REPUBLIC OF TÜRKİYE
HARRAN UNIVERSITY
INSTITUTE OF GRADUATE EDUCATION

DOCTORATE THESIS

EVALUATION OF BIOCHEMICAL AND HISTOPATHOLOGICAL EFFECTS OF OLIVE LEAF TEA ON EHRLICH ASCITES CARCINOMA-BEARING MICE

AWAT OMER SABR

BIOLOGY

Şanlıurfa
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İÇİNDEKİLER

ABSTRACT

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This study investigates the biochemical and histopathological effects of olive leaf tea (OLT) in mice bearing Ehrlich Ascites Carcinoma (EAC), a widely used experimental tumor model that mimics aggressive breast cancer behavior and systemic oxidative stress. The primary objective of the study was to evaluate the potential protective and supportive role of OLT, detoxification enzyme systems, and tissue integrity, as well as its modulatory effects on chemotherapy-induced toxicity, depending on the timing of administration. Olive leaf tea, rich in phenolic compounds such as oleuropein and hydroxytyrosol, was administered orally at a dose of 400 mg/kg before, during, or after tumor inoculation. Experimental animals were divided into groups, including control, tumor, OLT-treated, 5-fluorouracil (5-FU)-treated, and combined OLT + 5-FU groups. At the end of the experimental period, liver, kidney, and brain tissues were harvested for biochemical analyses, while liver, stomach, duodenum, kidney, and bladder tissues were examined histopathologically. Biochemical evaluations focused on reduced glutathione (GSH) levels, glutathione S-transferase (GST), and carboxylesterase (CaE) activities, which are critical indicators of oxidative balance and xenobiotic detoxification. The results demonstrated that tumor burden caused a significant depletion of hepatic GSH levels, reflecting pronounced oxidative stress. OLT administration, particularly when applied prophylactically before tumor induction, partially restored GSH levels and significantly enhanced GST activity, indicating an improved phase II detoxification capacity. Tumor-associated elevations in CaE activity showed a trend toward normalization in OLT-treated groups, suggesting a beneficial modulation of xenobiotic metabolism. Histopathological examination revealed that OLT administration alone did not induce any pathological alterations in the examined organs. Moreover, combined treatment with OLT and 5-FU markedly reduced chemotherapy-associated hepatic degeneration and gastric lymphocytic infiltration compared to 5-FU treatment alone. These findings indicate that OLT exerts a protective effect against tissue damage induced by both tumor progression and chemotherapeutic stress. In conclusion, this study provides experimental evidence that olive leaf tea supports antioxidant defense systems, enhances detoxification enzyme activity, and preserves tissue morphology in an EAC mouse model. The findings suggest that OLT may serve as a promising nutraceutical adjuvant, particularly when used preventively, to mitigate oxidative stress and reduce chemotherapy-related organ toxicity in cancer management.

KEYWORDS: Breast cancer, Olive leaf tea, hepatoprotective effect, Detoxification enzymes, Ehrlich Ascites Carcinoma

ABSTRACT

DOKTORA TEZİ

ZEYTİN YAPRAĞI ÇAYININ EHRLİCH ASİT KARSİNOMU TAŞIYAN FARELER ÜZERİNDEKİ BİYOKİMYASAL VE HİSTOPATOLOJİK ETKİLERİNİN DEĞERLENDİRİLMESİ

AWAT OMER SABR

HARRAN ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ
BİYOLOJİ

Tez Danışmanı:Doç. Dr. HATİCE AKTAŞ
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Bu çalışma, agresif meme kanseri davranışını ve sistemik oksidatif stresi taklit eden yaygın olarak kullanılan deneysel bir tümör modeli olan Ehrlich Asit Karsinomu (EAC) taşıyan farelerde zeytin yaprağı çayının (OLT) biyokimyasal ve histopatolojik etkilerini araştırmaktadır. Çalışmanın birincil amacı, OLT'nin, detoksifikasyon enzim sistemlerinin ve doku bütünlüğünün potansiyel koruyucu ve destekleyici rolünün yanı sıra kemoterapi kaynaklı toksisite üzerindeki düzenleyici etkilerini uygulama zamanına bağlı olarak değerlendirmektir. Oleuropein ve hidroksitirozol gibi fenolik bileşikler açısından zengin olan zeytin yaprağı çayı, tümör aşılamasından önce, sırasında veya sonra 400 mg/kg dozunda oral olarak uygulandı. Deney hayvanları kontrol, tümör, OLT ile tedavi edilen, 5-florourasil (5-FU) ile tedavi edilen ve OLT + 5-FU kombinasyonlu gibi gruplara ayrıldı. Deneysel dönemin sonunda, biyokimyasal analizler için karaciğer, böbrek ve beyin dokuları alınırken, karaciğer, mide, duodenum, böbrek ve mesane dokuları histopatolojik olarak incelendi. Biyokimyasal değerlendirmeler, oksidatif denge ve ksenobiyotik detoksifikasyonun kritik göstergeleri olan indirgenmiş glutatyon (GSH) seviyeleri, glutatyon S-transferaz (GST) ve karboksil esteraz (CaE) aktivitelerine odaklandı. Sonuçlar, tümör yükünün karaciğer GSH seviyelerinde önemli bir azalmaya neden olduğunu ve belirgin oksidatif stresi yansıttığını gösterdi. OLT, özellikle tümör indüksiyonundan önce profilaktik olarak uygulandığında, GSH seviyelerini kısmen geri kazandırdı ve GST aktivitesini önemli ölçüde artırarak faz II detoksifikasyon kapasitesinde iyileşme olduğunu gösterdi. Tümörle ilişkili CaE aktivitesindeki artışlar, OLT ile tedavi edilen gruplarda normalleşme eğilimi göstererek ksenobiyotik metabolizmasının faydalı bir şekilde modüle edildiğini düşündürmektedir. Histopatolojik inceleme, tek başına OLT uygulamasının incelenen organlarda herhangi bir patolojik değişikliğe neden olmadığını ortaya koydu. Dahası, OLT ve 5-FU'nun birlikte uygulanması, sadece 5-FU tedavisine kıyasla kemoterapiye bağlı karaciğer dejenerasyonunu ve mide lenfositik infiltrasyonunu önemli ölçüde azalttı. Bu bulgular, OLT'nin hem tümör ilerlemesi hem de kemoterapötik stresin neden olduğu doku hasarına karşı koruyucu bir etki meydana getirdiğini göstermektedir. Sonuç olarak, bu çalışma, zeytin yaprağı çayının antioksidan savunma sistemlerini desteklediğini, detoksifikasyon enzim aktivitesini artırdığını ve bir EAC fare modelinde doku morfolojisini koruduğunu deneysel olarak kanıtlamaktadır. Bulgular, OLT'nin özellikle önleyici olarak kullanıldığında, oksidatif stresi hafifletmek ve kanser yönetiminde kemoterapiye bağlı organ toksisitesini azaltmak için umut vadeden bir besin takviyesi olarak hizmet edebileceğini düşündürmektedir.

ANAHTAR KELİMELER: Meme kanseri, Zeytin yaprağı çayı, hepatoprotektif etki, Detoksifikasyon enzimleri, Ehrlich Ascites Karsinomu

ŞEKİLLER DİZİNİ

ÇİZELGELER DİZİNİ

ABBREVIATIONS

5-FU	5-Fluorouracil
BSS	Balanced Salt Solution
CaE	Carboxylesterase
EAC	Ehrlich Ascites Carcinoma
EAT	Ehrlich Ascites Tumor
GSH	Glutathione
GST	Glutathione S-Transferase
OLT	Olive leaf tea

1. INTRODUCTION

Cancer develops when genetic and epigenetic alterations accumulate in cellular DNA, disrupting normal regulatory mechanisms that control cell growth, differentiation, and survival. These mutations cause cells to proliferate uncontrollably and acquire malignant characteristics, ultimately leading to tumor formation (Hanahan & Weinberg, 2011). At the cellular level, cancer progression is closely linked to an imbalance between cell proliferation and programmed cell death. Many anticancer therapies exert their effects by reactivating apoptotic pathways and inducing cell death in cancer cells (Reed, 2000). Dysregulation of apoptotic signaling, including impaired caspase activation, allows genetically abnormal cells to survive and proliferate, thereby promoting tumor growth and disease progression (Nagata, 1997; Kakoli, 2015). One of the principal apoptotic mechanisms involved in cancer is the extrinsic death receptor pathway mediated by Fas (CD95) and its ligand, FasL, which triggers caspase cascades leading to apoptotic cell death (Ashkenazi & Dixit, 1998; Vaux & Korsmeyer, 1999).

Breast cancer is a particularly complex and heterogeneous malignancy, exhibiting wide variability in molecular, histological, and clinical features. Epidemiological evidence indicates that approximately one in eight women will be diagnosed with breast cancer in their lifetime, although the disease can also occur in men. Breast tumors may arise in different anatomical regions of the breast and possess the ability to invade local tissues and metastasize to distant organs (Siegel et al., 2023). Histopathological examination remains fundamental to breast cancer diagnosis and management, providing critical information on tumor morphology, grade, invasion patterns, and prognostic indicators (Walker et al., 1997).

Experimental tumor models play a crucial role in advancing cancer research by enabling the investigation of tumor biology and therapeutic responses under controlled conditions. Ehrlich ascites carcinoma (EAC) is a rapidly proliferating experimental breast tumor model derived from spontaneous mouse mammary adenocarcinoma. Following intraperitoneal transplantation, EAC cells proliferate aggressively within the peritoneal cavity, leading to ascitic fluid accumulation and host mortality within approximately 15-20 days (Ozaslan et al., 2011). This model is widely used to study tumor growth, inflammation, angiogenesis, and antitumor interventions. The ascitic fluid generated during EAC progression provides a nutrient-rich microenvironment that supports rapid tumor expansion and survival, while also inducing marked inflammatory responses characterized by increased vascular

permeability and immune cell infiltration (Nunes & Ricardo, 2022).

Current cancer treatment strategies include chemotherapy, radiotherapy, surgical intervention, or combinations thereof. Although these approaches have improved survival outcomes, many conventional anticancer drugs are associated with significant adverse effects, such as gastrointestinal toxicity and systemic complications. Consequently, increasing attention has been directed toward complementary strategies aimed at reducing treatment-related toxicity and enhancing therapeutic efficacy (Sun et al., 1990; Newman & Cragg, 2020).

Oxidative stress plays a central role in carcinogenesis by inducing DNA damage, genomic instability, and dysregulated cell signaling. Antioxidants can neutralize reactive oxygen species (ROS), chelate metal ions, and limit oxidative damage to cellular macromolecules, thereby potentially modulating carcinogenesis (Sun et al., 1990; Valko et al., 2006; Halliwell, 2007). Diets rich in antioxidant-containing foods have been shown to reduce oxidative DNA damage, an early and critical step in tumor development.

Within this context, plant-derived products and herbal preparations have gained increasing interest due to their diverse biological activities, environmental sustainability, and perceived lower toxicity compared with synthetic compounds (Willett et al., 2019). Diet and nutrition play a fundamental role in maintaining health and preventing chronic diseases. Alongside lifestyle modifications, dietary patterns rich in bioactive compounds are strongly associated with reduced disease risk and improved longevity. Herbal teas, in particular, have long been incorporated into traditional diets and are increasingly recognized for their potential health-promoting properties (Hodgson & Croft, 2010). Among these, olive leaf tea has attracted growing scientific interest due to its high phenolic content. Olive leaves contain substantial amounts of oleuropein, hydroxytyrosol, and tyrosol, which exhibit potent antioxidant, anti-inflammatory, antimicrobial, and cardioprotective activities. Experimental and clinical studies have demonstrated that olive leaf extracts and teas can exert beneficial effects at molecular, biochemical, histological, and immunological levels, supporting their potential role as complementary agents in health promotion and disease management (Shahidi & Ambigaipalan, 2015; Gorzynik-Debicka et al., 2018).

Given the nutritional value of nutraceutical agents that support the body’s functions, improve health, and protect against various diseases, the present study

aims to elucidate the role of olive leaf tea-based nutraceuticals as chemosensitizers in breast cancer treatment.

In this study, the biochemical and histopathological changes in tissues from mice with Ehrlich ascites carcinoma following treatment with olive leaf tea and 5-fluorouracil (5-FU) chemotherapy have been assessed. This study is particularly relevant as breast cancer can metastasise to various organs, including the liver, kidneys, stomach, duodenum, and bladder. The results are critical for evaluating the adjuvant potential of olive leaf tea in breast cancer, especially in processes associated with oxidative stress and inflammation.

2. PREVIOUS STUDIES

2.1. Breast Cancer

Breast cancer is a biologically diverse disease that develops through complex cellular, molecular, and histopathological changes (Hortobagyi et al., 2017). Multiple interacting factors influence the incidence and clinical outcome of breast cancer. These include age, genetic susceptibility, hormonal exposure, environmental conditions, and lifestyle habits. Breast cancer susceptibility arises from both non-modifiable factors, such as inherited genetic mutations, and modifiable lifestyle-related factors. Genetic predisposition plays a critical role in a subset of breast cancer cases. Germline mutations in high-risk genes, particularly BRCA1 and BRCA2, substantially increase lifetime breast cancer risk. Beyond inherited mutations, tumor development and progression are driven by somatic genetic and epigenetic alterations, disrupted RNA regulatory mechanisms, metabolic reprogramming, and pronounced tumor heterogeneity. These changes contribute to aggressive tumor behavior, resistance to therapy, and clinical complexity (Nik-Zainal et al., 2016).

From a histopathological perspective, breast cancer includes several distinct subtypes. These comprise invasive ductal carcinoma, invasive lobular carcinoma, mucinous carcinoma, papillary carcinoma, and tubular or cribriform carcinoma. Certain subtypes, such as mucinous and tubular carcinomas, are often associated with lower histological grade, reduced lymph node involvement, higher hormone receptor expression, and more favorable clinical outcomes (Lakhani et al., 2012).

