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ORIGINAL ARTICLE

Effect of *Olea oleaster* and *Juniperus procera* leaves extracts on thioacetamide induced hepatic cirrhosis in male albino mice

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Abstract The effect of *Olea oleaster* and *Juniperus procera* leaves extracts and their combination on thioacetamide (TAA)-induced hepatic cirrhosis were investigated in male albino mice. One hundred sixty mice were used in this study and were randomly distributed into eight groups of 20 each. Mice of group 1 served as controls. Mice of group 2 were treated with TAA. Mice of group 3 were exposed to TAA and supplemented with *O. oleaster* leaves extracts. Mice of group 4 were treated with TAA and supplemented with *J. procera* leaves extracts. Mice of group 5 were subjected to TAA and supplemented with *O. oleaster* and *J. procera* leaves extracts. Mice of groups 6, 7 and 8 were supplemented with *O. oleaster*, *J. procera*, and *O. oleaster* and *J. procera* leaves extracts respectively. Administration of TAA for six and twelve weeks resulted in a decline in body weight gain and increased the levels of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and total bilirubin. Histopathological evaluations of hepatic sections from mice treated with TAA showed severe alterations including increase of fibrogenesis processes with structural damage. Treatment of mice with these extracts showed a pronounced attenuation in TAA induced hepatic cirrhosis associated with physiological and histopathological alterations. Finally, this study suggests that the supplementation of these extracts may act as antioxidant agents and could be an excellent adjuvant support in the therapy of hepatic cirrhosis.
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1. Introduction

Liver, the largest gland in body, is vulnerable to a vast variety of harmful endogenous and exogenous agents. Therefore, liver is one of the most frequently injured organs in the body (Sivaraj et al., 2011). A report of The World Health Organization (WHO) indicates that 10% of the world population has chronic liver disease, in addition about two million people worldwide die each year from hepatic failure (Schuppan and

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Afdhal, 2008). Liver or hepatic fibrosis is a reversible physiological wound-healing process. When damage is sustained, however, this process becomes exacerbated and irreversible, leading to cirrhosis (Ramachandran and Iredale, 2012). Hepatic fibrosis after hepatocyte injury is a pathological process with deposition of extracellular matrix (ECM) proteins such as collagens (Lang et al., 2011). Despite the increasing burden of this condition, treatment options for liver fibrosis and its advanced lesion, cirrhosis, remain very limited. Following liver damage, there is trans-differentiation of hepatic stellate cells (HSCs) into ECM secreting myofibroblasts (Friedman, 2008).

Thioacetamide (TAA) is a thiono-sulfur containing compound. It has been used as a fungicide, organic solvent, accelerator in the vulcanization of rubber, and as a stabilizer of motor oil (Lee et al., 2003). TAA was first reported as a hepatotoxic agent by Fitzhugh and Nelson (1948). The hepatic toxic chemical TAA has been widely used in the study of the underlying mechanisms of hepatic fibrogenesis and the therapeutic effects of potential antifibrosis drugs. Additionally, many experimental investigations showed that TAA induced hepatic fibrosis and cirrhosis in rats and mice (Al-Attar, 2011, 2012; Ali et al., 2014; Abdou et al., 2015; Al-Attar and Shawush, 2015; Meng et al., 2015; Wang et al., 2015).

Plants are a rich source of bioactive components that have desirable health benefits and are traditionally known to be useful for prevention of chronic diseases (Yogalakshmi et al., 2010). Herbal medicines have been reported to show protective effects from liver fibrosis and injury (Hsieh et al., 2008; Yuan et al., 2008; Al-Attar, 2012; Saravanan et al., 2013; Ali et al., 2014; Abdou et al., 2015; Al-Attar and Shawush, 2015). *Olea oleaster* corresponds to *Olea europaea* subsp. and those trees originated in the eastern Mediterranean and south west of kingdom of Saudi Arabia. It contains secoiridoids such as oleuropein, ligostroside, dimethyl oleuropein and oleoside, flavonoids, phenolic compounds, such as caffeic acid, tyrosol (El and Karakaya, 2009). All previous studies carried out on *O. oleaster* show that olive extracts are used for nutrients that help fight against a variety of illnesses and control fat and weight loss (Flynn and Reinert, 2010). *Juniperus* is one of the major genera of Cupressaceae family. It is estimated that 70 species of *Juniperus* are distributed throughout the world (Topçu et al., 1999). *Juniperus* species are used for the treatment of hyperglycemia, tuberculosis, bronchitis, pneumonia, ulcers, intestinal worms, to heal wounds and cure liver diseases (Burits et al., 2001; Loizzo et al., 2007). Moreover, the medical use of *Juniperus* is well known in the Bosnian, Lebanese, and Turkish folk medicine and the berries are used to treat skin diseases like skin rash and eczema in addition to a wide range of respiratory tract diseases, like asthma, common cold, cough, bronchitis, throat inflammation, pneumonia and tuberculosis (El et al., 2008; Öztürk et al., 2011), urinary tract inflammations, renal and gall bladder stones, and rheumatism (Šarić et al., 2011). Therefore, the aim of this study was to find if the administration of *O. oleaster*, *J. procera* leaves extracts and their combination could have beneficial effects on experimental liver cirrhosis induced by TAA in male albino mice.

