ORIGINAL ARTICLE

Chemoprotective effect of omega-3 fatty acids on thioacetamide induced hepatic fibrosis in male rats

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Received 21 November 2015; revised 2 January 2016; accepted 14 January 2016
Available online 21 January 2016

KEYWORDS
Hepatic fibrosis; Thioacetamide; Omega-3 fatty acids; Rats

Abstract The current study was designed to investigate the possible protective effect of omega-3 fatty acids from fish oil on hepatic fibrosis induced by thioacetamide (TAA) in male rats. The experimental animals were divided into four groups. The first group was received saline solution and served as control. The second group was given 250 mg/kg body weight of TAA. The third group was treated with omega-3 fatty acids and TAA. The fourth group was given saline solution and supplemented with omega-3 fatty acids. Treatment of rats with TAA for three and six weeks resulted in a significant decrease in body weight gain, while the value of liver/body weight ratio was statistically increased. Furthermore, the levels of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase and total bilirubin were significantly increased. After three weeks of exposure to only TAA, liver sections showed an abnormal morphology characterized by noticeable fibrosis with the extracellular matrix collagen contents and damage of liver cells’ structure. Liver sections from rats treated with only TAA for six weeks revealed an obvious increase in extracellular matrix collagen content and bridging fibrosis. Treating TAA-intoxicated rats with omega-3 fatty acids significantly attenuated the severe physiological and histopathological changes. Finally, the present investigation suggests that omega-3 fatty acids could act against hepatic fibrosis induced by TAA due to its antioxidant properties, thus supporting its use in hepatic fibrosis therapy.

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1. Introduction

Hepatic fibrosis is a dynamic wound-healing response to chronic hepatic injuries. Without effective treatments, reversible liver fibrosis at an early stage leads to irreversible cirrhosis. Chronic liver injury leads to a progressive wound healing response that eventually results in liver fibrosis characterized by both quantity and quality alterations of hepatic extracellular matrix, ECM, (Liu et al., 2006). Moreover, liver fibrosis represents the response of the liver to diverse chronic insults such as parasitic disease, chronic viral infection (hepatitis B...
and C), immunologic attack (autoimmune hepatitis), hereditary metal overload, toxic damage, etc. Because of the worldwide prevalence of these insults, liver fibrosis is common and is associated with significant morbidity and mortality (Chen et al., 2002; Han, 2002; Shen et al., 2003).

Thioacetamide (TAA) is a chemical originally widely used as a fungicide, a curing ingredient, a chemical reagent, a raw medicine, a textile dye, and a finishing auxiliary (Chen et al., 2006).

TAA is a model hepatotoxicant and responsible of severe damage of the cells with significant toxic effects on the biosynthesis of macromolecules including proteins and nucleic acids (Yang et al., 1998). Furthermore, a different studies showed that the exposure to TAA caused liver injury, fibrosis and cirrhosis in experimental animals (Al-Attar, 2011, 2012; Abramovitch et al., 2015; Al-Attar and Shawush, 2015; Al-Attar et al., 2016; Ali et al., 2015; Luo et al., 2015).

The medical management of liver diseases or disorders including liver fibrosis is currently inadequate, so far no therapy has successfully prevented the progression of hepatic disease. Even though newly developed drugs have been used to treat chronic liver diseases, often these drugs have side effects. It is therefore necessary to search for alternative drugs for the treatment of liver diseases to replace currently used drugs of doubtful efficacy and safety (Bruck et al., 1996; Dhiman et al., 2012). Additionally, there is an increasing interest in the alternative medicines for the treatment of liver diseases and associated metabolic derailments which include herbal remedies and dietary supplements (Handa et al., 1985; Connor, 2000; Sharma and Shukla, 2010).

