Original Article

Hepatoprotective effect of *Olea europaea* L. seeds extracts against methotrexate induced liver injury in mice

Nada Sahib Shaker[1], Zeena Ayad Hussein[2*], Nibras Jamal Tahseen[2], Amjed Shawqy Al-Musalahi[2], Hayder Bahaa Sahib[3]

[1]Department of Pharmacology, College of Medicine, Al-Nahrain University, Baghdad, Iraq. [2]Department of Pharmacy, Al-Rasheed University College, Baghdad, Iraq. [3]Department of Pharmacology, College of Pharmacy, Al-Nahrain University, Baghdad, Iraq.

Correspondence: Zeena Ayad Hussein, Department of Pharmacy, Al-Rasheed University College, Baghdad, Iraq. zeena.kanada@gmail.alrasheedcol.edu.iq

ABSTRACT

The present study aimed to determine the hepatoprotective effect of different extracts of Olea europaea L. seeds in mice induced with MTX-induced liver injury and find out the most effective extract. Olea europaea L. (olive) seeds powder was extracted with three solvents with different polarities in a sequential manner (chloroform, methanol, and 50% aqueous ethanol) using the cold method. 48 male albino mice were divided into six groups. Mice were pretreated orally with silymarin (standard) and the test extracts for 5 consecutive days at a dose of 200mg/kg and 500mg/kg consecutively. Mice received methotrexate (20mg/kg) IP single dose on day 5. On day 6, blood samples were collected for biochemical analysis of serum liver indices; then animals were sacrificed to collect liver tissues for histological examination. The antioxidant activity for each extract was assessed using the DPPH assay. The results indicated that MTX successfully induced liver damage that was observed through marked elevation of serum GPT, GOT, and ALP as well as alterations in the cellular structure of liver tissue. All extracts of olive seeds were able to reduce serum levels of liver indices, although 50% ethanol extract was revealed to be the best in reducing levels of GPT, GOT, and ALP and preserve liver tissue cellular structure, as well as having the highest free radical scavenging activity in DPPH assay. The study suggests that 50% ethanol extract of Olea europaea L. seeds can be safely used with MTX therapy to reduce its hepatic toxic effect.

Keywords: Hepatoprotective, MTX, Olive seeds, Liver indices, Liver injury, DPPH assay

Introduction

Liver is the most important organ in the body that carries out vital physiological functions including metabolism of essential nutrients (carbohydrates, proteins, and lipids), energy utilization, metabolism and detoxification of various xenobiotics and medications, and excretion of waste substances [1, 2]. Liver metabolizes xenobiotics that lead to drug-induced liver injury (DILI) and is a possible complication of various therapeutic medications [3]. DILI is usually classified into two types: idiosyncratic and intrinsic; idiosyncratic DILI is and mostly dose-independent unpredictable while intrinsic DILI is predictable and dose-dependent (eg: toxicity of acetaminophen) [4]. DILI is one of the main reasons for the withdrawal of many drugs; Mechanism of hepatotoxicity could be mediated through mitochondrial dysfunction and DNA damage; such dysfunction could be caused by the drug itself or its cytochrome P450 mediated metabolite [5]. Various factors can attribute to hepatotoxicity like: genetics, environmental, gender variation, some drugs (eg; anticancer, antiviral, acetaminophen and some NSAIDs, and anti-mycobacterial), and certain disorders (eg; TB, HIV, and hepatitis) [6].

Methotrexate is a folic acid antagonist that is, extensively used as a chemotherapeutic agent to treat different cancerous stages such as acute lymphoblastic leukemia and other autoimmune inflammatory disorders (eg: multiple sclerosis, dermatomyositis, psoriasis, and rheumatoid arthritis) [7]. MTX is associated with many side effects including; gastrointestinal, hepatic and renal dysfunction, hematologic side effects, central and peripheral

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nervous system toxicity as well as respiratory toxicity [8]. Both high and low-dose therapy can lead to hepatotoxicity. Methotrexate at a very low dosage can affect the liver and cause changes in liver histology. Also, prolonged use or high-dose methotrexate (as in leukemia) can cause hepatotoxicity that may result in progressive fibrosis and cirrhosis. Hepatotoxicity resulting from the long-term use of methotrexate, will always remain a constant restriction of using this medication in the desired doses. It seems inhibiting the formation of tetrahydrofolate is responsible for both the toxic and therapeutic effects of methotrexate [9].