2. Materials and methods

2.1. Experimental animals

One hundred sixty male albino mice of the SWR strain, weighing 15.0–25.0 g used in this study were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Mice were housed 10 per cage in a room with 12/12-h light/dark cycle at ambient temperature of 20 ± 1 °C and humidity of 65%. The experimental animals were acclimatized to the laboratory conditions for one week prior to the initiation of experimental treatments. Mice 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. Extraction of *O. oleaster* and *J. procera* leaves

Fine qualities of *O. oleaster* and *J. procera* leaves were directly collected from the outskirts of Albaha region of Saudi Arabia. The leaves were scientifically defined by the herbarium of Biological Sciences Department, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. The collected leaves were completely washed, air dried at room temperature and stored in a dry plastic container until use for extraction processes. The method of Al-Attar and Abu Zeid (2013) was used to prepare the extracts. The dried leaves of *O. oleaster* (50 g) were powdered, added to 2 liters of cold water and mixed using an electric mixer for 20 min. Also, the dried leaves of *J. procera* (50 g) were powdered, added to 2 liters of cold water and mixed using an electric mixer for 20 min. Thereafter, the solutions of *O. oleaster* and *J. procera* leaves were gently filtered. Finally, the filtrates were evaporated in an oven at 40 °C to produce dried residues (active principles). With references to the powdered samples, the mean yield of *O. oleaster* and *J. procera* extracts was 19.3% and 17.8% respectively. Furthermore, these extracts were stored in a refrigerator for subsequent experiments.

2.3. Experimental protocol

The mice were randomly distributed into eight groups of 20 each. Mice of group 1 were served as controls and intraperitoneally injected with saline solution (0.9% NaCl), twice weekly. Mice of group 2 were given 150 mg/kg body weight of TAA (Sigma–Aldrich Corp., St. Louis, MO, USA) by intraperitoneal injection, twice weekly. Mice of group 3 were intraperitoneally injected with TAA at the same dose given to group 2 and were orally supplemented with *O. oleaster* leaves extract at a dose of 200 mg/kg body weight/day. Mice of group 4 were intraperitoneally injected with TAA at the same dose given to group 2 and were orally supplemented with *J. procera* leaves extract at a dose of 200 mg/kg body weight/day. Mice of group 5 were intraperitoneally injected with TAA at the same dose given to group 2 and were orally supplemented with *O. oleaster* leaves extract (100 mg/kg body weight/day) and *J. procera* leaves extract (100 mg/kg body weight/day). Mice

of group 6 were intraperitoneally received saline solution at the same dose given to group 1 and were orally supplemented with *O. oleaster* leaves extracts at the same dose given to group 3. Animals of group 7 intraperitoneally received saline solution at the same dose given to group 1 and were orally supplemented with *J. procera* leaves extract at the same dose given to group 4. Mice of group 8 intraperitoneally received saline solution at the same dose given to group 1 and were supplemented with *O. oleaster* and *J. procera* leaves extracts at the same dose given to group 5. The body weights of mice were determined at the start of the experimental period, after six and twelve weeks using a digital balance. These weights were measured at the same time during the morning (Al-Attar and Zari, 2010) and the percentage changes of body weight after six and twelve weeks were calculated. Moreover, the experimental animals were observed for signs of abnormalities throughout the period of study. After six and twelve weeks food was withdrawn from the mice and they were fasted for 8 h but had free access to water, and then anesthetized with diethyl ether. Blood specimens were collected from orbital venous plexus in non-heparinized tubes. Blood specimens were centrifuged at 2500 rpm for 15 min, and the clear samples of blood serum were separated and stored at −80 °C. These serum samples were used to determine the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin. The method of Reitman and Frankel (1957) was used to determine the levels of serum ALT and AST. The method of Szasz (1969) was used to measure the level of serum ALP. Serum level of total bilirubin was estimated using the method of Doumas et al. (1973). For histological examinations after six and twelve weeks, mice were dissected after blood sampling. Liver tissues were quickly isolated from each group, fixed in 10% buffered formalin, sectioned and stained with haematoxylin and eosin. Additionally, liver sections were stained using Masson's trichrome stain. All liver sections were examined using light microscope (Olympus BX61 – USA) connected to motorized controller unit (Olympus bx-ucb – USA) and photographed by a camera (Olympus DP72 – USA).

2.4. Statistical analysis

The data were expressed as mean ± standard deviation (SD) and were analyzed using the Statistical Package for Social Sciences (SPSS for windows, version 12.0). Statistical comparisons were performed by a two-way analysis of variance (ANOVA). The results were considered statistically significant if the *P*-values were less than 0.05.