Breast cancer is commonly categorized using molecular markers that reflect hormone and growth factor signaling, most notably estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression. Based on these biomarkers, tumors are grouped into clinically relevant subtypes such as Luminal A, Luminal B, HER2-enriched, and triple-negative breast cancer, each exhibiting distinct patterns of tumor biology, therapeutic sensitivity, and clinical outcome (Perou et al., 2000). Triple-negative breast cancer lacks ER, PR, and HER2 expression and is considered one of the most aggressive subtypes. Limited targeted treatment options, high recurrence rates, and significant molecular heterogeneity characterize it. This subtype more frequently affects younger women and certain ethnic populations (Abramson & Mayer, 2014). Recent studies have highlighted the importance of competing endogenous RNA (ceRNA) networks in breast cancer biology. Dysregulation of long non-coding RNAs, microRNAs, circular RNAs, and pseudogenes can alter oncogene and tumor suppressor gene

expression. These changes promote cancer hallmarks such as uncontrolled proliferation, invasion, and metastasis (Qi et al., 2020).

Breast cancer progression follows a multistep process involving cumulative genetic and epigenetic damage, impaired DNA repair, hormonal imbalance, immune dysregulation, and aberrant intracellular signaling. Together, these mechanisms drive tumor initiation, progression, and resistance to therapy (Majidinia & Yousefi, 2017). In addition, the tumor microenvironment, composed of immune cells, cancer-associated fibroblasts, endothelial cells, and extracellular matrix, plays a decisive role in shaping tumor growth and therapeutic response. Advances in understanding microenvironmental interactions have contributed to the development of novel therapeutic strategies, including immunotherapy. Moreover, circular RNAs have emerged as stable molecular biomarkers with potential applications in breast cancer diagnosis, prognosis, and monitoring of treatment resistance (Huang et al., 2023).

2.1.1. Molecular Pathogenesis of Breast Cancer

Breast cancer arises from a gradual accumulation of molecular abnormalities that disturb the normal regulatory balance of mammary epithelial cells. These abnormalities involve both genetic and epigenetic changes and affect fundamental cellular processes such as proliferation control, differentiation, DNA damage repair, and programmed cell death. As a result, cells acquire malignant characteristics and gain the ability to grow uncontrollably. Contemporary molecular research has clearly established that breast cancer is not a single disease entity but rather a collection of biologically diverse subtypes, each shaped by distinct oncogenic signaling pathways and interactions with the surrounding tumor microenvironment (Hortobagyi et al., 2017).

2.1.1.1. Genetic Alterations and Genomic Instability

The onset and advancement of breast cancer are propelled by the persistent accumulation of somatic genetic modifications affecting oncogenes, tumor suppressor genes, and DNA repair mechanisms. Errors during DNA replication and exposure to environmental stressors progressively increase the mutational burden, ultimately resulting in genomic instability, a defining feature of malignant cells. Large-scale genomic studies have revealed recurrent mutations in such genes as TP53, PIK3CA, GATA3, MAP3K1, and BRCA1/2, highlighting their central roles in breast cancer development (The Cancer Genome Atlas Network, 2012).

In aggressive breast cancer subtypes, especially triple-negative breast cancer, loss-of-function mutations in TP53 are frequently observed and are linked to impaired cell cycle regulation and defective apoptotic responses. Conversely, activating mutations in oncogenes such as PIK3CA are more prevalent in hormone receptor–positive tumors and lead to abnormal activation of the PI3K/AKT/mTOR signaling axis, thereby promoting tumor cell survival and proliferation (Nik-Zainal et al., 2016).

Inherited genetic alterations also contribute substantially to breast cancer susceptibility. Germline mutations in BRCA1 and BRCA2 disrupt homologous recombination–mediated DNA repair, causing chromosomal instability and a markedly increased lifetime risk of breast cancer. Tumors arising in BRCA-mutated individuals often display unique molecular features and exhibit increased sensitivity to targeted treatments such as PARP inhibitors (Roy et al., 2012).

2.1.1.2. Epigenetic Dysregulation

Epigenetic abnormalities, e.g., alterations in DNA methylation patterns, histone modifications, and chromatin structure, regulate gene expression without modifying the DNA sequence (Esteller, 2008). Epigenetic dysregulation often acts in concert with genetic alterations to enhance malignant behavior. Changes affecting genes involved in cell adhesion, apoptosis, and differentiation facilitate invasive growth and metastatic dissemination. Importantly, epigenetic modifications are reversible, which has generated significant interest in the development of epigenetic therapies as novel treatment strategies for breast cancer (Song et al., 2025).

2.1.1.3. Hormonal Signaling and Endocrine Regulation

Hormonal signaling represents a central component of breast cancer pathogenesis, particularly in hormone receptor-positive tumors. Estrogen signaling, mediated mainly through estrogen receptors ERα and ERβ, plays a key role in tumor initiation and progression by stimulating transcriptional programs that regulate cell cycle progression and cell survival (Ali & Coombes, 2002). Aberrant estrogen signaling may arise through multiple mechanisms, including estrogen receptor overexpression, increased local estrogen synthesis, and functional interactions with oncogenic signaling pathways such as HER2 and PI3K/AKT. These signaling interactions contribute to endocrine resistance, which remains a major clinical challenge in the treatment of ER-positive breast cancer. Progesterone receptor (PR) signaling further modulates estrogen-driven transcriptional activity, and PR status

serves as an essential indicator of functional ER signaling. The interplay between hormonal pathways and oncogenic signaling networks underscores the complexity of breast cancer pathogenesis (Musgrove & Sutherland, 2009).

2.1.1.4. Growth Factor Signaling Pathways

Dysregulated growth factor signaling is a key contributor to breast cancer development and progression. One of the most prominent alterations involves overexpression or amplification of human epidermal growth factor receptor 2 (HER2/ERBB2), which is detected in approximately 15–20% of breast cancer cases and is associated with aggressive tumor behavior and unfavorable clinical outcomes (Yarden & Sliwkowski, 2001). Activation of HER2 triggers multiple intracellular signaling cascades, particularly the MAPK and PI3K/AKT pathways. These pathways promote uncontrolled cell proliferation, enhanced survival, and increased metastatic potential (Pan et al., 2024). The identification of HER2-driven tumors has led to the development of targeted therapies that have significantly improved patient prognosis in this subgroup. Despite these advances, therapeutic resistance frequently emerges. Resistance mechanisms include activation of alternative growth factor pathways and mutations in downstream signaling components, which continue to pose major challenges in long-term disease control (Yarden & Sliwkowski, 2001).

2.1.1.5. Apoptosis and Cell Survival Pathways

One of the fundamental mechanisms underlying breast cancer development is the ability of tumor cells to evade apoptosis. Under physiological conditions, mammary epithelial cells undergo programmed cell death in response to irreparable DNA damage or oncogenic stress. In breast cancer, however, this protective mechanism is frequently disrupted, allowing abnormal cells to survive and accumulate (Reed, 2000). Alterations in apoptotic signaling often involve imbalances in the expression of BCL-2 family proteins. Overexpression of anti-apoptotic members such as BCL-2 and MCL-1 enhances tumor cell survival, whereas reduced activity of pro-apoptotic factors facilitates disease progression and resistance to therapy. In addition, impaired caspase activation and defects in death receptor–mediated pathways further weaken apoptotic responses in breast cancer cells. Because many anticancer treatments rely on restoring apoptosis, dysregulation of these pathways plays a critical role in both disease progression and treatment failure (Winder & Campbell, 2022).

2.1.1.6. Tumor Microenvironment and Immune Interactions

Breast cancer pathogenesis is strongly influenced by the tumor microenvironment (TME), which consists of cancer-associated fibroblasts, immune cells, endothelial cells, and extracellular matrix components. Rather than serving as a passive scaffold, the TME actively regulates tumor growth, invasion, and metastatic spread through complex cell-cell and cell-matrix interactions (Hanahan & Weinberg, 2011). Within the TME, chronic inflammation promotes genomic instability and supports tumor progression by releasing cytokines, chemokines, and growth factors that promote proliferation and angiogenesis. Immune surveillance initially acts to suppress tumor development; however, breast cancer cells can gradually evade immune control by altering antigen presentation and inducing immunosuppressive signaling. This dynamic interaction between tumor cells and immune components contributes to disease progression and has become a major focus in the development of immunotherapeutic strategies (De Visser & Joyce, 2023).

2.1.2. Biochemical Changes of the Body in Breast Cancer

Breast cancer is not only a localized malignancy of breast tissue but also a systemic disease that induces profound biochemical alterations throughout the body. These changes arise from complex interactions between tumor cells, host metabolism, immune responses, and endocrine regulation. As the disease progresses, disruptions in redox balance, energy metabolism, inflammatory signaling, lipid and protein homeostasis, and hormonal pathways become increasingly evident, reflecting both tumor burden and host adaptation to malignancy (Xiong et al., 2025).

2.1.2.1. Oxidative Stress and Redox Imbalance

Disruption of cellular redox balance represents a key biochemical alteration in breast cancer. Malignant cells generate excessive levels of reactive oxygen species (ROS) as a consequence of mitochondrial dysfunction, oncogenic signaling, chronic inflammation, and altered metabolic demands. This persistent ROS overproduction results in oxidative damage to DNA, lipids, and proteins, thereby facilitating mutagenesis, genomic instability, and tumor progression (Valko et al., 2006; Bel'skaya & Dyachenko, 2024). Both clinical and experimental studies have consistently reported elevated oxidative stress markers in breast cancer, including increased malondialdehyde (MDA) and protein carbonyl content. In parallel, the activity of endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase, is often diminished, indicating a compromised antioxidant defense mechanism. Together, these findings indicate a pronounced imbalance between oxidant production and antioxidant capacity in breast cancer

pathology (Tas et al., 2005; Halliwell, 2007; Bel'skaya & Dyachenko, 2024).

2.1.2.2. Lipid Metabolism Dysregulation

Lipid metabolism is markedly altered in breast cancer. Tumor cells display enhanced de novo lipogenesis to support membrane synthesis, signaling molecule production, and energy storage. The enzymes involved in lipid synthesis (e.g., fatty acid synthase (FASN) and acetyl-CoA carboxylase) are frequently overexpressed and have been linked to more aggressive disease and unfavorable clinical outcomes (Menendez & Lupu, 2007). At the systemic level, breast cancer is often accompanied by changes in circulating lipid profiles, including altered cholesterol, triglycerides, and lipoprotein levels. These alterations may reflect both tumor-driven metabolic demands and host inflammatory responses. Dysregulated lipid metabolism also contributes to oxidative stress through lipid peroxidation, further exacerbating cellular damage (Zipinotti dos Santos et al., 2023).

2.1.2.3. Protein Metabolism and Cachexia-Related Changes

In advanced breast cancer, protein metabolism is often disrupted, with enhanced protein breakdown and altered amino acid turnover contributing to muscle loss and cancer-related cachexia. Increased amounts of inflammatory cytokines such as TNF-α and IL-6 further accelerate muscle protein degradation and suppress protein synthesis (Fearon et al., 2012). Additionally, abnormal expression and post-translational modification of plasma proteins, including acute-phase reactants and transport proteins, have been observed in breast cancer patients. Proteomic studies have identified altered levels of albumin, transferrin, and C-reactive protein, highlighting systemic biochemical responses to malignancy and inflammation (Hanash et al., 2005).

2.1.2.4. Hormonal and Endocrine Alterations

Hormonal imbalance represents a key biochemical feature of breast cancer, particularly in hormone receptor-positive tumors. Estrogen promotes breast cancer development by activating estrogen receptor-dependent pathways that enhance cell growth and survival. Long-term exposure to endogenous or exogenous estrogen is a recognized risk factor for disease onset and progression (Missmer et al., 2004; Tin Tin et al., 2021). In addition to local estrogen signaling within breast tissue, breast cancer is associated with systemic endocrine disturbances. Alterations in circulating estrogen metabolites, insulin, insulin-like growth factor-1 (IGF-1), and adipokines

such as leptin and adiponectin have been reported. These changes are especially pronounced in obese individuals, in whom adipose tissue acts as a major source of estrogen production and pro-inflammatory cytokine release, thereby linking metabolic dysregulation to breast cancer pathophysiology (Cleary & Grossmann, 2009).

2.1.2.5. Inflammatory and Immune-Related Biochemical Changes

Persistent low-grade inflammation represents a key biochemical feature of breast cancer. Tumor progression promotes the production of inflammatory mediators, such as cytokines, chemokines, and growth factors, which reshape immune responses and facilitate disease advancement. Increased systemic concentrations of IL-6, IL-1β, and TNF-α have been associated with more advanced disease stages and unfavorable clinical outcomes (Balkwill & Mantovani, 2001; Coussens & Werb, 2002). Inflammation-driven biochemical changes also influence iron metabolism, coagulation pathways, and oxidative stress, further contributing to systemic complications in breast cancer patients (Karbakhsh Ravari et al., 2025; Obeagu & Maibouge Tanko, 2025). These immune-metabolic interactions highlight the interconnected nature of biochemical dysregulation in cancer.

2.1.2.6. Alterations in Energy Metabolism

Breast cancer involves metabolic reprogramming, with tumor cells preferentially utilizing glycolysis instead of oxidative phosphorylation, even in oxygen-sufficient environments, a phenomenon referred to as the Warburg effect. This metabolic shift results in higher glucose consumption, increased lactate formation, and changes in systemic metabolite profiles (Warburg, 1956; Vander Heiden et al., 2009). Systemically, breast cancer patients often exhibit altered serum glucose, lactate, and amino acid levels. This situation pictures increased tumor consumption and metabolic adaptation of the host. Additionally, changes in mitochondrial metabolism and tricarboxylic acid (TCA) cycle intermediates have been reported, due to the systemic nature of cancer-associated metabolic dysregulation (Pavlova & Thompson, 2016).