Olea europaea L., also known as Olive, belongs to the family Oleaceae which includes about 30 genera. Olive trees are native in temperate, tropical, and subtropical countries worldwide; and best known in Asia and Malaysia, especially the temperate and tropical regions of Asia [10]. Olive trees are distributed normally in the coastal regions of southeastern Europe and the eastern Mediterranean region, northern Africa, western Asia, and northern Iran at the south end of the Caspian Sea. The olive tree and fruit are important in the religion, and they have been mentioned in the Holy Quran and Bible [11]. *O. europaea* has some traditional medicinal uses; it is extensively used for various ailments in different countries. For example, olive leaves extract has cardioprotective and hypotensive effects; olive oil can be used orally as a laxative or applied externally for inflammation; and sometimes the oil can be used with lemon juice to treat urinary tract infection [12]. The extracts of the fruit and leaves are orally used as mouth cleanser, as well as to treat some intestinal and stomach discomfort, urinary tract and respiratory infections, and diarrhea. Continuous use of olive oil to the scalp can also usefully prevent hair loss. Also, different parts of olive trees possess anticarcinogenic, anti-oxidant, anti-inflammatory, anti-diabetic, and neuroprotective activities [13]. Phytochemical analysis carried out on different parts of *O. europaea* using various analytical methods like; HPLC, HPLC-MS, LC-MS, GC-MS, etc. revealed that they possess phenolic compounds, flavonoids, secoiridoids, secoiridoid glycosides, benzoic acid derivatives, sterols and triterpenes [14].

This study aimed to determine the hepatoprotective effect of different extracts of Olea europaea L. seeds in mice induced with liver injury by MTX and find out the most effective extract.

Materials and Methods

Materials and chemicals

Olive seeds (*Olea europaea* L.) were obtained from the local market (Baghdad, Iraq) and their authenticity was confirmed by the Department of Pharmacognosy, College of Pharmacy, Al-Mustansiriyah University. Methotrexate (MTX) vials for injection (5mg/2ml) were obtained commercially as MTX vial from Mylan pharmaceuticals (UK). Silymarin was obtained commercially as Legalon® capsules 135mg from Madaus pharmaceuticals (Greece). Ascorbic acid was obtained as powder for analytical purposes from Romil (UK). DPPH was obtained commercially as 99% DPPH powder for analysis produced in the USA.

Extraction process

Olive seeds were cleansed from fruit flesh, washed with tap water, and left to dry. Then, they were dried in an over at 40 °C for 0.5h then left to cool down, grounded into a fine with a final weight of 92 g. *Olea europaea* seeds powder was extracted with three solvents with varying polarities in a sequential manner (chloroform, methanol, and 50% aqueous ethanol), starting with low polar solvent and ending with the highest polarity using the cold method (maceration). The powder was soaked with each solvent at a ratio of (1:4 W/V) and left in a shaking water bath for 24h at 40 °C. Then it was filtered using Whatman filter paper no. 1for 3 times, concentrated using the rotary evaporator (Buchi, Switzerland), and put in a freeze dryer to obtain the lyophilized powder. The yield (%) for each extract was then calculated [15].

Experimental animals and grouping

The study was conducted in Pharmacology and Toxicology Lab. / Al-Rasheed University College / Pharmacy Department, Baghdad; Iraq after obtaining the ethical committee approval. All experiments were performed according to the National Institute of Health Guidelines for the care and use of laboratory animals and the European Council Directive on 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), and approved by the Local Ethics Committee. 48 male albino mice aged 8 – 10 weeks old with a mean weight of (32.5 $\pm$ 2 gm) were bought from the National Center for Drug Control and Research, Ministry of Health, Baghdad; Iraq. The mice were divided into 6 groups each with 8 mice (N = 8) housed under specific pathogen-free conditions at 23 $\pm$ 2 °C and 12 hr light/dark cycle, and left to acclimatize for at least 1 week. They were fed with water and formal forage ad libitum in the animal house of Al-Rasheed University College, Pharmacy Department, Baghdad; Iraq. Group (1) represents the apparently normal animals and were considered as the reference group; Group (2) represents the liver injury-induced animals in which methotrexate (MTX) was given as a single dose (20mg/kg) / IP [16], Group (3) represent the animals treated with standard silymarin at a dose of (200mg/kg per day) and a single dose of MTX [17], Groups (4, 5, and 6) represents animals treated with *Olea europaea* chloroform (OE-CE), methanol (OE-ME) and 50% ethanol (OE-50%EE) extracts respectively with a daily dose of (500mg/kg) and a single dose of MTX [18]. All animal groups except the normal group received a single dose of MTX (20mg/kg/IP) on day 5 of the experiment; and all animal groups except for the normal group received the test substances at their mentioned respective doses starting from day 1 till day 5 of the experiment through oral gavage. On day 6 of the experiment, mice were sacrificed under anesthesia and samples were collected for further analysis.

Biochemical analysis

At the end of the experiment (day 6), mice were put under anesthesia with diethyl ether, blood was collected through heart puncture in plain tubes and then the samples were put in a centrifuge (LabTech, Korea) adjusted to 5000 rpm for 10 minutes to separate plasma. Plasma was then collected in separate tubes to be used in the biochemical analysis required to assess liver indices including; serum AST (GOT), ALT (GPT), ALP, total serum protein (TSP), and total serum bilirubin (TSB). The analysis was carried out using an automation analyzer according to the manufacturers kit.

