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The Prophylactic Anti-Inflammatory Effect of Omega-7 Against Paracetamol-Induced Liver Injury in Rats

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A B S T R A C T

Paracetamol poisoning, whether intentionally or accidentally, is one of the main public health problems since the prevalence of its toxicity increased significantly in many countries. Currently, paracetamol is one of the primary causes of acute liver failure worldwide. The aim of this study was to investigate the potential prophylactic effect of omega-7 fatty acid in protecting male rats' livers from paracetamol-induced liver damage. Thirty albino male rats were divided randomly and equally into five groups and then treated as follows: Group 1 (negative control) rats were orally given liquid paraffin for seven consecutive days. Group 2 (positive control) rats were orally given liquid paraffin for seven consecutive days and a single injection of paracetamol (500 mg/kg) intraperitoneally on day eight of the experiment. Group 3 rats were orally given omega-7 (300 mg/kg) for seven consecutive days. Group 4 rats were orally given a single dose of omega-7 (100 mg/kg/day) for 7 days and a single injection of paracetamol (500 mg/kg) intraperitoneally on day eight of the experiment. Group 5 rats were orally given a single dose of omega-7 (300 mg/kg/day) for 7 days and a single injection of paracetamol (500 mg/kg) intraperitoneally on day eight of the experiment. After 24 h of the endpoint of treatment (on day 9), blood samples were collected, and serum was prepared for the evaluation of tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10). This study found that rats given paracetamol had a significant (P<0.05) increase in serum levels of TNF-α and IL-10, whereas rats previously given oral administration of omega-7 fatty acid before injection of paracetamol resulted in a significant decrease (P<0.05) of these cytokines. Oral omega-7 fatty acid supplementation may help to prevent liver damage caused by paracetamol.

Keywords: liver injury, omega-7, paracetamol, anti-inflammatory, rat

INTRODUCTION

Paracetamol (PCM) is one of the most commonly used medications as an analgesic and antipyretic. In comparison with other non-steroidal anti-inflammatory medications, PCM is relatively safe. Overdoes of PCM can cause a variety of liver injuries, ranging from elevated liver enzymes to sudden liver failure and hepatic encephalopathy (1). At the therapeutic level, PCM is conjugated with glucuronic acid and sulfate, the primary metabolic pathways of PCM, and then they are eliminated in the urine (2). A minor amount of PCM is oxidized by the cytochrome P450 (CYP450) enzyme, mainly metabolized by CYP2E1 (3), to form a potentially reactive compound, *N*-acetyl-*p*-benzoquinone imine (NAPQI), which is generally harmless as it is combined with hepatic glutathione and then excreted with urine (4). Although high doses of PCM cause saturation of its main metabolic pathways (glucuronidation and sulfation) and glutathione depletion due to excessive NAPQI formation (3, 5), NAPQI is bound to

macromolecules in the liver, resulting in irreversible hepatic necrosis (6, 7).

Liver damage is accompanied by a severe inflammatory process, distinguished by increased cell influx and enhanced generation and release of inflammatory mediators mainly tumor necrosis factor-alpha (TNF-α) and free radicals (reactive oxygen species) (8–10). TNF-α is a key regulator that has diverse effects on the inflammatory response, it induces the release of other cytokines and chemokines. Nevertheless, uncontrolled and excess production of TNF-α can be dangerous because it induces some systemic disorders as autoimmune diseases (11, 12). In addition, interleukin-10 (IL-10) is a cytokine with a critical function in the modification and suppression of pro-inflammatory mediators and in the regeneration of cells, as an increase in IL-10 synthesis then release which can reduce exacerbation of TNF-α level. it was demonstrated that synergistic anti-inflammatory cytokine protects animals from PCM-induced liver damage (13).