2.1.2.7. Alterations in Tissue Antioxidant and Detoxification Enzymes: CaE, GSH, and GST

Breast cancer is associated with significant changes in tissue-level antioxidant and detoxification systems. Enzymes involved in xenobiotic metabolism and redox

regulation, such as carboxylesterases (CaE), glutathione (GSH), and glutathione S-transferases (GST), play critical roles in maintaining cellular homeostasis and protecting tissues from oxidative and chemical damage (Laizure et al., 2013; Vašková et al., 2023). Dysregulation of these systems contributes to tumor progression, oxidative stress, and altered drug responses.

Carboxylesterases (CaEs) are a family of serine hydrolases involved in the detoxification of xenobiotics, lipid metabolism, and the activation or inactivation of ester-containing drugs. In cancerous tissues, altered CaE activity has been reported, reflecting disrupted detoxification capacity and metabolic reprogramming. Reduced CaE activity in tumor tissues may impair the hydrolysis and clearance of potentially harmful compounds, thereby increasing cellular susceptibility to oxidative damage and carcinogen accumulation. Conversely, aberrant CaE expression may also influence the local activation or inactivation of chemotherapeutic agents, contributing to variable treatment responses in breast cancer (Redinbo & Potter, 2005; Satoh & Hosokawa, 2006; Wen et al., 2025).

Glutathione (GSH), the most abundant intracellular non-enzymatic antioxidant, plays a central role in cellular defense against oxidative stress. GSH directly scavenges reactive oxygen species and serves as a critical cofactor for detoxification enzymes, including GST and glutathione peroxidase. In breast cancer tissues, GSH levels are frequently altered, with many studies reporting a depletion of GSH in tumor-bearing organs due to excessive ROS generation and increased antioxidant demand. Reduced GSH availability compromises redox balance, enhances lipid peroxidation, and promotes DNA damage, thereby facilitating tumor progression (Traverso et al., 2013). However, in specific tumor contexts, elevated intracellular GSH levels have also been observed, particularly in therapy-resistant cancer cells. Increased GSH content in malignant tissues may confer a survival advantage by enhancing antioxidant capacity and detoxification potential, thereby reducing sensitivity to chemotherapy-induced oxidative stress. This dual role highlights the complex and context-dependent nature of GSH regulation in breast cancer pathophysiology (Wu et al., 2004).

Glutathione S-transferases (GSTs) are a family of phase II detoxification enzymes that promote the elimination of electrophilic substances by catalyzing their conjugation with glutathione (Vašková et al., 2023). Numerous studies have reported dysregulated GST expression and activity in breast cancer tissues and experimental models. In particular, elevated levels of specific isoenzymes, such as GSTP1, have

been linked to enhanced detoxification potential, reduced sensitivity to chemotherapeutic drugs, and unfavorable clinical outcomes. Conversely, reduced GST activity in normal tissues surrounding tumors may exacerbate oxidative damage and inflammatory responses (Townsend & Tew, 2003; Hayes et al., 2005).

Collectively, dysregulation of CaE, GSH, and GST systems reflects a disturbed balance between oxidative stress generation and detoxification capacity in breast cancer. These biochemical alterations not only contribute to tumor initiation and progression but also influence therapeutic efficacy and toxicity. Therefore, evaluating tissue-specific changes in CaE activity, GSH levels, and GST expression provides valuable insight into breast cancer-associated oxidative stress, detoxification mechanisms, and potential targets for adjunctive therapeutic strategies.

2.1.3. Histopathology of Breast Cancer

Histopathological evaluation remains fundamental to the diagnosis, classification, prognostic assessment, and therapeutic planning of breast cancer. Despite remarkable advances in molecular and genomic profiling, microscopic examination of breast tissue remains the cornerstone of breast cancer evaluation. Histopathological analysis provides essential information regarding tumor type, grade, invasion patterns, lymphovascular involvement, and margin status, all of which directly influence clinical decision-making.

The normal breast is organized into terminal duct-lobular units (TDLUs), composed of epithelial cells arranged in ducts and lobules and supported by fibrous and adipose stroma. These epithelial units are composed of a luminal epithelial layer surrounded by a myoepithelial cell layer. The intact presence of the myoepithelium, together with the basement membrane, is a key histological criterion for distinguishing non-invasive breast lesions. Malignant transformation occurs when neoplastic epithelial cells penetrate the basement membrane and infiltrate the surrounding stroma, marking the transition from in situ to invasive carcinoma (Lakhani et al., 2012; Tan et al., 2020).

Non-invasive breast carcinomas include ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS). DCIS is characterized by malignant epithelial proliferation confined to the ductal-lobular system with intact basement membrane and myoepithelial cells. It displays variable architectural patterns, such as solid, cribriform, papillary, and comedo types. High nuclear grade and central necrosis,

particularly in comedo-type DCIS, are associated with a greater likelihood of progression to invasive disease (Allred, 2010). LCIS, in contrast, is composed of small, dyscohesive cells filling and expanding lobules, often lacking E-cadherin expression. It is considered both a marker of increased breast cancer risk and a non-obligate precursor lesion, frequently detected incidentally and often presenting as multifocal or bilateral disease (Rakha et al., 2010).

Histological grading is a critical prognostic component in breast cancer pathology. The Nottingham grading system evaluates tubule formation, nuclear pleomorphism, and mitotic count to classify tumors into grades I, II, or III. Higher histological grade correlates with increased tumor aggressiveness, higher proliferative activity, and poorer clinical outcomes (Elston & Ellis, 1991). Additional histopathological parameters with prognostic relevance include lymphovascular invasion, tumor necrosis, stromal reaction, and surgical margin status. Lymphovascular invasion is associated with an increased risk of regional and distant metastasis, whereas clear surgical margins are essential for effective local disease control (Houvenaeghel et al., 2021; Lee et al., 2023).

Immunohistochemistry (IHC) serves as an essential adjunct to routine histopathology. Evaluation of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression is an essential component of invasive breast cancer assessment, as these markers play a key role in determining therapeutic strategies and predicting clinical outcomes (Wolff et al., 2018; Allison et al., 2020). Proliferation markers such as Ki-67 provide additional prognostic information and are used alongside histological and molecular features to classify tumors into intrinsic-like subtypes. IHC is also critical for identifying myoepithelial cells using markers such as p63 and smooth muscle myosin, enabling reliable distinction between in situ and invasive lesions and aiding in the characterization of special histological variants (Dowsett et al., 2011; Nielsen et al., 2021).

The breast cancer microenvironment significantly influences tumor behavior and progression. Histopathological analysis reveals dynamic interactions between malignant cells and surrounding stroma, including cancer-associated fibroblasts, immune infiltrates, and extracellular matrix remodeling. Tumor-infiltrating lymphocytes (TILs), particularly in triple-negative and HER2-positive breast cancers, have emerged as important prognostic and predictive indicators, reflecting the host immune response to the tumor (Maley et al., 2025).

2.2. Risk Factors for Breast Cancer

Breast cancer is a multifactorial disease whose onset and progression are shaped by the combined influence of genetic susceptibility, hormonal exposure, environmental conditions, and lifestyle-related factors. Identifying these risk determinants is essential for effective prevention strategies, risk assessment, and early diagnosis. Among non-modifiable risk factors, sex and age are the most significant. Breast cancer predominantly affects women, and its incidence increases markedly with advancing age, reflecting cumulative genetic damage and prolonged hormonal exposure. Inherited genetic mutations also contribute substantially to disease risk. Germline alterations in high-penetrance genes such as BRCA1 and BRCA2 markedly increase lifetime breast cancer risk, while mutations in additional susceptibility genes, including TP53, PTEN, and PALB2, account for a smaller yet significant proportion of hereditary cases (Mavaddat et al., 2010; Roy et al., 2012).

Reproductive and hormonal factors that extend lifetime exposure to estrogen are well-established contributors to breast cancer risk. Early onset of menarche, delayed menopause, nulliparity, and late age at first full-term pregnancy are associated with increased susceptibility, mainly due to sustained estrogen-driven proliferation of mammary epithelial cells (Collaborative Group on Hormonal Factors in Breast Cancer, 2012).

Several modifiable lifestyle factors play a critical role in breast cancer development. Obesity, particularly after menopause, is strongly linked to higher breast cancer incidence. Higher body mass index in premenopausal women may exert a modest protective effect, underscoring the complexity of the adiposity-breast cancer relationship (Cleary & Grossmann, 2009).

Alcohol consumption is one of the most consistently documented lifestyle-related risk factors. Even moderate intake has been shown to elevate breast cancer risk in a dose-dependent manner, potentially through mechanisms involving increased estrogen levels, oxidative stress, and DNA damage. Physical inactivity further exacerbates risk by contributing to weight gain, insulin resistance, and chronic low-grade inflammation (Boyle & Boffetta, 2009; Fanfarillo et al., 2024).

Exposure to ionizing radiation, especially during adolescence and early adulthood when breast tissue is highly sensitive, significantly increases breast cancer

risk. This association is well established in individuals exposed to atomic bomb radiation and in patients who received chest radiotherapy at a young age for conditions such as Hodgkin lymphoma (Ronckers et al., 2005).

Use of exogenous hormones also influences breast cancer risk. Long-term use of combined estrogen-progestin hormone replacement therapy is associated with an apparent increase in breast cancer incidence. In contrast, estrogen-only therapy appears to carry a lower risk in women who have undergone hysterectomy. Oral contraceptive use has been linked to a slight, transient increase in risk during current or recent use, which diminishes after cessation (Chlebowski et al., 2010; Chlebowski et al., 2015).

High mammographic breast density is another well-established independent risk factor for breast cancer, with women exhibiting dense breast tissue facing a markedly higher risk than those with predominantly fatty breasts. This association is attributed to the increased amount of epithelial and stromal components that are more vulnerable to malignant change. Moreover, a prior diagnosis of benign proliferative breast lesions or a history of breast cancer substantially increases the likelihood of subsequent disease (Boyd et al., 2007).

2.3. Nutrition and Breast Cancer

Nutrition is a key determinant of breast cancer risk, disease progression, and survivorship, acting through its influence on hormonal regulation, metabolic homeostasis, oxidative stress, inflammation, and immune function. A substantial body of epidemiological and experimental evidence indicates that both overall dietary patterns and individual nutritional components can modulate breast cancer susceptibility and may also affect clinical outcomes following diagnosis (Clinton et al., 2020). In breast cancer survivors, adequate nutritional status is particularly important for lowering the risk of recurrence, alleviating treatment-related adverse effects, and enhancing quality of life. Accordingly, nutritional strategies emphasizing body weight control, balanced macronutrient distribution, and sufficient micronutrient intake are increasingly recognized as integral elements of comprehensive cancer care (Coussens & Werb, 2002; Cleary & Grossmann, 2009; Rock et al., 2020).

Among nutrition-related determinants, energy balance and body weight represent some of the most influential factors associated with breast cancer. Excess

caloric intake leading to obesity, especially in postmenopausal women, is consistently linked to increased breast cancer incidence. After menopause, adipose tissue becomes a major site of estrogen production and a source of pro-inflammatory cytokines and adipokines that support tumor initiation and progression. In contrast, maintaining a healthy body weight through appropriate diet and physical activity is associated with reduced breast cancer risk and improved prognosis (Cleary & Grossmann, 2009; Clinton et al., 2020).

The contribution of dietary fat to breast cancer has been widely examined. While total fat intake alone shows variable associations, the quality of fat appears to be more relevant. Diets rich in saturated and trans fats have been linked to chronic inflammation and insulin resistance, whereas diets containing higher proportions of monounsaturated and omega-3 polyunsaturated fatty acids, such as those found in olive oil, nuts, and fatty fish, are associated with anti-inflammatory effects and potential protective roles against breast cancer (Chajès & Romieu, 2014).

Carbohydrate quality also plays an important role in breast cancer development. High consumption of refined carbohydrates and diets with a high glycemic index may elevate breast cancer risk by promoting hyperinsulinemia and enhanced insulin-like growth factor-1 (IGF-1) signaling. Insulin and IGF-1 stimulate cell growth and inhibit apoptosis, thereby creating a metabolic environment favorable for tumor growth. Conversely, diets rich in whole grains, dietary fiber, and low-glycemic foods are associated with improved insulin sensitivity and reduced inflammatory burden (Gunter et al., 2009).

Oxidative stress is a central mechanism in breast cancer pathogenesis, contributing to DNA damage and genomic instability. Diets abundant in antioxidant-rich foods, including fruits, vegetables, legumes, and whole grains, provide vitamins, carotenoids, polyphenols, and trace elements that strengthen endogenous antioxidant defenses and help limit oxidative damage. These bioactive compounds exert protective effects through redox regulation and modulation of cell signaling pathways relevant to cancer development (Bel'skaya & Dyachenko, 2024; Li et al., 2025). However, evidence on high-dose antioxidant supplementation remains inconclusive, particularly during active cancer treatment, as excessive antioxidant intake may compromise the therapeutic efficacy of chemotherapy or radiotherapy. Consequently, antioxidants obtained from whole foods rather than supplements are generally recommended (Rock et al., 2020).

Rather than focusing on isolated nutrients, growing evidence underscores the importance of overall dietary patterns. There is growing evidence that some diet patterns, such as the Mediterranean diet, have been associated with reduced breast cancer risk and improved survival among patients. These dietary patterns exert synergistic effects by concurrently modulating inflammation, oxidative stress, and metabolic pathways involved in tumor progression (Schwingshackl et al., 2017).

2.3.1. Herbal Teas

Herbal teas are widely consumed for their perceived health benefits and are frequently used by patients with breast cancer as complementary approaches during or after conventional treatment. However, evidence regarding their efficacy and safety in breast cancer remains heterogeneous and highly dependent on the specific plant species, preparation methods, and bioactive constituents involved. Consequently, careful evaluation of their biological effects and potential interactions with standard therapies is essential (Ross & Kasum, 2002; Poswal et al., 2019).