Dietary fats and oils are known as macronutrients which provide concentrated source of energy for the metabolic processes. In addition, fats are the main source of fat-soluble vitamins (Sanchez-Muniz and Bastida, 2006). Dietary fats are composed of different types of fatty acids which are saturated, monounsaturated and polyunsaturated. Evidence has been shown that dietary fats can have important effects on human health. Fish oil is most widely used as food supplements. Owing to its wide array of biological actions public and scientific interest has been directed toward the role of fish oil in health promotion and disease prevention. Dietary fish oil, which provides omega-3 fatty acids, has been shown to have beneficial effects on some chronic degenerative diseases such as cardiovascular disease (Car and Webel, 2012; Xin et al., 2012), rheumatoid arthritis (Miles and Calder, 2012), diabetes, other autoimmune diseases (Calder, 2007; Chapkin et al., 2009), and cancer (Cockbain et al., 2012; Vaughan et al., 2013). The beneficial effects of fish oil seem to be due to its high content of the omega-3 polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Scientific evidence revealed that a diet rich in long chain omega-3 fatty acids help in the development of healthy brain, heart and immune system. It has a role in joint movement, balanced mood, a sense of well being, strength, stamina and maintaining cholesterol levels within the normal range. Omega-3 fatty acids contain about 60% of long chain omega-3 fatty acids DHA and EPA as combined. The most widely available source of EPA and DHA is cold water oily fish such as salmon, herring, mackerel, anchovies and sardines (Bolles and Begg, 2000). In recent years, fish oil is gaining attention as a nutraceutical and source of potential pharmaceuticals (Kaur et al., 2014). Therefore, the present study was conducted to investigate the effect of omega-3 fatty acids from fish oil on hepatic fibrosis induced by TAA.

2. Materials and methods

2.1. Animals

Male albino rats of the Wistar strain (Rattus norvegicus), weighing 94.2–134.6 g were used in the present study. The experimental animals were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Rats were acclimatized to the laboratory conditions for one week prior to the initiation of experimental treatments. The experimental animals were housed in standard plastic cages and maintained under controlled laboratory conditions of humidity (65%), temperature (20 ± 1 °C) and 12:12 h light: dark cycle. Rats were fed ad libitum on normal commercial chow and had free access to water. The experimental treatments were conducted in accordance with ethical guidelines of the Animal Care and Use Committee of King Abdulaziz University.

2.2. Experimental design

A total of sixty rats were randomly divided into four experimental groups, fifteen rats each. The experimental groups were treated as follows:

(1) Rats of group 1 were served as controls and intraperitoneally injected with saline solution (0.9% NaCl), twice weekly.

(2) Rats of group 2 were given 250 mg/kg body weight of TAA (Sigma–Aldrich Corp., St. Louis, MO, USA) by intraperitoneal injection, twice weekly.

(3) Rats of group 3 were orally supplemented with omega-3 fatty acids from fish oil (Pharma Nord ApS, Denmark) at a dose of 800 mg/kg body weight/day. Moreover, they were intraperitoneally injected with TAA at the same dose given to group 2.

(4) Rats of group 4 were intraperitoneally injected with saline solution (0.9% NaCl), twice weekly and were orally supplemented with omega-3 fatty acids from fish oil at a dose of 800 mg/kg body weight/day.

2.3. Body weight determinations

The body weights of rats were determined at the start of the experimental period, after three and six weeks using a digital balance. These weights were measured at the same time during the morning (Al-Attar and Zari, 2010). Moreover, the experimental animals were observed for signs of abnormalities throughout the period of study.

2.4. Blood serum analyses

After three and six weeks, the experimental animals were fasted for 12 h, water was not restricted, and then anaesthetized with diethyl ether. Blood samples were collected from orbital venous plexus in non–heparinized tubes, centrifuged at
2500 rpm for 15 min and blood sera were then collected and stored at –80 °C. Serum samples were used to determine the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and total bilirubin. The levels of serum ALT and AST were measured using the method of Reitman and Frankel (1957). The methods of MacComb and Bowers (1972), Szasz (1969), Doumas et al. (1973) were used to estimate the levels of serum ALP, GGT and total bilirubin respectively.

2.5. Weight changes of liver

After three and six weeks, livers were carefully excised and weighed for the evaluation of their ratios/body weight. The ratio was calculated by the following equation (Al-Attar, 2010):

\[
\text{Ratio} = \frac{\text{Liver weight}}{\text{Body weight}} \times 100
\]

2.6. Histopathological examinations

After blood sampling, rats were dissected and the livers were preserved in 10% formalin immediately after removal from the animals. Paraffin sections were stained with hematoxylin and eosin, and Masson’s trichrome. Liver sections were examined microscopically and photographed.

