



Research article

Histoprotective Efficacy of *Olea europaea* Leaf Extract Against Sodium Benzoate-Induced Hepato-Renal Damage in Male Wistar Rats

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ABSTRACT

Background: preservative that induces dose-dependent cumulative toxicity in tissues following high-dose or long-term exposure. Oleuropein (OLE), a bitter glycoside from *Olea europaea*, contains many phenolic compounds with known antioxidant and cytoprotective activities.

Objective: The present study was designed to examine the protective effect of hydroethanolic *Olea europaea* leaf extract (OLE) against sodium benzoate (SB)-induced hepatic and renal tissue histopathological alteration with an accompanying serum pro-oxidant and antioxidant defense status in male Wistar rats.

Methods: Thirty-six adult male Wistar rats were randomly divided into six equal groups (n = 6/group) and treated daily via oral gavage for 45 consecutive days: Control (distilled water, 1 mL/rat), OLE alone (200 mg/kg b.wt.), SB100 (100 mg/kg b.wt.), SB100 + OLE (100 mg/kg SB + 200 mg/kg OLE), SB200 (200 mg/kg b.wt.), and SB200 + OLE (200 mg/kg SB + 200 mg/kg OLE). At the end of the experiment, liver and kidney tissues were collected and subjected to histopathological examination for evaluation of structural changes and lesion severity. **Results:** Administration of SB produced dose-dependent histological lesions in both hepatic and renal tissues. Hepatic alterations were characterized by hepatocellular necrosis, intersinusoidal edema, nuclear pyknosis, and extensive vacuolar degeneration. Renal damage manifested as periglomerular edema, shrinkage of glomerular tufts, tubular dilatation, hemorrhage, and epithelial necrosis. Co-treatment with OLE (200 mg/kg) attenuated the severity of these pathological changes, markedly reducing hepatocellular degeneration and preserving tubular and glomerular structural integrity.

Conclusion: Co-administration of hydroethanolic *Olea europaea* leaf extract significantly reduces the severity of SB-induced histopathological lesions in hepatic and renal tissues of male Wistar rats, indicating its potential protective role against chemical additive-induced organ injury.

1. Introduction

The global food industry has experienced a significant increase in the use of synthetic chemical additives, such as preservatives, colorants, and antioxidants over the past decades. Although these compounds are key in the food industry to extend their preservation, preventing microbial spoilage and preserving organoleptic properties, prolonged exposure by humans at high concentrations have been a major issue

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of concern for health authorities worldwide (Piper, 2011). Evidence of chronic intake of synthetic food additives has established a wide range of toxicological effects influenced by the overproduction of reactive oxygen species (ROS), elicitation of cellular oxidative stress, and thereafter histopathological damage to essential metabolic organs (Carocho & Ferreira, 2013).

Sodium Benzoate (SB) is one of the most frequently used synthetic preservatives, found in soft drinks, acidic foods, fruit juices as well as processed sauces and pharmaceutical formulations to prevent fungal and bacterial growth (Lennerz et al., 2015). However, the application provided data to indicate that SB was safe within specific legal parameters, but toxicological findings accumulated during assessment evidenced that high or prolonged intake of SB adversely influences biological systems (Walczak-Nowicka and Herbet, 2019). Sodium benzoate is readily absorbed from the gastrointestinal tract following oral ingestion, and it is primarily metabolized in the liver, where it is conjugated with glycine to form hippuric acid for renal excretion (Abo- El-Sooud et al., 2018). Nevertheless, high doses will saturate the metabolic pathways and lead to intracellular accumulation and mitochondrial dysfunction (Oyewole et al., 2012).

Oxidative stress, as a result of an imbalance between the production of free radicals and the cellular endogenous antioxidant defense system (Afshar et al., 2013), is the dominant mechanism in SB-induced tissue injury. Such a broad oxidative cascade causes cytoplasmic lipid peroxidation, protein oxidation, and fragmentation of nuclear DNA (truncation), which finally ends up with cellular apoptosis and necrosis in the tissue (Qin et al., 2026). Since the liver is the major organ for drug metabolism and detoxification while kidneys are the major excretion organs, both hepatic and renal tissues are highly likely to be affected by SB toxicity (Al-Salmani et al., 2015). Various biochemical studies revealed that, the SB exposure caused hepatocellular vacuolization, pyknosis, focal necrosis and periglomerular edema with tubular degeneration (Khaled 2011). Natural products and plant-derived bioactive extracts have thus received utmost importance as therapeutic counter-agents against the toxic effects exerted by synthetic food additives and chemicals of environmental concern (Erbil, 2021). They are also rich in phytochemicals like polyphenols, flavonoids, tannins, and terpenoids which have strong radical-scavenging, anti-inflammatory and cytoprotective properties [Xu et al., 2017]. Natural antioxidants reduce the toxic nature of chemicals due to their potent action mechanisms including direct neutralization of free radicals either by scavenging or trapping, chelation of transition metal ions, up-regulation of endogenous antioxidant enzymes (e.g. superoxide dismutase, catalase and glutathione peroxidase) and downregulations to pro-inflammatory signaling pathways (Abdel- Daim et al., 2019).

Olea europaea (olive) leaf extract (OLE) is one of the most promising natural therapeutic agents due to its unique comprehensive source and concentrated phytochemical composition. The bioactive phenolic compounds found in olive leaves exist at high concentrations and are dominated by oleuropein, hydroxytyrosol, luteolin apigenin and caffeic acid (Omar, 2010). As a wide-spectrum antioxidant agent, OLE is responsible for inhibiting xenobiotic-mediated toxicity by scavenging hydroxyl and superoxide radicals, preventing the propagation of lipid peroxidation chains and stabilizing cellular structural integrity (Boss et al., 2016). In addition, OLE has some powerful anti-inflammatory and anti-apoptotic actions through inhibition of nuclear factor kappa B (NF- κ B) activation as well as regulation of apoptotic proteins (Hashim et al., 2015). However, although the influence of these preservatives has been investigated in relation to their overall biochemical toxicity, studies looking into detailed dose-dependent pathohistological changes of hepatic and renal microarchitecture following chronic sodium benzoate

exposure have largely remained unfulfilled and a precise quantification of structural protection provided by hydroethanolic *Olea europaea* leaf extract (OLE) is lacking. While OLE is widely known for its antioxidant activity, its specific restoring effects against sodium benzoate-induced toxicologically-irreversible damage at the tissue level have yet to be elucidated. We proposed that the repeated use of polyphenol-rich OLE can diminish sodium benzoate-induced damage in examined tissues and protect hepatic and renal microstructures against prescribed doses. Thus, the precise aim of this research was to investigate the protective effect of OLE (200 mg/kg) on dose-dependent hepatonephrohistopathological injury induced by Sodium Benzoate (SB)-treatment (100 and 200 mg/kg) in adult male Wistar rats applied once daily for 45 consecutive days.