3. Results

The body weights after six weeks of all experimental groups are represented in Fig. 1. There was a gradual increase in the body weight of normal control mice (122.8%) at the end of six weeks compared with their initial body weights. Significant decreases in the values of body weight gain were observed in mice treated with TAA, TAA plus *O. oleaster* leaves extract, TAA plus *J. procera* leaves extracts, and TAA plus *O. oleaster* and *J. procera* leaves extracts. The minimum body weight gain was noted in TAA-intoxicated mice (36.7%). Supplementation with the tested extracts showed a remarkable lowering effect on the percentage changes of body weight in mice treated with TAA plus *O. oleaster* leaves extracts, TAA plus *J. procera* leaves extracts, and TAA plus *O. oleaster* and *J. procera* leaves extracts which amounted 40.5%, 43.6% and 59.6% respectively. Oral administration of tested extracts to normal mice caused significant increases in body weight gain. The change of body weight gain was 108.6% in normal mice supplemented with *O. oleaster* leaves extract. Supplementation with *J. procera* leaves extract in normal mice showed a remarkable increase in the percentage change of body weight (112.6.4%). The percentage change of body weight gain in normal mice fed with *O. oleaster* and *J. procera* leaves extracts is 105.4%. Fig. 2 demonstrates the body weights of all experimental groups after twelve weeks. The maximum body weight gain was noted in normal mice supplemented with *J. procera* leaves extract (105.2%) followed by normal control mice (98.3%) and mice treated with TAA plus *J. procera* leaves extract (96.1%). The minimum body weight gain was noted in normal mice treated with only TAA (79.7%). Notable decreases in the values of body weight gain were observed in mice subjected to TAA plus *O. oleaster* extract (85.3%), TAA plus *O. oleaster* and *J. procera* leaves extracts (86.1%), *O. oleaster* extract (88.2%) and *O. oleaster* and *J. procera* leaves extracts (86.6%).

Fig. 3A–D shows the level of serum ALT, AST, ALP and total bilirubin in control, TAA, TTA plus *O. oleaster* leaves extract, TAA plus *J. procera* leaves extract, TAA plus *O. oleaster* and *J. procera* leaves extracts, *O. oleaster* leaves extract, *J. procera* leaves extract, and *O. oleaster* and *J. procera* leaves extracts treated mice after six weeks. Administration of TAA (150 mg/kg) for six weeks resulted in a markedly high increase (2046.3%) in the level of serum ALT compared with control mice and other treated groups (Fig. 3A). A significant increase in the level of serum ALT was noted in mice treated with TAA plus *O. oleaster* leaves extract (127.6%), TAA plus *J. procera* leaves extract (236.4%) and TAA plus *O. oleaster* and *J. procera* leaves extracts (296.7%) compared with control mice. The levels of serum AST after six weeks were statistically increased in rats exposed to TAA (839.9%), TAA plus *O. oleaster* leaves extract (179.2%) and TAA plus *J. procera* leaves extract (195.0%) TAA plus *O. oleaster* and *J. procera* leaves extracts (296.7%) as compared with control mice. In comparison with control group, the level of serum ALP was significantly increased after six weeks in mice exposed to TAA (465.6%), TAA plus *O. oleaster* leaves extract (334.1%) and TAA plus *J. procera* leaves extract (418.9%) TAA plus *O. oleaster* and *J. procera* leaves extracts (299.0%). The levels of serum total bilirubin were statistically increased after six weeks in mice subjected to TAA (133.5%), TAA plus *O. oleaster* leaves extract (29.6%), TAA plus *J. procera* leaves extract (62.9%) and TAA plus *O. oleaster* and *J. procera* leaves extracts (59.3%) compared with control mice.

Fig. 4A–D shows the level of serum ALT, AST, ALP and total bilirubin in control, TAA, TAA plus *O. oleaster* leaves extract, TAA plus *J. procera* leaves extract, TAA plus *O. oleaster* and *J. procera* leaves extracts, *O. oleaster* leaves extract, *J. procera* leaves extract, and *O. oleaster* and *J. procera* leaves extracts treated mice after twelve weeks. Serum ALT level was statistically enhanced after twelve weeks in mice treated with TAA (1632.0%), TAA plus *O. oleaster* leaves extract (348.2%), TAA plus *J. procera* leaves extract (390.2%) and TAA plus *O. oleaster* and *J. procera* leaves extracts (422.5%) compared with control mice. In comparison with control

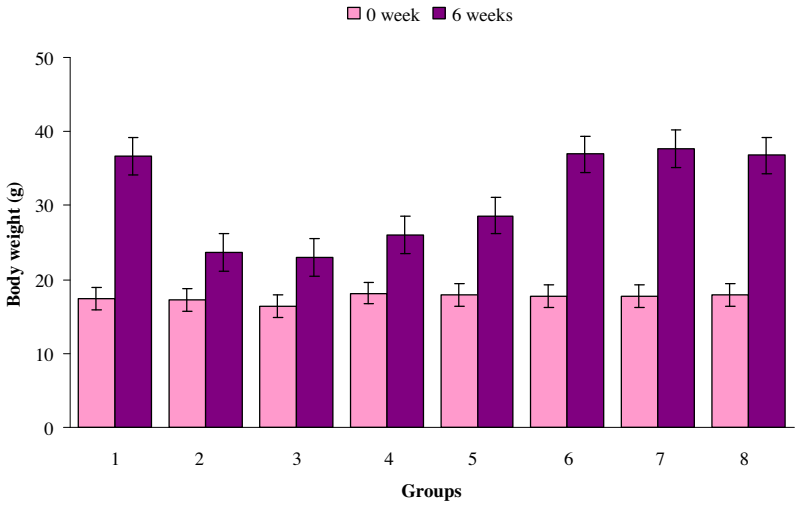

Figure 1 Changes of body weight after six weeks in control (group 1), TAA (group 2), TAA plus *O. oleaster* leaves extract (group 3), TAA plus *J. procera* leaves extract (group 4), TAA plus *O. oleaster* and *J. procera* leaves extracts (group 5), *O. oleaster* leaves extract (group 6), *J. procera* leaves extract (group 7), and *O. oleaster* and *J. procera* leaves extracts (group 8) treated mice.

