PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(2): 1-10
https://doi.org/10.14719/pst.6955

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Comparative therapeutic efficacy of *Olea europaea* L. leaf extract and bone marrow-derived mesenchymal stem cells in ameliorating streptozotocin-induced hepatic and splenic damage in pregnant Rats: Mechanisms of antioxidant and anti-apoptotic protection

Mervat Ahmed AbdRabou[1*], Ahmed B M Mehany[2], Zahrah R Alrayes[1], Diaa Massoud[1], Aml M Asran[3], Fawzyah A Alghamdi[4], Suad Kausara[1] & Ahmed Atwa[2]

[1]Olive Research Center, Department of Biology, College of Science, Jouf University, P.O. Box 2014, Sakaka, Saudi Arabia

[2]Zoology Department, Faculty of Science (Boys), Al-Azhar University, Cairo 11884, Egypt

[3]Olive Research Center, Common First Year Dean's Office, Jouf University, El Jouf, Saudi Arabia

[4]Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, Jeddah 21589, Saudi Arabia

*Email: mababdraboh@ju.edu.sa

OPEN ACCESS

ARTICLE HISTORY
Received: 25 December 2024
Accepted: 28 February 2025
Available online
Version 1.0 : 10 March 2025
Version 2.0 : 01 April 2025

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Mervat Ahmed A, Ahmed BMA, Zahrah RA, Diaa M, Aml MA, Fawzyah AA, Suad K, Ahmed A. Comparative therapeutic efficacy of *Olea europaea* L. leaf extract and bone marrow-derived mesenchymal stem cells in ameliorating streptozotocin-induced hepatic and splenic damage in pregnant Rats: Mechanisms of antioxidant and anti-apoptotic protection. Plant Science Today. 2025; 12(2): 1-10. https://doi.org/10.14719/pst.6955

Abstract

Mesenchymal stem cells (MSCs) are multipotent stromal cells with remarkable plasticity, enabling them to differentiate into various tissue-specific cell types. MSCs play a pivotal role in tissue repair, hematopoiesis and immunomodulation. This study aimed to evaluate and compare the protective effects of olive leaf extract (OLE), bone marrow-derived mesenchymal stem cells (BM-MSCs) and their combination against hepatic and splenic toxicity in a rat model of gestational diabetes induced by streptozotocin (STZ). Histopathological and immunohistochemical analyses of liver and spleen tissues were conducted to assess these effects. Methods: Pregnant female rats were divided into five groups (n = 10 per group): Control group: Rats received STZ at a dose of 35 mg/kg body weight. OLE group: Rats were administered olive leaf extract (OLE) at 200 mg/kg body weight. GD + OLE group: Rats with STZ-induced gestational diabetes (GD) were treated with OLE (200 mg/kg body weight). GD + MSCs group: Rats with STZ-induced GD were treated with BM-MSCs. GD + OLE + MSCs group: Rats with STZ-induced GD were treated with both OLE and BM-MSCs. Results: STZ administration induced significant histopathological and immunohistochemical alterations in the liver and spleen tissues of pregnant femal rats. Treatment with OLE, BM-MSCs, or their combination markedly ameliorated these STZ-induced deteriorations, with the combined treatment showing the most pronounced protective effects. Conclusion: The findings demonstrate that both BM-MSCs and OLE exert protective effects against hepatotoxicity and splenic toxicity in a rat model of gestational diabetes. The combination of OLE and BM-MSCs exhibited synergistic benefits, highlighting their potential as therapeutic agents for mitigating organ damage in gestational diabetes.

Keywords

apoptosis; gestational diabetes; hepatotoxicity; histopathology; immunotoxicity; spleen

Introduction

Diabetes mellitus is characterized by elevated blood sugar levels and is associated with long-term complications affecting the eyes, kidneys, nerves and blood vessels. While the exact mechanisms driving these complications are

not fully understood, oxidative stress has been proposed as a key contributor to their pathogenesis (1).

The predominant catalyst for liver impairment among diabetic individuals appears to stem from heightened oxidative stress resulting from hyperglycemia. This heightened oxidative stress precipitates disturbances in the metabolism of carbohydrates, proteins and lipids (2). Consequently, these events trigger additional oxidative stress and activate inflammatory cascades (3). The likelihood of developing serious liver conditions, including liver failure and cirrhosis, is rising due to the prevalence of liver conditions like non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD) and metabolic liver disorders (4, 5). Emerging evidence suggests that hyperglycemia-induced oxidative stress also impacts spleen function, leading to altered immune responses and systemic inflammation, which further exacerbate liver damage (6). Additionally, stem cells, particularly mesenchymal stem cells (MSCs), play a crucial role in modulating oxidative stress and inflammation. MSCs have been shown to promote liver regeneration and mitigate fibrosis by secreting anti-inflammatory cytokines and enhancing antioxidant defenses (7). Thus, a mechanistic link between diabetes, liver-spleen axis dysfunction and stem cell-mediated repair mechanisms underscores the complex interplay in diabetic liver pathology.

Current approaches to treating diabetes primarily aim to lower high blood sugar levels using oral medications and insulin injections. However, the gradual development of resistance to these treatments and their adverse effects emphasize the need for alternative therapies that are gentler on diabetic patients, or with minimal side effects (6). Numerous health benefits, such as antimicrobial, anti-inflammatory, antihypertensive and antioxidant qualities, have been demonstrated for *Olea europea* olive leaf extract (OLE) (7, 8). The main active ingredient found in the *Olea europea* and its extract is oleuropein, belonging to the secoiridoid group of natural products. Oleuropein exhibits a widespread array of pharmacological and health-enhancing features. These include its abilities as spasmolytic, an antiarrhythmic, immune - stimulating, hypotensive, cardioprotective, antioxidant, anti-inflammatory and anti–thrombotic agent (7). Many of these properties are attributed to the antioxidant characteristic so oleuropein (9). However, the specific mechanism by which oleuropein attenuates hyperglycemia and cardiovascular risk parameters is not well understood (10, 11). This cell population's thorough characterization remains insufficient, primarily due to the absence of specific biomarkers.

The International Society for Cellular Therapy has established precise criteria for designating Mesenchymal Stem Cells (MSCs). These criteria include adhesion to plastic surfaces, the capability for in vitro transformation into osteoblasts, adipocytes and chondrocytes and the presence of distinct markers such as CD73 (ecto-50-nucleotidase), CD90 (Thy1) and CD105 (endoglin, SH2). It's crucial to note that MSCs must not display the expression of markers like CD45, CD19, CD79, CD14, or CD11b, alongside the absence of the human leukocyte antigen-DR isotype (HLA-DR) hematopoietic markers (12, 13). One of the most popular cell types in clinical trials and experimental research is bone-marrow-derived MSCs, or BM-MSCs. They are being researched and tested right now to treat a variety of illnesses (14). MSCs have demonstrated a defensive function against injury in various systems and organs, such as the nervous system, cardiac tissue and cartilages (15). Studies on MSCs' capacity for regeneration in the treatment of acute renal impairment have been conducted (16) and further studies examined their ability to regenerate in liver stem-cell-conditioned medium (CM-MSC). Also known as the secretome of the MSCs, CM-MSCs are thought to be a rich source of paracrine factors and are the subject of extensive investigation for a variety of regenerative therapeutic approaches, including the treatment of wound healing, myocardial infarction, stroke, bone regeneration and hair growth. Numerous analyses have evaluated the importance of paracrine aspects secreted by SCs as the primary mechanism underlying stem cells' ability to regenerate (17). Research have shown that MSCs downregulated fibrogenic growth factors by acting in a paracrine manner (18), pro-inflammatory cytokines. Additionally, they increased the levels of anti-apoptotic and anti-inflammatory factors (19, 20).