2. Materials and Methods

2.1. Chemicals and Reagents:

Sodium Benzoate (SB) powder of a purity $\geq 99.0\%$ was obtained from Sigma-Aldrich (St. Louis, MO, USA). Materials: Formalin (10% neutral buffered) was obtained from standard commercial sources, as were paraffin wax, ethanol and Xylene. Hematoxylin and Eosin (H&E) stains were obtained from new laboratory resources.

2.2. Plant Material and Extraction Protocol

Young leaves of *Olea europaea* L., were collected then washed with distilled water and shade dried at room temperatures (25 ± 2 °C) to a constant weight. Dried leaves were ground with an electric blender into a fine powder. One hundred grams of leaf powder was macerated with 1000 mL of a 70% (v/v) ethanol in water solution for 72 h at room temperature, agitating each 12 h. The filtrate was concentrated at 40 °C under reduced pressure by a rotary evaporator (Rotavapor R-210, BÜCHI). The obtained crude hydroethanolic extract was lyophilized within a freeze-dryer, providing in a dry powder and yielding an overall total extract (%) of 18.5% (w/w) when compared to the starting dry plant material. The final extract was kept in brown, sealed vials at -20 °C until the administration for the experimentation purposes.

2.2. Experimental Animals and Housing

The present study was carried out using adult male Wistar rats (180–210 g) obtained from the Animal House Unit of NRC (Dokki, Giza, Egypt). Experimental and animal handling procedures were in accordance with the guidelines of National Research Centre (Giza, Egypt) animal care unit. Experiments were conducted 14 days after animals had been maintained in laboratory conditions. The experiments were carried out in controlled environmental conditions (22 ± 2 °C, 50–60% relative humidity, and a 12 h light/dark cycle) with the rats housed in conventional polypropylene cages with free access for standard rodent diet and water ad libitum. All animal procedures were approved by local IACUC committee and are in compliance with standards for use of laboratory animals. During the 45-day experimental period, all rats were carefully observed twice a day for any clinical sign of toxicity, behavioral changes or change in physical appearance as well as mortality. The lethality and severe physical distress in any of the experimental groups (% mortality none). Rats in the OLE-alone, SB 100 + OLE and Control groups showed normal activity, regular feed/water intake and appropriate grooming behavior during the 7 days evaluation period. In contrast, high-dose sodium benzoate administered singly (SB 200 mg/kg) resulted in mild transient lethargy, reduced food consumption

during the first two weeks and slightly piloerection in animals. Nevertheless, all these clinical signs were significantly reduced in the co-treated animals (SB 200 mg/kg + OLE), where wild-type appearances and behavioural features were maintained throughout the duration of the experiment.

2.3. Experimental Design

In this study, an initial 288 adult male Wistar rats (total n = 36 per group) were randomly assigned to six experimental groups (n = 6 per group) utilizing a computer-generated random sequence method from October, up to January of the following year. Standard resource equation and previous toxicological power analyses provided justification for the sample size (n = 6) to maintain adequate statistics while minimizing animal use. Grouped by treatment, all interventions were provided daily by oral gavage using a fixed volume of 5 mL/kg body weight (b.w) days 01 to 45 at the time interval between 09:00 AM and 10:00 AM.

Group 1 (Control): Distilled water (vehicle, PO; 5 ml/kg b.wt.) daily.

Group 2 (OLE 200) is given hydroethanolic *Olea europaea* leaf extract at a dose of 200 mg/kg b.wt. suspended in distilled water. daily.

Group 3 (SB 100), administered Sodium Benzoate diluted in distilled water at a dosage of 100 mg/kg b.wt. daily.

Group 4 (SB 100 + OLE): Received Sodium Benzoate (100 mg/kg b.wt.) followed 1 hour later by OLE (200 mg/kg b.wt.) daily.

Group 5 (SB 200): Received Sodium Benzoate dissolved in distilled water at a dose of 200 mg/kg b.wt. daily.

Group 6 (SB 200 + OLE): Received Sodium Benzoate (200 mg/kg b.wt.) followed 1 hour later by OLE (200 mg/kg b.wt.) daily.

The doses of sodium benzoate (SB; 100 mg/kg b.wt. The highest doses administered (1000 mg/kg b.w./day) were established according to preclinical toxicological models demonstrating sub-chronic oral exposure without acute lethality, associated with mild-to-moderate target organ toxicity in rodents (El-Sawy et al., 2023). These doses are also representative of multiples of human daily exposure attained through excessive intake of preservatives from industrially processed foods.

The study was carried on the male Wistar rats were divided into six groups as:≤. Gonzalez-Burgos & Gomez-Serranillos, 2022) without intrinsic hepatorenal toxicity and within the range established in previous pharmacological studies showing optimal antioxidant, anti-inflammatory and cytoprotective activities.

2.4. Preparation of Hydroethanolic *Olea europaea* Leaf Extract (OLE)

Collection and authentication of fresh leaves of *Olea europaea*. Leaves were thoroughly washed with distilled water, shade dried at room temperature, and powdered. Using a plant-to-solvent (1:10 w/v) ratio, powder was extracted with are maceration using 70% ethanol at room temperature for 72 h with intermittent manual shaking. The filtered this mixture through Whatman No. 1 filter paper and the resulting filtrate was reduced under vacuum at 40° C using a rotary evaporator. The crude hydroethanolic extract was then dried and kept at -20°C until use (Omar,2010). Mature fresh leaves of *Olea europaea* L. were obtained from a commercial farm, October 2023 in Giza, Egypt . A botanist classifying and identifying the plant material taxonomically. A voucher specimen was deposited (No. OLE-2023- 11) in the institutional herbarium for further reference. The leaves were extensively rinsed with distilled water, shade dried at room temperature (25 ± 2) for two weeks and pulverized into a coarse powder in an electric blender. The powder (500 g) was macerated with 70% ethanol at a plant to solvent ratio of 1:10 (gram per milliliter, total volume 5 L, w/v) at room temperature for 72 h with constant gentle stirring. The extract was filtered through a double layer of gauze followed by Whatman No. 1 filter paper. The filtrate was evaporated under reduced pressure using a rotary evaporator (Büchi R-210, Switzerland) at 40 C to remove solvent traces and then lyophilized, yielding approximately 68.5 g of dry extract (extraction yield: 13.7% w/w). The dry crude hydroethanolic extract was stored in airtight, light-resistant containers at -20 C until administration. For experimental dosing, the OLE residue was freshly reconstituted daily in distilled water (vehicle) prior to oral gavage.