Herbal teas and plant extracts are commonly employed as complementary, not alternative, strategies in breast cancer management (Sartippour et al., 2006). While they should not replace evidence-based oncological treatments, certain herbal preparations may contribute to overall well-being, symptom relief, and supportive care when used appropriately. Nonetheless, clinical evidence supporting direct anticancer efficacy in humans remains limited, and benefits observed in vitro or in animal models do not always translate to clinical outcomes (Li et al., 2017; Poswal et al., 2019).

Several herbal teas and botanicals, including green tea, white tea, olive leaf, turmeric, flaxseed, rosemary, and burdock, have been investigated for their potential roles in breast cancer prevention or as adjuncts to conventional therapy. Among these, green tea catechins, particularly epigallocatechin-3-gallate (EGCG), have received considerable attention for their ability to modulate estrogen signaling, inhibit tumor cell proliferation, and induce apoptosis in breast cancer models (Sartippour et al., 2006). Olive leaf derived phenolics such as oleuropein and hydroxytyrosol have also demonstrated antioxidant, anti-inflammatory, and pro-apoptotic effects in experimental systems (Chajès & Romieu, 2014; Gorzynik-Debicka et al., 2018).

At the molecular level, certain phytochemicals derived from medicinal plants

have shown the capacity to interact with hormone receptors and apoptosis-related pathways. For example, plant-derived compounds with affinity for estrogen or progesterone receptors may influence hormone-dependent breast cancer signaling. In contrast, others can activate intrinsic apoptotic pathways by modulating caspases, p53, and BCL-2 family proteins in experimental models. Although these findings highlight promising mechanistic pathways, they largely stem from preclinical studies and should be interpreted cautiously in the clinical context (Reed, 2000; Schroeder et al., 2009). Therefore, integration of herbal teas into breast cancer care should be guided by evidence-based recommendations, clinician oversight, and careful consideration of potential risks and benefits.

2.3.2. Olive Leaf Tea

Olive leaf tea, prepared from the leaves of Olea europaea L., is a rich source of bioactive compounds, including oleuropein, hydroxytyrosol, flavonoids, and triterpenes, which collectively contribute to its broad spectrum of biological activities (Shahidi & Ambigaipalan, 2015; Aktas et al., 2025). These phytochemicals have been extensively studied for their antioxidant, anti-inflammatory, antihypertensive, hypoglycemic, and hypocholesterolemic properties, underpinning the long-standing use of olive leaf preparations as traditional remedies in Mediterranean regions (El & Karakaya, 2009).

The phenolic constituents of olive leaves exhibit potent antioxidant activity and have been shown to reduce oxidative stress and inflammation, two key processes implicated in metabolic disorders and cancer pathogenesis. In addition, olive leaf polyphenols demonstrate antiglycation properties by inhibiting the formation of advanced glycation end-products, largely through their capacity to scavenge reactive oxygen species and modulate inflammatory signaling pathways (Silvestrini et al., 2023; Vasarri et al., 2024).

Although numerous in vitro studies report promising biological effects, further in vivo investigations and well-designed clinical trials are required to fully elucidate the metabolic fate, bioavailability, and therapeutic efficacy of olive leaf bioactives in humans. Olive leaf tea and its derivatives, such as standardized extracts and powders, have been explored for potential applications in chronic conditions, including cancer, diabetes, and cardiovascular diseases. Nevertheless, the precise molecular mechanisms and long-term health effects of sustained olive leaf tea consumption in both healthy individuals and patients remain incompletely

understood (Milanizadeh et al., 2014; Boss et al., 2016; Ferdousi et al., 2019).

Emerging preclinical evidence suggests that olive leaf tea and its major phenolic compounds may exert anticancer effects, particularly in breast cancer models, through multiple histopathological, molecular, biochemical, and cellular mechanisms. Oleuropein, one of the most abundant secoiridoids in olive leaves, has been shown to induce apoptosis and cause S-phase cell cycle arrest in triple-negative breast cancer cells, accompanied by alterations in gene expression profiles related to cell survival and proliferation. These findings indicate a potential role for oleuropein in targeting aggressive breast cancer subtypes at the molecular level (Messeha et al., 2020). In addition, olive leaf extracts have demonstrated synergistic anticancer effects when combined with established metabolic drugs such as metformin. In estrogen receptor-positive MCF-7 breast cancer cells, combined treatment resulted in reduced cell viability through activation of distinct apoptotic pathways, suggesting that olive leaf-derived compounds may enhance the efficacy of conventional therapeutic agents (Isleem et al., 2020). Similar observations have been reported for other phenolic-rich plant extracts, supporting the concept that polyphenols can sensitize cancer cells to standard treatments by modulating redox balance and apoptotic signaling (Oriol-Caballo et al., 2025).

Evidence from animal models further supports the anticancer potential of olive leaf extracts. In vivo studies have demonstrated that olive leaf–derived compounds can reduce tumor volume, suppress angiogenesis, and enhance apoptosis through caspase activation and regulation of pro- and anti-apoptotic proteins. These effects highlight the capacity of olive leaf bioactives to interfere with tumor growth and vascularization, which are critical processes in cancer progression (Milanizadeh & Bigdeli, 2019).

2.4. Ehrlich Ascites Carcinoma (EAC) Model

Experimental models of ascitic tumors are extensively utilized in cancer research, particularly for examining tumor growth kinetics, host-tumor interactions, and therapeutic responses in controlled in vivo environments. The Ehrlich Ascites Carcinoma (EAC) (syn: Ehrlich Ascites Tumor, EAT) model is among the most established and well-characterized transplantable murine tumor models. It is frequently used in studies of cancer biology, inflammation, angiogenesis, and cancer drug screening (Calixto-Campos et al., 2013; Saleh et al., 2022; Radulski et al., 2023).

EAC originated from a spontaneous murine mammary adenocarcinoma and has been maintained through serial intraperitoneal transplantation in mice (Ehrlich & Apolant, 1905; Loewenthal & Jahn, 1932). It is classified as an undifferentiated carcinoma with high transplantability, rapid proliferation rate, and aggressive biological behavior. When inoculated intraperitoneally, EAC cells proliferate freely within the peritoneal cavity, leading to the accumulation of ascitic fluid rich in tumor cells, inflammatory mediators, and metabolic by-products (Andersson & Agrell, 1972; Ozaslan et al., 2011; Feitosa et al., 2021).

One of the defining advantages of the EAC model is its short and predictable tumor latency, with untreated hosts typically succumbing within 15 to 20 days post-inoculation. The formation of ascites is driven by increased vascular permeability, impaired lymphatic drainage, and pronounced inflammatory responses (Hartveit, 1965). These factors collectively contribute to a nutrient-rich microenvironment that fosters tumor growth and facilitates immune evasion, mirroring characteristics observed in advanced human cancers (Ertekin et al., 2016; Mello-Andrade et al., 2017).

Histologically, EAC ascitic fluid is rich in viable tumor cells and inflammatory components, further emphasizing its relevance as a model for malignant ascites (Gupta et al., 2004). In addition to its primary function as an ascitic tumor model, EAC displays metastatic behavior when introduced through alternative routes, with tumor infiltration observed in organs such as the liver, spleen, lungs, and lymph nodes. EAC-bearing animals often exhibit systemic effects, including oxidative stress, immune suppression, anemia, cachexia, and metabolic disruptions, reflecting the multi-systemic impact of advanced malignancy (Gupta et al., 2004; Mello-Andrade et al., 2017).

The EAC model has been widely used in preclinical investigations of synthetic chemotherapeutics, natural compounds, and nanomedicine-based therapies. Researchers commonly measure endpoints such as ascitic fluid volume, total tumor cell count, survival duration, and histopathological alterations in vital organs. This model is also instrumental in exploring apoptosis-related mechanisms, including caspase activation, mitochondrial dysfunction, and BCL-2 family protein interactions (Baral & Chattopadhyay, 2004; Gupta et al., 2004; Gumushan & Musa, 2008; Alotaibi et al., 2020; Hashem et al., 2020; Mohamed, 2021; Saleh et al., 2022).

3. MATERIALS AND METHODS

3.1. Animals

Fifty-four mice were utilized in this study. The animals were housed in standard laboratory conditions (4 mice per cage) with a 12-hour light/dark cycle and an ambient temperature of 22 ± 4°C. Throughout the experiment, all mice had ad libitum access to tap water and standard laboratory feed. The study was conducted at the Animal Experiment Application and Research Center, Harran University (Şanlıurfa, Türkiye), between September and November 2020. This study was approved by Harran University Animal Experiments Local Ethics Committee with number 2019/002/02.

3.2. Preparation of Olive Leaf Tea

To prepare olive leaf tea (OLT), 250 mg of dried olive leaves were manually crushed and infused in 5 mL of distilled water (boiled and then cooled to 95°C) for 10 minutes, yielding a final concentration of 50 mg/mL. The tea was filtered through sterile filter paper and further sterilized using a 0.45 μm syringe-tip bacteriological filter. The administered OLT volume was adjusted to 400 mg/kg body weight per mouse, calculated based on daily weight measurements. Considering our in vitro experimental results, a concentration of 400mg/kg, much lower than the LD_{50} values reported in the literature (Duke, 2002: 3000 mg/kg, Amabeoku and Bamuamba, 2010: 3475 mg/kg), was selected (Duke, 2002; Amabeoku and Bamuamba, 2010). Freshly prepared OLT was administered daily via gastric gavage at the same time each day.

3.3. Experimental Design

The Ehrlich ascites carcinoma (EAC) model was established by intraperitoneal inoculation of 3×10^5 EAC cells into each mouse (Gumushan & Musa, 2008). Different experimental groups received either olive leaf tea (OLT), balanced salt solution (BSS), 5-Fluorouracil (5-FU), or a combination of both, as detailed in Table 1.

Commercially available 5-FU was injected intraperitoneally as a single dose at 20 mg/kg body weight (Hossain et al., 2012). The injection volume for each mouse was adjusted according to its current body weight, as specified in Table 1.

Table 3.1. The groups used for the experiments

Groups		Regime	Number of mice
Sham control		No operation	4
Untreated control (EAC)		3×10^5 EAC cells were inoculated intraperitoneally (i.p) on day 0	7
BSS (healthy) controls	BSS (8d)	No tumor + Balanced Salt Solution (BSS) was injected into periton on day 0 + OLT (400 mg/kg/day) was given orally, day 0-7 (for 8 days)	4
	BSS (15d)	No tumor + Balanced Salt Solution (BSS) was injected into periton on day 0 + OLT (400 mg/kg/day) was given orally, day 0-14 (for 15 days)	4
Protective effect	OLT (15d)	OLT (400 mg/kg/day) was given orally, day 0-14 (for 15 days) + 3×10^5 EAC cells were inoculated (i.p.) on day 7	7
Tumor initiation	OLT (8d)	3×10^5 EAC cells were inoculated (i.p.) on day 0 + OLT (400 mg/kg/day) was given orally, day 0-7 (for 8 days)	7
Tumor growth	OLT (6d)	3×10^5 EAC cells were inoculated (i.p.) on day 0 + olive leaf tea (OLT, 400 mg/kg/day) was given orally, day 2-7 (for 6 days)	7
	5-FU (Positive control)	3×10^5 EAC cells were inoculated (i.p) on day 0 + 5-fluorouracil (5-FU) was injected into periton on day 2 (single dose)	7
	5-FU + OLT (6d)	3×10^5 EAC cells were inoculated (i.p) on day 0 + 5-fluorouracil (5-FU) was injected into periton on day 2 (single dose) + OLT (400 mg/kg/day) was given orally, day 2-7 (for 6 days)	7

3.4. Biochemical Experiments

3.4.1. Tissue Sample Preparation

Liver, brain, and kidney tissues from rats were weighed and blended using a Teflon-headed homogenizer (Heidolph RZR 2021) in a potassium phosphate buffer (0.1 M, made from 0.5 M K_2HPO_4 and 0.5 M KH_2PO_4) at pH 7.4. The homogenates were centrifuged at 16,000 × g for 20 min at 4 °C (Hettich 460 R). The supernatant was collected after centrifugation and transferred to clean microcentrifuge tubes. Enzyme activity in the postmitochondrial fractions was then examined. Total protein content and enzyme activity readings were performed in three replicates using a microplate reader (Thermo Varioscan Flash 2000). Without refreezing the assay samples, all of the assays were completed on the same day.

3.4.2. Determination of Activity and Levels of Enzymatic and Non-enzymatic Biomarkers

All enzyme activity and total protein measurement procedures were performed using a microplate reader system (Thermo, Varioscan Flash 2000) immediately after tissue homogenization and centrifugation, without waiting for the samples to be processed. In enzyme activity and total protein measurements, three repeated absorbance readings were performed for each sample. When a correlation difference greater than 10% was observed between the values obtained for the same sample, the reading procedure was repeated. The activities of all enzymes were expressed in terms of specific activity (nmol/min/mg total protein) after measuring the total protein levels in the samples.

3.4.2.1. Carboxylesterase (CaE) Activity

To measure CaE activity, the protocol of Santhoshkumar and Shivanandappa (1999) was modified to work with a microplate reader. PNPA (p-nitrophenol acetate) was used as the substrate. Two hundred fifty microliters of Trizma buffer (pH 7.4) with 5 µL of sample were placed in microplate wells and incubated at 25 °C for 3 min. Five microliters of PNPA were pipetted onto this mixture, and the absorbance value at 405 nm was measured.

3.4.2.2. Glutathione S-Transferase (GST) Activity

GST activity was measured as the enzymes responsible for detoxification. To determine the GST activity, the method developed by Habig et al. (1974) was used, adapted for use with a microplate reader. CDNB (1-chloro-2,4-dinitrobenzene) and reduced glutathione were used as the substrate and cofactor, respectively. A mixture of 100 µL of GSH, 100 µL of phosphate buffer, 10 µL of CDNB, and 10 µL of supernatant was pipetted into microplate wells, and an absorbance reading was performed at 344 nm.

3.4.3. Determination of Reduced Glutathione (GSH) Level

The GSH level was determined according to the method developed by Moron et al. (1979), adapted for use with a microplate reader system. The GSH level was calculated as nmol GSH mg protein^{-1} by reading the absorbance value of the samples at 412 nm against the GSH standard curve.