Histological examination

Liver tissues were collected from all animal groups, fixed in 10% formalin for 48 h, and then processed to obtain paraffin-embedded liver tissue. Paraffin-embedded tissues were sectioned later on into layers with 5 µm thickness, which was then used to make the histological slide. Finally, the histological section was stained with hematoxylin & eosin stains and the section was viewed under a light microscope with 40X.

Free radical scavenging activity by (DPPH) assay

DPPH method was selected to measure the free radical scavenging activity of *Olea europaea* seeds extracts. A stock solution of 0.1mM DPPH was prepared by dissolving (0.04mg/ml) with absolute methanol. The extracts of *Olea europaea* seeds were tested at six different concentrations (500, 250, 125, 62.5, 31.25, & 15.63 µg/ml) by diluting each concentration to half; the procedure was carried out in a 96-well plate in which 100 µl of each concentration of each test extract was mixed with 200 µl of DPPH solution (each concentration was tested in 3 wells) and then incubated in a dark place for 30 minutes. The absorbance was measured at 517 nm using ELISA micro-plate reader (LabTech, Korea). A stock solution of each extract was prepared by dissolving (1mg/ml) in absolute methanol then diluted to the mentioned concentrations using absolute methanol as well. Vitamin C (ascorbic acid) was utilized as the positive control, absolute methanol alone was used as the blank, and 200 µl of DPPH solution plus 100 µl methanol was the negative control. The antioxidant activity (AA) percentage was calculated using the formula below [19]:

$$\%AA = 1-(A_S - A_B / A_C - A_B) \times 100 \quad (1)$$

Where A_S, A_B, and A_C represent the absorbance of the test sample, blank, and control, respectively.

Statistical analysis

Data were analyzed using the SPSS 16.0 and Microsoft Office Excel 2019. The results of (N = 8) are presented as means ± SD and the comparison of the mean difference between groups was done by One-Way Analysis of Variance (ANOVA) test followed by Post-hoc Tukey test and the significance value was set at ($P \leq 0.05$) for significant difference, and ($P \leq 0.001$ and 0.0001) for a highly significant difference. The concentration of the active extract that inhibited 50% of free radicals was determined according to the logarithmic equation by Microsoft Office Excel 2019.

Results and Discussion

Extraction process

Powder of *Olea europaea* L. was extracted with three solvents (chloroform, methanol, and 50% ethanol) using the cold method (maceration) for extraction. The final yield weight of crude extract and percentage of 92 grams of the powder are shown in **Table 1**.

Table 1. final yield weight and percentage of *Olea europaea* L. seeds extracts using (maceration) for the extraction

No.	Type of Extract	Yield Weight (gm)	Yield Percentage (%)
1	Chloroform Extract (OE-CE)	5.85 gm	6.36%
2	Methanol Extract (OE-ME)	3.2 gm	3.48%
3	50% Ethanol Extract (OE-EE)	4.15 gm	4.51%

Biochemical analysis of liver indices

Liver indices were measured using serum levels of transaminases (ALT & AST) as well as ALP. Such biomarkers are useful for distinguishing early acute liver injury. Results of the study revealed a significant sharp elevation ($P \leq 0.0001$) in serum transaminases (ALT & AST) in the MTX group [receiving 20mg/kg MTX only as a single dose], suggesting a successful induction of acute liver injury when compared to the normal animal group. Animal group pretreated with silymarin served as animals receiving standard hepatoprotective agent, where the results revealed a significant reduction in serum values of transaminases (ALT & AST) ($P \leq 0.001$) when compared to the MTX group. Animal groups pretreated with *Olea europaea* extracts (group 4, 5, and 6) receiving *Olea europaea* chloroform extract, methanol extract and 50% ethanol extract respectively at 500mg/kg once daily dosing showed a statistically significant reduction in serum levels of transaminases (ALT & AST), ($P \leq 0.05$) for OE-CE and ($P \leq 0.0001$) for OE-ME and OE-EE when compared to MTX group as shown in **Table 2** and **Figure 1**. Although, animals pretreated with 50% ethanol extract of *Olea europaea* (OE-EE) showed a better reduction in liver indices when compared to OE-CE and OE-ME pretreated groups ($P \leq 0.05$). Serum alkaline phosphatase (ALP) remained within normal levels among all animal groups; although the MTX group showed a mild elevation in ALP value, these values remained within normal intervals [20].