This study was motivated by previous research highlighting the therapeutic potential of BM-MSCs and OLE in mitigating organ toxicity. However, gaps remain in understanding their combined effects on gestational diabetes-induced hepatotoxicity and spleen toxicity. The rationale was to explore this synergy and address the lack of comprehensive histopathological and immunohistochemical evidence in existing literature.

Materials and Methods

The chemicals used in this study included streptozotocin (STZ) (Sigma-Aldrich, USA) to induce gestational diabetes and olive leaf extract (OLE) (Al-Jouf Saudia Arabia) as a therapeutic agent. Bone marrow-derived mesenchymal stem cells (BM-MSCs) were cultured using Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) supplemented with fetal bovine serum (FBS) (Invitrogen Australia Pty Ltd., Mount Waverley, Victoria, Australia) and penicillin/streptomycin (Gibco, USA). Immunohistochemical staining involved primary antibodies (Abcam, UK) for P53 and PCNA detection, with visualization via the biotin-streptavidin (BSA) system (Dako, Denmark). Tissue processing utilized a Leica EM TP tissue processor (Leica Biosystems, Germany), microtome, microscope, incubator (Thermo Fisher Scientific, USA), centrifuge (Eppendorf, Germany) and microwave for antigen retrieval. A gavage needle facilitated OLE administration, while blood glucose levels were monitored using a blood glucose meter (Accu-Chek, Roche, Germany).

Experimental animals

Female albino rats (n=50) were provided by Biological Products & Vaccines Co., Helwan, Egyp. The rats, aged 8-9 weeks and weighing 150-170 g, were housed in a controlled environment at 25°C with a 12-hr light-dark cycle (21). Two groups were formed: a control group (12-hr light-dark cycle) and a gestational diabetes mellitus (GDM) group (18-r light-4-hour dark cycle), a model known to induce oxidative stress (22). The study was conducted at animal house facility,

Zoology Dept, Faculty of Science, Al-Azhar University, Cairo, Egypt. Ethically approved by Fayoum University Institutional Animal Care and Use Committee (FU-IACUC, Code No. AEC 2304), in accordance with the guidelines set forth by the National Institute of Health (NIH).

Preparation of the Olive Leaf Extract (OLE)

Olive leaves extract (OLE) was obtained from the leaves of the olive tree. The resulting dry residue was carefully weighed, dissolved in water and stored at -20°C until its use as a therapeutic agent. OLE was administered to rats orally via gavage at a dosage of 200 mg/kg body weight once daily. The treatment commenced following the confirmation of stable hyperglycemia, which was verified through blood glucose measurements taken 72 hours after STZ injection (23).

Isolation and characterization Mesenchymal Stem Cells (BM -MSCs)

Bone marrow-derived mesenchymal stem cells (BM-MSCs) were isolated from the bone marrow of 4-week-old male Sprague-Dawley rats following previously established protocols (24). Briefly, the rats were anesthetized and their femurs and tibiae were excised and carefully cleaned to remove muscle and connective tissue. After removing the epiphyses, the bone marrow was flushed out and cultured in low-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS; Invitrogen Australia Pty Ltd., Mount Waverley, Victoria, Australia) and 1 % penicillin/streptomycin. The cultures were maintained at 37 °C in a humidified incubator with 5 % CO_2, with the medium being refreshed twice weekly. Cells were expanded until they reached approximately 80 % confluence and for all experiments, BM-MSCs from the third and fourth passages were utilized (13).

Experimental design

The research methodology encompassed the use of 50 pregnant female rats. These rodents underwent vaginal smear assessments to identify their estrous cycle phases, then put with male. After the pregnancy sure by the presence of sperms in the next morning and this consider the day zero of pregnancy (25). The rats were divided into five groups (n=10/group): (a) Pregnant control (control group), receiving no treatment; (b) Gestational diabetes (GD group), induced by a single intraperitoneal (ip) injection of Streptozotocin (STZ, 35 mg/kg) on day 0 of pregnancy, with hyperglycemia confirmed 72 hours post-injection (26), (c) GD + Mesenchymal stem cells (MSCs), receiving 1×10^6 MSCs intravenously on day 7 of pregnancy; (d) GD + Olive leaf extract (OLE), administered 200 mg/kg/day of standardized OLE (containing 20% oleuropein) orally throughout pregnancy; and (e) GD + MSCs + OLE, receiving both treatments at the aforementioned doses.

Histological analysis

The liver and spleen tissue specimens from adult female rats, preserved in formalin, underwent automated processing using the EM TP Leica® tissue processor, Germany. The processing involved a two-step fixation and dehydration procedure. Fixation entailed immersing the tissue in 10 % buffered formalin for 48 hr, followed by the removal of the fixative through a 30 min distilled water rinse. Dehydration was then carried out by sequentially exposing the tissues to a graded series of alcohol concentrations (70 %, 90 % and 100 %). The tissue first underwent a 120 min exposure to 70 % alcohol, followed by 90 % alcohol for 90 min and two cycles of absolute alcohol, each lasting one hour. Following dehydration, the samples underwent clearing in multiple changes of xylene. This process involved immersing the tissue in a mixture of 50 % alcohol and 50 % xylene for one hr, followed by pure xylene for one and a half hours. Subsequently, the samples were impregnated with molten paraffin wax, then embedded and blocked out. The resulting paraffin-embedded sections, measuring 4-5 micrometers, were stained with hematoxylin and eosin (27).

Immunohistochemical staining

The tissue samples were subjected to microwave processing. The detection of antigens within these tissues involved an immunostaining method that followed a two-step process. Initially, the primary antibody was directed towards its specific antigen and the subsequent reaction was observed using a biotin-streptavidin (BSA) system for visualization (28).

Results

Histopathological and Immunohistochemical Assessment of Liver Sections

Hepatocytes were distributed in hexagonal plates in the liver serial sections of control animals, according to histopathological analysis using Hematoxylin and Eosin staining. The functional unit, the liver acinus, was oval, with a short arm created by the common border of neighboring lobules and portal canals. An imaginary line that joined two nearby major veins was known as the long arm. Large and polyhedral, hepatocytes make up 75–80 % of liver cells. They frequently have two or four spherical nuclei at the cell center (Fig. 1A). A hepatocyte has an average life span of about five months. Hepatocyte villi that extended into the perisinusoidal vascular space, allowing fluid exchange. By contrast, liver samples obtained from diabetic rats (GD) exhibited notable biliary proliferation spreading into interlobular tissue, accompanied by bile stagnation, particularly around the portal area. Various necrotic cells, characterized by condensed nuclei and reddish cytoplasm, were observed. These were accompanied by moderate degenerative changes in hepatocytes such as cloudy swelling, hydropic degeneration, vacuolations and occasional signet-ring fatty alterations. Some portal blood vessels and hepatic sinusoids showed moderate dilation, with infiltration of round cells (including lymphocytes and plasma cells) in both interstitial and intravascular spaces (Fig. 1B).