3.4.4. Total Protein Analysis

Total protein concentrations were determined according to the Bradford (1976) method. In this method, after the supernatants of the samples were diluted, 5 µL of them were pipetted into microplate wells, and 250 µL of Bradford solution was added. The reaction mixture was incubated at room temperature in the dark for 15 minutes. The absorbance value was measured at a 595 nm wavelength, depending on the color change. Then, the total protein values in the supernatants were calculated by taking the dilution factor into account. The activities of all enzymes were expressed in terms of specific activity (nmol min^{-1} mg total protein^{-1}) after measuring the total protein levels.

3.5. Histopathologic Evaluation

Tissues, including the liver, stomach, small intestine (duodenum), kidneys, and bladder, obtained from experimental groups, were evaluated histopathologically using light microscopy. According to the tissue staining protocol optimized in our laboratory based on Cardiff et al. (2014), tissue sections were stained using a standard hematoxylin and eosin (H&E) protocol. Briefly, after fixation in 10% neutral buffered formalin, tissues were processed into paraffin-embedded sections (4 μm thickness). The slides were rehydrated through a graded alcohol series (95% to 50%) and rinsed in distilled water. Nuclei were stained by immersing the slides in hematoxylin for 5 min, then rinsing under running water. Cytoplasmic counterstaining was performed using eosin (1 min). Excess stain was removed by washing under running water. The slides were then dehydrated through ascending graded alcohols (70% to 96%). Subsequently, the slides were cleared in aceton and xylene and mounted with entellan under a 22 × 50 mm coverslip. Stained sections were examined under a microscope, and images were captured for analysis.

3.6. Statistical Analysis

Statistical evaluations were conducted using GraphPad Prism software (version 10.5.0, GraphPad Software, USA). The distribution of data was first assessed with the Shapiro-Wilk test. For datasets following a normal distribution, either one-way ANOVA with Tukey's multiple comparison procedure or an unpaired Student's *t*-test was employed. In cases where the data were non-normally distributed, the Kruskal-Wallis test was used, followed by Dunn's multiple comparison test.

4. FINDINGS

4.1. Biochemical Parameters Results

4.1.1. Liver

Biochemical analysis of liver tissue samples revealed significant differences in detoxification and antioxidant markers, including Glutathione S-transferase (GST), Carboxylesterase (CaE), and Glutathione (GSH), in response to olive leaf tea (OLT) in different treatment regimes.

As presented in Table 4.1. and Figure 4.1., CaE enzyme activity was measured as 1630.27 nmol min^{-1} mg $protein^{-1}$ and 1580.31 nmol min^{-1} mg $protein^{-1}$ in the untreated control and the sham control groups, respectively. An increase was observed in all groups compared to sham and untreated controls ($p<0.05$-0.0001). The OLT (15d) (1798.41 nmol min^{-1} mg $protein^{-1}$) and OLT (6d) groups (1898.14 nmol min^{-1} mg $protein^{-1}$) showed inhibition compared to the BSS control groups ($p<0.0001$) (15d: 1883.98 nmol min^{-1} mg $protein^{-1}$, and 8d: 2571.75 nmol min^{-1} mg $protein^{-1}$).

In Figure 4.1., asterisks indicate statistically significant differences between groups (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$).

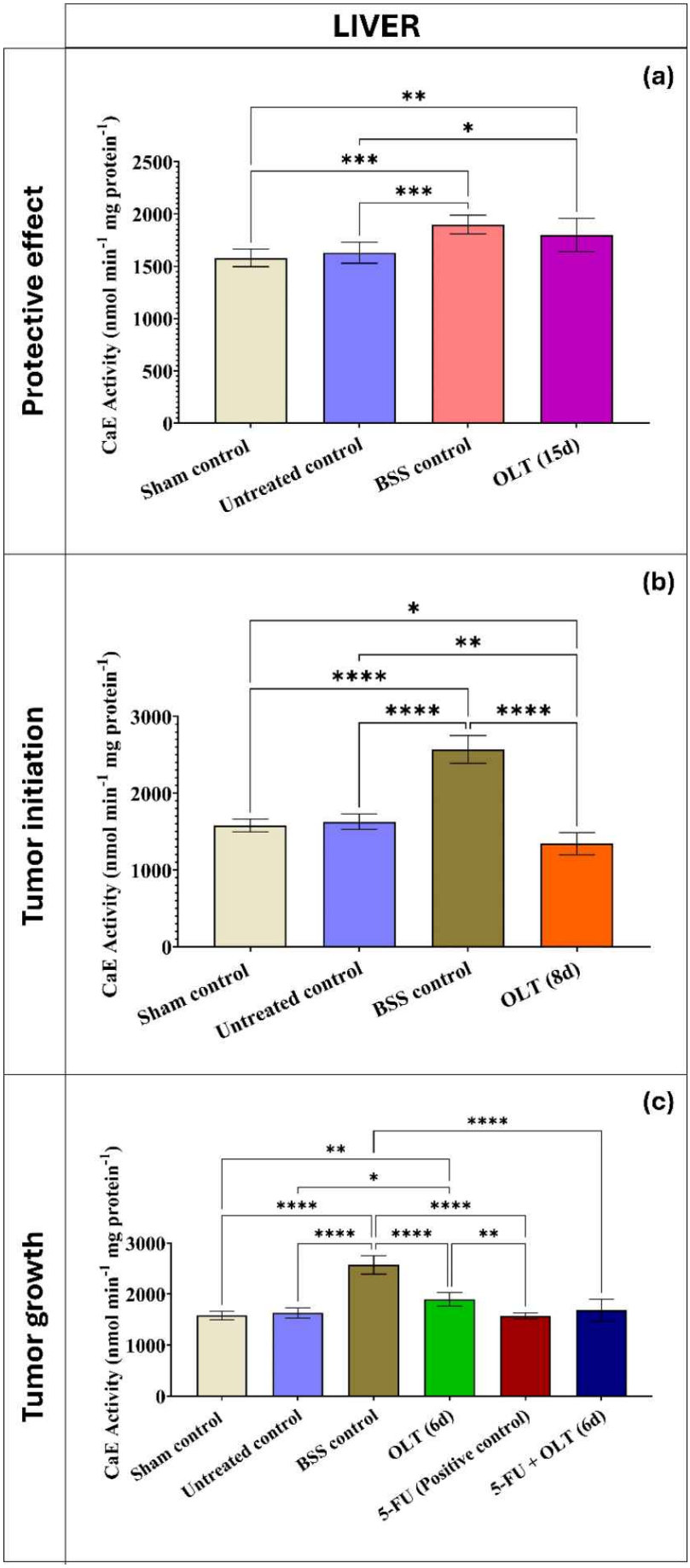
LIVER
Protective effect
(a)
CaE Activity (nmol min-1 mg protein-1)
2500
2000
1500
1000
500
0
**
*
***
***
Sham control
Untreated control
BSS control
OLT (15d)
Tumor initiation
(b)
CaE Activity (nmol min-1 mg protein-1)
3000
2000
1000
0
*
**
****
****
****
Sham control
Untreated control
BSS control
OLT (8d)
Tumor growth
(c)
CaE Activity (nmol min-1 mg protein-1)
3000
2000
1000
0
****
**
*
****
****
****
****
**
Sham control
Untreated control
BSS control
OLT (6d)
5-FU (Positive control)
5-FU + OLT (6d)

Figure 4.122. CaE levels measured in the liver tissues of mice treated with 400 mg/kg OLT for (a) 15, (b) 8, or (c) 6 days.

Table 4.1. Biochemical parameter [Carboxylesterase (CaE), Glutathione S Transferase (GST), Glutathione (GSH)] levels measured in the liver tissues of mice treated with 400 mg/kg olive leaf tea (OLT) for (a) 15, (b) 8, or (c) 6 days.

Groups	Biochemical Parameters		
	CaE	GST	GSH
Sham control	1580.31 ± 31.63	98.33 ± 3.30	0.121 ± 0.007
Untreated control	1630.27 ± 38.22	105.58 ± 3.53	0.067 ± 0.004
BSS Control (8d)	2571.75 ± 67.85^{cz}	116.62 ± 3.49^{a}	0.121 ± 0.006^{z}
BSS Control (15d)	1883.98 ± 42.60^{cz}	93.39 ± 2.52	0.073 ± 0.004^{cz}
5-FU (Positive control)	1575.36 ± 22.62	70.97 ± 2.67^{cz}	0.136 ± 0.017^{z}
OLT (6d) (Tumor growth)	1345.66 ± 54.74^{ay}	111.52 ± 5.40	0.08 ± 0.004^{c}
5-FU + OLT (6d)	1638.30 ± 81.48	83.19 ± 4.70^{az}	0.083 ± 0.011^{a}
OLT (8d) (Tumor initiation)	1345.66 ± 54.74^{ay}	111.52 ± 5.40	0.08 ± 0.004^{c}
OLT (15d) (Protective effect)	1798.41 ± 60.29^{bx}	142.82 ± 7.96^{cz}	0.094 ± 0.006

Data are expressed as mean ± standard error (n = 7). CaE and GST activities were expressed as nmol min^{-1} mg protein^{-1}, and GSH levels were expressed as nmol GSH mg protein^{-1} ± standard error of the mean.
Statistically significant difference compared to the sham control group a:$p<0.05$, b:$p<0.01$, c:$p<0.001$
Statistically significant difference compared to the untreated control group x:$p<0.05$, y:$p<0.01$, z:$p<0.001$

GST activity in liver tissue showed no difference between sham and untreated controls ($p>0.05$) (Table 4.1., Figure 4.2.). GST activity was measured as 105.58 nmol min^{-1} mg protein^{-1} in the untreated control group. This value was relatively higher than the sham control value of 98.33 nmol min^{-1} mg protein^{-1}. In the protective effect group, the OLT (15d) group showed a significant increase (142.82 nmol min^{-1} mg protein^{-1}; $p<0.0001$). As a result of administering OLT to healthy individuals (BSS control group) for 15 days, the enzyme activity was found to be slightly lower than the sham control value of 93.39 nmol min^{-1} mg protein^{-1} ($p>0.05$). OLT (6d) had a similar value (97.37 nmol min^{-1} mg protein^{-1}; $p>0.05$) to the sham control. Marked reductions were observed in the 5-FU (70.97 nmol min^{-1} mg protein^{-1}; $p<0.0001$) and 5-FU + OLT (6d) (83.19 nmol min^{-1} mg protein^{-1}; $p<0.001$) groups. In Figure 4.2., asterisks indicate statistically significant differences between groups (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$).

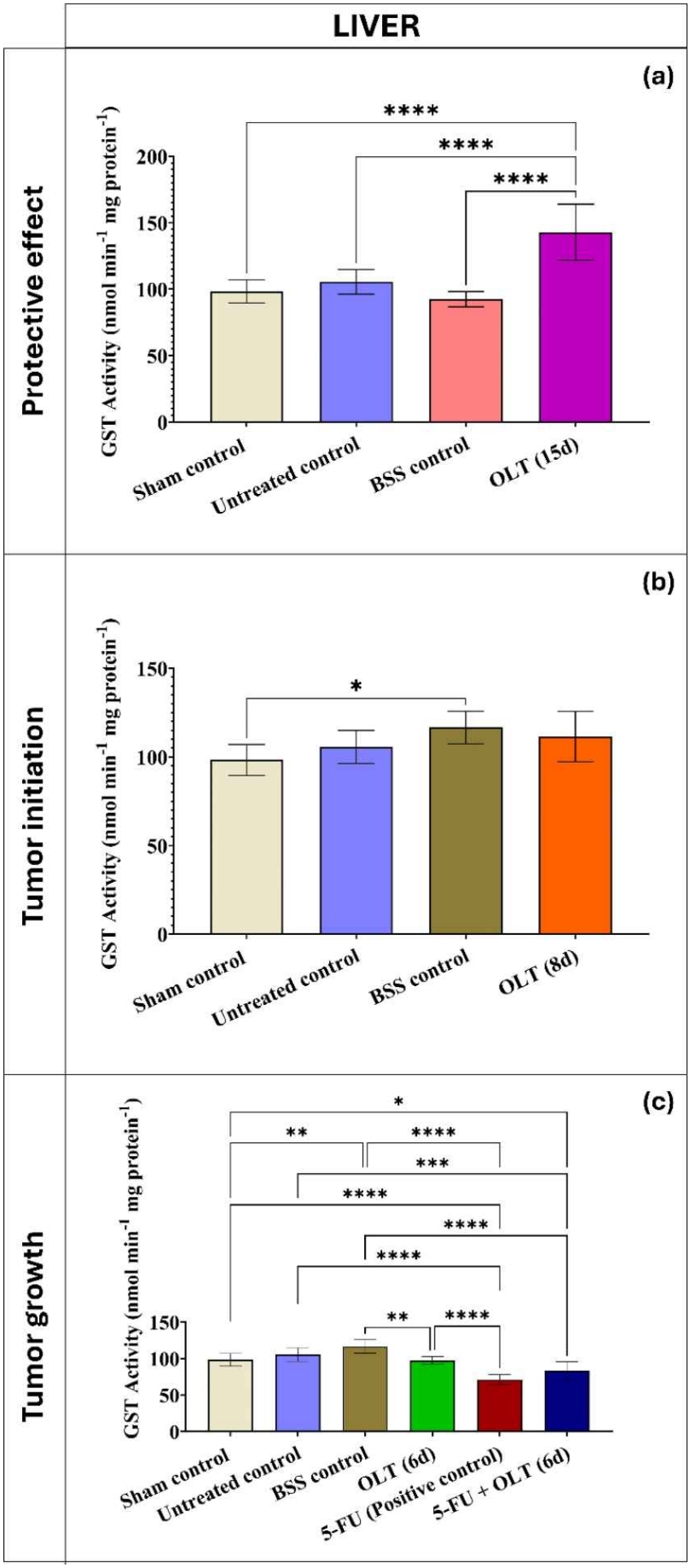
LIVER
Protective effect
(a)
GST Activity (nmol min-1 mg protein-1)
****
****
****
200
150
100
50
0
Sham control
Untreated control
BSS control
OLT (15d)
Tumor initiation
(b)
GST Activity (nmol min-1 mg protein-1)
*
150
100
50
0
Sham control
Untreated control
BSS control
OLT (8d)
Tumor growth
(c)
GST Activity (nmol min-1 mg protein-1)
*
**
****
***
****
****
****
**
****
150
100
50
0
Sham control
Untreated control
BSS control
OLT (6d)
5-FU (Positive control)
5-FU + OLT (6d)

Figure 4.123. GST levels measured in the liver tissues of mice treated with 400 mg/kg OLT for (a) 15, (b) 8, or (c) 6 days.