As for serum total protein (STP) and serum total bilirubin (TSB), the results of the experiment revealed that their values among all animal groups remained within normal intervals. Although; regarding STB, the MTX group showed a statistically significant increase in STB (P≤0.05) when compared to the normal group, still that elevation remained within normal intervals. As well as animals pretreated with (OE-EE) revealed a statistically significant reduction in STB (P≤0.001) when compared to the MTX group and the others pretreated groups as shown in **Figure 2**. Data analysis of STP revealed no significant difference among all animal groups (P = 0.46) as shown in **Figure 3**, and the values of TSP for all animal groups remained within normal intervals [20].

Table 2. effect of MTX and pretreatments with *Olea europaea* L. extracts on values of liver indices in albino mice

Groups	GPT(ALT) IU/L	GOT(AST) IU/L	ALP (IU/L)	TSB (mg/dl)	TSP (mg/dl)
Normal	40 + 9.23	56.13 + 3.87	31.75 + 10.98	0.14 + 0.07	6.29 + 0.55
MTX only (20mg/kg)	158.63+51.37**	419.5 + 93.63**	85.5 + 22.62**	0.16 + 0.07	5.54 + 0.51
MTX & Silymarin	73 + 9.71	197.25+12.79	65.75 + 11.93	0.13 + 0.05	6.31 + 0.66
MTX & OE-CE	116.88+20.49	296.25+50.19	80.88 + 8.99	0.16 + 0.07	6.31 + 0.66
MTX & OE-ME	88.25 + 14.39	210.63+27.09	72 + 10.24	0.13 + 0.05	6.34 + 0.5
MTX & OE-50%EE	73.5 + 9.32**	147 + 22.16**	49.25 + 10.61**	0.11 + 0.04	6.43 + 0.46

Results are expressed as mean ± SD, (n = 8), the significance level was set at P≤0.05.

** represents highly significant difference (P≤0.0001)

GPT(ALT)= glutamic pyruvic transaminase (Alanine Transaminase), **GOT (AST)**= glutamic oxaloacetic transaminase (Aspartate Transaminase), **ALP**= Alkaline Phosphatase, **TSB**= Total Serum Bilirubin, **TSP**= Total Serum Protein, **MTX**= methotrexate, **OE-CE**= Olea europaea chloroform extract, **OE-EE**= Olea europaea 50% ethanol extract**, OE-ME**= Olea europaea methanol extract.

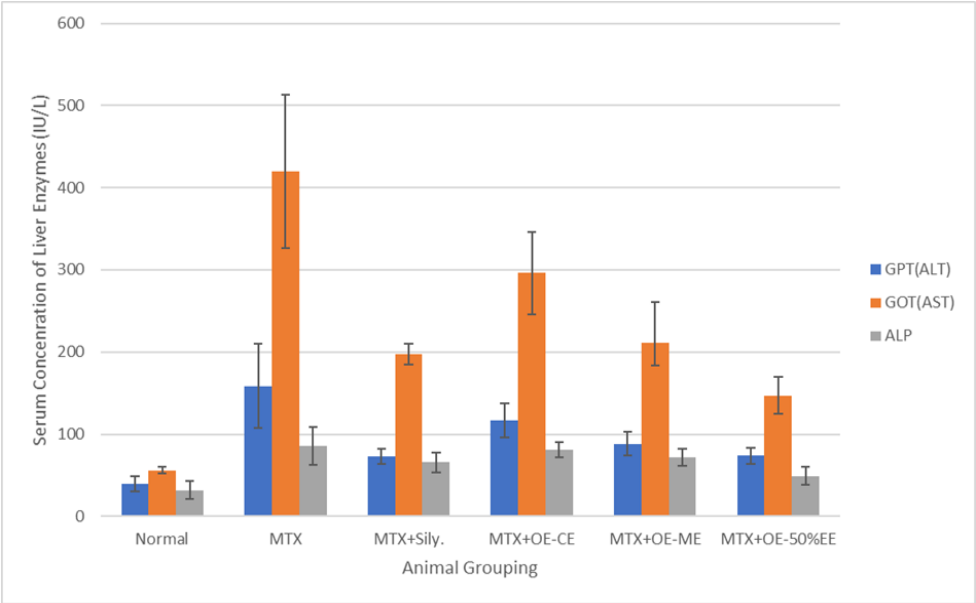

Figure 1. Effect of test substances on values of liver enzymes among animal groups. Results are presented as mean ± SD, (n = 8), the significance level was set at P≤0.05.
GPT(ALT)= glutamic pyruvic transaminase (Alanine Transaminase), **GOT (AST)**= glutamic oxaloacetic transaminase (Aspartate Transaminase), **ALP**= Alkaline Phosphatase, **MTX**= methotrexate, **OE-CE**= Olea europaea chloroform extract, **OE-EE**= Olea europaea 50% ethanol extract **OE-ME**= Olea europaea methanol extract,.