Omega-7 are monounsaturated fatty acids (at 7 carbon atom), that are present in plants including sea buckthorn berries, macadamias and fishes like cold water fish. Vaccenic acid and palmitoleic acid are the main two forms of omega-7 in nature (14, 15). In addition to the above, numerous studies have shown that palmitoleic acid (POA) has a variety of potential health benefits, because it is accompanied with a reduced risk of cardiovascular disease, diabetes, and inflammation (16–18). Particularly in the liver, POA reduces inflammation (19), enhances cholesterol metabolism (20) and improves insulin sensitivity (21). This study aims to assess the prophylactic effect of omega-7 fatty acid against paracetamol-induced liver damage in male rats.

MATERIALS AND METHODS

In this study, thirty adults male Albino rats (8 weeks old, weighting 180-200 g) were involved. They were obtained and kept in the College of Pharmacy's Animal House at the University of Baghdad under circumstances of constant temperature, humidity, and light/dark cycles 12/12 h. The animals were given regular pellet food and unlimited access to tap water. The College of Pharmacy, University of Baghdad's scientific and ethical committee examined and approved the experimental protocol.

Paracetamol and Omega-7

Paracetamol ampoule of 600 mg/5 mL was purchased from BS Pharma (France), and omega-7 POA soft gel (210 mg) from Source and Naturals (USA).

Experimental Design

Thirty Albino male rats were divided randomly and equally into five groups of six, then tested for the following protocols: Group 1 (negative control) rats were orally administered liquid paraffin for seven consecutive days; Group 2 (positive control) rats were orally administered liquid paraffin for seven consecutive days, followed by a single intraperitoneal injection (IP) of 500 mg/kg paracetamol on the eighth day of the experiment (22); Group 3 rats were orally administered omega-7 at 300 mg/kg/day for seven consecutive days (23); Group 4, rats were orally administered once daily omega-7 at 100 mg/kg/day for 7 days then a single injection of paracetamol at 500 mg/kg IP at the eighth day of the experiment (23); Group 5, rats were orally administered once daily omega-7 at 300 mg/kg/day for 7 days then a single injection paracetamol at 500 mg/kg IP on the eighth day of the experiment (23).

After 24 h of the endpoint of the administration (day 9), a heart puncture was performed to obtain blood samples after anesthetizing the rats using diethyl ether anesthesia; then serum was separated to be used for TNF-α and IL-10 evaluations (24).

An enzyme-linked immunosorbent assay was used to measure TNF-α and IL-10 according to the instructions given on the kit by the manufacturer (Bioassay Technology Laboratory BT LAB, China).

Statistical Analysis

The Statistical Package for Social Science was used to conduct the study (SPSS, version 26). This study presented the data by using descriptive statistics (mean, standard deviation); unpaired *t*-test and one-way analysis of variance (ANOVA).

RESULTS

In this study, paracetamol was proven to affect negatively on the liver, whereas omega-7 was found to correct that effect.

Figure 1 shows that the PCM group had significantly higher serum TNF-α levels than the negative control group ($P<0.05$). Simultaneously, when the omega-7 group was compared to the negative control group, TNF-α was found to be nonsignificantly lower ($P>0.05$). Furthermore, the comparison of the omega-7-group and the PCM group revealed a significant decrease in TNF-α in omega-7 group ($P<0.05$). Omega-7 (100 mg/kg) as a prophylactic treatment, produced significant reduction in TNF-α as compared to PCM group ($P<0.05$). The (300 mg/kg) of omega-7 was administered shows a significant reduction in TNF-α when compared to PCM group ($P<0.05$). There were no statistically significant differences in TNF-α levels between groups received omega-7.

In this study, administration of PCM at 500 mg/kg BW significantly increased ($P<0.05$) the serum IL-10 level in comparison to negative control group and omega-7 treated group at 300 mg/kg BW (Figure 2). Furthermore, omega-7 treated groups at 100 and 300 mg/kg BW significantly reduced the serum IL-10 levels when compared to PCM treated group. omega-7 treated groups at 100 and 300 mg/kg BW have no significant ($P>0.05$) differences in IL-10.