Liver sections from diabetic rats transplanted with MSCs predominantly displayed healthy hepatocytes, with a few showing signs of hydropic degeneration and apoptotic changes. In most areas, portal triads appeared normally, exhibiting minor vascular dilatations, aggregations of round cells and mild cholangitis. Within certain regions of hepatic lobules, sinusoids showed slight dilation, with prominent Von Kupffer cells (Fig. 1C). Additionally, liver tissues of diabetic rats treated with (OLE) showed mild congestion in portal veins, central veins and dilated sinusoids with noticeable Von

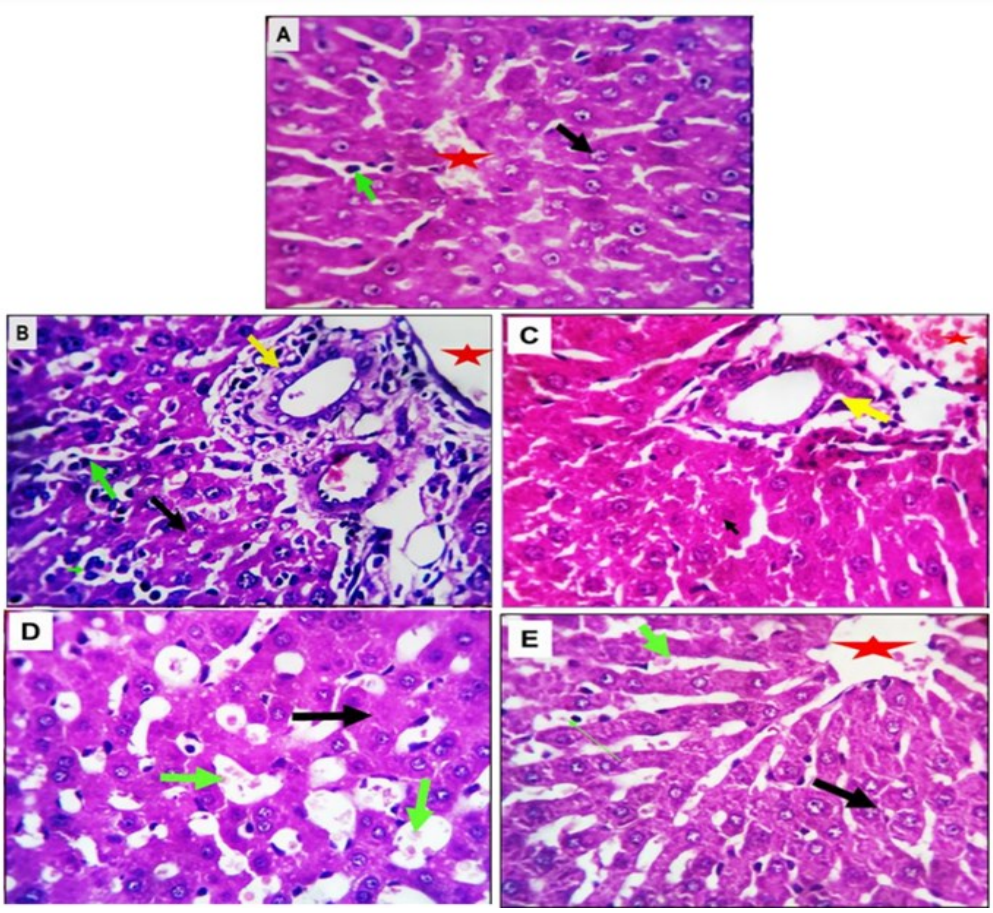

Fig. 1. (A-E). Photomicrographs of the liver tissue of control pregnant rats and treated groups stained with Hematox442 ylin and eosin. A (control group), a normal central vein is marked with a red asterisk, hepatic cords are indicated by 443 black arrows and hepatic sinusoids are denoted by green arrows. B (GD group), there is evidence of portal biliary 444 proliferation, lymphocytic cholangitis (yellow arrow), moderately dilated blood vessels (red asterisk), sinusoids with 445 associated lymphocytosis (green arrow) and degenerated/necrotic hepatocytes (black arrow). C (GD+MSCs group), 446 displays aggregations of round cells and mild cholangitis (yellow arrow). D&E (DG+OLE & DG+OLE+MSCs groups), 447 hepatic lesions are evident, characterized by mild congestion of portal veins, central veins (red asterisk) and dilated 448 sinusoids with prominent Von-Kupffer cells (green arrows). The hepatocytes in these groups appear normal (black 449 arrows). X 100.

Kupffer cells. Many hepatocytes appeared normal following these treatments (Fig. 1D&E).

Sections from liver denoted positively reactive apoptotic cells with marked to moderate intensity in variable numbers of Von Kupffer cells and inflammatory cells besides a few weak positive hepatocytes in the GD group. Control rats were negatively reacted. A few reactive positive Von Kupffer cells and inflammatory cells of moderate intensity were recorded in the treatment groups after diabetes (Fig. 2 A-E).

In hepatic tissue, a minimal number of weakly positive cells for PCNA are observed in the control rats (Fig. 3A) and all treatment groups after diabetes (Fig. 3C-E). Meanwhile, in GD group (Fig. 3B), a few to a moderate number of strongly positive hepatic and inflammatory cells are detected in the portal triads (Fig. 3D& E)

Fig. 3 (A-E) displays photomicrographs of liver tissue from control pregnant rats and treated groups, immunostained for Proliferating Cell Nuclear Antigen (PCNA). In control group (A), a few weakly positive cells are observed. In contrast, the gestational diabetes (GD) group (B) shows a moderate number of strongly positive hepatic and inflammatory cells in the portal triads. All treatment groups (C-E) exhibit varying degrees of PCNA-positive cells after diabetes induction. Positive cells are indicated by red arrows, while negative cells are marked by light blue arrows.

Histopathological and Immunohistochemical Assessment of spleen Sections

According to the results of the current study, the spleen is made up of a red pulp that makes up the bulk of the stromal tissue and is made up of the splenic sinusoids and Billroth cords. The cellular aggregations held up by the reticular connective tissue are known as the cords of Billroth, or splenic cords. They are made up of blood cells, plasmocytes and macrophages and have the appearance of stripes. The spaces between the Billroth cords are home to the splenic sinusoids. The characteristic crimson color of the red pulp is a result of its blood-filled interior. Blood passes through the sinusoids slowly, waiting for foreign antigens to teemingly emerge in the blood to be exposed to macrophages from the Billroth cords. The red pulp acts as a blood filter for different toxins, eliminating them before they have a chance to enter the bloodstream, spread throughout the body and harm other organs. The spleen's white pulp is composed of three distinct compartments: the border zone, lymphoid follicles and the periarterial lymphoid sheath (PALS). The PALS is made up of a sheath of lymphoid tissue encircling the central artery, which is a branch of the splenic artery. There are two layers in the lymphoid tissue: an inner layer and an outer layer. The inner layer is known as T lymphocytes or T-zone since T cells make up most of its composition. T and B-lymphocytes can be found