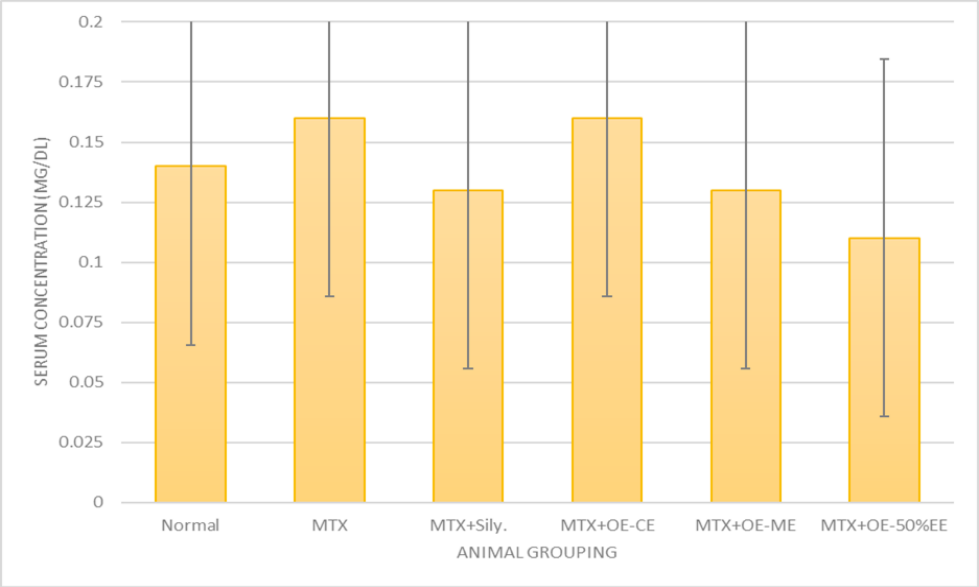

Figure 2. Effect of test substances of serum concentration of total bilirubin (TSB) among animal groups. Data are presented as mean + SD, (n = 8), the significance level was set at P≤0.05

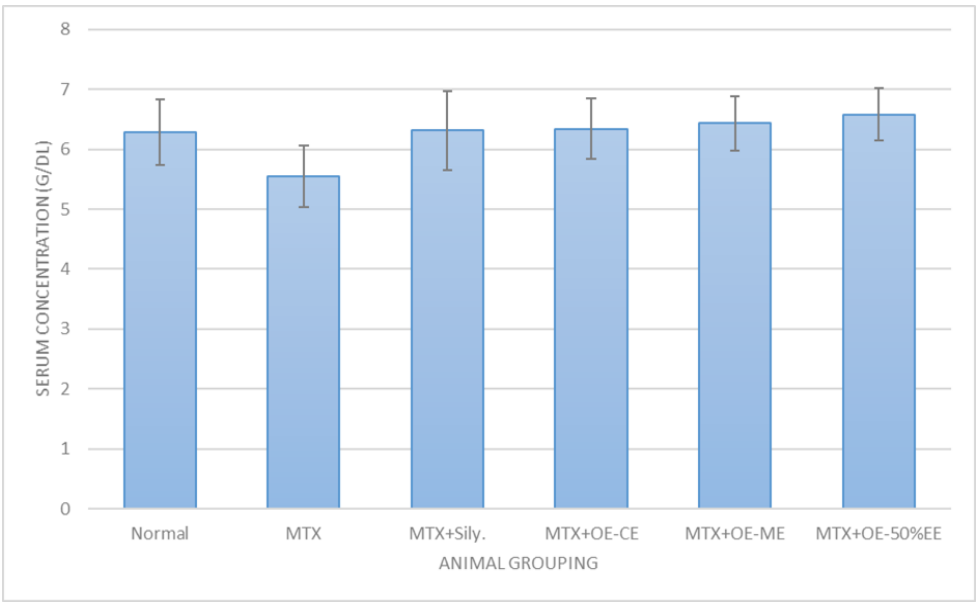

Figure 3. Effect of test substances on serum concentration of total proteins (TSP) among animal groups. Data are presented as mean + SD, (n = 8), the significance level was (P=0.46)

Histopathology description

The results of the examination revealed; liver section of the normal group showing the normal histological structure, which consists of central vein and arrangement of hepatocyte cells as a thread like in between sinusoid. The liver section of the animal group that received MTX only revealed a focal necrotic area with an accumulation of inflammatory cells with necrosis of hepatocyte cells, as well as accumulation of fat droplets and apoptotic cells are present around central veins. Histological liver section of animals pretreated with standard silymarin showing focal necrosis of hepatocytes with infiltration of inflammatory cells around the central vein. As for liver sections from animals pretreated with olive seeds (*Olea europaea*) extracts revealed varying results; animals pretreated with chloroform extract (OE-CE) showed marked inflammatory infiltrate, necrosis of hepatocytes and abundant accumulation of glycoprotein granules and dilated sinusoids. While in animals pretreated with methanol extract (OE-ME), the liver section revealed focal necrosis of hepatocytes around congested central vein but to a lesser extent with fewer inflammatory infiltration. Animals pretreated with 50% methanol extract (OE-EE) revealed the best results where the histological section showing a near-normal structure with a mild accumulation of lipid materials, these results are properly explained in **(Figure 4)**. Stained liver sections were viewed under a light microscope with 40X, and the histological results obtained solidify and support the results of the biochemical analysis of liver indices **(Figure 1)**.