The GSH level, which was determined to be 0.121 nmol GSH mg protein^{-1} in the sham control group, decreased dramatically in the untreated control group with tumors and was recorded as 0.067 nmol GSH mg protein^{-1} ($p<0.0001$) (Table 4.1., Figure 4.3.). When OLT was given to healthy individuals for 8 days, the GSH level remained the same as that of the sham group. When OLT was continued for 15 days, the GSH level (0.073 nmol GSH mg protein^{-1}) was found to decrease compared to the sham group and approached the level of the untreated control. When tumor-bearing animals were treated with OLT for 15 days (0.099 nmol GSH mg protein^{-1}), GSH level increased significantly compared to untreated control ($p<0.05$). In Figure 4.3., asterisks indicate statistically significant differences between groups (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$).

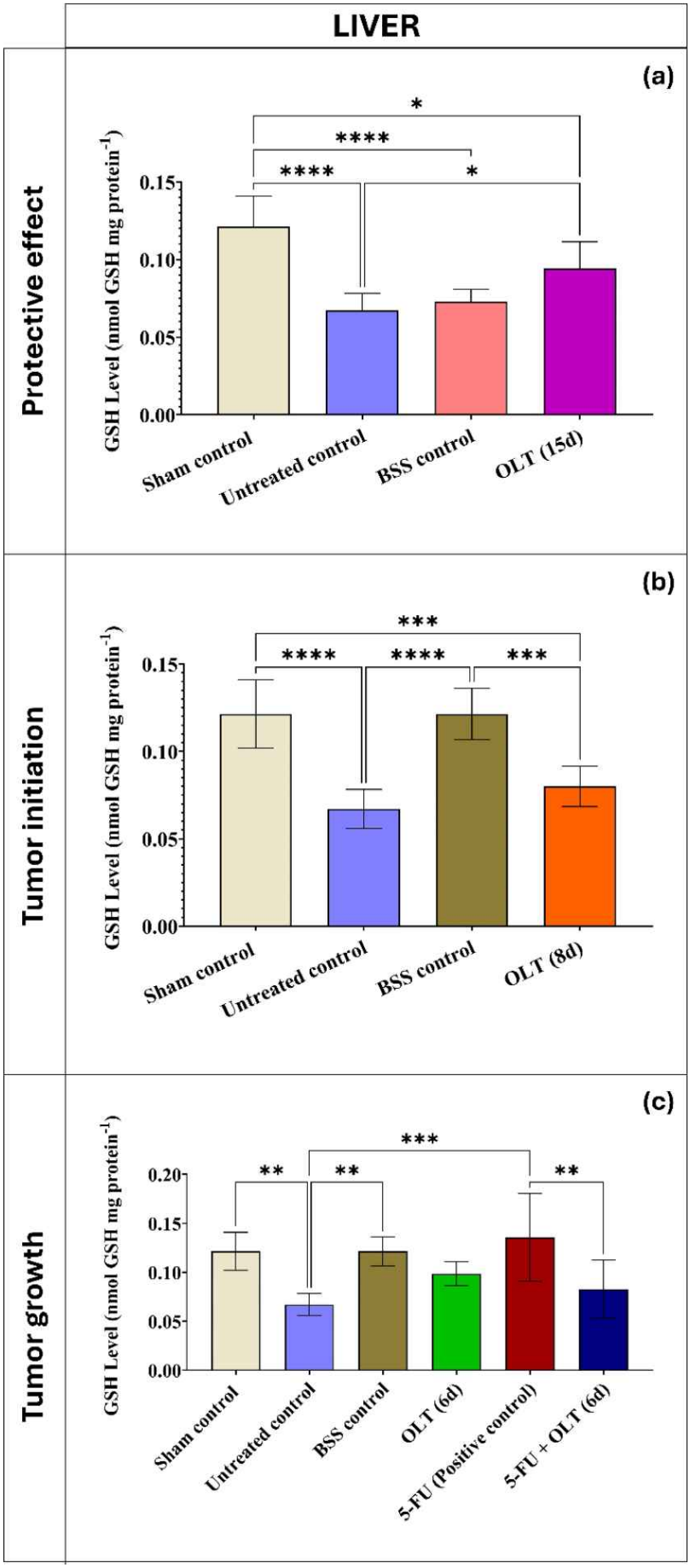
LIVER
Protective effect
(a)
GSH Level (nmol GSH mg protein-1)
0.15
0.10
0.05
0.00
*
****
****
*
Sham control
Untreated control
BSS control
OLT (15d)
Tumor initiation
(b)
GSH Level (nmol GSH mg protein-1)
0.15
0.10
0.05
0.00
***
****
****
***
Sham control
Untreated control
BSS control
OLT (8d)
Tumor growth
(c)
GSH Level (nmol GSH mg protein-1)
0.20
0.15
0.10
0.05
0.00
***
**
**
**
Sham control
Untreated control
BSS control
OLT (6d)
5-FU (Positive control)
5-FU + OLT (6d)

Figure 4.124. GSH levels measured in the liver tissues of mice treated with 400 mg/kg OLT for (a) 15, (b) 8, or (c) 6 days.

4.1.2. Kidney

As shown in Table 4.2. and Figure 4.4., the CaE activity was significantly elevated in the untreated control group (1975.30 nmol min^{-1} mg $protein^{-1}$) compared to the sham control group (1447.38 nmol min^{-1} mg $protein^{-1}$), with the highest values in the BSS control groups ($p<0.0001$). In the tumor growth study group, the CaE activity level was found to be 2032 in the OLT (6d) group. Treatment with 5-FU alone reduced this elevation (1828.67 nmol min^{-1} mg $protein^{-1}$) ($p<0.01$), whereas co-administration of 5-FU and OLT (6d) further reduced CaE activity (1763.10 nmol min^{-1} mg $protein^{-1}$) ($p<0.0001$), suggesting a partial renoprotective effect. For tumor initiation and protective effect study groups, the most notable reductions were observed in the OLT-treatment groups: 1645.42 nmol min^{-1} mg $protein^{-1}$ (OLT 8d) and 1720.21 nmol min^{-1} mg $protein^{-1}$ (OLT 15d), both of which approached the level of the sham control. In Figure 4.4., asterisks indicate statistically significant differences between groups (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$).

Table 4.1. Biochemical parameter [Carboxylesterase (CaE), Glutathione S-transferase (GST), Glutathione (GSH)] levels measured in the kidney tissues of mice treated with 400 mg/kg olive leaf tea (OLT) for (a) 15, (b) 8, or (c) 6 days.

Groups	Biochemical Parameters		
	CaE	GST	GSH
Sham control	1447.38 ± 12.78^{c}	60.36 ± 0.87^{a}	0.147 ± 0.004
Untreated control	1975.30 ± 41.06	72.21 ± 1.69	0.121 ± 0.008
BSS Control (8d)	2475.36 ± 54.90^{cz}	89.50 ± 1.57^{cz}	0.173 ± 0.004^{c}
BSS Control (15d)	2205.62 ± 72.62^{az}	76.74 ± 2.44^{z}	0.157 ± 0.007^{b}
5-FU (Positive control)	1828.67 ± 54.65^{z}	73.72 ± 4.51^{x}	0.144 ± 0.01
OLT (6d) (Tumor growth)	2032.48 ± 44.50^{z}	82.93 ± 2.67^{z}	0.161 ± 0.006^{b}
5-FU + OLT (6d)	1763.10 ± 78.21^{z}	65.80 ± 3.29	0.114 ± 0.006^{x}
OLT (8d) (Tumor initiation)	1645.42 ± 42.20^{cx}	74.15 ± 2.08^{z}	0.139 ± 0.005
OLT (15d) (Protective effect)	1720.21 ± 81.58ax	90.83 ± 1.37cz	0.146 ± 0.006

Data are expressed as mean ± standard error (n = 7). CaE and GST activities were expressed as nmol min^{-1} mg $protein^{-1}$, and GSH levels were expressed as nmol GSH mg $protein^{-1}$ ± standard error of the mean.
Statistically significant difference compared to the sham control group a:$p<0.05$, b:$p<0.01$, c:$p<0.001$
Statistically significant difference compared to the untreated control group x:$p<0.05$, y:$p<0.01$, z:$p<0.001$

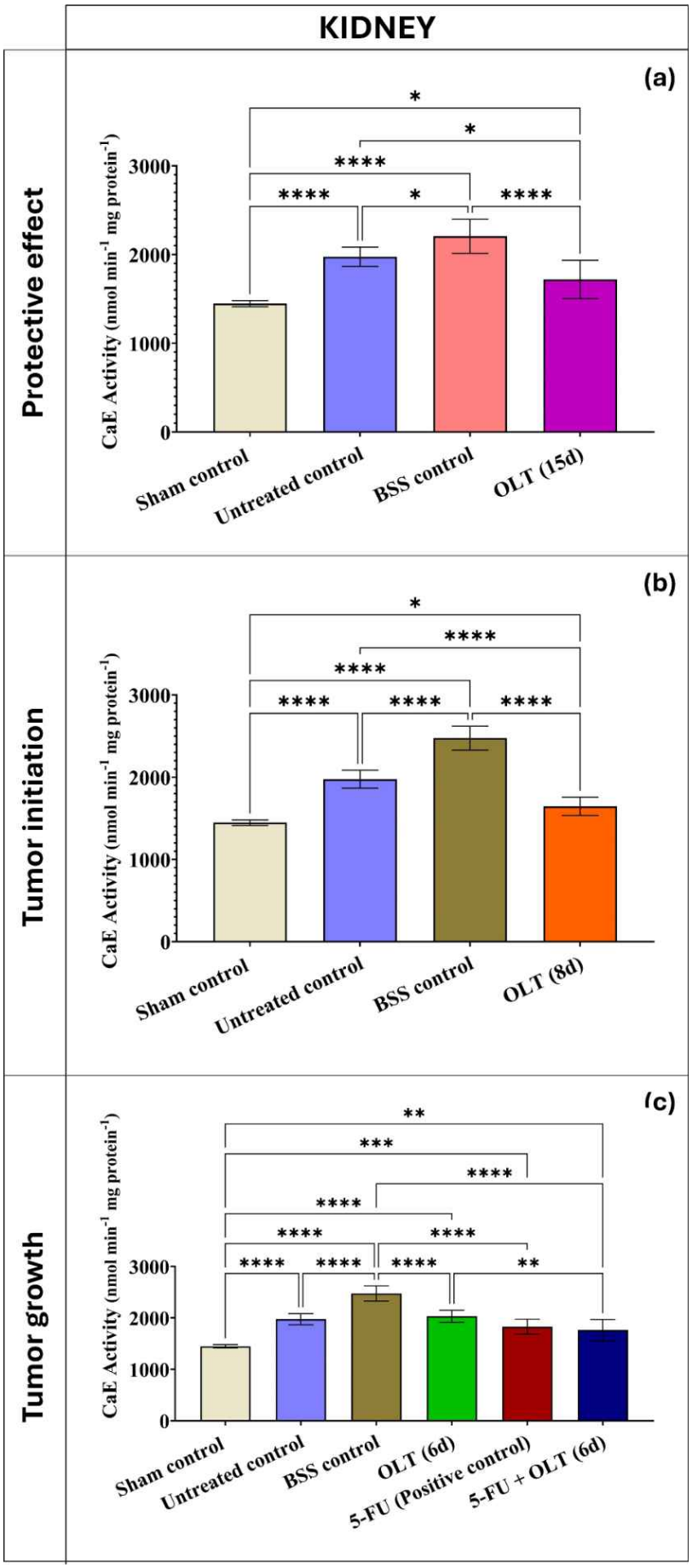
KIDNEY
Protective effect
(a)
CaE Activity (nmol min-1 mg protein-1)
3000
2000
1000
0
*
*
****
****
*
****
Sham control
Untreated control
BSS control
OLT (15d)
Tumor initiation
(b)
CaE Activity (nmol min-1 mg protein-1)
3000
2000
1000
0
*
****
****
****
****
****
Sham control
Untreated control
BSS control
OLT (8d)
Tumor growth
(c)
CaE Activity (nmol min-1 mg protein-1)
3000
2000
1000
0
**
***
****
****
****
****
****
****
****
**
Sham control
Untreated control
BSS control
OLT (6d)
5-FU (Positive control)
5-FU + OLT (6d)

Figure 4.125. CaE levels measured in the kidney tissues of mice treated with 400 mg/kg OLT for (a) 15, (b) 8, or (c) 6 days.

When the data were compared to the sham control (60.36 nmol min^{-1} mg $protein^{-1}$), GST activity, an essential marker of detoxification, was elevated in the untreated control group (72.21 nmol min^{-1} mg $protein^{-1}$) (Table 4.2. and Figure 4.5.). Administration of 5-FU alone caused an increase (73.72 nmol min^{-1} mg $protein^{-1}$) ($p>0.05$), whereas 5-FU + OLT (6d) reduced GST activity (65.80 nmol min^{-1} mg $protein^{-1}$). OLT treatment alone for 15 days produced the most significant increase (90.83 nmol min^{-1} mg $protein^{-1}$). Moreover, BSS controls showed higher GST activity than tumor-bearing groups, with values of 89.50 nmol min^{-1} mg $protein^{-1}$ (8d) and 76.74 nmol min^{-1} mg $protein^{-1}$ (15d), respectively. In Figure 4.5., asterisks indicate statistically significant differences between groups (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$).

