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Research Article
Hepatoprotective Properties of Olive Extract on Methotrexate-Induced Liver Damage

[1,2]Ali Hassan A. Ali, [1]Muhammad Aslam Siddiqui, [3]Salman Khalid Alghamdi, [3]Abdulelah Feraih Alenazey, [3]Bader Musaed Alharbi, [3]Talal Habis Almutairi and [4]Shatha Shayan Almutairi

[1]Basic Medical Science Department, College of Medicine, Prince Sattam bin Abdulaziz University, Al-Kharj 11942, Kingdom of Saudi Arabia
[2]Anatomy Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
[3]College of Medicine, Prince Sattam bin Abdulaziz University, Al-Kharj, Kingdom of Saudi Arabia
[4]College of Medicine and Medical Science, Arabian Gulf University, Manama, Kingdom of Bahrain

Abstract

Background and Objective: A common folate antagonist used to treat neoplastic conditions is methotrexate (MTX); the drug's biotransformation in the liver results in active metabolites that increase hepatotoxicity. Olive leaf extract is one type of antioxidant that may shield our bodies from the damaging effects of free radicals. The current study aimed to assess olive extract's hepato-protective potential against MTX-induced liver injury in rats. **Materials and Methods:** Twenty-four male rats were divided into three groups of eight. The control group received no treatment, the methotrexate (MTX) group was administered a single 20 mg/kg dose of MTX intraperitoneally and the third group received olive leaf extract (1 mL/100 g body weight) daily for one month after the same MTX injection. Serum globulin levels were measured and histological, histochemical and immunohistochemical investigations were performed. Statistical analysis was conducted using ANOVA with $p<0.05$ considered significant. **Results:** With a highly significant decrease in mean total proteins and albumin levels and an insignificant decrease in globulin values, the second group showed a highly significant increase in mean total bilirubin and hepatic enzyme levels. Additionally, compared to the control group, this one showed worsened microscopic alterations. In addition to noticeably better microscopical results than the second group, the third group also showed reversed biochemical results. **Conclusion:** This research found that administering olive extract to individuals receiving methotrexate is important for managing and shielding them from the drug's serious hepatotoxic side effects.

Key words: Hepatotoxicity, protective action, oxidative stress, methotrexate, olive

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Corresponding Author: Ali Hassan A. Ali, Department of Basic Medical Science, College of Medicine, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Kingdom of Saudi Arabia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One popular anti-neoplastic medication that is thought to be the first line of treatment for cancer patients is methotrexate (MTX)[1]. Further conditions for which MTX is recommended include autoimmune diseases (such as rheumatoid arthritis), psoriasis, Crohn's disease, immunological disorders and systemic inflammation. Hepatotoxicity and renal toxicity are two of methotrexate's most well-known side effects. It is unknown what the main mechanism is by which methotrexate affects the liver. Nevertheless, studies have shown that methotrexate treatment may result in changes to the liver's histology and a hepatic folate shortage[2]. Conversely, the liver is essential for the metabolism of substances, medications and food. Therefore, the primary organ affected by drug-induced organ damage is the liver. The primary cause of several diseases during liver damage is oxidative stress, which is demonstrated by the rise in reactive oxygen and nitrogen species as well as the decrease in endogenous antioxidants[3].

Methotrexate therapy also increases liver oxidative stress because it increases lipid peroxidation and decreases antioxidant levels[4]. Antioxidants may shield cells from the harm that free radicals, which are implicated in several illnesses, do. An appropriate antioxidant may prevent liver damage and lessen the toxicity of free radicals. It has been observed that several dietary antioxidants reduce the damage that free radicals cause to biomolecules. The leaf of the olive tree (*Olea europaea*) is known as an olive leaf. Ancient Egyptian and Mediterranean cultures employed olive leaf extract to cure a variety of illnesses, including fever, pain and infections[5]. Because olive leaves contain significant antioxidant and phenolic components including oleuropein, tyrosol and hydroxytyrosol to prevent oxidative damage, they are commonly utilized in Mediterranean nations for conventional therapies[6]. Additionally, the olive leaf contains rutin, verbascoside, luteolin, p-coumaric acid, vanillin, vanillic acid and caffeic acid, all of which have numerous pharmacologically advantageous qualities. Research has indicated that olive leaves can be used as a medication for several illnesses that cause pathological, physiological and biochemical alterations[7]. Numerous potent medicinal ingredients, including oleuropein, oleuropeoside and hydroxytyrosol, are present in the raw olive leaf extract. These medicinal ingredients have been shown in earlier studies to possess antiviral, anti-inflammatory, anti-cancer, anti-microbial, anti-atherogenic and antioxidant properties[8].