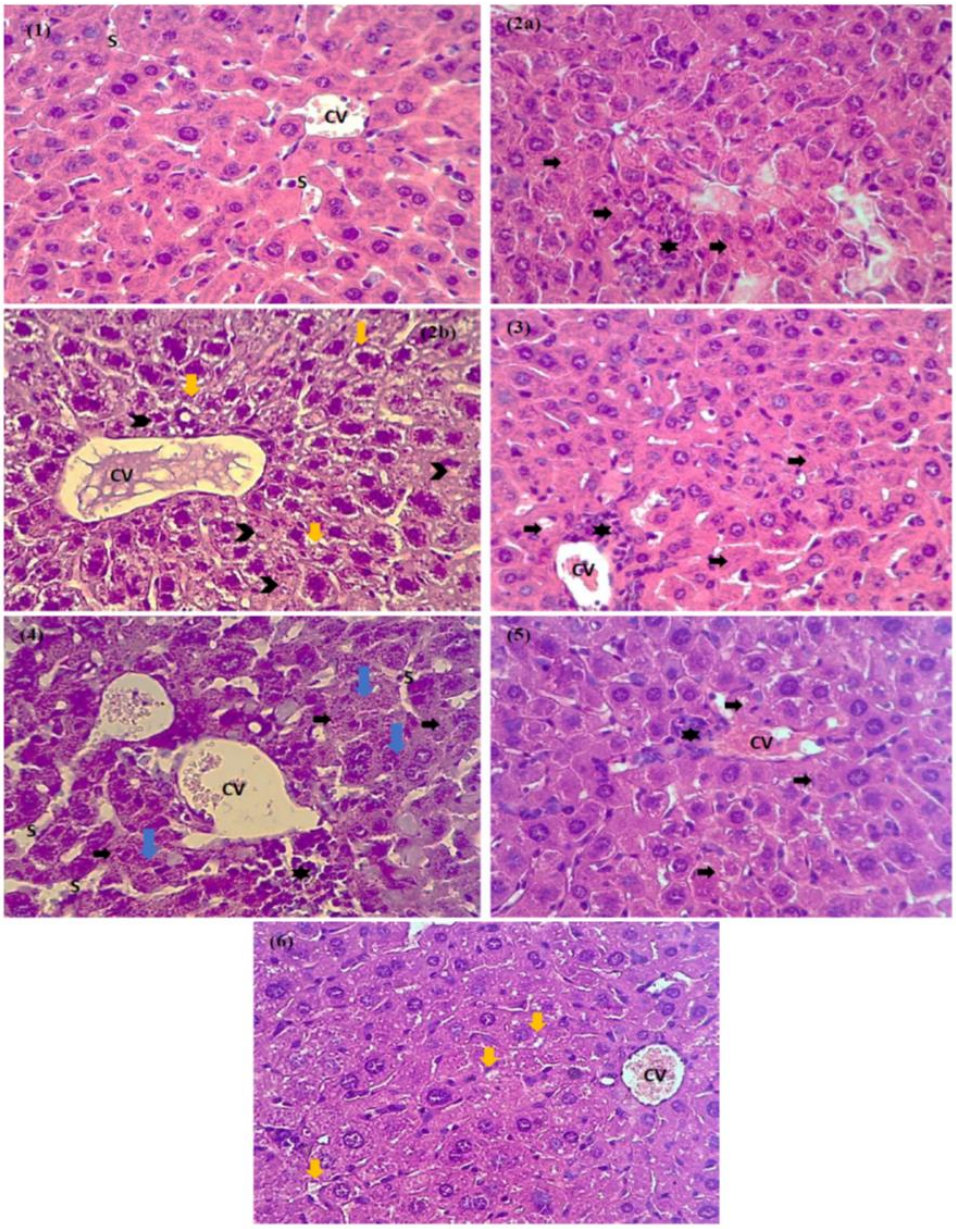

Figure 4. Histological examination of liver tissue. **(1)** represents normal group, **(2a & 2b)** represents animals treated with MTX only (20mg/kg) single dose, **(3)** animal pretreated with silymarin, **(4)** animals pretreated with Olea europaea chloroform extract, **(5)** animals pretreated with Olea europaea methanol extract and **(6)** animals pretreated with Olea europaea 50% methanol extract.
Star: inflammatory cells infiltrate, **black arrow:** necrotic hepatocyte cells, **arrow head:** apoptotic cells, **yellow arrow:** fat accumulation, **blue arrow:** glycoprotein granules accumulation.
CV: central vein and **S:** Sinusoid. (H & E) at 40X