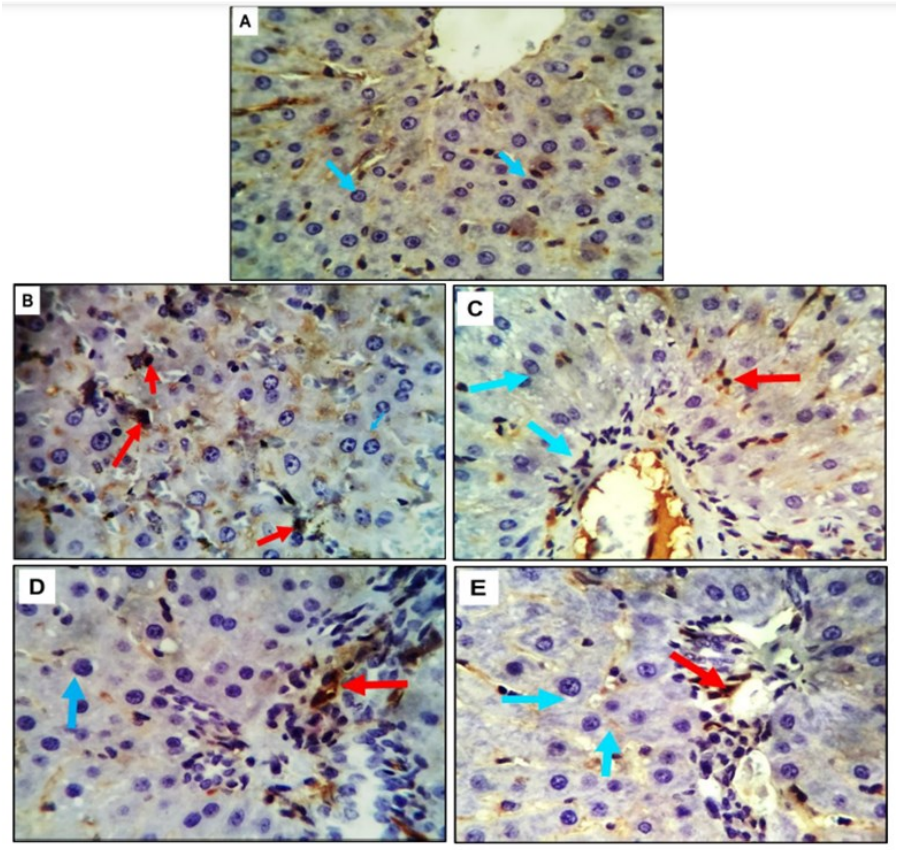

Fig. 2. (A-E). Photomicrographs of liver tissue of control pregnant rats and treated groups immune-stained with the 457 apoptotic marker P53 showing; A (Control) appears negatively reacted (light blow arrows) .B(GD group), positively re458 active apoptotic cells with marked to moderate intensity in variable numbers of Von Kupffer cells and inflammatory cells 459 beside a few weak positive hepatocytes. C-E (DG+OLE & DG+ MSCs & GD+OLE+MSCs groups), a few reactive posi460 tive Von Kupffer cells and inflammatory cells of moderate intensity. Positive cells pointed by red arrows and negative 461 cells pointed by light blow arrows. X 400.

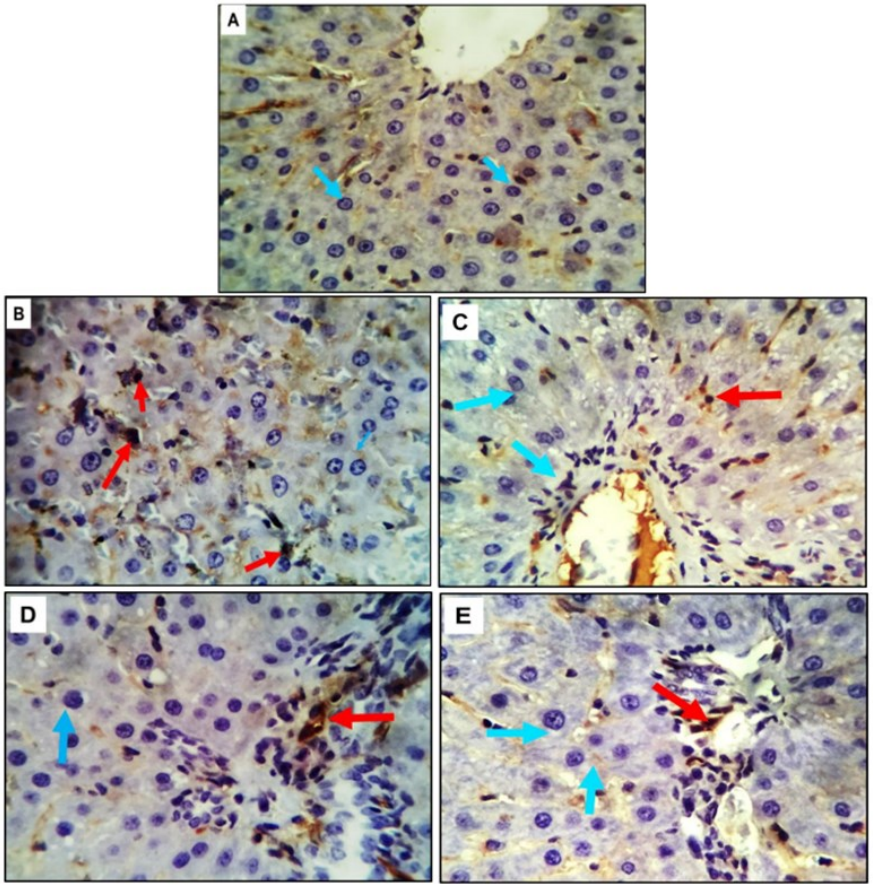

Fig. 3. (A-E). Photomicrographs of liver tissue of control pregnant rats and treated groups immune-stained with the 463 Proliferating cell nuclear antigen (PCNA) showing a few weak positive cells in the control group (A) and all treatment 464 groups after diabetes (D-E), meanwhile a few to moderate number of strongly positive hepatic and inflammatory cells 465 in the portal triads are seen in GD (B). Positive cells are pointed by red arrows and negative cells are pointed by light 466 blue arrow. X 400.

in the outer layer, which have a more varied cellular shape. The distinct regions of B-lymphocytes, which include the lymphoid follicles of the spleen, encircle the branches of central arterioles. Depending on the characteristics of the B-lymphocytes that make them up, there are two different kinds of lymphoid follicles: primary follicles and secondary nodules. A primary follicle is one that is mostly made up of tiny, immature lymphocytes. Many nodules in the spleen, however, are secondary nodules that develop from primary follicles when the lymphocytes mature and enlarge. They are distinguished from primary follicles by the presence of a unique zone in the center known as the germinal center. Germinal centers are sites where lymphocytes mature and acquire the ability to produce antibodies. Therefore, the presence of the germinal center indicates an antigen-stimulated response in lymphoid tissue. In addition to B-lymphocytes, follicular dendritic cells (FDC) are present in the germinal centers and proliferate following antigen stimulation. They stimulate and direct B-lymphocytes immunological responses. A germinal center is surrounded by an outside ring of tiny lymphocytes known as the mantle zone. The marginal zone, which is located just on the border of lymphoid follicles, is home to a variety of immune cells that are prepared to initiate the proper immune response. Neither inflammatory, granulomatous nor degenerative, necrotic or apoptotic changes were recorded in the control group (Fig. 4A).

Meanwhile sections from diabetic rats demonstrated marked lymphoid depletion of the cellular component of the white pulp which was restricted on a narrow zone of germinal centers aggregated lymphocytes. The red pulp was markedly congested with dilated sinusoids and depleted splenic cord lympho-histiocytic components (Fig. 4B). Spleen of GD+MSCs group) showed gradual restoration of the white pulp lymphoid components with discrimination of germinal centers and marginal zones. The red pulp also regains its structural configurations with still presence of mildly dilated sinusoids (Fig. 4C). Keeping features of Billroth cords were detectable. Spleen of GD+OLE & GD+OLE+MSCs showed complete rearrangement of the white pulp lymphoid structures with a near normal occurrence of germinal centers, mantle and marginal zones. The red pulp sinusoids and Billroth cords were seen in good structural arrangement and cellular components (Fig. 4D & E).