The goal of the current investigation was to find out how well olive extract worked to treat MTX-induced liver damage in rats.

MATERIALS AND METHODS

Study area and duration: The experimental study was conducted from August, 2023 to September, 2024.

Ethical consideration: The Al-Kharj Ethical Committee of PSA University accepted our work, which met with regulations regarding the use and care of animals in research (SCBR-150-2023).

Methods: Twenty-four adult male Albino rats weighing between 185 and 200 g from PSA University's Pharmacy Faculty. Each was housed in an animal house at 21-22 fed a conventional rat chow diet and given unrestricted access to water. Two weeks before the start of the trial, the rats were acclimated to the lab environment. Three groups of eight rats each were created from the animals. After soaking 5.5 g of olive leaf powder in 100 mL of hot water and keeping it covered for 10 min, the mixture was allowed to cool to room temperature before being filtered. Using a stomach tube, the extract was administered orally once daily at a quantity of 1 mL/100 g of body weight. This dosage is the same as the 500 mg therapeutic human dose[9]. The second and third groups received methotrexate hydrate intraperitoneally, from Sigma Chemical Company, St. Louis, USA. The Roche Biodiagnostic Company, Boehringer, Mannheim, Germany, provided the biochemical assay kits for Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and total bilirubin. At the same time, albumin and total protein kits were purchased from Biosystems S.A., Costa Brava 30, Barcelona, Spain. Serum albumin value was subtracted from serum total protein value to determine globulins. Throughout the trial, the control group of healthy animals did not receive any medication. For nine weeks, the methotrexate group received a single intraperitoneal injection of 0.5 mg/kg body weight twice a week. The last group of rats was given a daily dosage of olive extract 1 mL/100 g of body weight through a gastric tube for nine weeks after receiving the same injection of methotrexate as the second group. Twenty-four blood samples (one from each sacrificed rat) from the venous plexus at the ocular canthus were collected at the end of the study, placed in sterile tubes and centrifuged for 20 to 30 min at

3000 rpm to extract serum. This serum was then stored in Eppendorf tubes at -20°C until it was time to analyze the results for total bilirubin, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP), which were calculated using a Roche 917 auto-analyzer. Additionally, the colorimetric approach was used to determine serum total proteins and albumin[10]. The values of the globulins were then obtained by subtracting the albumin values from the total protein values.

Liver specimens were prepared for light microscopy analysis by embedding them in paraffin, cutting them into 5 µm slices, dehydrating them in ethyl alcohol, cleaning them in xylene and staining them with Hematoxylin and Eosin (H&E) to assess histopathological alterations and structural morphology. In addition, to identify collagen fibers Mallory's trichrome stain was used. Moreover, to measure the amount of cytoplasmic glycogen periodic acid schiff stain (PAS) with diastase was also used. Toluidine blue stain was used to identify cellular infiltration.

Statistical analysis: Analytical statistics variables were carried out by SPSS 15.0 and various statistical tests such as ANOVA. They were employed across various groups to compare changes in both immunohistochemistry and histopathology. Values of $p<0.05$ were considered significant.