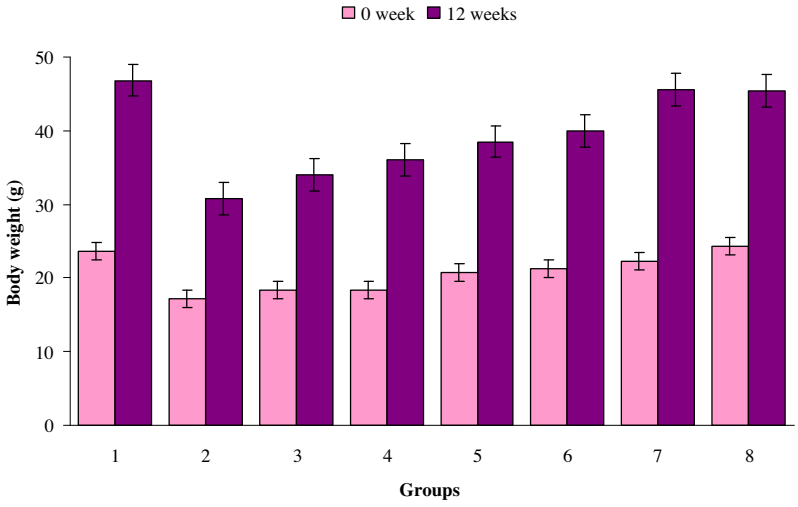

Figure 2 Changes of body weight after twelve weeks in control (group 1), TAA (group 2), TAA plus *O. oleaster* leaves extract (group 3), TAA plus *J. procera* leaves extract (group 4), TAA plus *O. oleaster* and *J. procera* leaves extracts (group 5), *O. oleaster* leaves extract (group 6), *J. procera* leaves extract (group 7), and *O. oleaster* and *J. procera* leaves extracts (group 8) treated mice.

values, the levels of serum AST were statistically increased after twelve weeks in TAA (280.3%), TAA plus *O. oleaster* leaves extract (88.9%) and TAA plus *J. procera* leaves extract (89.1%) TAA plus *O. oleaster* and *J. procera* leaves extracts (62.3%) treated mice. Significant elevations in the level of serum ALP were observed in mice exposed to TAA (1146.5%), TAA plus *O. oleaster* leaves extract (206.2%), TAA plus *J. procera* leaves extract (270.9%), and TAA plus *O. oleaster* and *J. procera* leaves extracts (299.0%) compared with control mice. There were significant increases after twelve weeks in the level of serum total bilirubin in mice subjected to TAA (271.7%), TAA plus *J. procera* leaves extract (27.8%) and TAA plus *O. oleaster* and *J. procera* leaves extracts (31.9%) compared with control mice, while this parameter was statistically unchanged in TAA plus *O. oleaster* leaves extracts (Fig. 4D). The levels of serum ALT, AST, ALP and total bilirubin were remarkably unchanged in normal mice supplemented with *O. oleaster* leaves extract, *J. procera* leaves extract, and *O. oleaster* and *J. procera* leaves extracts after six (Fig. 3A–D) and twelve (Fig. 4A–D) weeks.

Histopathological examination of liver sections from control mice showed a normal hepatocellular architecture (Fig. 5A). Similar observations were noted in *O. oleaster* leaves extract (Figs. 5H and 6F), *J. procera* leaves extract (Figs. 5I and 6G), and *O. oleaster* and *J. procera* leaves extracts (Figs. 5J and 6H) treated mice after six and twelve weeks. Photomicrographs from liver sections of mice exposed to TAA for six weeks showed a structural damage with the extracellular matrix collagen (Fig. 5B–D). Conversely, photomicrographs of liver sections from mice subjected to TAA plus *O. oleaster* leaves extract (Fig. 5E), TAA plus *J. procera* leaves extract (Fig. 5F), and TAA plus *O. oleaster* and *J. procera* leaves extracts (Fig. 5G) shows a decreased development of fibrogenesis processes. Liver sections from