Immunohistochemical analysis of P53 antibody expression in the liver of Stained spleen tissue for P53 demonstrated a few moderately stained red pulp cells in the

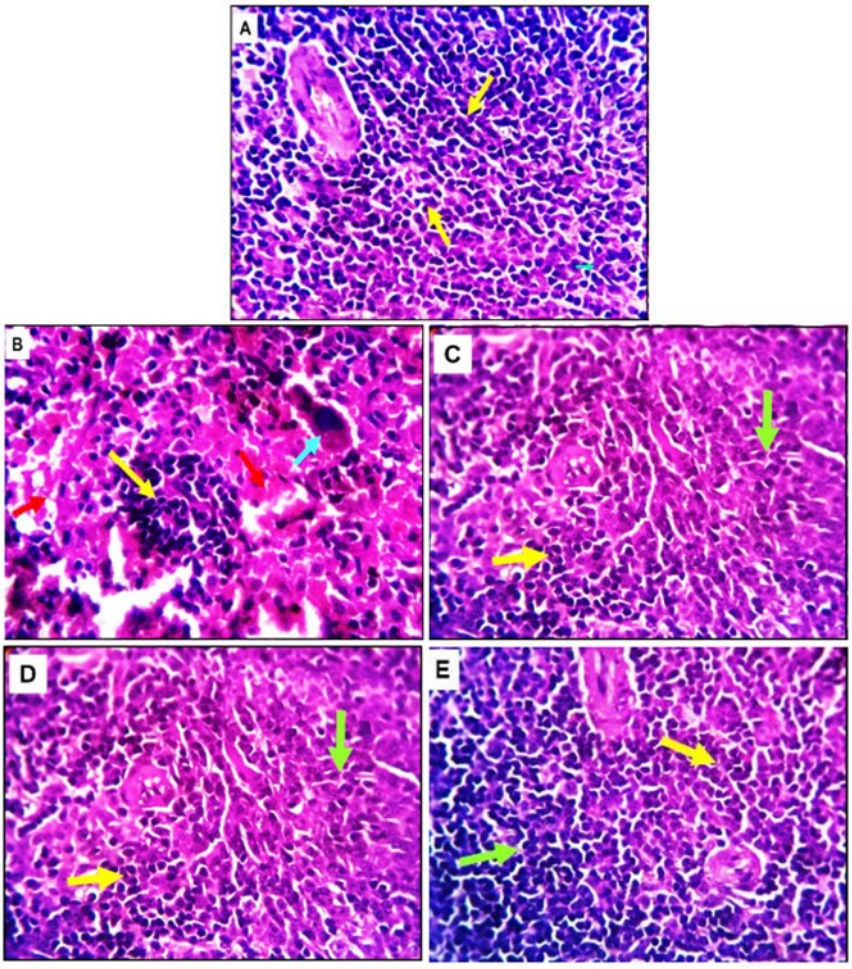

Fig. 4. (A-E). Photomicrographs of the spleen tissue of control pregnant rats and treated groups stained with Hematox468 ylin and eosin. A (control group), red pulp which occupies the majority of the stromal tissue and consists of the cords of 469 Billroth and splenic sinusoids. The germinal centers (yellow arrow) are the sites where lymphocytes mature and acquire 470 the ability to produce antibodies. B (GD group), demonstrated marked lymphoid depletion of the cellular component of 471 the white pulp which was restricted on a narrow zone of germinal centers aggregated lymphocytes (yellow arrow). The 472 red pulp was markedly congested with dilated sinusoids and depleted splenic cord lympho-histiocytic components (red 473 arrow). C (GD+MSCs group) shows gradual restoration of the white pulp lymphoid components with discrimination of 474 germinal centers and marginal zones (yellow arrow). E&E (DG+OLE & DG+OLE+MSCs groups), shows complete re475 arrangement of the white pulp lymphoid structures with a near normal occurrence of germinal centers, mantle and mar476 ginal zones (yellow arrows and green arrow). The red pulp sinusoids and Billroth cords are seen in a good structural 477 arrangement and cellular components. X 400.

control negative rats (Fig. 5A) but the reactive cells were moderate and intensely stained and involved both red and white pulp in diabetic rats (Fig. 5B). A variable number of moderately stained red pulp cells were seen in GD+MSCs group (Fig. 5C) and a very few positive cells were seen randomly distributed in GD+OLE& GD+OLE+MSCs groups (Fig. 5. D & E).

Splenic tissue reactivities to PCNA are peculiar as negative reaction was seen in the control rats (Fig. 6A), A few moderately stained follicular and red pulp cells are seen in GD (Fig. 6B), however a strongly positive germinal and mantle zone follicular cell beside a few red pulp lymphocytes were evident in the three treatment groups (Fig. 6C-E).

Fig. 6 (A-E) displays photomicrographs of spleen tissue from control pregnant rats and treated groups, immunostained for Proliferating Cell Nuclear Antigen (PCNA). In the control group (A), PCNA expression is negative. In contrast, the gestational diabetes (GD) group (B) shows a few to moderately stained follicular and red pulp cells. Strongly positive PCNA reactions are observed in the germinal and mantle zone follicular cells, along with a few red pulp lymphocytes, in the three treatment groups (C-E). Positive cells are indicated by red arrows, while negative cells are marked by yellow arrows

Discussion

The present study revealed significant lymphoid depletion in the spleen tissue of diabetic rats, characterized by a narrowed zone of germinal centers, aggregated lymphocytes and moderate to intense apoptosis in both red and white pulp. In the liver tissue, STZ-induced diabetes resulted in various necrotic changes, including condensed nuclei and reddish cytoplasm, alongside positively reactive apoptotic cells in Von Kupffer cells, inflammatory cells and a few weakly positive hepatocytes. These findings align with previous studies demonstrating that STZ and ALX induce pancreatic beta-cell destruction, leading to Type 1 Diabetes Mellitus (T1DM) and subsequent multi-organ damage, including the liver and spleen (29). Hyperglycemia-induced oxidative stress is a well-documented mechanism contributing to hepatic dysfunction, disrupting carbohydrate, protein and lipid metabolism (30). While diabetes is a recognized contributor to chronic liver diseases, its role is often overshadowed by other factors such as obesity, dyslipidemia and metabolic syndrome in the context of Non-Alcoholic Fatty Liver Disease (NAFLD).