2.7. Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS for windows, version 22.0). Each value is expressed as mean ± standard deviation (S.D.) and values were analyzed using a two-way analysis of variance (ANOVA) to determine differences between the mean values of experimental groups. \( P \)-values of less than 0.05 were considered as significant.

3. Results

The body weights after three weeks of all experimental groups are represented in Fig. 1. A gradual increase in the body weight gain of normal control rats and those supplemented with omega-3 was recorded, which amounted to 99.4% and 75.4%, compared with their initial body weights. Significant decreases in the values of body weight gain were observed in rats treated with TAA and omega-3 plus TAA. The minimum body weight gain was noted in TAA-intoxicated rats (52.7%) followed by omega-3 plus TAA treated rats (66.7%). Fig. 2 demonstrates the body weights of all experimental groups after six weeks. The maximum body weight gain was noted in normal control rats (181.4%). The minimum body weight gain...
was noted in TAA-intoxicated rats (106.7%) followed by omega-3 plus TAA treated rats (124.1%). Supplementation with omega-3 in normal rats showed a remarkable increase in the percentage change of body weight (149.3%). After three weeks, the values of liver/body weight ratio were statistically increased in rats treated with TAA (10.6%) and omega-3 plus TAA (21.3%) compared with control rats (Fig. 3). The values of liver/body weight ratio were significantly elevated after six weeks in rats exposed to TAA (40.5%) and omega-3 plus TAA (21.4%) compared with control rats. Additionally, there was no significant difference in the value of liver/body weight ratio in normal rats supplemented with omega-3 (Fig. 4).

The levels of serum ALT, AST, ALP, GGT and total bilirubin in control, TAA, omega-3 plus TAA and omega-3 treated rats after three weeks are shown in Fig. 5(A–E). Statistical increases in the level of serum ALT were observed in rats subjected to TAA (1153.5%) and omega-3 plus TAA (167.5%) compared with control rats. Notable increases in the level of serum AST were observed in TAA (1058.1%) and omega 3 plus TAA treated rats (221.1%) as compared with control rats. The levels of serum ALP were statistically elevated in rats treated with TAA (295.4%) and omega-3 plus TAA (73.2%) compared with control rats. Serum GGT level was statistically enhanced in rats exposed to TAA (951.8%) and omega-3 plus

![Figure 5](A–E) The levels of ALT, (A) AST, (B) ALP, (C) GGT (D) and total bilirubin (E) in serum from control, TAA, omega 3 plus TAA and omega 3 treated rats for three weeks. *Indicates a significant difference between control and treated groups. **Indicates a significant difference between rats treated with TAA and omega 3 plus TAA and omega 3. ***Indicates a significant difference between rats treated with omega 3 plus TAA and omega 3.
TAA (158.4%) compared with control rats. TAA administration to normal rats significantly increased the level of serum total bilirubin (1110.9%) compared with control rats. A statistical increase in the level of serum total bilirubin was observed in omega-3 plus TAA treated rats (249.4%) compared with control rats. On the other hand, no statistically significant difference was observed in the level of serum ALT, AST, ALP, GGT and total bilirubin in omega-3 treated rats.

The levels of serum ALT, AST, ALP, GGT and total bilirubin in control, TAA, omega-3 plus TAA and omega-3 treated rats after six weeks are shown in Fig. 6(A–E). A highly significant increase in the level of serum ALT was observed in rats exposed to TAA (853.6%) compared with control and omega-3 treated rats. Administration of omega-3 plus TAA resulted in a marked increase (398.3%) in the level of serum ALT compared with control rats. TAA administration to normal rats significantly elevated the level of serum AST (559.2%) compared with control rats. The level of serum AST was significantly increased in rats treated with omega-3 plus TAA (312.7%). According to the data shown in Fig. 6C, significant elevations in the level of serum ALP were noted in rats treated with TAA (360.3%) and omega-3 plus TAA (174.8%).

