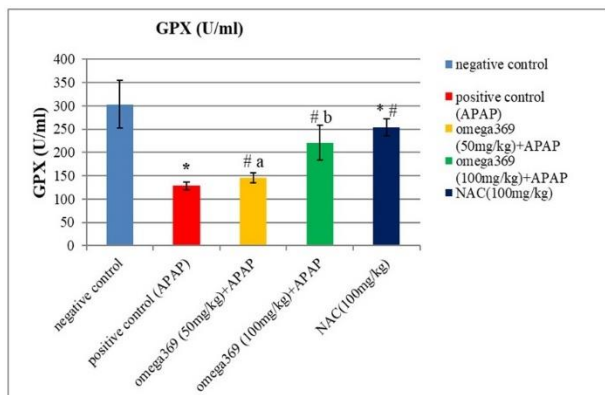
mice in each group were euthanized with anesthetic ether, their livers were obtained, and liver tissue homogenates were prepared in accordance with a standard procedure [21] for assessing glutathione peroxidase (GP-X), superoxide dismutase (SOD) activities, and malondialdehyde (MDA) concentrations in the liver tissue homogenate. The activities of SOD, and MDA levels were determined using an enzyme-linked immunosorbent assay (ELISA) kit (Pars Biochem Co., Ltd., China) according to the manufacturer's instructions. When measuring GP-X activity, the manufacturer's (MyBiosource, Inc., USA) recommendations were followed.

**Statistical analysis**

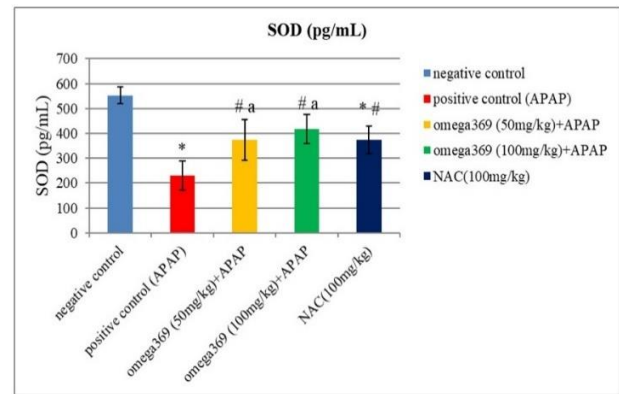
The data are displayed as the mean and standard deviation (SD). Statistical analysis was performed using the statistical package for the social sciences (SPSS version 25). An unpaired *t*-test was carried out in order to determine the statistical significance between groups. A *P* value of less than 0.05 was used to determine significant differences.

**RESULTS**

The results demonstrated that APAP has a negative impact on the liver and that the use of omega-369 can counteract the toxic effects. Figures 1, 2, and 3 revealed that GP-X and SOD activities in group 2 (positive control) were significantly decreased ( $p < 0.05$ ) compared to those in group 1 (negative control).

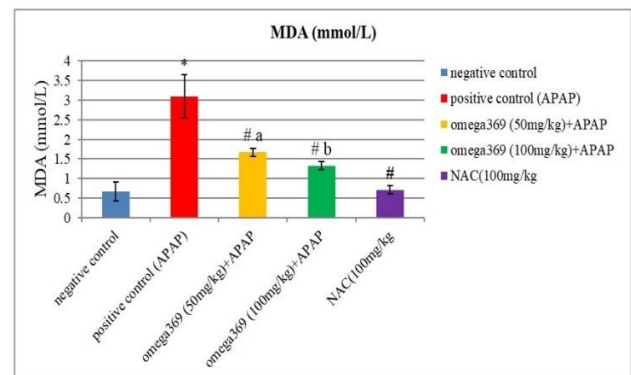


**Figure 1:** The effect of APAP, omega-369 and NAC on glutathione-peroxidase (GP-X) activity (U/ml) in mice's liver tissue homogenate. Values are mean±SD; n=7; \* significant difference compared to negative control ( $p < 0.05$ ); # significant difference compared to group positive control ( $p < 0.05$ ). Values with different superscripts a and b are significantly different ( $p < 0.05$ ).



**Figure 2:** The effect of APAP, omega-369 and NAC on superoxide dismutase (SOD) activity (U/ml) in mice's liver tissue homogenate. Values are mean±SD; n=7; \* significant difference compared to negative control ( $p < 0.05$ ); # significant difference compared to group positive control ( $p < 0.05$ ). Values with superscript "a" are not significantly different ( $p > 0.05$ ).

Meanwhile, MDA levels in group 2 (positive control) were significantly higher ( $p < 0.05$ ) when compared to those in group 1 (negative control).



**Figure 3:** The effect of APAP, omega-369 and NAC on malondialdehyde (MDA) concentrations (mmol/L) in mice's liver tissue homogenate. Values are mean±SD; n=7; \* significant difference compared to negative control ( $p < 0.05$ ); # significant difference compared to group positive control ( $p < 0.05$ ). Values with different superscripts a and b are significantly different ( $p < 0.05$ ).