RESULTS

When comparing the mean values of all biochemically measured components in the methotrexate-olive managed group (G3) to the control group, Table 1 revealed negligible statistical differences. As demonstrated by statistically significant increases in the mean values of liver enzymes (AST, ALT and ALP) and mean total bilirubin. In addition, there were highly significant decreases in the mean values of total proteins and albumin and a statistically insignificant decrease ($p>0.05$) in the mean values of globulins, the methotrexate-treated group (G2) exhibited significant hepatotoxicity when compared to the control group. On the other hand, when compared to the second group, the third group showed statistically significant increases in the mean values of albumin and total proteins ($p<0.05$). In addition, there were statistically insignificant increases in the mean values of globulins ($p>0.05$) and highly statistically significant decreases in the mean values of liver enzymes (AST, ALT and ALP) and mean total bilirubin ($p<0.05$).

According to histopathology, control hepatic sections (G1) showed a typical architecture with anastomotic liver cell cords organized radially, extending from the central veins and divided by blood sinusoids (Fig. 1a-d).

Figure 1 illustrates the histological architecture of an adult albino rat's liver under various staining techniques. Figure 1a shows the normal lobular pattern with a portal triad comprising the portal vein (PV), hepatic artery (HA) and bile duct (BD), along with the centrilobular vein (C.V). Figure 1b highlights strong PAS-positive granules in hepatocytes, while Fig. 1c demonstrates the arrangement of the central vein (C.V) and hepatocytes. Figure 1d reveals minimal collagen fiber distribution.

However, group 2 (G2) showed significant dilatation and congestion of the central venous and blood sinusoids, fluid exudate formation, enlarged von Kupffer cells within the dilated congested sinusoids, hepatocytic fatty accumulation, especially in the peri-portal area and a noticeable inflammatory cellular infiltration, particularly in the portal tract areas, after receiving methotrexate (Fig. 2a-d).

In Fig. 2 methotrexate (MTX) group was described. Figure 2a shows moderate PAS-positive granules in most hepatocytes. Figure 2b depicts dilated and congested central and portal veins with lymphocyte infiltration. Figure 2c demonstrates mild cellular infiltration in the portal triad while Fig. 2d highlights marked caspase-3 immunostaining expression in the cytoplasm of hepatocytes.

Table 1: Biochemical examination of serum to various chemicals in each group

	Groups		
Analyzed substances	G1	G2	G3
AST (U/L)	29.2±1.19649	85.75±1.65036	30.05±1.31689
ALT (U/L)	24.45±0.82558	90.6±1.3917	25±0.97333
ALP (U/L)	46.7±0.8645	120.1±1.41049	47.3±1.68897
Total bilirubin (mg/dL)	0.3725±0.042037	1.0725±0.039454	0.383±0.047473
Total proteins (g/dL)	7.47±0.10809	4.915±0.06329	7.3825±0.17865
Albumin (g/dL)	5.037±0.03496	2.535±0.08751	4.96±.0.25215
Globulins (g/dL)	2.433±0.11939	2.38±0.10377	2.4225±0.24734

AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase and ALP: Alkaline Phosphatase, ±: Mean±Standard Deviation (SD), showing the average value and the variability or dispersion of data around the mean, G1: Control group with no dose, G2: Methotrexate (MTX) group at a single dose (20 mg/kg) MTX intra-peritoneally and G3: Last group of rats, was given oral intake of olive leaf extract with a dose of 1 mL/100 g of body weight every day for one month after receiving the same injection of methotrexate as the second group