While chromatographic profiling was not performed in the present study, hydroethanolic extracts of *Olea europaea* leaves are widely recognized in literature to be rich in bioactive polyphenolic compounds, predominantly secoiridoids (such as oleuropein and hydroxytyrosol), flavonoids (including luteolin and apigenin), and phenolic acids (Omar, 2010; Boss et al., 2016). These literature-reported constituents are well-documented to account for the prominent radical-scavenging and tissue-protective properties associated with olive leaf extracts.

2.5. Tissue preparation

The rats were appreciated at the end of the active drug treatment period. Liver and kidneys were isolated, washed with saline, and samples of choice organs for histological study were fixed in Formalin (10%). Embedded in paraffin wax, sections of 5 µm were cut using a microtome. The stained morphology was then assessed by microscopy, following established procedures (Drury and Wallington, 1980), on H&E-stained sections of the corpus. Throughout the 45-day experimental period, behavioral changes, and mortality. Body weights were recorded weekly to adjust dosage volumes accordingly. Daily food and water intake were monitored per cage. No mortality or severe overt signs of systemic toxicity were observed in any of the experimental groups during the study period.

2.6. Quantitative Histopathological Evaluation and Blinding

For each animal (n= 6 per group), three non-consecutive tissue sections (5µm thick) per organ (liver and kidney) . Qualitative and semi-quantitative histological assessments were conducted across ten randomly selected, non-overlapping microscopic fields per section (totaling 30 fields per animal) at ×100, ×200, and ×400 magnifications using a light microscope equipped with a digital camera. Field selection was performed in a systematic zigzag pattern to avoid examiner bias. Pathological lesions were identified based on standardized diagnostic criteria:

Hepatic lesions: Evaluated for hepatocellular necrosis (loss of nuclear architecture, eosinophilic cytoplasm), intersinusoidal edema, nuclear pyknosis (chromatin condensation), and vacuolar degeneration (cytoplasmic clear vacuoles).

Renal lesions: Evaluated for glomerular atrophy/shrinkage, periglomerular edema, renal tubular dilatation, epithelial necrosis and sloughing, and hyaline cast formation.

To eliminate evaluation bias, all histopathological examinations were performed blindly by an experienced board-certified veterinary pathologist who was completely blinded to the experimental group allocations and treatment codes.

The severity of histological lesions in hepatic and renal tissues was semi-quantitatively assessed based on the percentage of tissue area or microscopic fields affected across 30 examined fields per rat. Scoring criteria were defined as follows:

Score 0 (–) [None/Normal]: Absence of lesions or structural alterations (<5% of examined fields affected).

Score 1 (+) [Mild]: Mild histological changes affecting 5-25% of examined fields.

Score 2 (++) [Moderate]: Moderate pathological alterations affecting 26-50% of examined fields.

Score 3 (+++) [Severe]: Severe pathological changes affecting >50% of examined fields. For each individual rat, lesion scores were averaged across all examined sections to derive a mean animal score. Group scores were subsequently calculated as the median score with interquartile range (IQR) per experimental group to represent ordinal categorical data accurately.

2.7. Ethical Statement

The study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Science / Veterinary Medicine (Approval Ref. No. IACUC/CU-EF-2023-89). All procedures were carried out in accordance with international guidelines for the care and use of laboratory animals (NIH Publication No. 85-23, revised 1985) and the ARRIVE guidelines to minimise suffering. At the end of the 45 days treatment period, all animals were fasted overnight (12 h) with free access to water. Animals were then euthanized by intraperitoneal injection (i.p) of sodium pentobarbital (150 mg/kg b.wt.). to cause an immediate and pain-free loss of consciousness before cervical dislocation, in strict accordance with the AVMA Guidelines for the Euthanasia of Animals (2020). The obtained tissue samples (liver and kidney) were then immediately dissected, washed with ice-cold saline, and fixed in 10% neutral buffered formalin from which histopathological processing was performed.

3.Results

Histology serves as a highly responsive instrument that facilitates the identification of dose effects and provides a profound understanding of the onset and mechanism(s) of action. Notably, histology offers a prompt technique for detecting the effects of pathogens in diverse organs, thereby serving as a reliable indicator for abnormal environmental conditions.

3.1. Liver Histology

Microscopic examination of liver sections from rats treated with Sodium Benzoate at a dose of 200 mg/kg b.wt. (SB 200) revealed severe vacuolar degeneration of hepatocytes, pyknosis, and extensive hepatocellular damage (Figure 5). In contrast, liver sections from rats co-treated with SB 200 mg/kg + OLE 200 mg/kg showed marked histological improvement, presenting only mild mononuclear inflammatory cell infiltration, increased Kupffer cell count, and localized mild hepatocyte degeneration compared to the SB 200 single-treatment group (Figure 6; Table 1).

In comparison, liver tissue from rats treated with Sodium Benzoate alone (SB 100 mg/kg) exhibited hepatocellular necrosis, intersinusoidal edema, pyknosis in some nuclei of the hepatocytes and mild vacuolar degeneration in some hepatocytes (Figure 3). The liver tissue of rats co-treated with SB 100 mg/kg + OLE exhibited localized hepatocyte degeneration, as well as maintained structural modulation and restoration of hepatic architecture, a proliferation of Kupffer cells (**Figure 4**).

Figures 5 (B) & (C): Liver tissue of rats subjected to SB treatment in doses of 200 and 400 mg/kg show intense vacuolar degeneration of hepatocytes. In the liver tissue of rats, SB 200 mg/kg + OLE was given once a day and at the end of treatment process revealed slight infiltration of mononuclear inflammatory cells, increased Kupffer cells count, mild hepatocyte degeneration and necrosis were noticed, but in a reduced area where not affected by sodium benzoate (**Figure 6; Table 1**).