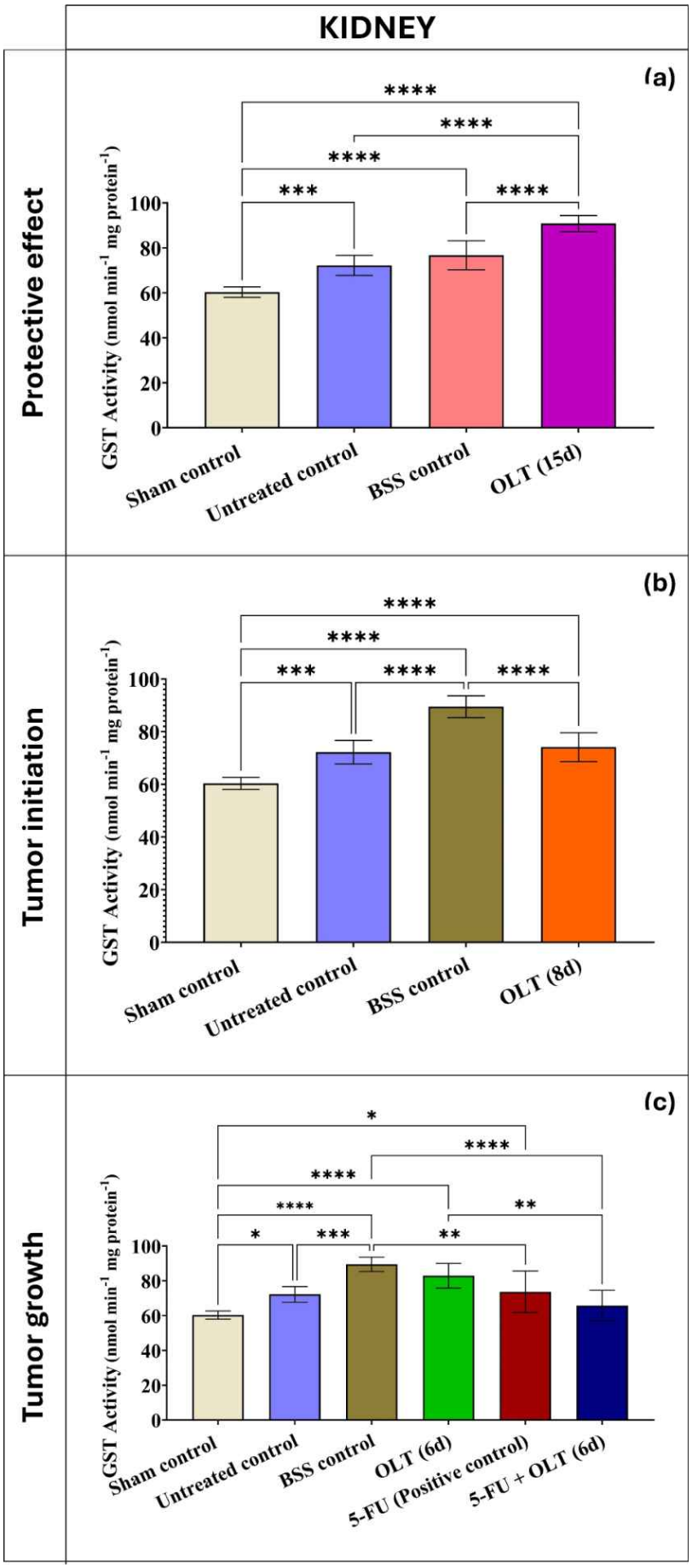
KIDNEY
Protective effect
(a)
GST Activity (nmol min-1 mg protein-1)
Sham control
Untreated control
BSS control
OLT (15d)
Tumor initiation
(b)
GST Activity (nmol min-1 mg protein-1)
Sham control
Untreated control
BSS control
OLT (8d)
Tumor growth
(c)
GST Activity (nmol min-1 mg protein-1)
Sham control
Untreated control
BSS control
OLT (6d)
5-FU (Positive control)
5-FU + OLT (6d)

Figure 4.126. GST levels measured in the kidney tissues of mice treated with 400 mg/kg OLT for (a) 15, (b) 8, or (c) 6 days.

A relative decrease in GSH levels (Table 4.2., Figure 4.6.) was found in the untreated control group (0.121 nmol GSH mg protein^{-1}) compared to sham controls (0.147 nmol GSH mg protein^{-1}) ($p>0.05$), and a statistically significant decrease was seen in the tumor initiation group ($p<0.05$). BSS control GSH levels increased significantly across all groups ($p<0.01$; $p<0.0001$). In the tumor growth group, OLT (6d) (0.161 nmol GSH mg protein^{-1}) significantly increased GSH levels ($p<0.01$), while relative increases were observed in the OLT (8d) (0.139 nmol GSH mg protein^{-1}) and OLT (15d) (0.146 nmol GSH mg protein^{-1}) groups ($p>0.05$). No significant differences were found in GSH levels among the 5-FU (0.144 nmol GSH mg protein^{-1}) and 5-FU+OLT (6d) (0.114 nmol GSH mg protein^{-1}) groups compared to untreated controls ($p>0.05$). In Figure 4.6., asterisks indicate statistically significant differences between groups (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$).

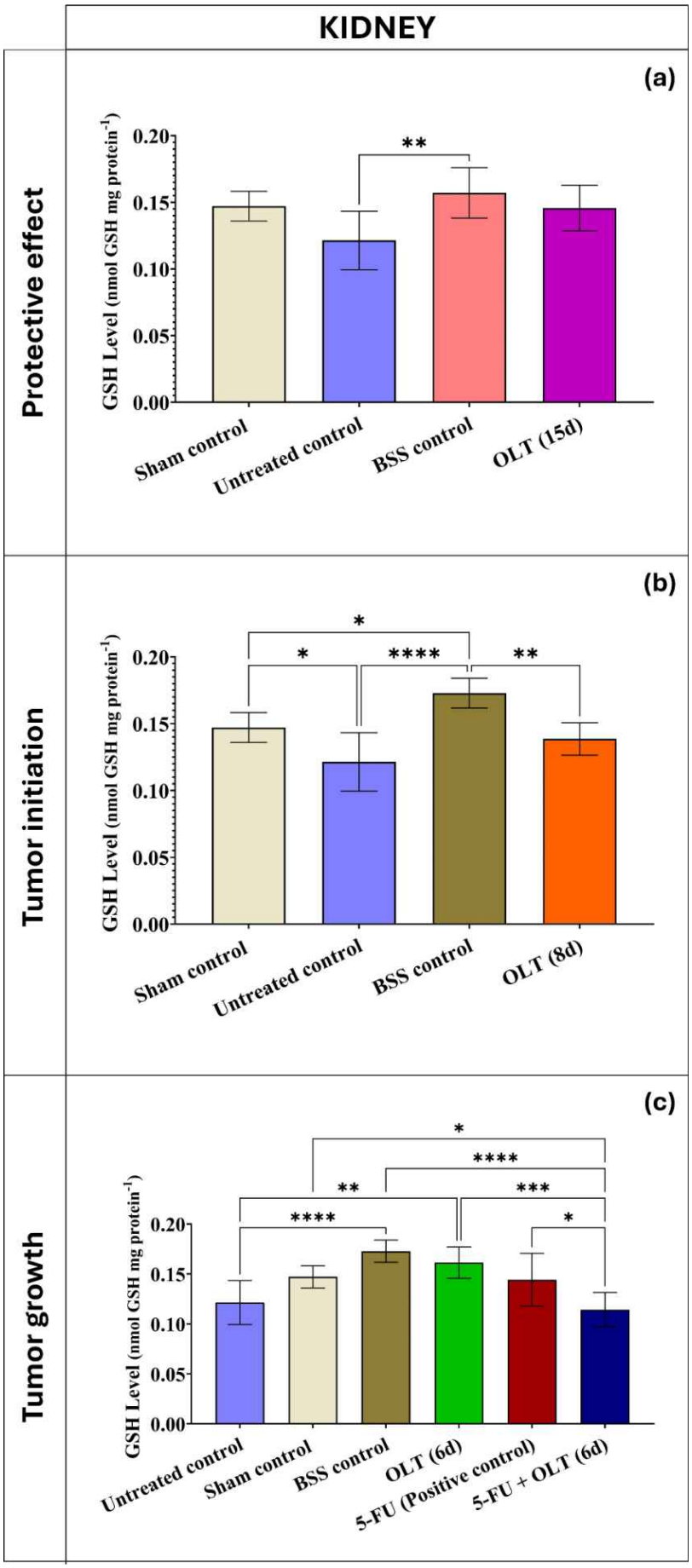
KIDNEY
Protective effect
(a)
GSH Level (nmol GSH mg protein-1)
0.20
0.15
0.10
0.05
0.00
**
Sham control
Untreated control
BSS control
OLT (15d)
Tumor initiation
(b)
GSH Level (nmol GSH mg protein-1)
*
*
****
**
Sham control
Untreated control
BSS control
OLT (8d)
Tumor growth
(c)
GSH Level (nmol GSH mg protein-1)
*
****
**
***
****
*
Untreated control
Sham control
BSS control
OLT (6d)
5-FU (Positive control)
5-FU + OLT (6d)

Figure 4.127. GSH levels measured in the kidney tissues of mice treated with 400 mg/kg OLT for (a) 15, (b) 8, or (c) 6 days.

4.1.3. Brain

As shown in Table 4.3. and Figure 4.7., CaE enzyme activity increased significantly in all groups compared to the sham control (0.086 nmol min^{-1} mg $protein^{-1}$) ($p<0.05$-0.0001), with the highest activity in the BSS control (15d) group (599 nmol min^{-1} mg $protein^{-1}$) ($p<0.0001$). In Figure 4.7., asterisks indicate statistically significant differences between groups (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$).

Table 4.1. Biochemical parameter [Carboxylesterase (CaE), Glutathione S-transferase (GST), Glutathione (GSH)] levels measured in the brain tissues of mice treated with 400 mg/kg olive leaf tea (OLT) for (a) 15, (b) 8, or (c) 6 days.

Groups	Biochemical Parameters		
	CaE	GST	GSH
Sham control	384.11 ± 13.04^{a}	95.63 ± 4.67	0.086 ± 0.007
Untreated control	482.13 ± 25.04	100.48 ± 3.15	0.109 ± 0.009
BSS Control (8d)	586.37 ± 14.93^{az}	123.54 ± 7.55^{x}	0.229 ± 0.020^{cz}
BSS Control (15d)	599.42 ± 20.99^{bz}	166.69 ± 2.68^{cz}	0.174 ± 0.011^{cz}
5-FU (Positive control)	424.43 ± 23.21	69.58 ± 2.59^{ax}	0.117 ± 0.014
OLT (6d) (Tumor growth)	486.18 ± 20.60^{x}	111.31 ± 6.79	0.147 ± 0.010^{x}
5-FU + OLT (6d)	474.63 ± 23.29^{x}	104.09 ± 9.70	0.120 ± 0.011
OLT (8d) (Tumor initiation)	503.59 ± 25.47^{y}	75.81 ± 5.09^{a}	0.099 ± 0.007
OLT (15d) (Protective effect)	403.03 ± 21.95^{a}	121.15 ± 5.83^{ay}	0.134 ± 0.013^{x}

Data are expressed as mean ± standard error (n = 7). CaE and GST activities were expressed as nmol min^{-1} mg $protein^{-1}$, and GSH levels were expressed as nmol GSH mg $protein^{-1}$ ± standard error of the mean.
Statistically significant difference compared to the sham control group a:$p<0.05$, b:$p<0.01$, c:$p<0.001$
Statistically significant difference compared to the untreated control group x:$p<0.05$, y:$p<0.01$, z:$p<0.001$

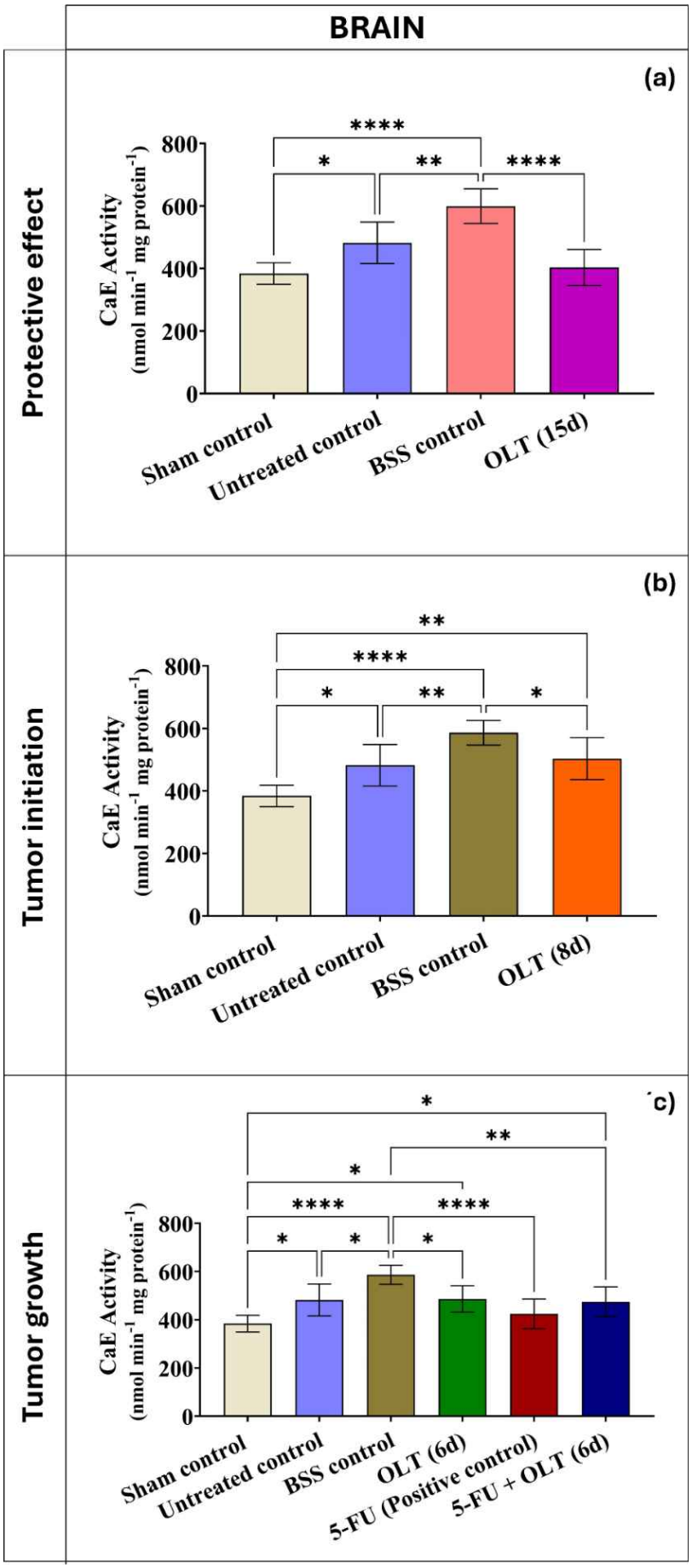
BRAIN
Protective effect
(a)
CaE Activity (nmol min-1 mg protein-1)
800
600
400
200
0
****
*
**
****
Sham control
Untreated control
BSS control
OLT (15d)
Tumor initiation
(b)
CaE Activity (nmol min-1 mg protein-1)
800
600
400
200
0
**
****
*
**
*
Sham control
Untreated control
BSS control
OLT (8d)
Tumor growth
´c)
CaE Activity (nmol min-1 mg protein-1)
800
600
400
200
0
*
**
*
****
****
*
*
*
Sham control
Untreated control
BSS control
OLT (6d)
5-FU (Positive control)
5-FU + OLT (6d)