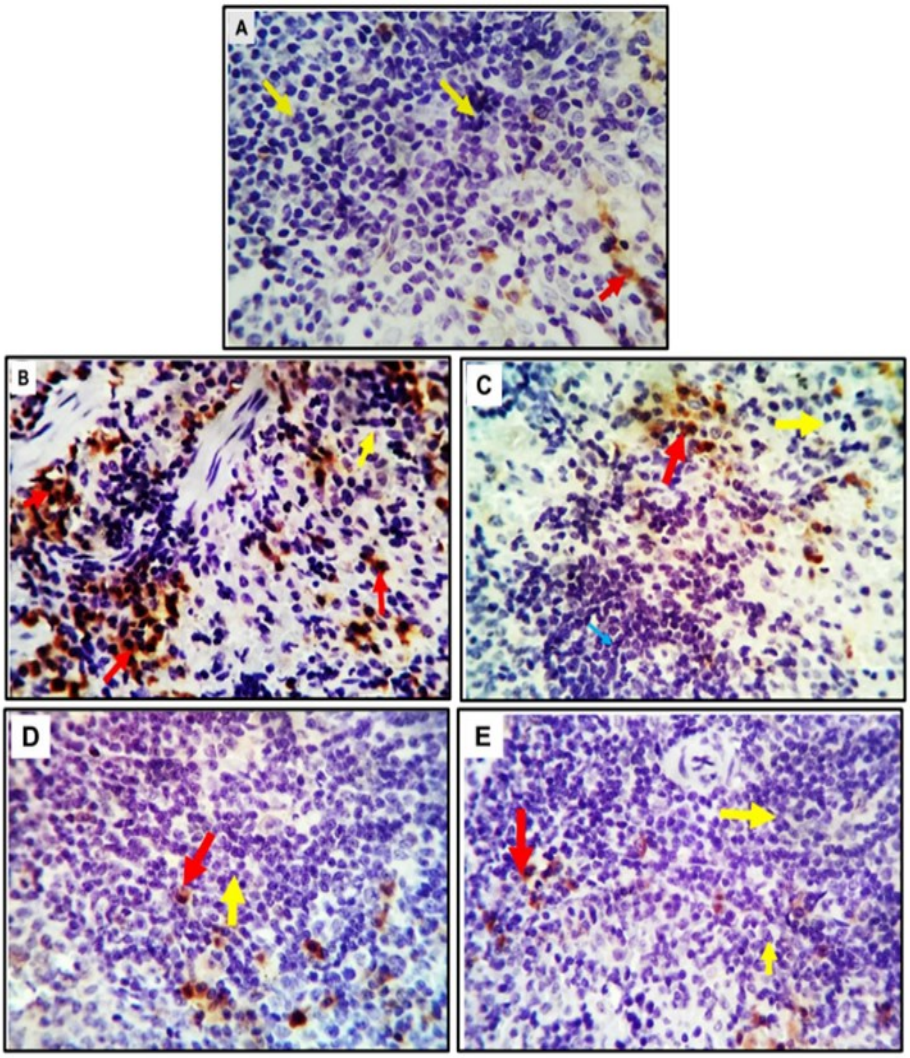

Fig. 5. (A-E). Photomicrographs of spleen tissue of control pregnant rats and treated groups immune-stained with the 479 apoptotic marker P53 showing; A (Control) , few moderately stained red pulp cells in the control negative rats (A), but 480 the reactive cells appears moderate , intensely stained and involve both red and white pulp in the diabetic rats (B). A 481 variable number of moderately stained red pulp cells are seen in GD+MSCs group (C) and a very few positive cells are 482 seen randomly distributed in GD+OLE & GD+OLE+MSCs groups (D&E). Positive cells pointed by red arrows and neg483 active cells are pointed by yellow arrow. X 400.

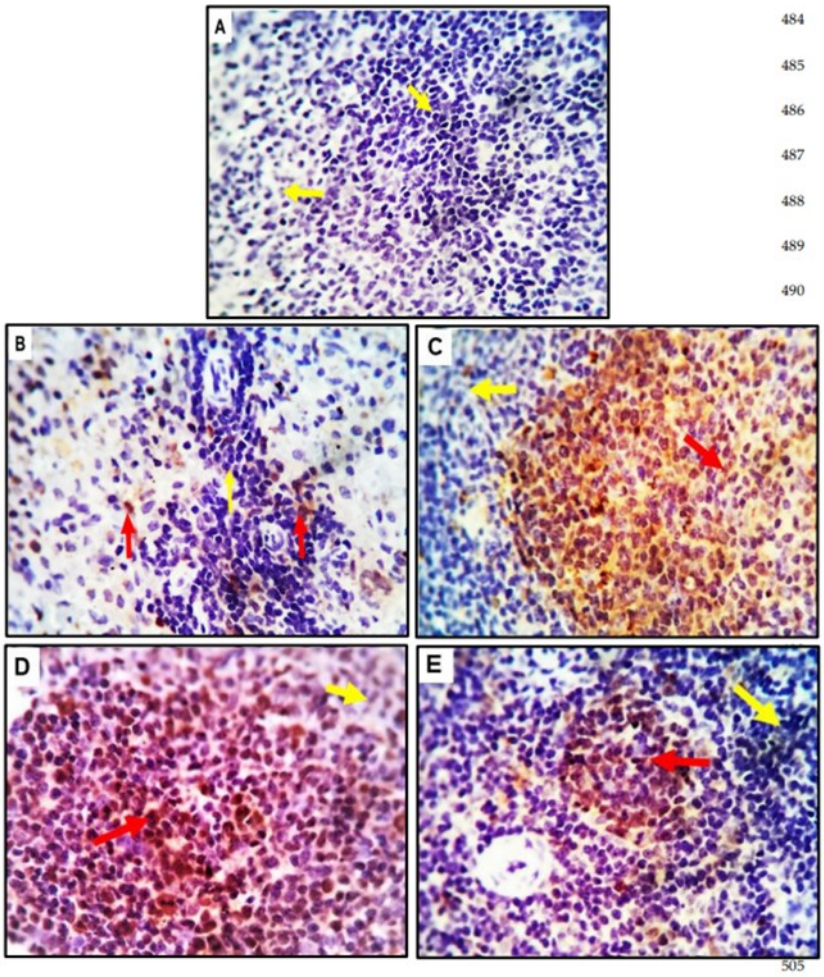

Fig. 6. (A-E). Photomicrographs of spleen tissue of control pregnant rats and treated groups immune-stained with the 507 Proliferating cell nuclear antigen (PCNA) showing negative reaction in the control rats (A), however a strongly positive 508 germinal and mantle zone follicular cell beside a few red pulp lymphocytes in the three treatment groups (C-E). A few to 509 moderate number of moderately stained follicular and red pulp cells are seen in GD (B). Positive cells are pointed by red 510 arrows and negative cells are pointed by yellow arrow.

Hyperglycemia-induced oxidative stress damages several internal organs, particularly the liver, disrupting the processing of carbohydrates, proteins and fats (31).

Olive leaf extract (OLE), rich in phenolic compounds such as oleuropein, flavonoids and tocopherols, demonstrated significant hepatoprotective and splenoprotective effects in this study. OLE attenuated STZ-induced oxidative stress, apoptosis and inflammation in hepatic and splenic tissues, consistent with prior research (32-34). The aqueous extraction method employed in this study yielded an OLE with high phenolic and flavonoid content, contributing to its potent antioxidative properties (35). Methanol/water extraction methods have also been shown to enhance polyphenol yield, further supporting the efficacy of OLE (36). Notably, oleuropein and hydroxytyrosol, key components of OLE, have been shown to mitigate organ enlargement and lipid deposition in high-fat and cholesterol-rich diet models (37). These findings underscore the therapeutic potential of OLE in combating diabetes-induced organ damage

The antioxidative properties of OLE are primarily attributed to its phenolic constituents, which exhibit redox activity due to their functional groups and hydroxyl positioning (38, 39). Synergistic interactions among these compounds enhance their overall antioxidant capacity (40). Similarly, bone marrow-derived mesenchymal stem cells (BM-MSCs) demonstrated protective effects against STZ-induced hepatic and splenic damage, likely mediated by their anti-inflammatory, anti-apoptotic and antioxidative properties (41). BM-MSCs have been shown to secrete cytokines and growth factors that promote tissue repair, reduce inflammation and inhibit apoptosis, consistent with our findings (42). The combination of OLE and BM-MSCs exhibited enhanced therapeutic efficacy, suggesting a potential synergistic interaction in mitigating diabetes-induced organ damage.

According to the current findings, STZ-induced cellular damage to the liver and spleen tissues was prevented by OLE, MSCs and their combination. The antioxidant and anti-apoptotic properties of the OLE and BM-MSCs employed, as well as their combination, may be responsible for the protective effects (41). The current investigation validated the anti-inflammatory, anti-apoptotic and antioxidant properties of BM-MSCs, which is consistent with previous research (43). It has been discovered that BM-MSCs reduce DNA damage in cardiac cells by secreting several cytokines and growth factors that encourage angiogenesis, lessen inflammation and slow down apoptosis. Moreover, they were successful in enlisting proteins that fix single- and double-strand breaks in DNA (44).