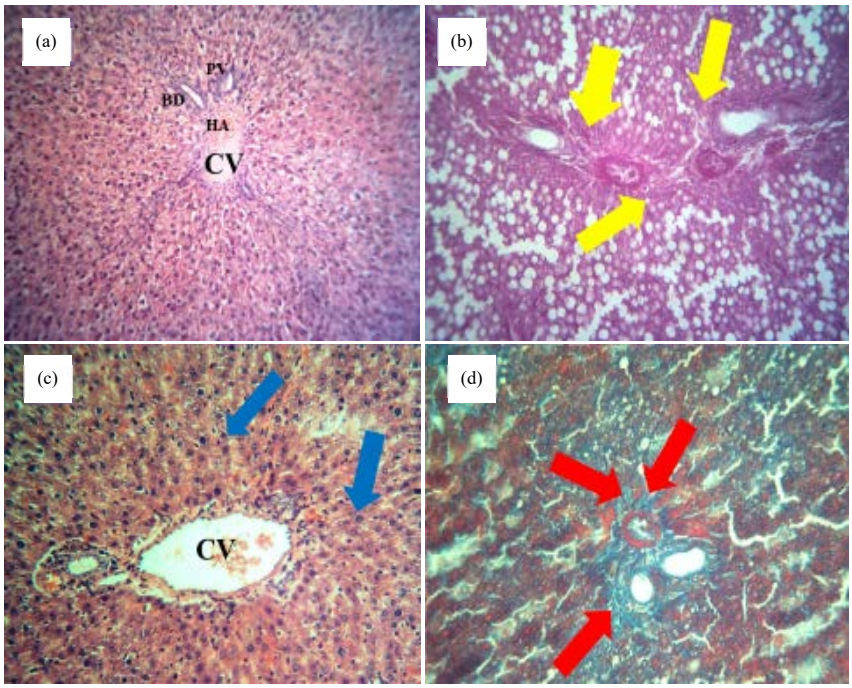

Fig. 1(a-d): Control group, (a) Normal lobular pattern normal portal triad consisting of a branch of the portal vein (PV), the branch of the hepatic artery (HA) and bile duct (BD) (CV) centrilobular vein (H&E ×200), (b) Strong PAS +ve granules are visible in the majority of the hepatocytes in this photomicrograph of an adult albino rat's control liver (yellow arrows) (PAS ×200), (c) Architecture of the central vein (CV) and hepatocytes (blue arrows) (H&E ×200) and (d) Normal minimal distributed collagen fibers (Mallory trichrome ×400)

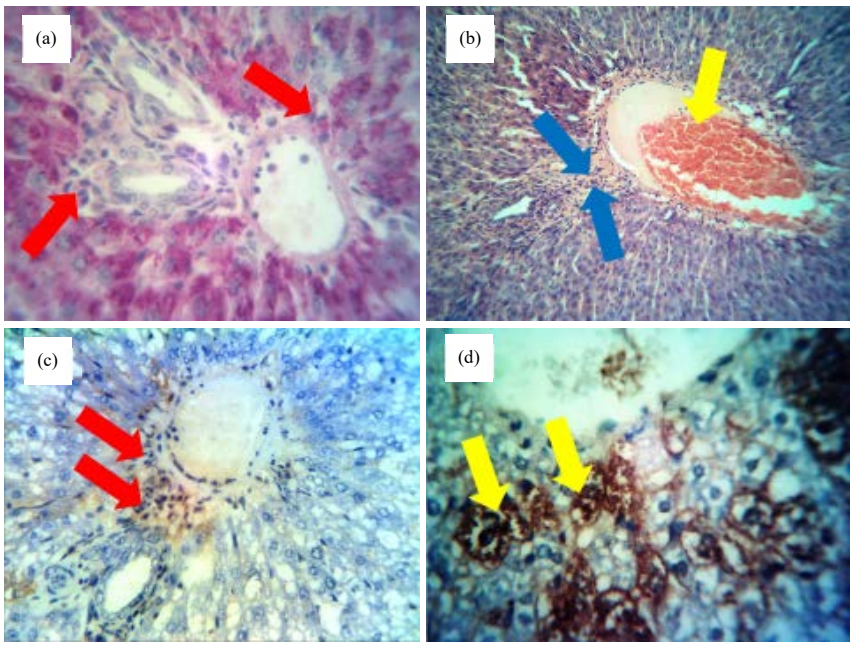

Fig. 2(a-d): Different images of the methotrexate (MTX) group, (a) Moderate PAS-positive granules in most of the hepatocytes (Red arrows) (PAS ×400), (b) Central and portal veins were dilated and congested (yellow arrows) and lymphocyte infiltration (blue arrows) (H&E ×200), (c) Mild cellular infiltration in the portal triad (red arrows) (T.B. x400) and (d) Marked expression of caspase-3 immunostaining in the cytoplasm of the hepatocytes (yellow arrows) (caspase-3 immunostaining ×400)