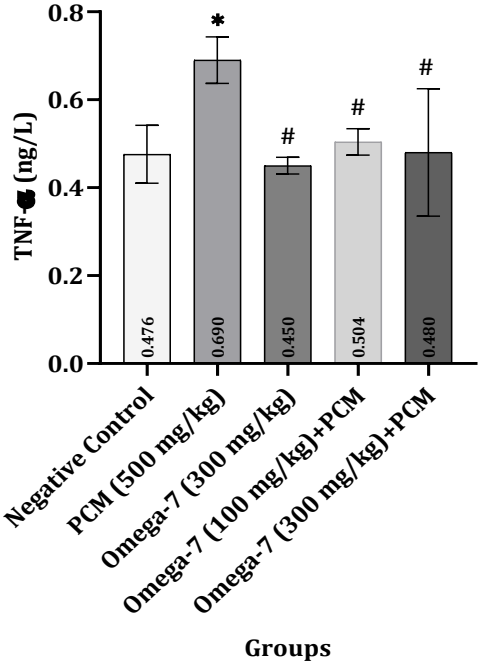

Figure 1. Effect of paracetamol (PCM) and omega-7 on serum tumor necrosis factor-alpha (TNF-α) ng/L. Values are mean±SD, n=6, *Indicates a significant difference when compared to the negative control group (P<0.05). #Indicates a significant difference when compared to the paracetamol group (P<0.05)

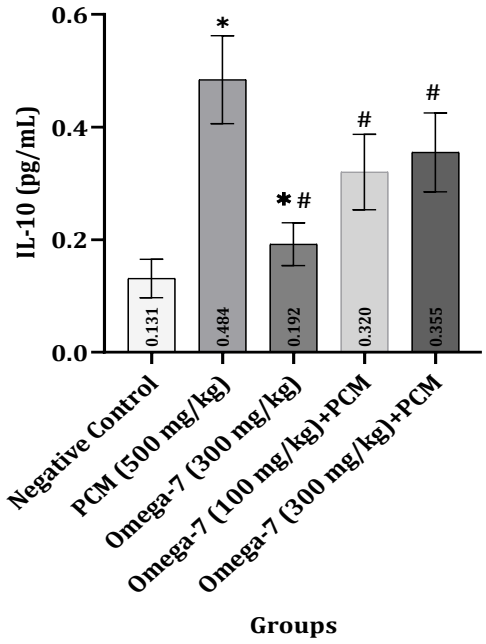

Figure 2. Effect of paracetamol (PCM) and omega-7 on serum interleukin-10 (IL-10) level (pg/ml). Values are mean ± SD, n=6, *Indicates a significant difference when compared to the negative control group (P<0.05). #Indicates a significant difference when compared to the paracetamol group (P<0.05)

DISCUSSION

PCM overdose is a major factor in self-poisoning due to its accessibility and availability. It was reported that PCM is the most frequent drug taken in overdose, either intentionally or accidentally, in many countries (24–26).

An earlier study demonstrated that oxidative stress is mainly linked to the development of PCM toxicity by the formation of the reactive compound NAPQI, which causes peroxidation of lipids, antioxidant attenuation, mitochondrial malfunction, and eventually DNA damage and cell death by necrosis (27).

The current study found that rats in the PCM group had significantly higher serum TNF- levels than the negative control group, indicating a severe inflammatory response. This finding supports previous research that found a significant increase in serum TNF- levels as a result of PCM-induced liver damage (28, 29). PCM-induced oxidative stress causes hepatocyte necrosis, which leads to inflammatory cell influx and the inflammatory process (30).

Damage-associated molecular patterns (DAMPs) are produced by necrotic hepatocytes and are determined by toll-like receptors (TLR4 and TRL9) (31), leading to innate immune system activation and releases excessive inflammatory mediators such as TNF-α and other cytokines and this eventually results in severe hepatic injury (32).

Additionally, this study showed a noticeable increase in the level of serum IL-10 in PCM group in comparison with the negative control group. IL-10 is a strong anti-inflammatory cytokine that is synthesized by different types of cells in the liver, involving hepatocytes, sinusoidal endothelial cells, and Kupffer cells. It is clearly associated with protective functions in chronic liver diseases and is upregulated in a variety of conditions during liver inflammation (33). In this study, up-regulation of IL-10 reflected the immunoreactive response of the liver to PCM-induced hepatotoxicity as it has been demonstrated that IL-10 protects the liver from drug-induced liver damage (34). This result is in consistence with previous research illustrated significant elevation in IL-10 level by high dose PCM administration to investigate the hepatoprotective effect of carnosine and histidine on PCM-induced liver injury in mice (35).