Determination of free radical scavenging activity

Free radical scavenging (antioxidant) activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Each extract of *Olea europaea* L. seeds was tested with the use of a standard solution of DPPH free radical to observe the ability of each extract in reducing the free radical. Result of the study revealed that, all the extracts were able to scavenge DPPH free radical when they were compared to the negative control (P≤0.05). although, 50% ethanol extract of Olea europaea L. seeds (OE-EE) exhibited the best free radical scavenging activity compared to the negative control and the other extracts (P≤0.05) as shown in **(Figure 5)** with IC_{50} of (5.48 µg/ml) according to the logarithmic equation. This could support the results obtained from the biochemical analysis of liver indices and the histological examination of liver tissues that 50% ethanol extract of *Olea europaea* L. seeds revealed a high statistical reduction in the levels of liver indices, as well as better histological results of livers tissue when compared to the other animal groups (P≤0.0001).

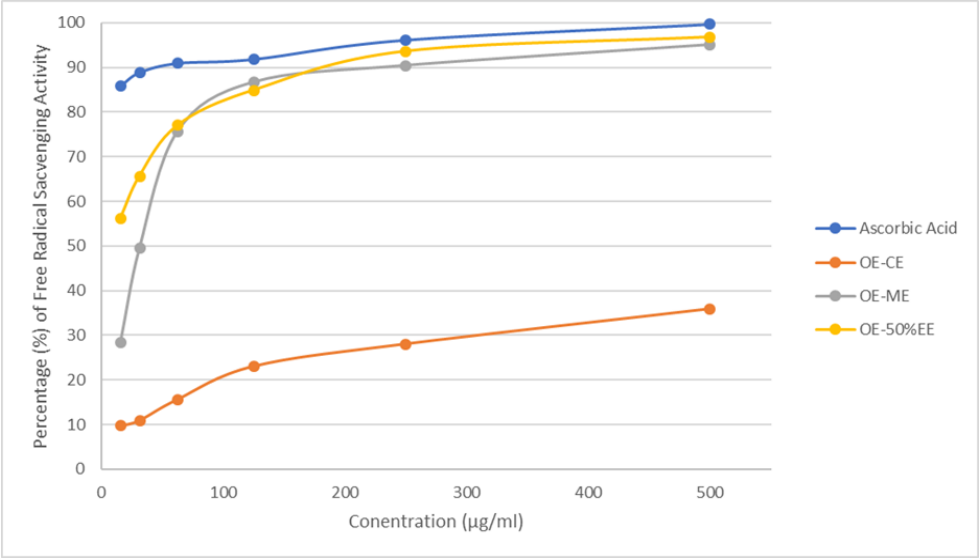

Figure 5. Free radical scavenging activity of *Olea europaea* L. extracts by DPPH assay, Vit C was used as the positive control.
OE-CE = Olea europaea chloroform extract, OE-ME = Olea europaea methanol extract, OE-50%EE = Olea europaea 50% ethanol extract

Liver is considered the main organ for the metabolism and detoxification of drugs; thus, a wide range of therapeutic medications could alter its normal physiological function like: anticancer agents, antiviral agents, some NSAIDs, and among others. The early signs or markers for altered liver function are the elevation in the serum level of transaminases (GPT and GOT) [21]; so, the search for naturally occurring herbs with medicinally active phytochemicals that could attenuate liver injury became an interesting field for research because most of the naturally occurring herbs or their crude extracts can be taken safely and with very minimal adverse effects.

Powder of *Olea europaea* L. seeds was extracted with different solvents of different polarities using the cold method (maceration) for the extraction process. The choice for such type of extraction method was to ensure that all the phytochemicals in the seeds were extracted according to their polarities by exhaustion through repetitive maceration with each solvent using low temperature during the extraction time, in order to avoid destruction of the essential phytochemical substances [22].