Table 1: Effects of of Sodium Benzoate and *Olea europaea* leaves extract on liver tissue

	Control	OLE	SB100	SB100+OLE	SBG200	SB200+OLE
Lymphocyte infiltration (inflammatory cells)	-	-	-	-	-	+
Interlobular connective tissue degeneration	-	-	-	-	-	-
Necrosis& degeneration	-	-	++	+	+++	+
Disruption of architecture hepatic cord	-	-	-	-	-	-
Swelling of hepatic cell	-	-	++	-	-	-
Fibrosis of Portal area	-	-	-	-	-	-

OLE; dose of *Olea europaea* leaves extract (200 mg/kg), SB 100; dose of Sodium Benzoate (100 mg/kg), SB 200; dose of Sodium Benzoate (200 mg/kg)

(-): Normal tissue, (+): Mild changes, (++) : Moderate changes, (+++) : Severe changes

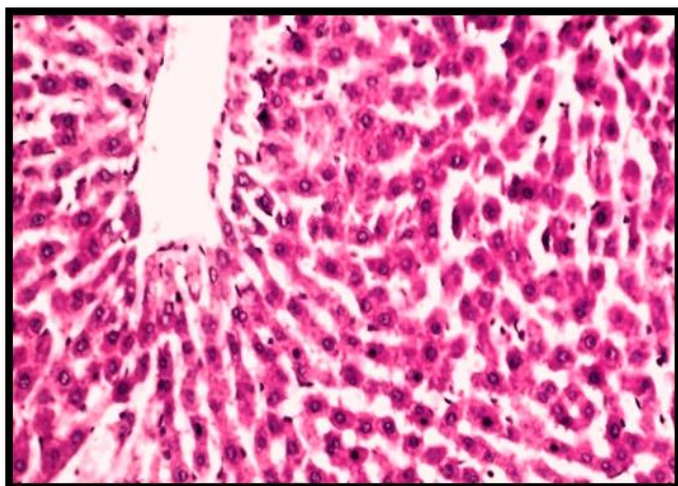


Figure 1: Photomicrographs of liver sections of male rats control group showing normal hepatic architecture, with normal hepatic cells, portal area and hepatic cords (H&E.X200).

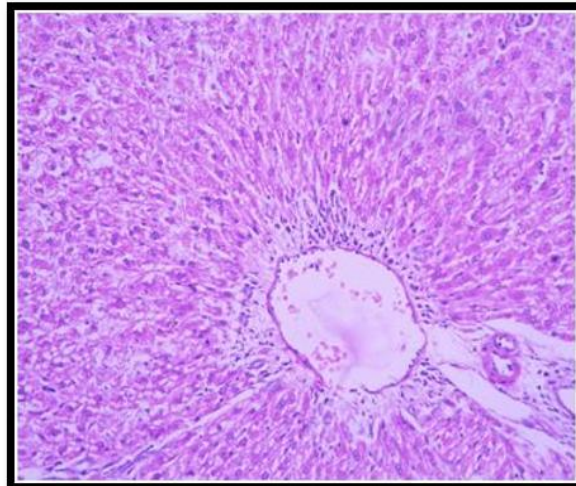


Figure 2: Photomicrographs of liver sections of male rats treated with Olea europaea leaf extract (OLE) (200mg/kg) Liver showing perivascular mononuclear cell infiltration and increased in number of kupffer cells (H&E. X 200)

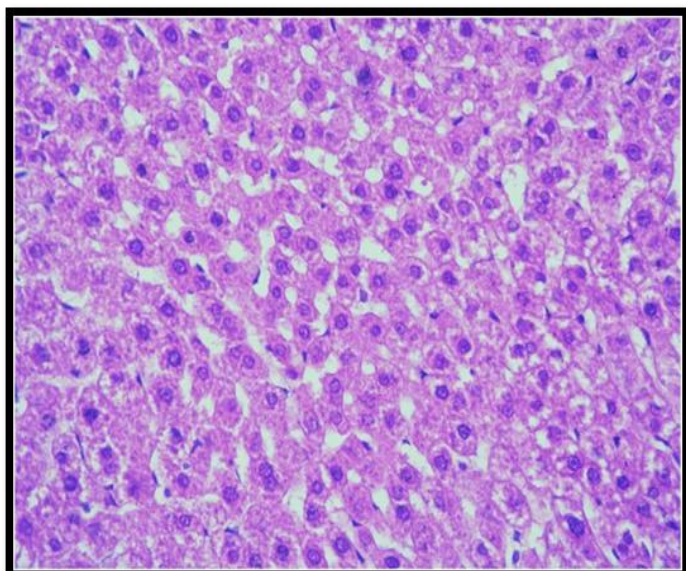


Figure 3: Photomicrographs of liver sections of male rats treated with Sodium Benzoate (SB) (100mg/kg) liver showing hepatocellular necrosis, edema in between sinusoids and pyknosis of some nuclei of hepatocytes and mild vacuolar degeneration in some hepatocytes H&E X 400

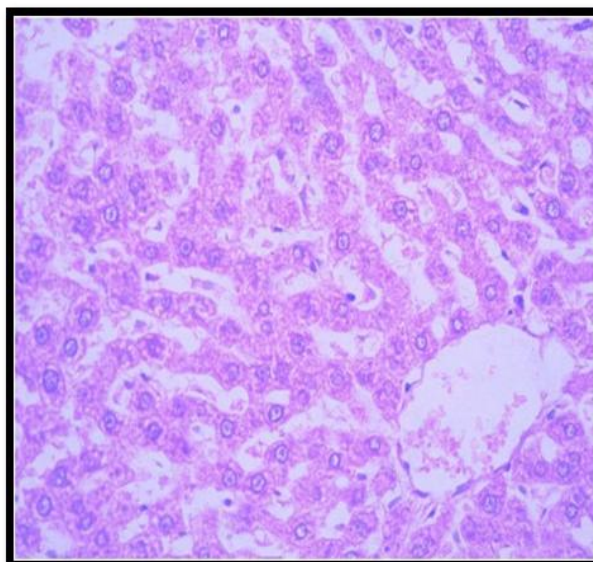


Figure 4: Photomicrographs of liver sections of male rats treated with SB100 + OLE. liver showing degeneration in hepatocytes, modulation in histological structure with an increase in the number of kupffer cells H&E X 400