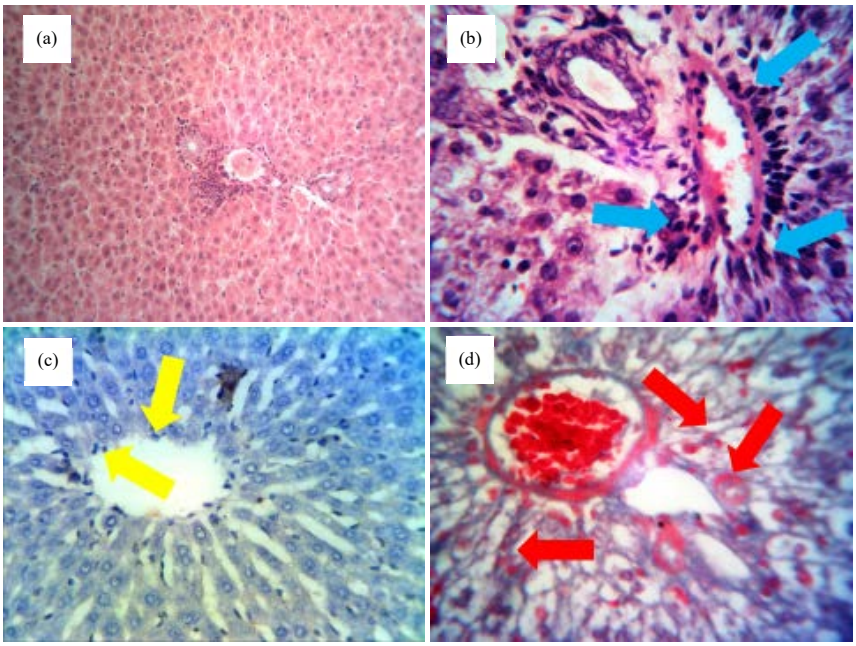

Fig. 3(a-d): Different images of the olive treated group, (a) Hepatic cords positioned radially around the major vein in a typical hepatic design similar to that of the control group (H&E ×200), (b) Distribution of fine threads of collagen fibers around the hepatic portal vein and bile duct (blue arrows) (Mallory's trichrome ×400), (c) Mild cellular infiltration in the portal triad(yellow arrows) (TB ×200) and (d) Typical distribution of collagen fibers in tiny threads surrounding the major vein (red arrows) (Mallory's trichrome ×400)

Furthermore, the liver sections of group 3 rats given olive showed significantly fewer aberrant toxic hepatic structural alterations, as shown by the architecture of most group 3 hepatocytes being somewhat similar to that of the control group, except a mildly dilated portal vein and a mild periportal mononuclear cellular infiltration (Fig. 3a-d).

Figure 3 presents details about the olive-treated group. Figure 3a illustrates hepatic cords arranged radially around the major vein, resembling the control group's typical hepatic design. Figure 3b shows the distribution of fine collagen fibers around the hepatic portal vein and bile duct. Figure 3c highlights mild cellular infiltration in the portal triad, while Fig. 3d demonstrates the distribution of collagen fibers in tiny threads surrounding the major vein.

Furthermore, the majority of group 2 hepatocytes showed an excessive intracellular vacuolar degeneration in addition to a strong eosinophilic granular cytoplasmic reaction when periodic acid schiff (PAS) with diastase stain was applied to the hepatocytes in both the control and third groups. This was due to an apparent decrease in hepatocytic glycogen, which was indicated by a weak cytoplasmic PAS-positive granular reaction. Additionally, the control group's portal tracts had blue collagen fibers that were regularly dispersed, according to Mallory's trichrome stain. However, compared to the relatively small perivenous (around the central vein) and portal (at portal tracts) collagen fibrous reactions seen in the group 3 sections, a significantly higher perivascular collagen fibrous reaction was observed in the group 2 portal tracts (Fig. 2a-d).