Methotrexate (MTX) was the drug of choice for the induction of liver injury in mice, because it is still being used effectively in the therapeutic management of rheumatoid arthritis, severe cases of psoriasis, leukemia, etc. Unfortunately, treatment with MTX has been associated with serious adverse effects; including myelosuppression, gastro-intestinal side effects, and the most predominant is liver toxicity [23], thus, encouraging the search for effective therapeutic medicines of natural sources to prevent or reduce its toxic effects during the treatment. Results of the current study revealed that MTX successfully induced liver injury as seen from the biochemical analysis, where there was a sharp increase in the serum level of the transaminases (AST and ALT) which is supported by the histological examination, where the liver tissue section showed marked appearance of necrosis, apoptotic bodies, inflammatory cells infiltrate, swelling and accumulation of fats, these findings are in consistence with previous studies [21, 24]. The pathogenesis of MTX liver toxicity is still not understood, but a certain hypothesis was focusing either on the oxidative stress and others explaining MTX toxic effect is brought by the inhibition of folate synthesis [25]. Most of the studies focusing on the oxidative stress produced by MTX; usually there is an equilibrium between ROS and the antioxidant mechanisms, but some medications can shift that balance towards excessive production of ROS [26]. MTX appears to indirectly affect MTHFR (methylene tetrahydro folate reductase) which is responsible for the conversion of homocysteine into methionine; thus, accumulation of homocysteine can stimulate oxidative stress or sensitize the hepatic cells to its effects, causing DNA alteration, increase cell membrane permeability and endoplasmic reticulum stress. All this leads to increased leakage of liver transaminases (an indicator of early cell injury) to fat accumulation and stimulation of proinflammatory cytokines [25], which explains the results mentioned previously. MTX hepatotoxicity can occur with a dose of more than 1.5 g but also can occur at lower doses and sometimes early in the treatment. In this study, a single dose of 20mg/kg MTX was used to produce liver injury because a higher dose may result in systemic toxicity and unwanted death of the animal [18, 27].

Results of the current study revealed that extracts of *Olea europaea* seeds were able to reduce the serum levels of liver transaminases (ALT and AST) with varying degrees; although 50% ethanol extract of *Olea europaea* seeds was the best in attenuating the signs of liver injury produced by MTX compared to the other extracts. *Olea europaea* L. was found to be a good source of different types of phytochemical constituents, which are oleuropein, secoiridoids glycosides, flavonoids, phenolics like tyrosol and hydroxytyrosol, nuzhenide as well as benzoic acid derivatives. Among these phytochemicals, oleuropein was found in all plant parts and according to different studies found to possess remarkable antioxidant and free radical scavenging activity both in-vivo and in-vitro studies [13]. While nuzhenide

was the most concentrated phenolic compound in the seeds and seemed to have potent antioxidant properties [28]. Such phenolics compounds are polar constituents and could be found in higher concentrations in the polar extracts (methanol and 50% ethanol); which could explain the significant hepatoprotective effect produced by the 50% ethanol extract of *Olea europaea* seeds as shown in the biochemical analysis **(Figure 1)** and liver histological examination **(Figure 4)** as well as its effect in reducing DPPH free radical which supports the previous findings. Animals were pretreated with the extracts at a dose of 500mg/kg according to previous study protocols as well as, toxicity profile studies of the pulp aqueous extract concluded that a dose as high as 2000mg/kg caused no adverse effect and was considered safe [18, 29]. Silymarin was used as the standard hepatoprotective agent; it is mainly extracted from the seeds of *Silybum marianum* (milk thistle) and has been proven effective in the management and prevention of liver injury caused by hepatitis, excessive alcohol consumption and drug induced injury. Its mechanism of hepatoprotective effect is still not clear but most likely produced by its antioxidant activity thus, regulating the intracellular levels of glutathione and membrane permeability by preventing the hepatotoxic agent from entering hepatocytes [30].

Since most researches focusing on oxidative stress as the pathogenesis of MTX liver injury, the use of a high dose of MTX resulted in the reduction of the levels of antioxidant enzymes (GSH, SOD and CAT) and elevated levels of MDA as shown in a study done by Heibatullah *et al*. [21]. 50% ethanol extract of *Olea europaea* seeds may produce its effect by its main constituents (oleuropein and nuzhenide) and their potent free radical scavenging activity; thus, reducing or preventing the oxidative stress and its deleterious effect on lipid peroxidation and alterations of intracellular components of hepatic cells.

Conclusion

The current investigation revealed that pretreatments with the extracts of *Olea europaea* L. seeds were able to reduce serum levels of liver indices (GPT, GOT and ALP) in mice and attenuate liver toxicity caused by MTX. Results of the study showed that all the extracts of *Olea europaea* L. seeds possess antioxidant activities and hepatoprotective effect to MTX-induced liver injury but with varying extents. Although, 50% ethanol extract of *Olea europaea* L. seeds showed the best reduction in serum liver indices, the liver histological section was almost close to normal and the best free radical scavenging activity. These results suggest that 50% ethanol extract of olive seeds could be a promising natural hepatoprotective agent and could be used safely with MTX therapy to reduce its toxic effects.

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Conflict of interest: None

Financial support: None

Ethics statement: Animal housing and handling, as well as scarification with the use of proper anesthesia to ensure minimal pain infliction was approved by the institutional ethical committee of Al-Rasheed University College / Pharmacy Department; Ref. No.: 2020/PD-009.

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