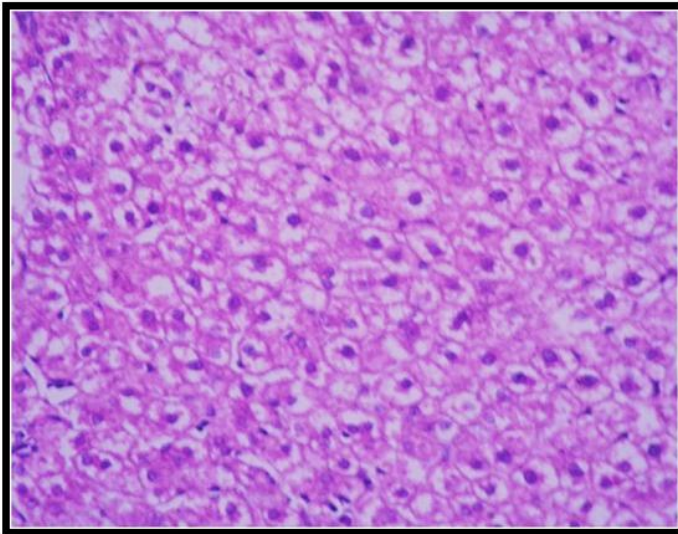


Figure 5: Photomicrographs of liver sections of male rats treated with Sodium Benzoate (SB) (200mg/kg) liver showing severe vacuolar degeneration of hepatocytes H&E X 200

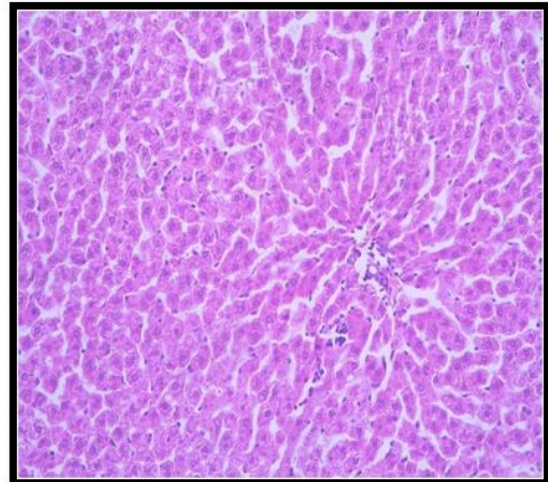


Figure 6: Photomicrographs of liver sections of male rats treated with SB200 + OLE. Liver showing slight infiltration with mononuclear inflammatory cells, increase in number of kupffer cell with mild degeneration and necrosis in some hepatocytes and modulation in hepatocytes rather than liver of group received Sodium Benzoate H&E X 200

3.2. Kidney Histology:

Microscopic examination of photomicrograph sections stained with Hematoxylin and Eosin (H&E) of the kidneys of control male rats revealed the presence of a normal renal cortex and normal renal medulla, with normal glomeruli and renal tubules. The kidney tissue exhibited a normal architecture with healthy glomeruli, as depicted in Figure 7 (A, B).

Administration of *Olea europaea* (olive) leaf extract (OLE 200 mg/kg) to the rats resulted in mild degenerative changes in the renal tubules. Eosinophilic material inside the tubular lumen (renal casts) was also observed, along with the dilatation of some tubules, as shown in Figure 8. On the other hand, the administration of Sodium Benzoate (SB 100 mg/kg) led to periglomerular edema, shrinkage of the glomerular tuft of some glomeruli, and degenerative and necrotic changes in some renal tubules. In addition, dilatation of some renal tubules with degenerative and necrotic changes in the tubular epithelium was also observed, as illustrated in Figure 9 (A, B).

In addition, in Figure 10, examination of kidney tissue of rats treated with SB 100 mg/kg + OLE demonstrated renal casts in some tubules, hypercellularity of renal glomeruli, and the glomerular tuft was hypercellular with necrobiotic changes within the renal tubules. Tissues of the kidney of rats treated with a higher dose of Sodium Benzoate (SB 200 mg/kg) induced periglomerular edema and degenerative changes in the glomerular tuft and degeneration in renal tubules. Other changes included dilatation of some renal tubules along with a degenerated glomerular tuft, severe hemorrhage and necrosis in renal tubules, complete sloughing of tubular epithelium of some tubules into their lumen, and vacuolar degeneration with slight hemorrhage between tubules (fig; 11 A, B, C, D). In comparison, the histological examination of kidney tissue from rats treated with SB 200 mg/kg + OLE revealed dilatation in some renal tubules (normal glomerular structure) and necrobiotic changes in only individual dilated renal tubules (Figure 12.). Details are shown in Table 2.

Table 2: Effects of of Sodium Benzoate and *Olea europaea* leaves extract on kidney tissue.

	Control	OLE	SB100	SB100+OLE	SBG200	SB200+OLE
Atrophy Glomeruli	-	-	++	-	++	-
Renal tubules dilation	-	+	++	-	++	+
Interstitial connective tissue	-	-	-	-	+++	-
Dilated blood vessel	-	-	-	-	++	-
Hyprertrophy (glomerulus)	-	-	+	+	++	-
Necrosis of epithelial lining	-	-	++	-	+++	+
Inflammatory cells	-	-	-	-	-	-

OLE; dose of *Olea europaea* leaves extract (200 mg/kg), SB 100; dose of Sodium Benzoate (100 mg/kg), SB 200; dose of Sodium Benzoate (200 mg/kg)

(-): Normal tissue, (+): Mild changes, (++) : Moderate changes, (+++) : Severe changes.