DISCUSSION

Methotrexate is regarded as one of the first cancer chemotherapeutic medications and is a commonly used treatment for rheumatoid arthritis[11]. In the group of rats given methotrexate, investigation showed several degenerative liver structural, morphological and biochemical alterations. Along with a highly significant increase in serum levels of total bilirubin and alkaline phosphatase, which indicate cholestasis, these morphostructural changes indicate toxicity to the liver cells and were linked to a highly significant elevation of serum alanine and aspartate aminotransferases that were released from the injured hepatocytes. Additionally, the study showed a highly significant fall in blood levels of albumin and total proteins, which previous studies had shown was caused by the liver's reduced ability to synthesize proteins as a result of

damaged hepatocytes[12]. These results were in line with those of another researcher who noted that while long-term treatment, advanced age, alcohol use, obesity, diabetes mellitus and prior hepatitis B or C were independent risk factors for the development of liver disease, methotrexate toxicity constituted a minor risk of severe hepatic disease in patients with rheumatoid arthritis[13]. The dilation and congestion of the portal and central hepatic veins, as well as the liver's blood sinusoids, may be the source of the hepatic degenerative outcomes observed in the group treated with methotrexate. While vascular congestion may have come from a loss of circulatory fluid followed by vascular engorgement with red blood cells, vascular dilatation had been caused by an increase in prostaglandin synthesis followed by smooth muscle relaxation. Furthermore, some researchers had previously observed inflammatory cellular infiltration and fatty degenerated hepatocytes, especially in the portal tract area. They attributed the inflammation to the microcirculation response that resulted in fluid and leukocyte migration from the bloodstream into the extravascular tissue[14,15]. The results show that MTX injection causes significant liver damage in rats, which is linked to elevated blood parameters, hepatic oxidative stress and inflammatory biomarkers. These results were in line with earlier research that showed epicatechin's ability to reduce methotrexate-induced hepatotoxicity in mice[16]. According to the study's findings, which are consistent with earlier research, methotrexate-induced liver and kidney damage is significantly decreased when using coconut oil nanoemulsion as a delivery mechanism[17].

Lastly, the current investigation found that group 3 had significantly improved liver function (biochemical) and morphology (structural), as well as protection against the severe degenerative alterations that had been previously identified in group 2. With a few minor morphological alterations, such as a slight dilation of the portal vein, a slight periportal mononuclear cellular infiltration and the development of very few perivenous and portal collagen fibers, there was an apparent improvement in the liver architecture. In addition to the morphostructural changes observed in group 3, the biochemical profile of the liver showed a highly significant decrease in serum alanine and aspartate aminotransferases, which indicate healthy liver cell membranes with no leakage and an improvement in hepatic toxicity. According to Ali *et al.*[18], olive oil may be a useful therapeutic approach for hepatic injury. This study encouraged to treatment of liver disease by these findings. The presence of physiologically active compounds that can scavenge free radicals is linked to the hepato-protective effect of olive oil.

CONCLUSION

This study concluded that olives had a protective effect on the structure and function of the liver. Liver dysfunctions brought on by methotrexate can be lessened by using olive extract supplements. Olive oil may be an appropriate and useful dietary supplement in lowering the consequences of MTX-induced liver injury and associated hepatotoxicity during cancer treatment because it may decrease the production of free radicals during MTX-induced toxicity. Therefore, the current study's findings imply that taking olives orally has a significant attenuating impact against oxidative stress and the resulting liver damage brought on by MTX and may be a viable therapeutic option. As a result, it should be standard practice to recommend olive supplements to patients receiving methotrexate to shield and heal their livers from the unpleasant side effects of the drug. These results could be confirmed by other research.

SIGNIFICANCE STATEMENT

This study discovered that the olives had a protective effect on the structure and function of the liver. The results therefore suggest that oral olive consumption has a strong attenuating effect against oxidative stress and the consequent liver damage caused by MTX and could be a promising treatment option. To identify the target chemicals from olive extract that provide liver protection, more research is required. Furthermore, it is advised that human studies be conducted to show whether comparable outcomes may be achieved.

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