In conclusion, this study highlights the protective effects of OLE and BM-MSCs against STZ-induced hepatic and splenic damage, mediated through antioxidative and anti-apoptotic mechanisms. These findings align with previous research and underscore the therapeutic potential of these interventions in diabetes-associated organ pathology.

Further studies are warranted to explore the synergistic effects of OLE and BM-MSCs in greater detail and to validate their clinical applicability

Conclusion

Our study indicated that rats induced with gestational diabetes mellitus through intravenous STZ display alterations in the liver and spleen histopathology and immunohistochemistry, however, the specific cause of these histopathological changes, whether attributed to the drug's toxic effects. OLE, MSCs and their combination might modulate hepatic and splenic injury through attenuation of oxidative stress and inflammatory. The decrease in cellular density observed in diabetes and the subsequent restoration of cellular components following therapeutic interventions suggest the promise of these interventions in alleviating diabetes-induced liver and spleen alterations. Given that MSCs have longer-lasting effects, this discovery could pave the way for novel approaches to the treatment of a range of illnesses.

Acknowledgements

The authors extend their appreciation to the Deanship of Scientific Research in cooperation with the Olive Research Center at Jouf University for funding this work through research grant no. DSR2022-RG-0165.

Funding: This work was funded by the Deanship of Scientific Research in cooperation with the Olive Research Center at Jouf University, under Grant Number (DSR2022-RG-0165).

Authors' contributions

Conceptualization was done by MA and AA. MA, AA and DM carried out the methodology. Formal analysis was carried out by ZA and FA. The investigation was done by MA. Resources were gathered by DM. Original draft was prepared by MA, AA and AM. Review and editing was done by MA, AA, ZA and AM. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: Fayoum University Institutional Animal Care and Use Committee (FU-IACUC) approved the entire research protocol ethically under Code No. AEC 2304. The Global Strategies for the Ethical Treatment and Utilization of Laboratory Animals were closely followed in the granting of this permit.

References

1. Jiang S, Liu A, Ma W, Liu X, Luo P, Zhan M, et al. *Lactobacillus gasseri* CKCC1913 mediated modulation of the gut–liver axis alleviated insulin resistance and liver damage induced by type 2 diabetes. Food Function. 2023;14:8504-20. https://doi.org/10.1039/D3FO01701J
2. Karigidi KO, Akintimehin ES, Omoboyowa DA, Adetuyi FO, Olaiya CO. Effect of *Curculigo pilosa*-supplemented diet on blood sugar, lipid metabolism, hepatic oxidative stress and carbohydrate metabolism enzymes in streptozotocin-induced diabetic rats. Journal of Diabetes & Metabolic Disorders 2020;19:1173-84. https://doi.org/10.1007/s40200-020-00618-w
3. Bolajoko EB, Akinosun OM, Khine AA. Hyperglycemia-induced oxidative stress in the development of diabetic foot ulcers. In: Preedy VR, editor. *Diabetes*. 2nd ed. Academic Press; 2020. p. 35-48.
4. Ott KC, Bi M, Scorletti F, Ranginwala SA, Marriott WS, Peiro JL, et al. The interplay between prenatal liver growth and lung development in congenital diaphragmatic hernia. Frontiers in Pediatrics 2022;10:213. https://doi.org/10.3389/fped.2022.983492
5. Owrangi S, Paik JM, Golabi P, de Avila L, Hashida R, Nader A et al. Meta-Analysis: Global Prevalence and Mortality of Cirrhosis in Metabolic Dysfunction-Associated Steatotic Liver Disease. Alimentary Pharmacology & Therapeutics. 2025;61:433-43. https://doi.org/10.1111/apt.18451
6. Fromenty B, Roden M. Mitochondrial alterations in fatty liver diseases Journal of Hepatology. 2023;78:415-29. https://doi.org/10.1016/j.jhep.2022.09.020
7. Şahin S, Bilgin M. Olive tree (*Olea europaea* L.) leaf as a waste by -product of table olive and olive oil industry: a review. Journal of the Science of Food and Agriculture. 2018;98:1271-79. https://doi.org/10.1002/jsfa.8619
8. Castro AJ, Lima-Cabello E, Alché JD. Identification of seed storage proteins as the major constituents of the extra virgin olive oil proteome. Food Chemistry X. 2020;7:100099. https://doi.org/10.1016/j.fochx.2020.100099
9. Susalit E, Agus N, Effendi I, Tjandrawinata RR, Nofiarny D, Perrinjaquet-Moccetti T, et al. Olive (*Olea europaea*) leaf extract effective in patients with stage-1 hypertension: Comparison with Captopril. Phytomedicine. 2011;18:251-58. https://doi.org/10.1016/j.phymed.2010.08.016
10. Acar-Tek N, Ağagündüz D. Olive leaf (*Olea europaea* L. *folium*): Potential effects on glycemia and lipidemia. Annals of Nutrition and Metabolism. 2020;76:10-15. https://doi.org/10.1159/000505508
11. Stevens Y, Winkens B, Jonkers D, Masclee A. The effect of olive leaf extract on cardiovascular health markers: a randomized placebo-controlled clinical trial. European Journal of Nutrition. 2021;60:2111-20. https://doi.org/10.1007/s00394-020-02397-9
12. Huang S, Leung V, Peng S, Li L, Lu FJ, Wang T, et al. Developmental definition of MSCs: new insights into pending questions. Cell Reprogram. 2011;13:465-72. https://doi.org/10.1089/cell.2011.0045
13. El-Sayed ME, Atwa A, Sofy AR, Helmy YA, Amer K, Seadawy MG, et al. Mesenchymal stem cell transplantation in burn wound healing: uncovering the mechanisms of local regeneration and tissue repair. Histochemistry and Cell Biology. 2023. https://doi.org/10.1007/s00418-023-02244-y
14. Bahsoun S, Coopman K, Akam EC. The impact of cryopreservation on bone marrow-derived mesenchymal stem cells: a systematic review. Journal of Translational Medicine. 2019;17:397. https://doi.org/10.1186/s12967-019-02136-7
15. Li XW, Feng LX, Zhu X, Liu Q, Wang HS, Wu X, et al. Human umbilical cord blood mononuclear cells protect against renal tubulointerstitial fibrosis in cisplatin-treated rats. Biomedicine & Pharmacotherapy. 2020;121:109310. https://doi.org/10.1016/j.biopha.2019.109310
16. Luo J, Zhao X, Tan Z, Su Z, Meng F, Zhang M. Mesenchymal-like progenitors derived from human embryonic stem cells promote recovery from acute kidney injury via paracrine actions. Cytotherapy. 2013;15:649-62. https://doi.org/10.1016/j.jcyt.2013.01.009

17. Gunawardena TNA, Rahman MT, Abdullah BJJ, Abu Kasim NH. Conditioned media derived from mesenchymal stem cell cultures: the next generation for regenerative medicine. Journal of Tissue Engineering and Regenerative Medicine. 2019;13:569-86. https://doi.org/10.1002/term.2806

18. Semedo P, Palasio CG, Oliveira CD, Feitoza CQ, Gonçalves GM, Cenedeze MA, et al. Early modulation of inflammation by mesenchymal stem cell after acute kidney injury. International Immunopharmacology. 2009;9:677-82. https://doi.org/10.1016/j.intimp.2008.12.008

19. Zhang Y, Ye C, Wang G, Gao Y, Tan K, Zhuo Z, et al. Kidney-targeted transplantation of mesenchymal stem cells by ultrasound-targeted microbubble destruction promotes kidney repair in diabetic nephropathy rats. BioMed Research International. 2013;2013:526367. https://doi.org/10.1155/2013/526367

20. Alkhathami AG, El-Fakharany EM, El-Sayed MH, Atwa A, Ali FK, Hamad N, et al. Chemopreventive effect of *Pistacia vera* leaf extract against mammary carcinoma induced by dimethyl-benz (a)anthracene *in vivo* and *in vitro*: potential role of antioxidant, anti-inflammatory and immune mechanisms. Food and Chemical Toxicology. 2025;196. https://doi.org/10.1016/j.fct.2024.115229

21. Abdel-Reheim ES, Abd-Elmoneim AA, Hosni AA. Fatty-sucrosed diet/minimal dose of streptozotocin-treated rat: a novel model of gestational diabetes mellitus, metabolic and inflammatory insight. Diabetes & Metabolism Journal. 2014;5(9).