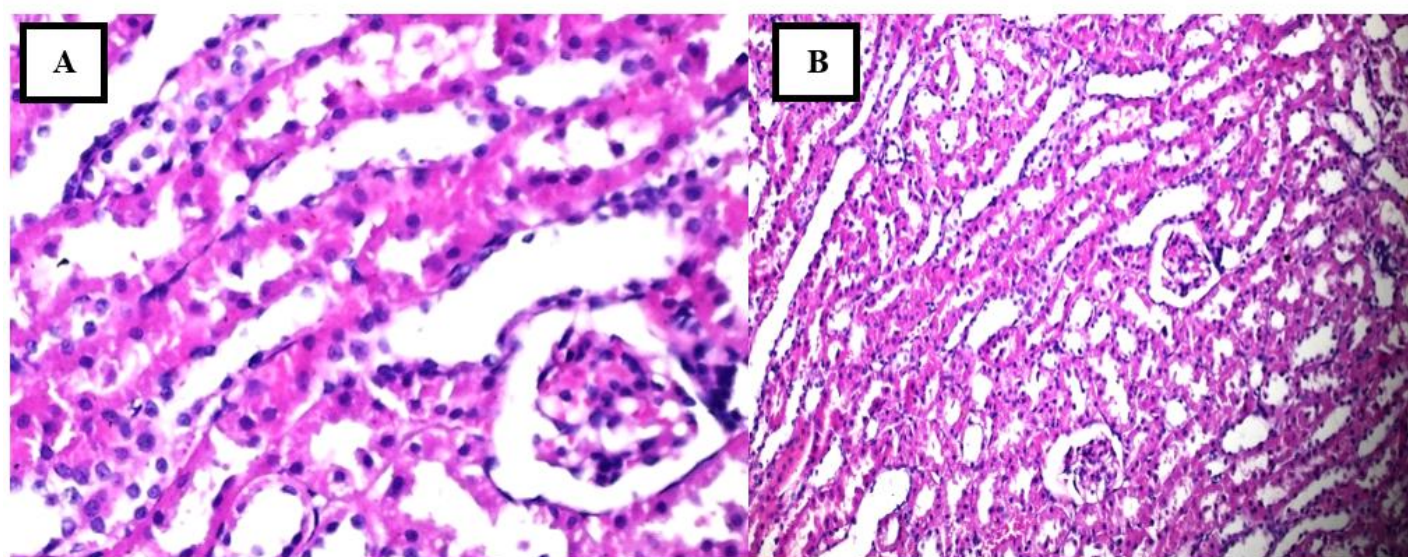


Figure 7 (A, B): Photomicrographs of control group kidney sections of male rats showing **(A):** normal renal cortex and normal renal medulla with normal glomeruli and normal renal tubules stained with (H&Ex100); **(B):** normal architecture of kidney tissue with normal glomeruli (H&Ex400)

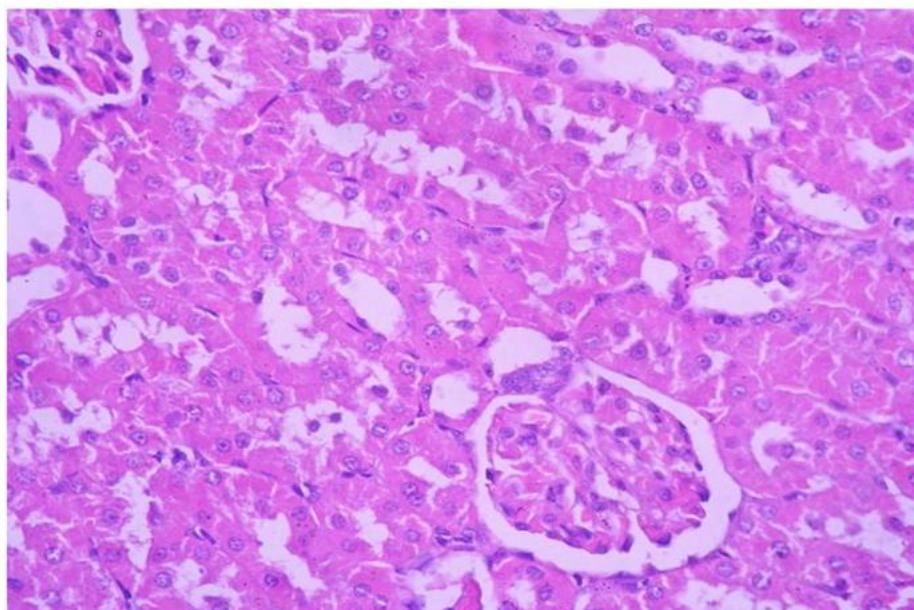


Figure 8: Photomicrographs of kidney sections of male rats treated with *Olea europaea* leaf extract (OLE) (200mg/kg). Kidney showing a mild degenerative change in the renal tubule, eosinophilic material inside tubular lumen (renal cast) with dilatation of some tubules H&E X400

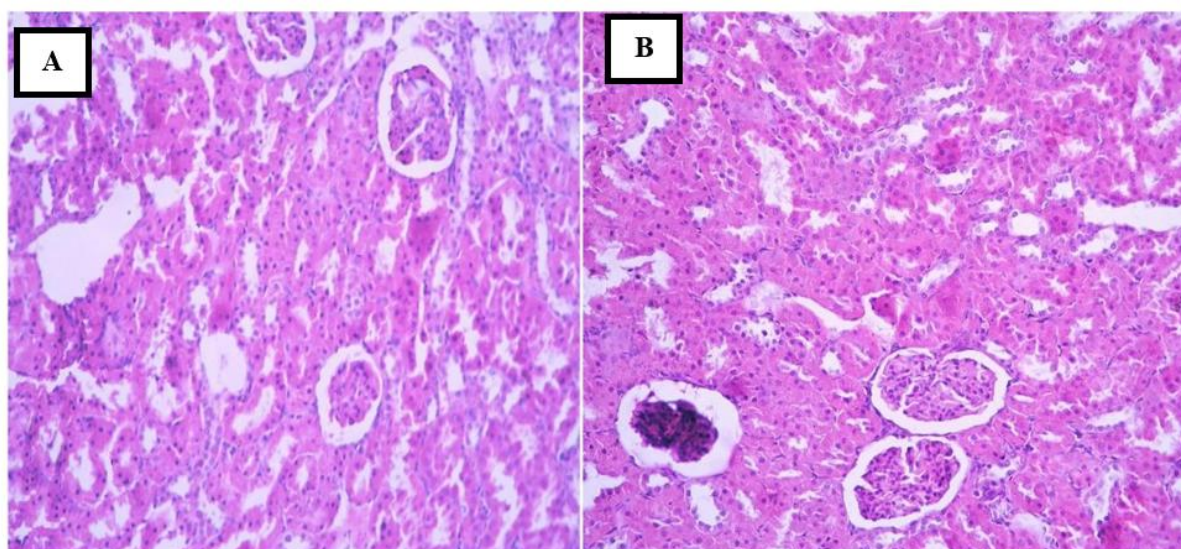


Figure 9 (A,B): Photomicrographs of kidney sections of male rats treated with Sodium Benzoate (SB) (100mg/kg) **(A)** kidney showing periglomerular edema with shrinkage of glomerular tuft of some glomeruli with degenerative or necrotic change in some renal tubules H&E X100 **(B)** kidney illustrating dilatation of some renal tubules with degenerative and necrotic change in tubular epithelium H&E X100