Figure 6  (A–E) The levels of ALT, (A) AST, (B) ALP, (C) GGT (D) and total bilirubin (E) in serum from control, TAA, omega 3 plus TAA and omega 3 treated rats for six weeks. *Indicates a significant difference between control and treated groups. **Indicates a significant difference between rats treated with TAA and omega 3 plus TAA and omega 3. ***Indicates a significant difference between rats treated with omega 3 plus TAA and omega 3.
compared with control rats. Relative to the control rats, the experimental rats exposed to TAA exhibited a significant increase in the level of serum GGT (467.1%). The level of serum GGT was statistically enhanced in omega-3 plus TAA treated rats (104.2%). As shown in Fig. 6E, a significant enhancement in the level of serum total bilirubin was observed in rats treated with TAA (891.6%) and omega-3 plus TAA (188.2%) as compared with control rats. Treatment of rats with omega-3 alone did not cause any significant change in the levels of serum ALT, AST, ALP, GGT and total bilirubin.

As shown in Fig. 7A and F, the control and omega-3 treated groups, normal hepatic architecture with central vein and radiating hepatic cords were seen. The liver sections of these groups showed normal liver cells or hepatocytes with preserved cytoplasm, prominent nucleus and nucleolus, and well brought out central vein. These cells are cuboidal epithelial cells arranged in anastomosing plates and cords. In classical lobules, the plates radiate from the central vein and cords alternate with sinusoids. After three weeks of exposure to TAA, liver sections showed an abnormal morphology characterized by noticeable fibrosis with the extracellular matrix collagen contents and damage of liver cells structure (Fig. 7B and C).

Liver sections from omega-3 plus TAA treated rats showed a reduced extent and development of fibrosis processes (Fig. 7D and E). Moreover, the liver cells showed slight alterations compared with liver cells structure of rats subjected to only TAA.

Liver sections from rats treated with only TAA for six weeks revealed an obvious increase in extracellular matrix collagen content and bridging fibrosis. Furthermore, there were bundles of collagen surrounding the lobules that resulted in large fibrous septa and distorted tissue architecture. Liver sections from TAA treated rats showed an evidence of severe histopathological alterations comprising nodular transformations in liver parenchyma similar to those found in human nodular fibrosis. Moreover, the parenchyma nodules were surrounded by the extension of fibrous septae which divided the liver into pseudolobules. Additionally, the administration of TAA caused centrilobular necrosis, hepatic cells surrounding central vein showed various degenerative changes like cloudy swelling, hydropic degeneration and necrosis with loss of nucleus (Fig. 8A–C). Liver sections from omega-3 plus TAA treated rats (Fig. 8D and E) showed improvements and reduction in fibrosis processes compared with liver sections of rats treated with only TAA.

Figure 7  (A–F) Photomicrographs of liver sections in each group. (A) Control (×200), (B and C) TAA (×100 and ×200), (D and E) omega 3 plus TAA (×100 and ×200) and (F) omega 3 (×200) treated rats for three weeks.
treated with only TAA. Moreover, the liver cells showed slight alterations compared with liver cells’ structure of rats treated with only TAA. Additionally, there are no any obvious features of hepatic fibrosis in rats treated with omega-3 plus TAA. Moreover, in omega 3 treated rats all sections of liver tissue had normal structure (Fig. 8F).

4. Discussion

Liver fibrosis is a characteristic of most types of chronic liver diseases. Liver fibrosis is a public health problem that results in significant morbidity and mortality (Sánchez-Valle et al., 2012). Liver fibrosis is a dynamic and highly integrated molecular, tissue cellular process that drives the progression of chronic liver diseases toward liver cirrhosis and hepatic failure (Ezhilarasan et al., 2014). Moreover, liver fibrosis occurs as a consequence of dynamic wound-healing response to acute or chronic hepatocellular damage(s), and it poses a high threat with significant morbidity and mortality (Hamza, 2010). The field of hepatic fibrosis is flourishing thanks to continued experimental advances complemented by exciting progress in the treatment of chronic liver disease (Friedman et al., 2007).

Additionally, no acceptable therapeutic strategies exist. There is a huge need and great significance to search for effective ways to inhibit liver fibrosis and prevent the development of cirrhosis (Zou et al., 2008). Conventional, drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Therefore, it is necessary to search for alternative drugs for the treatment of liver disease in order to replace currently used drugs of doubtful efficacy and safety (Ozbek et al., 2004). In the absence of reliable liver protective drugs in allopathic medical practices, natural products, herbs and dietary antioxidants play a role in the management of various liver disorders (Bjelakovic et al., 2011; Buraimoh et al., 2011). Therefore, it is very important and prudent to seek for new sources for a safe and effective treatment of liver diseases.