The present study also revealed that omega-7 pretreated rats in group 4 and 5 had considerably lower serum levels of TNF-α and IL-10, suggesting that omega-7 has strong anti-inflammatory activity against PCM-induced liver injury. These findings are consistent with an earlier study illustrated that repeated administration of omega-7 suppresses pro-inflammatory gene expression by reducing mRNA expression of TNF-α (36), omega-7 attenuating

inflammation-induced by high fat diet in mice by decreasing inflammatory cytokine expression (37), in addition, another study revealed that omega-7 had an anti-inflammatory effect by decrease inflammation when used topically as wound treatments in rats (38).

It is concluded that omega-7 fatty acid could be highly effective supplement in the prevention of liver injury caused by paracetamol.

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N/A.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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التأثير الوقائي المضاد للالتهابات للأوميغا ٧ ضد ضرر الكبد المحدث بوساطة الباراسيتامول في الفئران

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الخلاصة

يعتبر التسمم بالباراسيتامول سواء كان متعمدا او غير متعمد مشكلة صحية عامة،حيث زادت اعداد المصابين بشكل كبيرفي العديد من البلدان. ويعد البارسيتامول حاليا احد اهم الاسباب الرئيسية لفشل الكبد الحاد في عديد من دول العالم. كان الهدف من هذه الدراسة هو دراسة التأثير الوقائي المحتمل لأحماض أوميغا 7 الدهنية في حماية كبد ذكور الجرذان من الاصابة الناجم عن الباراسيتامول. ثلاثون من ذكور الجرذان المختبرية قسمت عشوائيا وبشكل متساوٍ الى خمس مجاميع وتم معاملتها على النحو التالي: المجموعة الاولى اعطيت البارافين السائل عن طريق الفم لمدة سبعة ايام متتالية. المجموعة الثانية اعطيت البارافين السائل عن طريق الفم لمدة سبعة ايام متتالية، وفي اليوم الثامن تم حقن الجرذان بجرعة مفردة داخل الصفاق من الباراسيتامول (٥٠٠ ملغم/كغم). المجموعة الثالثة اعطيت الاوميغا-٧ (٣٠٠ ملغم/كغم) عن طريق الفم لمدة سبعة ايام متتالية. المجموعة الرابعة اعطيت الاوميكا-٧ (١٠٠ ملغم /كغم) عن طريق الفم لمدة سبعة ايام متتالية، وفي اليوم الثامن تم حقن الجرذان بجرعة مفردة داخل الصفاق من الباراسيتامول (٥٠٠ ملغم/كغم). المجموعة الخامسة اعطيت الاوميكا-٧ (٣٠٠ ملغم/كغم) عن طريق الفم لمدة سبعة ايام متتالية، وفي اليوم الثامن تم حقن الجرذان بجرعة مفردة داخل الصفاق من الباراسيتامول (٥٠٠ ملغم/كغم).في نهاية التجربة، في اليوم التاسع، تم جمع عينات الدم وفصل المصل الدم لغرض تحليل عامل نخر الورم الفا والانترلوكين-١٠. وجدت هذه الدراسة ان اعطاء الباراسيتامول للجرذان ادى الى زيادة معنوية في مستوى المصل لكل من عامل نخر الورم الفا والانترلوكين-١٠ فضلا عن انخفاض معنوي في مستوى المصل لهذه السيتوكينات في الجرذان التي اعطيت الاوميكا-٧ مقارنة بالجرذان التي اعطيت الباراسيتامول. كاستنتاج ، قد تساعد مكملات احماض الاوميكا-٧ في الوقاية من اصابة الكبد التي يسببها الباراسيتامول.

الكلمات المفتاحية: اصابة الكبد، الاوميكا-٧،الباراسيتامول، جرذ