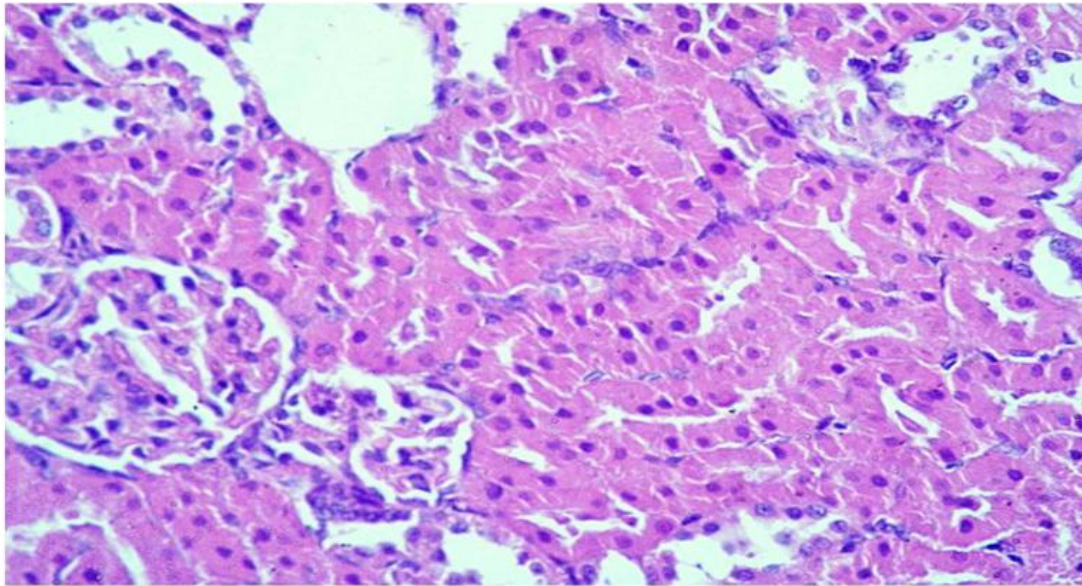


Figure 10: Photomicrographs of kidney sections of male rats treated with SB100 + OLE. Kidney showing the presence of renal cast in some tubules, hypercellularity of renal glomeruli and hypercellularity in glomerular tuft with necrobiotic changes in renal tubule H&E X 200

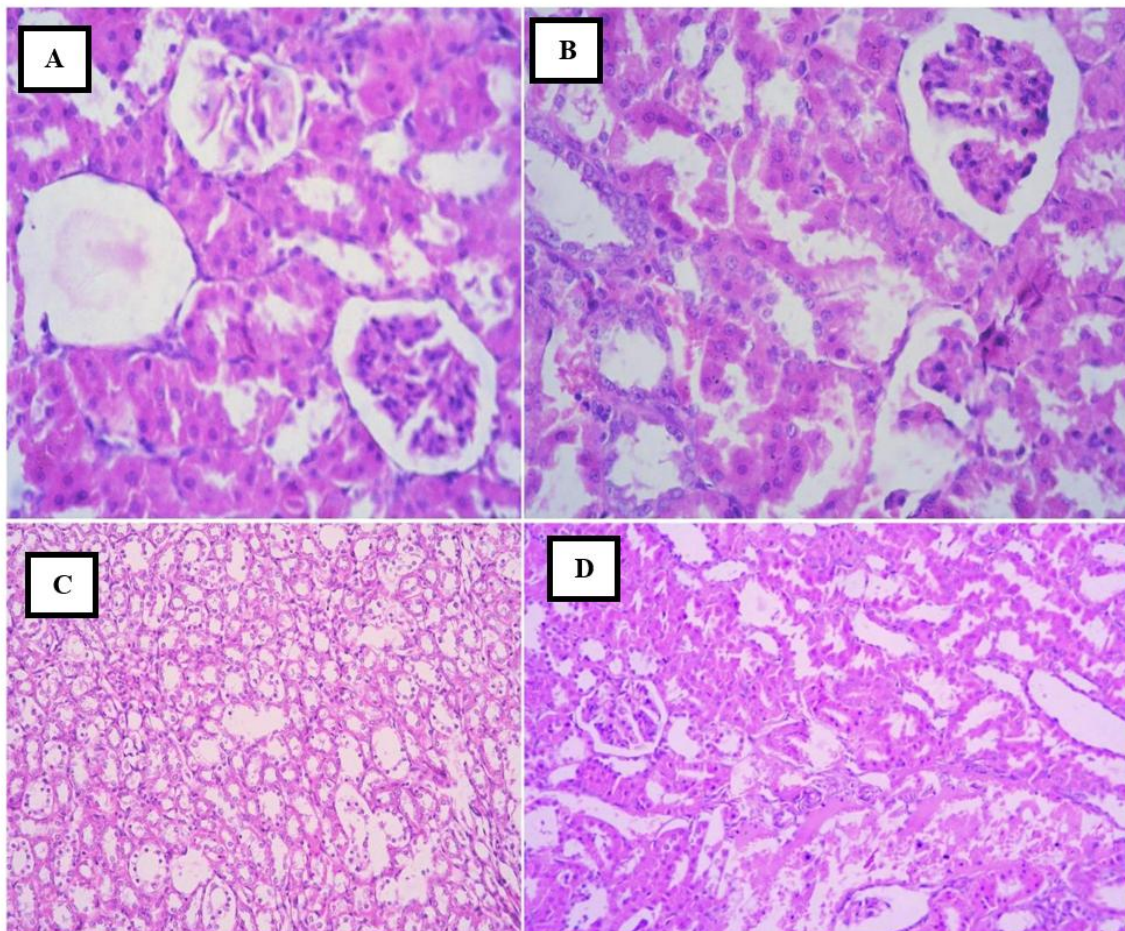


Figure 11 (A,B,C,D): Photomicrographs of kidney sections of male rats treated with Sodium Benzoate (SB) (200mg/kg) **(A)** kidney showing periglomerular edema with degenerative change in glomerular tuft and degeneration in renal tubules H&E X 200 **(B)** kidney illustrating dilatation of some renal tubules with degeneration in glomerular tuft H&E X 200 **(C)** kidney showing hemorrhage , severe degeneration and necrosis in renal tubules H&E X 100 **(D)** kidney revealing complete sluffing of renal tubular epithelium of some tubules in its lumen and vacuolar degeneration with slight haemorrhage in between tubules H&E X 200