Physiologically, it is known that TAA toxicity is generally associated with hepatic fibrosis induction, complicated metabolic disorders and health problems (Al-Attar and Shawush, 2014). The present results showed that the maximum increases in body weight were observed in control rats. The significant increase in body weight in the control rats may be representative of the normal pattern of growth in rodents. On the contrary, the minimum increases in body weight were noted in TAA treated rats accompanied with a notable increase in
liver/body ratio. These observations were also noted by other studies (Wong et al., 2012; Kadir et al., 2013; Kabiri et al., 2014; Zargar, 2014; Al-Attar and Shawush, 2015; Al-Attar et al., 2016)

An increase in body weight implies that anabolic effects have overridden the catabolic ones. No variation means protection against weight loss. A decrease in body weight would mean that catabolism has persisted. The body weight decrease as a result of TAA injection was considered to be the result of direct toxicity of TAA and/or indirect toxicity related to the liver damage. Moreover, the relative decrease in mean body weight gain recorded in TAA treated rats may be adduced to malnutrition resulting from reduced absorption of nutrients from the intestine of treated rats. Furthermore, reduced adipose tissue and proteins may be the foremost cause of lower body weight. Additionally, the observed decrease in body weight could be due to the direct effect of TAA on the food intake behavior of the rats. TAA might have increased the protein catabolism and hampered the utilization of food consumed during the intoxication period, thereby causing a decrease in body weight. Such a reduction in body weight in TAA-treated rats might be in part due to gastrointestinal toxicity and concomitant loss of the animal appetite with subsequent reduction of food ingestion or due to excessive loss of water, salts and proteins as a result of renal injury with subsequent dehydration and weight loss.

Measurement of liver body weight ratio is a more accurate approach to determine the changes in liver size compared to the measurement of liver weight alone as the liver weight largely depends on the size of the rat. The enlargement of livers in TAA treated rats signified hepatic lesions and liver injury associated with the toxicological effects of TAA. Studies that relate to liver weight change are complex (Rizzo et al., 1997). Many factors are involved in the pathogenesis of this interaction, including control of feeding behavior, fat storage mechanisms, regulation of energy intake and energy expenditure, as well as hormonal, genetic and psychological influences (Lissner, 1993). When the liver is damaged, it can initiate regenerative actions (Yamada and Fausto, 1998), thus increasing the weight of liver. If it was heavily damaged, however, liver fibrosis and cirrhosis appear resulting in liver atrophy (Pérez Tamayo, 1983). Therefore, the change in weight of liver cannot directly predict the pathological process in chronic liver injuries (Lin and Lin, 2006).

The present study showed that the administration of TAA for three and six weeks induced an elevation in the levels of serum ALT, AST, ALP, GGT and total bilirubin in rats, since necrosis or membrane damage releases these enzymes into circulation, which agrees with the previously reported results (Kew, 2000). Moreover, many investigations showed that these parameters were significantly enhanced in experimental animals exposed to TAA (Al-Attar, 2011, 2012; Ali et al., 2014; Kim et al., 2014; Zargar, 2014; Abdou et al., 2015; Al-Attar and Shawush, 2015; Al-Attar et al., 2016; Luo et al., 2015). Damage of hepatocytes is reflected by an elevation in the levels of hepatic specific enzymes (ALT, AST, and ALP), these are cytoplasmic in lection and are released into circulation after cellular damage (Sallie et al., 1991). The increase in serum total bilirubin may be owing to blockage of bile ductules as the inflammation and fibrosis in the portal triads and/or due to the regurgitation of conjugated bilirubin from the necrotic hepatocytes to sinusoids (Ahmed, 2001). Additionally, ALT, AST, ALP and total bilirubin will leak into the serum resulting in the elevation of their serum concentrations. Serum levels of these parameters are very sensitive markers employed in the diagnosis of liver diseases (de David et al., 2011).