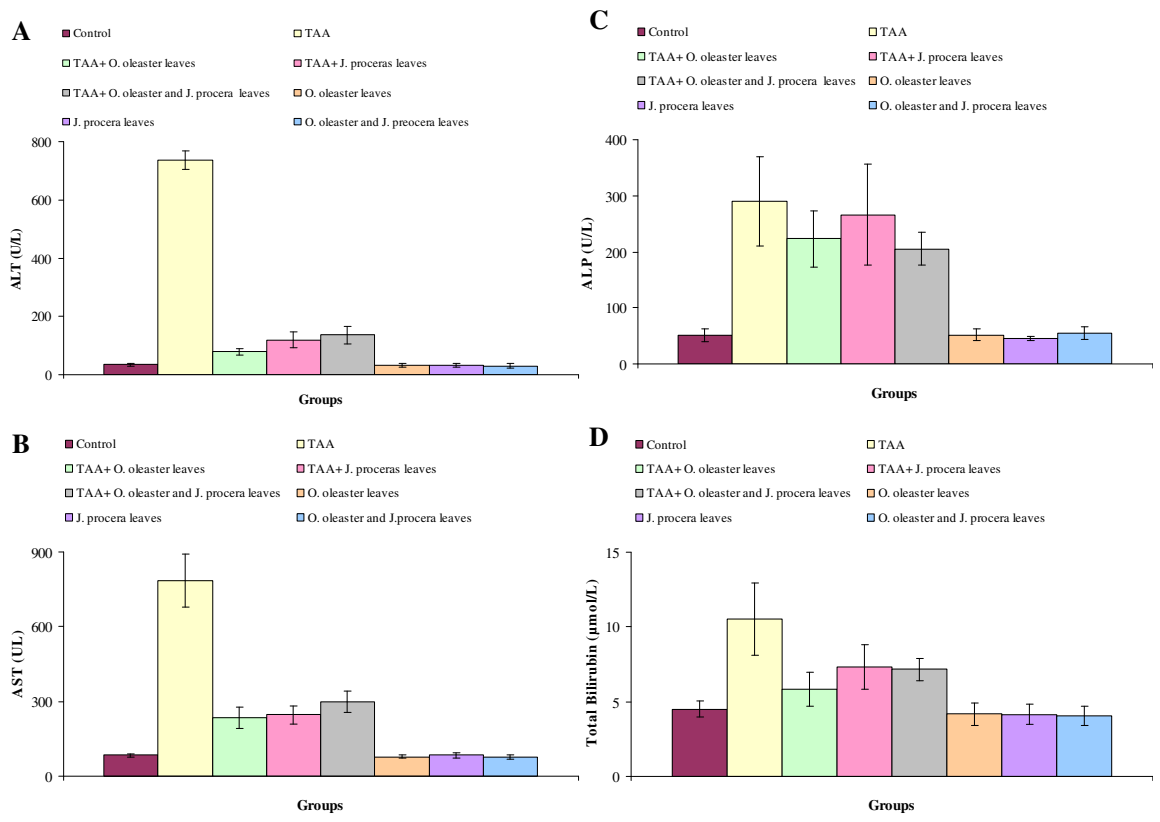

Figure 3 (A–D) The levels of ALT (A), AST (B), ALP (C) and total bilirubin (D) in serum from control, TAA, TAA plus *O. oleaster* leaves extract, TAA plus *J. procera* leaves extract, TAA plus *O. oleaster* and *J. procera* leaves extracts, *O. oleaster* leaves extract, *J. procera* leaves extract, and *O. oleaster* and *J. procera* leaves extracts treated mice for six weeks.

mice intoxicated with TAA for twelve weeks showed extensive alterations of tissue architecture and advanced fibrosis with increases of extracellular matrix collagen content (Fig. 6A and B). Administration of the studied extracts attenuated the development of hepatic fibrosis induced by TAA after twelve weeks (Fig. 6C–E).

4. Discussion

Liver or hepatic diseases resulting from liver damage is a global problem. Among hepatic diseases, cirrhosis is an important and common cause of human mortality in many countries. It is the end-stage of most liver pathologies of different etiologies and leads to chronic liver dysfunction, accompanied by important metabolic alterations (Galisteo et al., 2006). Cirrhosis of liver is a common end consequence of a variety of chronic liver diseases. Its underlying pathology, fibrosis, represents the common response of liver to toxic, infectious, or metabolic agents (Schuppan and Kim, 2013). Currently, hepatic fibrosis still contributes to the high incidence and morbidity rates of cirrhosis as the latter is irreversible. Thus, researchers are dedicated to find out specific treatment targets that contribute to the development of hepatic fibrosis (Qin et al., 2014).

The present study is the first experimental investigation designed to evaluate whether supplementation of *O. oleaster* leaves extract *J. procera* leaves extract, and *O. oleaster* and *J. procera* leaves extracts would have protective influences on TAA induced hepatic fibrosis and cirrhosis with physiological disturbances and histological injuries in male mice. TAA-induced hepatic fibrosis animal models must resemble human ones in the hemodynamics, morphology and biochemical metabolism aspects (Pietrangelo et al., 2007; Friedman, 2008), and also be similar to virus-induced cirrhosis (Peng and Wang, 2010). 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). As seen in the present study, the administration of TAA for six and twelve weeks that TAA induced a notable decrease of body weight gain and significantly increase of serum ALT, AST, ALP and total bilirubin. Histopathologically, severe alterations of liver structure with fibrogenesis processes were noted. Similar observations were detected in many experimental studies on TAA induced relative influences (Al-Attar, 2011, 2012; Mustafa et al., 2013; Ali et al., 2014; Abdou et al., 2015; Al-Attar and Shawush, 2015; Wang et al., 2015).