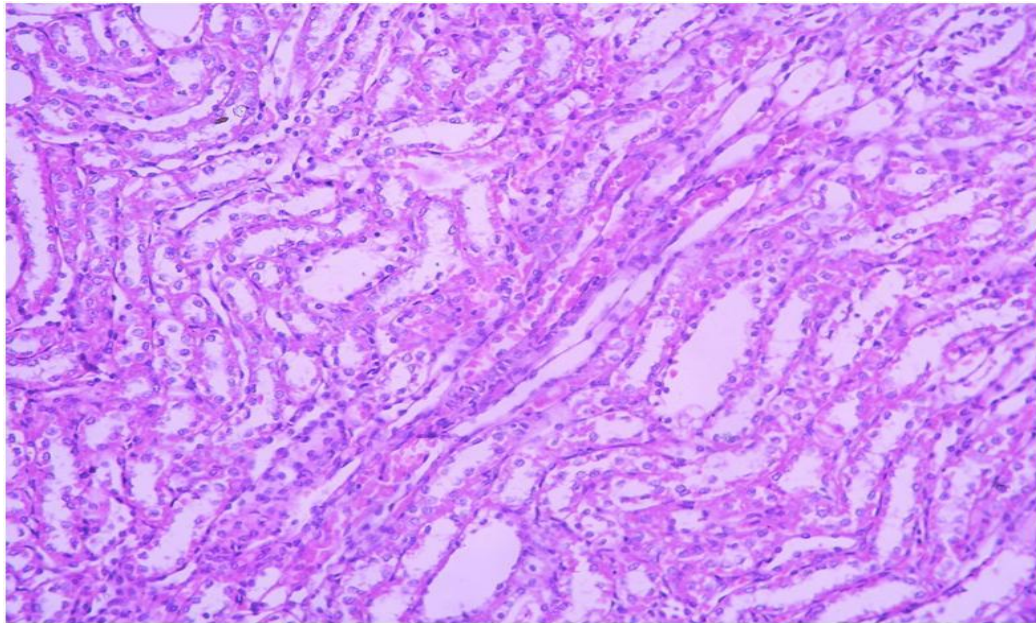


Figure 12: Photomicrographs of kidney sections of male rats treated with SB200 + OLE. Kidney showing dilatation in some renal tubules with normal glomerular structure, dilatation renal tubules with a necrobiotic change in some tubules H&E X 200

4. Discussion

In summary, the current results indicate that hydroethanolic *Olea europaea* leaf extract (OLE) offered noticeable protection against structural changes of hepatic and renal tissues induced by sodium benzoate (SB). While typically considered a mechanical barrier, the structural preservation noted is biologically compatible with the attenuation of intracellular oxidative stress, lipid peroxidation, and secondary inflammatory cascades usually stimulated by chronic preservative bioaccumulation. Microscopically, results demonstrated that Sodium Benzoate (SB) elicited a dose-dependent hepatic effect by which SB 100 mg/kg exhibited hepatocellular necrosis and pyknosis while SB 200 mg/kg led to severe vacuolar degeneration. These toxicological changes correlate with earlier reports for food additives inducing cellular damage and oxidative stress on hepatic tissue (Albrahim & Binobead, 2018). Simultaneous treatment with *Olea europaea* leaf extract (OLE 200 mg/kg) significantly ameliorated all degenerative lesions, salvaging hepatic architecture and activating Kupffer cell proliferation. This hepatoprotective potential supports the earlier data from another major food additive, Polyethylene Glycol (PEG), in which plants endowed with antioxidant properties markedly reverted PEG-induced hepatic disequilibrium and cellular damage (Irwandi et al., 2023). This is

possibly due to the fact that OLE includes many polyphenolics and flavonoids which act as scavengers of reactive oxygen species (ROS) as well as protect cellular membrane integrity (Xu et al. 2017). Renal histological examination demonstrated that Sodium Benzoate induced severe renal toxicity, manifested by periglomerular edema, glomerular shrinkage, complete tubular epithelial sloughing, and necrosis at higher doses (SB 200 mg/kg). These structural changes have also been well-supported in the literature to have toxicological effects on intrinsic renal tubules and filtration barrier integrity (Pareta et al., 2011).

Importantly, the concurrent treatment using *Olea europaea* leaf extract demonstrated substantial protection of renal histoarchitecture and preserved normal glomerular architectures, showing only mild tubular dilatation. This nephroprotective activity is similar to the mode of protection against renal injury induced by PEG, again supporting the fact that natural plant extracts can ameliorate nephrotoxicity caused by synthetic food additives (Yousef et al., 2022). The renal recovery by OLE also accompanies very potent antioxidant properties, as well as anti-inflammatory effects of these compounds in Kidney tissues (Hamed, & El-Sayed, 2019).

5. Conclusion

Objective To investigate the effect of sodium benzoate on liver and kidney papilloma in male f Wistar rats. The underlying mechanism for this toxicity is oxidative stress, which leads to mitochondrial dysfunction and lipid peroxidation as well as global necrosis through damage of DNA. Combination with *Olea europaea* leaf extract (OLE) protects against SB-induced hepato-renal impairment at the dose of 200 mg/kg. OLE is a potent free-radical scavenger and anti-inflammatory agent as it contains high concentrations of various bioactive phenolics and flavonoids, particularly oleuropein, hydroxytyrosol and luteolin. The structural tissue integrity was correctly maintained by OLE and cellular degeneration was suppressed, while modulation of non-alcoholic fatty liver disorder results in normalized architecture. In summary, these results highlight the therapeutic properties of *Olea europaea* leaf extract as a new natural candidate for preventing chemical-induced oxidative damage and organ injury.

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