Omega-369 at doses of 50 mg/kg and 100 mg/kg, and NAC (100 mg/kg) when used as a prophylactic treatment, produced a significant increase ( $p < 0.05$ ) in GP-X and SOD activities compared to those in group 2 (positive control). Whereas the levels of malondialdehyde (MDA) were significantly decreased ( $p < 0.05$ ) in mice treated with omega-369 at a dosage of 50 mg/kg, omega-369 at a dose of 100 mg/kg, and NAC at a dose of 100 mg/kg compared to those in group 2 (positive control). Furthermore, GP-X and MDA levels in mice receiving omega-369 (50 mg/kg and 100 mg/kg) were significantly different ( $p < 0.05$ ).

when compared with each other, while SOD activity in mice receiving omega-369 (50 mg/kg and 100 mg/kg) was not significantly different when compared with each other.

## DISCUSSION

Due to its availability and ease of administration, APAP overdose plays a key role in self-poisoning. According to research, APAP is the medicine that people in many different countries purposefully or unintentionally overdose on the most. [22,23]. An earlier study showed that oxidative stress is connected to the emergence of APAP toxicity through the formation of the reactive compound NAPBQI, which results in peroxidation of lipids, a decrease in anti-oxidants, mitochondrial dysfunction, and ultimately DNA damage and necrosis-induced cell death [24]. According to the results of the current study, group 2 (the positive control) GP-X activity dramatically decreased when compared to group 1 (the negative control), as shown in Figure 1. This result is in line with past research that found acetaminophen intoxication was linked to a considerable drop in GP-X levels in the liver tissue homogenate of mice as a result of liver damage brought on by APAP [25,26]. GP-X, a crucial antioxidant enzyme, detoxifies hydrogen peroxide by changing it into water [27]. In comparison to group 2 (positive control), mice treated with omega-369 (50 mg/kg and 100 mg/kg) had significantly higher hepatic GP-X activity. Several distinct pathways can be used to explain how long-chain unsaturated fatty acid supplementation affects the rise in GP-X activity. Previous cellular studies have demonstrated that long-chain unsaturated fatty acid supplementation increases SIRT1 and PGC1 gene expression via increasing AMPK activation, lowers the expression of pro-inflammatory genes, and activates the production of antioxidant genes such as GP-X [28]. These outcomes are in line with those that were mentioned in an earlier study [29]. GP-X levels in the liver significantly increased when NAC (100 mg/kg) was compared to group 2 (a positive control). These results are consistent with past studies that demonstrated NAC can boost glutathione synthesis and may directly eliminate reactive oxygen species to protect cells from oxidative damage [30]. The current investigation also revealed that, as indicated in Figure 2, group 2 (the positive control) had much lower hepatic SOD activity than group 1 (the negative control). These findings are in line with earlier research that showed acetaminophen toxicity lowered hepatic SOD levels [31,32], whereas therapy with omega-369 (50 mg/kg and 100 mg/kg) significantly increased hepatic SOD activity compared to group 2 (positive control). This result is in line with a previous investigation into the antioxidant advantages of long-chain unsaturated fatty acids, which revealed a link between oleic acid intake and reduced oxidative damage as well as an increase in the activity of

antioxidant enzymes [33]. Comparing group 2 (positive control) and NAC (100 mg/kg), there was a significantly higher increase in hepatic SOD activity in group 5. This outcome is consistent with research suggesting that NAC protects against acetaminophen toxicity, which was presented in a study. Additionally, because thiol groups are present, it reacts with reactive oxygen species (ROS) more quickly [34]. According to this study, group 2 (the positive control) had considerably higher hepatic MDA levels than group 1 (the negative control), as shown in Figure 3. The findings are consistent with previous studies that have established a correlation between elevated hepatic MDA levels in Group 2 and the excessive formation of free radicals, as well as the incapacity of antioxidant enzymes to eliminate them, resulting in lipid peroxidation [35,36]. However, administering omega-369 (50 mg/kg) and Omega-369 (100 mg/kg) to mice resulted in a marked reduction in hepatic MDA levels compared to Group 2 (positive control). This result is in line with a previous investigation that found high long-chain unsaturated fatty acid diets modify the structure of membrane phospholipids, offer protection from acetaminophen-induced liver toxicity, and lessen vulnerability to free radical damage [37]. Comparing group 2 (positive control) to NAC (100 mg/kg), there was a significantly lower level of hepatic MDA. This outcome was consistent with earlier research that demonstrated NAC's antioxidant properties by scavenging ROS, decreasing the activity of cyclooxygenase-2, and preventing the oxidation of membrane lipids brought on by inflammation [38]. Omega-369 may lessen liver damage by preventing various oxidation processes, according to pretreatment studies in mice using dosages of 50 and 100 mg/kg against APAP-induced acute hepatotoxicity [15,39,40].

## Conclusion

According to the findings of this investigation, it can be inferred that omega-369 has antioxidative effects, as reflected by a significant increase in GP-X and SOD activities in mice's liver tissue homogenate, accompanied by a considerable drop in the concentrations of MDA in the liver tissue homogenate of mice.

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## Conflict of interests

The author declares no conflict of interests.

## Source of fund

No specific fund received.

**Data sharing statement**

Data can be provided based on a reasonable request to the corresponding author.

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