The present work showed that the treatment of mice with *O. oleaster* and *J. procera* leaves extracts and their combination attenuated the physiological and histopathological changes induced by TAA administration. Moreover, the most improvements were observed in mice supplemented with *O. oleaster* leaves extract followed by *J. procera* leaves extract and their combination. This indicated the effectiveness of these extracts in prevention of TAA toxicity. The main constituent of the olive leaves is oleuropein, one of iridoide monoterpenes, which is thought to be responsible for pharmacological effects. Furthermore, the olive leaves contain triterpenes (oleanolic

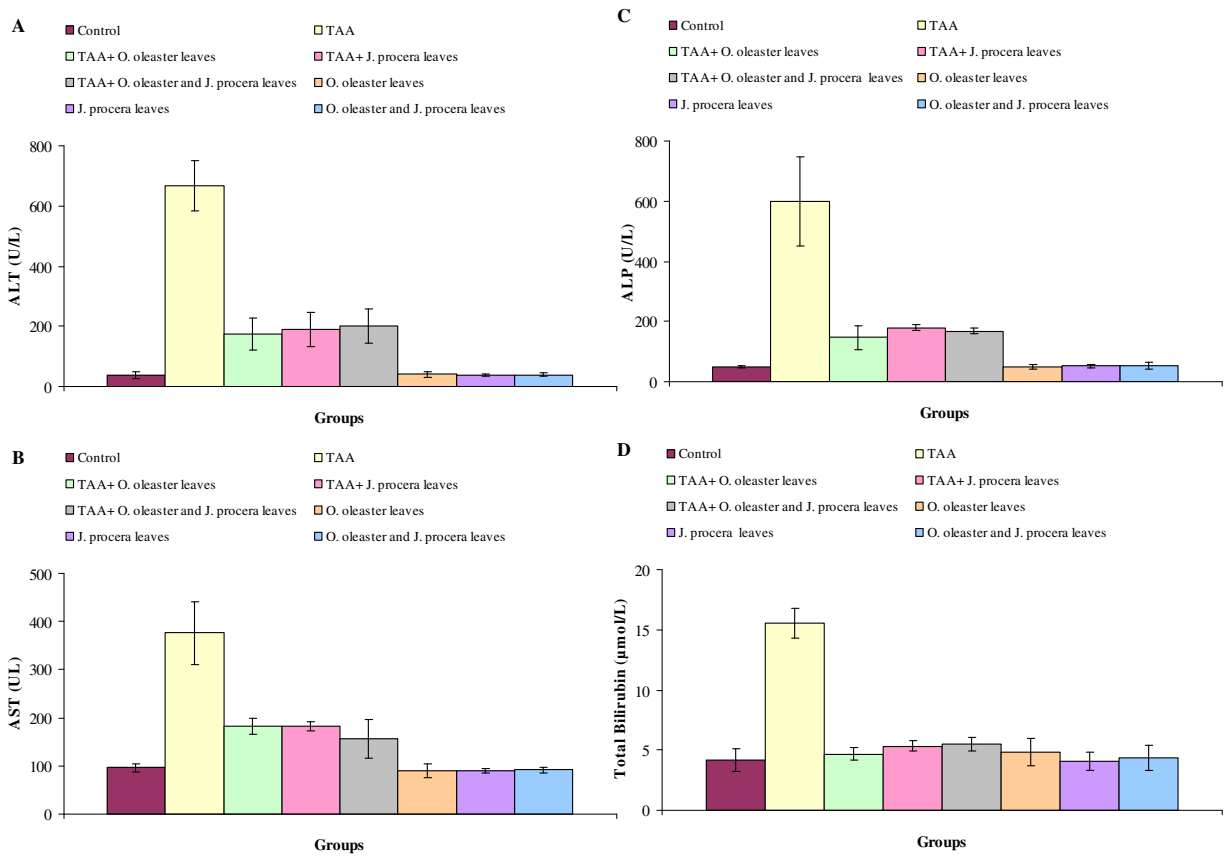

Figure 4 (A–D) The levels of ALT (A), AST (B), ALP (C) and total bilirubin (D) in serum from control, TAA, TAA plus *O. oleaster* leaves extract, TAA plus *J. procera* leaves extract, TAA plus *O. oleaster* and *J. procera* leaves extracts, *O. oleaster* leaves extract, *J. procera* leaves extract, and *O. oleaster* and *J. procera* leaves extracts treated mice for twelve weeks.

and maslinic acid), flavonoids (e.g., luteolin, apigenin, rutin), and chalcones (olivine, olivine-diglucoside) (Meirinhos et al., 2005; Pereira et al., 2007). It is its chemical content that makes olive leaves one of the most potent natural antioxidants. Oleuropein has high antioxidant activity *in vitro*, comparable to a hydrosoluble analog of tocopherol (Speroni et al., 1998), as do other constituents of olive leaves (Briante et al., 2002). It was shown that total olive leaves extract had antioxidant activity higher than vitamin C and vitamin E, due to the synergy between flavonoids, oleuropeosides and substituted phenols (Benavente-Garcia et al., 2000).