Histopathological evaluations of the present study demonstrated that severe alterations of liver structure were observed in rats subjected to only TAA. However, the impact of TAA depends on several factors such as its concentration, number of doses, administration period, administration methods, animal’s gender, strain, age, weight and physiological case. TAA is a toxin widely used for fibrosis-induction. It damages both zone 1 and 3 hepatocytes with periportal injury being more prominent than with other toxins. It is repeatedly injected intraperitoneally or administered in the drinking water (at a concentration adapted to water consumption). A long time is needed for significant fibrosis (Salguero et al., 2008), but this model is non invasive and requires minimal handling of the animals. Cholangiocarcinoma and hepatocellular carcinoma have been described in rats after a long time exposure to TAA (Newell et al., 2008). The choice of the fibrosis-inducing stimulus in an animal model is based on the expectation that the response will engage mechanisms that are similar to those occurring in the related human disease. In this study, TAA induced severe hepatic damage as shown in histopathological examination which coupled with markedly elevated levels of liver hematobiochemical markers (ALT, AST, ALP, GGT and total bilirubin).

The present study showed that the treatment with omega-3 fatty acids attenuated the physiological and histopathological alterations induced by TAA in rats. This indicated the effectiveness of omega-3 fatty acids in prevention of TAA toxicity. The possible mechanism of the studied omega-3 fatty acids as hepatoprotective factor may be due to its antioxidant effect which impairs the activation of TAA into the reactive form. Bruck et al. (2004) stated that the chronic of TAA administration induced liver cirrhosis and oxidative stress. Cruz et al. (2005) reported that TAA induced liver fibrosis, oxidative stress with extensive tissue damage and enhanced alpha-smooth muscle actin expression in rat liver. Al-Attar (2012) reported that the levels of liver superoxide dismutase (SOD) and glutathione (GSH) were significantly decreased in mice treated with TAA. Luo et al. (2015) reported that the level of liver malondialdehyde (MDA) was significantly elevated in TAA-treated rats while contents of SOD and GSH were decreased compared with the control rats. Furthermore, Mansour et al. (2015) showed that TAA induced hepatic oxidative damage as indicated by an increase in lipid peroxidation, a decrease in GSH and SOD activity as well as the increased nitric oxide levels in experimental rats.

Atakisi et al. (2013) studied the effect of omega-3 fatty acids on diethylnitrosamine (DEN) toxicity in rats. They suggested that omega-3 fatty acids could ameliorate the toxic effects of DEN in part by means of its free radical scavenging activity and may be of therapeutic value in the protection of liver against toxic effects of DEN. Kajikawa et al. (2011) assessed the therapeutic effect of EPA and its mechanisms in an animal model of nonalcoholic steatohepatitis (NASH). They concluded that EPA attenuates the progression of hepatic fibrosis in developed steatohepatitis, and this effect is likely mediated by the inhibition of reactive oxygen species (ROS) production. Alaraj and Qiblawi (2015) evaluated the role of fish oil in modulating carbon tetrachloride (CCL₄) induced hepatotoxicity in
Fish oil has a protective activity against CCl₄-induced hepatoxicity and it can be proposed that fish oil can serve as a potent hepatoprotective agent. Hassanen and Ahmed (2015) investigated the hepatoprotective effect of fish oil against hepatoxicity of DEN in rats. Significant increments in the activities of serum SOD, catalase (CAT), glutathione peroxidase (GSPx) and GSH were observed in rats treated with DEN and fish oil, while the level of MDA was significantly decreased. Furthermore, treatment with fish oil reduces the histopathological liver abnormalities associated with hepatotoxicity. Moreover to the anti hepatotoxicity effect and possess antioxidant potential that may be used for therapeutic purposes suggested. Collectively, the present study provided evidence indicating that omega-3 fatty acids significantly exert noticeable biological and pharmacological effects. From the present study, it is obvious that the pretreatment of rats with omega-3 fatty acids attenuated the highly physiological and histopathological alterations induced by TAA intoxication. Moreover, the present results justify the development of additional physiological, pharmacological and biochemical investigations in order to clarify the exact mechanism of action of omega-3 fatty acids against the effect of TAA. Finally, further experimental studies are needed to pursue the influence of different doses of omega-3 fatty acid on hepatic fibrosis induced by TAA.

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