22. Suzuki YJ, Jain V, Park AM, Day RM. Oxidative stress and oxidant signaling in obstructive sleep apnea and associated cardiovascular diseases. Free Radical Biology and Medicine. 2006;40:1683-92. https://doi.org/10.1016/j.freeradbiomed.2006.01.008

23. Giacometti J, Žauhar G, Žuvić M. Optimization of ultrasonic-assisted extraction of major phenolic compounds from olive leaves (*Olea europaea* L.) using response surface methodology. Foods. 2018.

24. Abd Rabou MA, Atwa A, Massoud D, Asran AM, Alrayes ZR, Al-Otaibi AM, et al. Therapeutic effect of mesenchymal stem cells and olive leaf extract against cytotoxicity induced by streptozotocin in gestational diabetes rats. Cellular Physiology and Biochemistry. 2024;58:702-21. https://doi.org/10.33594/000000744

25. Abd Rabou MA, Alhumaidi Alotaibi M. Toxicity of Somadril compound on fetal ileum tissues of albino rats. Pakistan Journal of Biological Sciences. 2021;24:72-79. https://doi.org/10.3923/pjbs.2021.72.79

26. Furman BL. Streptozotocin-induced diabetic models in mice and rats. Current protocols in pharmacology. 2015;70(1):5-47. https://doi.org/10.1002/0471141755.ph0547s70

27. Suvarna KS, Layton C, Bancroft JD. Bancroft's Theory and Practice of Histological Techniques. 8th ed. Elsevier Health Sciences; 2018.

28. Basha M. Immuno-techniques. In: Basha M, editor. *Analytical Techniques in Biochemistry*. New York, NY: Springer US; 2020. p. 77-102. https://doi.org/10.1007/978-1-0716-0134-1_7

29. Queiroz LAD, Assis JB, Guimarães JPT, Sousa ESA, Milhomem AC, Sunahara KKS, et al. Endangered lymphocytes: the effects of alloxan and streptozotocin on immune cells in Type 1 induced diabetes. Mediators of Inflammation. 2021;2021:9940009. https://doi.org/10.1155/2021/9940009

30. Nandini HS, Naik PR. Action of corilagin on hyperglycemia, hyperlipidemia and oxidative stress in streptozotocin-induced diabetic rats. Chemico-Biological Interactions. 2019;299:186-93. https://doi.org/10.1016/j.cbi.2018.12.012

31. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology and the American Gastroenterological Association. Hepatology. 2012;55:2005-23. https://doi.org/10.1002/hep.25762

32. Badr AB, Fouad DF. Anti-apoptotic and anti-inflammatory effects of olive leaf extract against cisplatin-induced nephrotoxicity in male rats. International Journal of Pharmacology. 2016;11:468-76.

33. Elgebaly HA, Mosa NM, Allach M, El-Massry KF, El-Ghorab AH, Al Hroob AM, et al. Olive oil and leaf extract prevent fluoxetine-induced hepatotoxicity by attenuating oxidative stress, inflammation and apoptosis. Biomedicine & Pharmacotherapy. 2018;98:446-53. https://doi.org/10.1016/j.biopha.2017.12.101

34. Vezza T, Rodríguez-Nogales A, Algieri F, Garrido-Mesa J, Romero M, Sánchez M, et al. The metabolic and vascular protective effects of olive (*Olea europaea* L.) leaf extract in diet-induced obesity in mice are related to the amelioration of gut microbiota dysbiosis and to its immunomodulatory properties. Pharmacological Research. 2019;150:104487. https://doi.org/10.1016/j.phrs.2019.104487

35. Franco MN, Galeano-Díaz T, López Ó, Fernández-Bolaños JG, Sánchez J, De Miguel C, et al. Phenolic compounds and antioxidant capacity of virgin olive oil. Food Chemistry. 2014;163:289-98. https://doi.org/10.1016/j.foodchem.2014.04.091

36. Lapornik B, Prošek M, Golc Wondra A. Comparison of extracts prepared from plant by-products using different solvents and extraction time. Journal of Food Engineering. 2005;71:214-22. https://doi.org/10.1016/j.jfoodeng.2004.10.036

37. Song L, Qu D, Zhang Q, Jiang J, Zhou H, Jiang R, et al. Phytosterol esters attenuate hepatic steatosis in rats with non-alcoholic fatty liver disease rats fed a high-fat diet. Scientific Reports. 2017;7:41604. https://doi.org/10.1038/srep41604

38. Choi HS, Lee MJ, Na MS, Lee MY, Choi D. Antioxidant properties of *Achyranthis radix* extract in rats. Journal of Industrial and Engineering Chemistry. 2009;15:275-80. https://doi.org/10.1016/j.jiec.2008.12.001

39. Petridis A, Therios I, Samouris G, Koundouras S, Giannakoula A. Effect of water deficit on leaf phenolic composition, gas exchange, oxidative damage and antioxidant activity of four Greek olive (*Olea europaea* L.) cultivars. Plant Physiology and Biochemistry. 2012;60:1-11. https://doi.org/10.1016/j.plaphy.2012.07.014

40. Lambert de Malezieu M, Courtel P, Sleno L, Abasq ML, Ramassamy C. Synergistic properties of bioavailable phenolic compounds from olive oil: electron transfer and neuroprotective properties. Nutritional Neuroscience. 2021;24:660-73. https://doi.org/10.1080/1028415X.2019.1666480

41. Ibrahim MA, Khalifa AM, Abd El-Fadeal NM, Abdel-Karim RI, Elsharawy AF, Ellawindy A, et al. Alleviation of doxorubicin-induced cardiotoxicity in rat by mesenchymal stem cells and olive leaf extract via MAPK/TNF-α pathway: preclinical, experimental and bioinformatics enrichment study. Tissue Cell. 2023;85:102239. https://doi.org/10.1016/j.tice.2023.102239

42. Rayat Pisheh H, Sani M. Mesenchymal stem cells derived exosomes: a new era in cardiac regeneration. Stem Cell Research & Therapy. 2025;16:16. https://doi.org/10.1186/s13287-024-04123-2

43. Sherif IO, Al-Gayyar MMH. Oleuropein potentiates anti-tumor activity of cisplatin against HepG2 through affecting proNGF/NGF balance. Life Sciences. 2018;198:87-93. https://doi.org/10.1016/j.lfs.2018.02.027

44. Gao S, Zhao Z, Wu R, Zeng Y, Zhang Z, Miao J, et al. Bone marrow mesenchymal stem cell transplantation improves radiation-induced heart injury through DNA damage repair in rat model. Radiation and Environmental Biophysics. 2017;56:63-77. https://doi.org/10.1007/s00411-016-0675-0