Alirezaei et al. (2012) evaluated the antioxidant properties of oleuropein on ethanol induced oxidative damage and its beneficial effects on liver function of Sprague–Dawley male rats. They reported that oleuropein during ethanol treatment in rats resulted in a higher antiperoxidative enzyme activity, catalase, and inhibited toxicity to the liver, as monitored by the reduction in ALT and AST levels and thiobarbituric acid reactive substance (TBARS) concentration. They suggested that oleuropein possesses beneficial antioxidant effects against ethanol-induced liver toxicity. Al-Attar and Shawush (2015) investigated the influence of olive (*Olea europaea*) leaves extract on TAA-induced hepatic cirrhosis in Wistar male rats. They demonstrated that the extract of olive possesses hepatoprotective properties against TAA-induced hepatic cirrhosis by inhibiting the physiological and histopathological alterations. Moreover, they suggested that the hepatoprotective effects of olive extract may be attributed to its antioxidant activity. The petroleum ether fraction of *J. procera* showed significant activity as hepatoprotective when investigated against CCl_4 induced liver injury Wistar male rats (Alqasoumi and Abdel-Kader, 2012). The hepatoprotective activity was evaluated through the quantification of biochemical parameters and confirmed using histopathology analysis. Different fraction obtained from the aerial parts of *J. phoenicea* showed significant activity as hepatoprotective when investigated against CCl_4 induced liver injury in Wistar male rats (Alqasoumi et al., 2013). The hepatoprotective activity was evaluated through the quantification of biochemical parameters and confirmed using histopathological study.

In general, one of the most important findings in the present study is the observation that the *O. oleaster* and *J. procera* leaves extracts and their combination were effective in attenuating the TAA induced hepatic fibrosis and cirrhosis that were proven by hematobiochemical examinations and histopathological evaluations. It may therefore be suggested from the evidence from the present study, that the supplementations of the studied extracts may give some beneficial results for people with some hepatic diseases. Moreover, this study suggests that the supplementation of these extracts may act as antioxidant agents and could be an excellent adjuvant support in the therapy of hepatic fibrosis and cirrhosis induced by TAA and different pathogens. Finally, further physiological, biochemical and histopathological investigations using

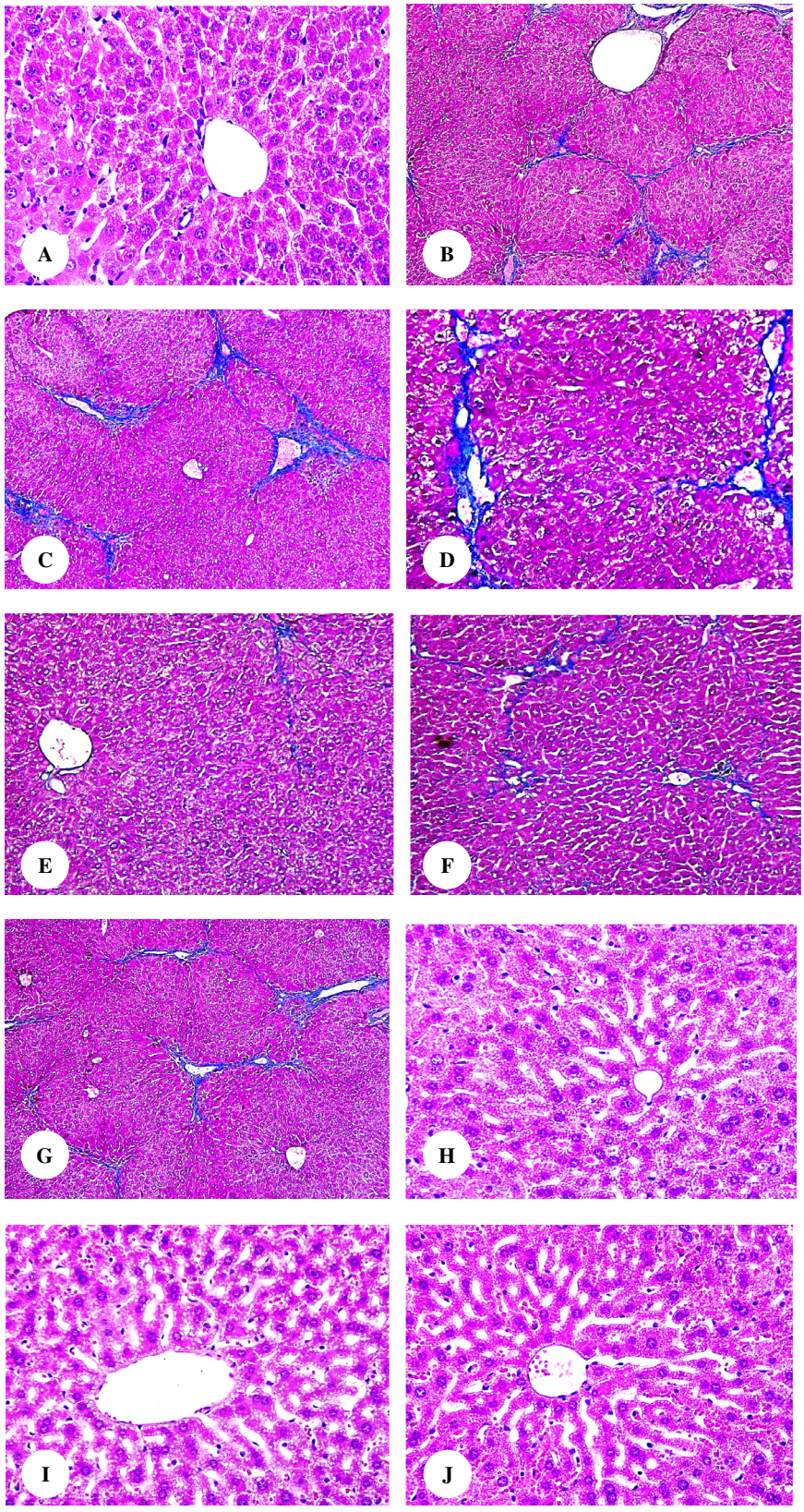

Figure 5 (A–J) Photomicrographs of liver sections in each group. (A) control (X400), (B, C and D) TAA (X100, X100 and X200), (E) TAA plus *O. oleaster* leaves extract (X200), (F) TAA plus *J. procera* leaves extract (X200), (G) TAA plus *O. oleaster* and *J. procera* leaves extracts (X200), (H) *O. oleaster* leaves extract (X400), (I) *J. procera* leaves extract (X400), and (J) *O. oleaster* and *J. procera* leaves extracts (X400) treated mice for six weeks.

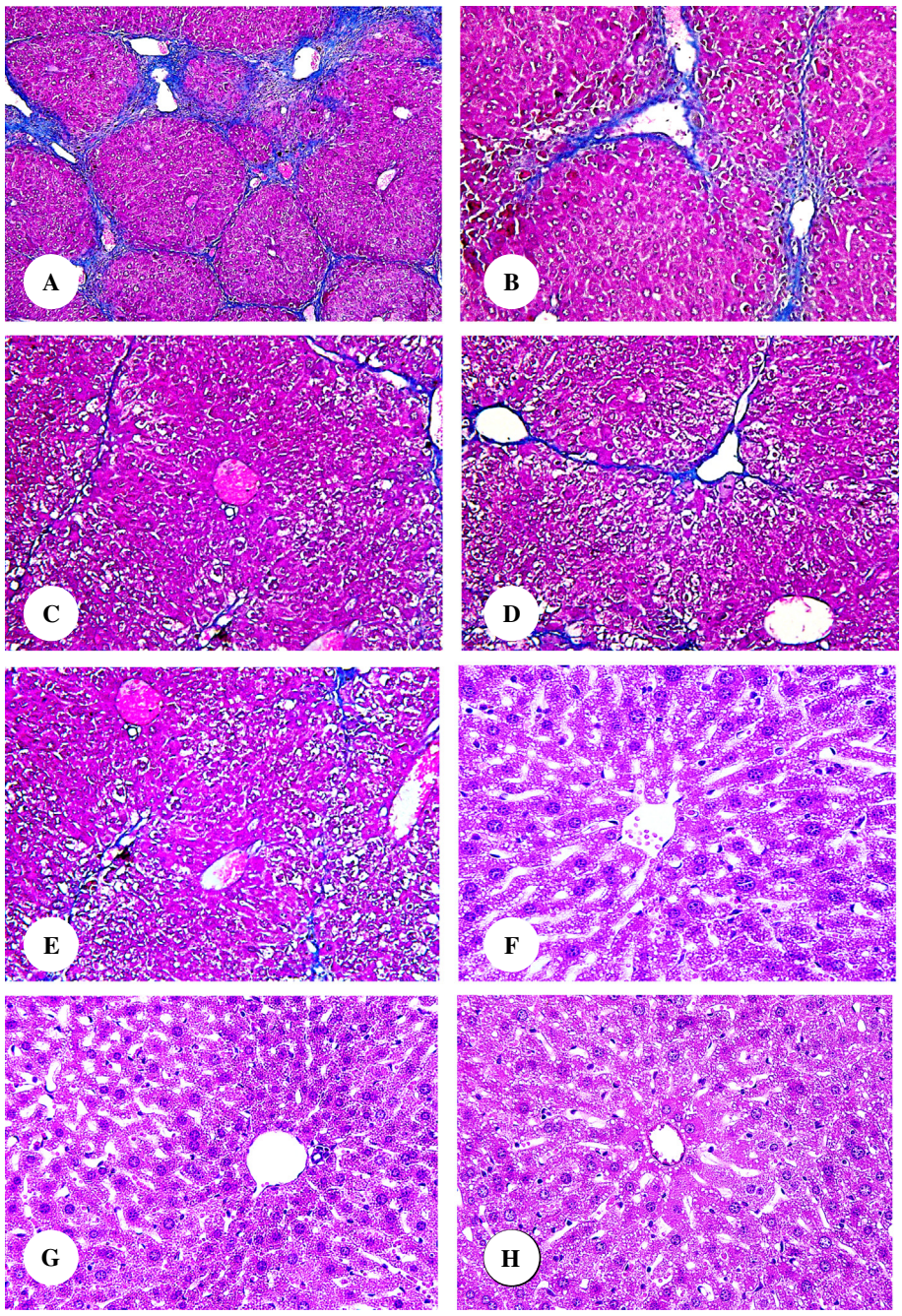

Figure 6 (A–H) Photomicrographs of liver sections in treated groups. (A and B) TAA (X100 and X200), (C) TAA plus *O. oleaster* leaves extract (X200), (D) TAA plus *J. procera* leaves extract (X200), (E) TAA plus *O. oleaster* and *J. procera* leaves extracts (X200), (F) *O. oleaster* leaves extract (X400), (J) *J. procera* leaves extract (X400), and (H) *O. oleaster* and *J. procera* leaves extracts (X400) treated mice for twelve weeks.

different doses of these extracts are needed to find out the optimal therapeutic doses and to explore the exact mechanism of these extracts and their natural chemical constituents against the fibrotic activity of TAA and its metabolites, and may be against other related fibrogenic and pathogenic factors.

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