Figure 4.128. CaE levels measured in the brain tissues of mice treated with 400 mg/kg OLT for (a) 15, (b) 8, or (c) 6 days.

GST activity showed the highest increase in all BSS control groups: 123.54 nmol min^{-1} mg $protein^{-1}$: 8 days; 166.69 nmol min^{-1} mg $protein^{-1}$: 15 days; ($p<0.05$-0.0001) (Table 4.3. and Figure 4.8.). No differences were found between the sham (95.63 nmol min^{-1} mg $protein^{-1}$) and untreated control (100.48 nmol min^{-1} mg $protein^{-1}$) groups ($p>0.05$). However, in the protective effect group, OLT (15d) showed increased GST activity (121.15 nmol min^{-1} mg $protein^{-1}$) compared to the sham group ($p < 0.01$). In the tumor growth study group, GST activity was lowest in the 5-FU group (69.58 nmol min^{-1} mg $protein^{-1}$) and increased in the 5-FU+OLT group (104.09 nmol min^{-1} mg $protein^{-1}$) ($p<0.01$). In Figure 4.8., asterisks indicate statistically significant differences between groups (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$).

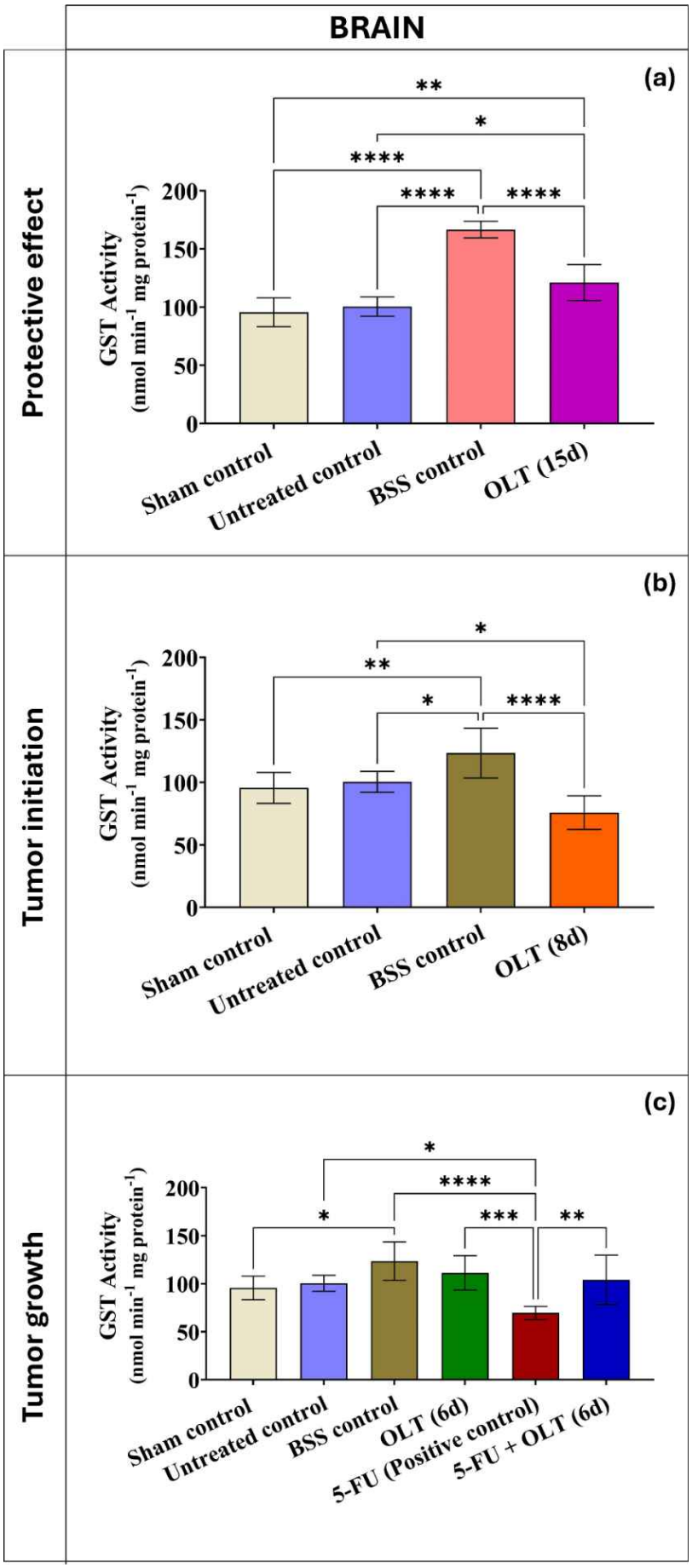
BRAIN
Protective effect
(a)
GST Activity (nmol min-1 mg protein-1)
200
150
100
50
0
**
*
****
****
****
Sham control
Untreated control
BSS control
OLT (15d)
Tumor initiation
(b)
GST Activity (nmol min-1 mg protein-1)
200
150
100
50
0
*
**
*
****
Sham control
Untreated control
BSS control
OLT (8d)
Tumor growth
(c)
GST Activity (nmol min-1 mg protein-1)
200
150
100
50
0
*
****
*
***
**
Sham control
Untreated control
BSS control
OLT (6d)
5-FU (Positive control)
5-FU + OLT (6d)

Figure 4.129. GST levels measured in the brain tissues of mice treated with 400 mg/kg OLT for (a) 15, (b) 8, or (c) 6 days.

In terms of GSH levels, no significant differences were observed between the sham (0.086 nmol GSH mg protein^{-1}) and untreated control (0.109 nmol min^{-1} mg protein^{-1}) groups ($p>0.05$) (Table 4.3., Figure 4.9.). In contrast, the BSS control groups across all studies showed increased GSH levels (8d: 0.229 nmol GSH mg protein^{-1}; 15d: 0.174 nmol GSH mg protein^{-1}) ($p<0.001$; $p<0.0001$). Relative or significant increases in GSH were observed in all OLT alone treatment groups. These values were measured as 0.147 nmol GSH mg protein^{-1} ($p<0.05$), 0.099 nmol GSH mg protein^{-1} ($p>0.05$), and 0.134 nmol GSH mg protein^{-1} ($p<0.05$) in groups OLT (6d), OLT (8d), and OLT (15d), respectively. No significant differences were determined in GSH levels between sham, untreated control, 5-FU (0.117 nmol GSH mg protein^{-1}), and 5-FU+OLT (6d) (0.120 nmol GSH mg protein^{-1}) groups ($p>0.05$). In Figure 4.9., asterisks indicate statistically significant differences between groups (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$).

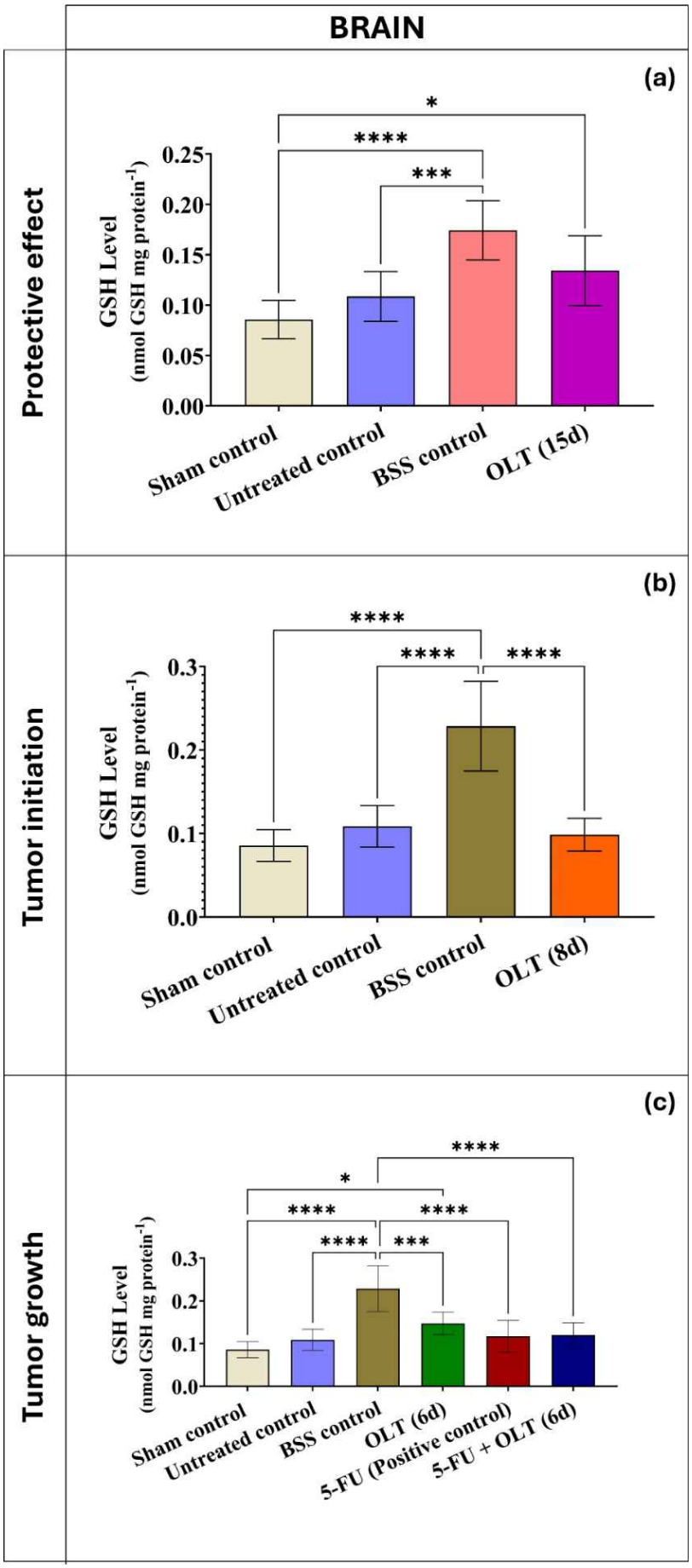
BRAIN
Protective effect
(a)
GSH Level
(nmol GSH mg protein-1)
0.25
0.20
0.15
0.10
0.05
0.00
*
****
***
Sham control
Untreated control
BSS control
OLT (15d)
Tumor initiation
(b)
GSH Level
(nmol GSH mg protein-1)
0.3
0.2
0.1
0.0
****
****
****
Sham control
Untreated control
BSS control
OLT (8d)
Tumor growth
(c)
GSH Level
(nmol GSH mg protein-1)
0.3
0.2
0.1
0.0
****
*
****
****
****
***
Sham control
Untreated control
BSS control
OLT (6d)
5-FU (Positive control)
5-FU + OLT (6d)

Figure 4.130. GSH levels measured in the brain tissues of mice treated with 400 mg/kg OLT for (a) 15, (b) 8, or (c) 6 days.

4.2. Histopathological Results

Histopathological evaluation was performed on liver, stomach, duodenum, kidney, and bladder tissues collected from all experimental groups. According to the pathological assessment, no structural abnormalities were detected in any groups except the 5-FU-treated animals, in which characteristic chemotherapy-related lesions were observed. The detailed organ-based findings are presented below.

4.2.1. Liver

Liver tissues from the sham control, untreated tumor control, BSS control, and OLT-treated groups exhibited normal hepatic architecture (Figure 4.10.a-c, e, g-i). Hepatocyte cords, sinusoidal spaces, and central vein morphology were well preserved, and no degenerative, necrotic, or inflammatory changes were observed in these groups. In contrast, the 5-FU-treated animals demonstrated distinct features of chemotherapy-induced hepatotoxicity. The liver sections showed cytoplasmic vacuolization and nuclear pyknosis in hepatocytes, indicated by arrows in the corresponding micrographs (Figure 4.10.d). These findings represent classic degenerative changes induced by antimetabolite chemotherapeutic agents. Importantly, co-administration of OLT with 5-FU did not exhibit these lesions, and liver morphology remained comparable to the non-5-FU groups, suggesting a protective effect of OLT (Figure 4.10.f).

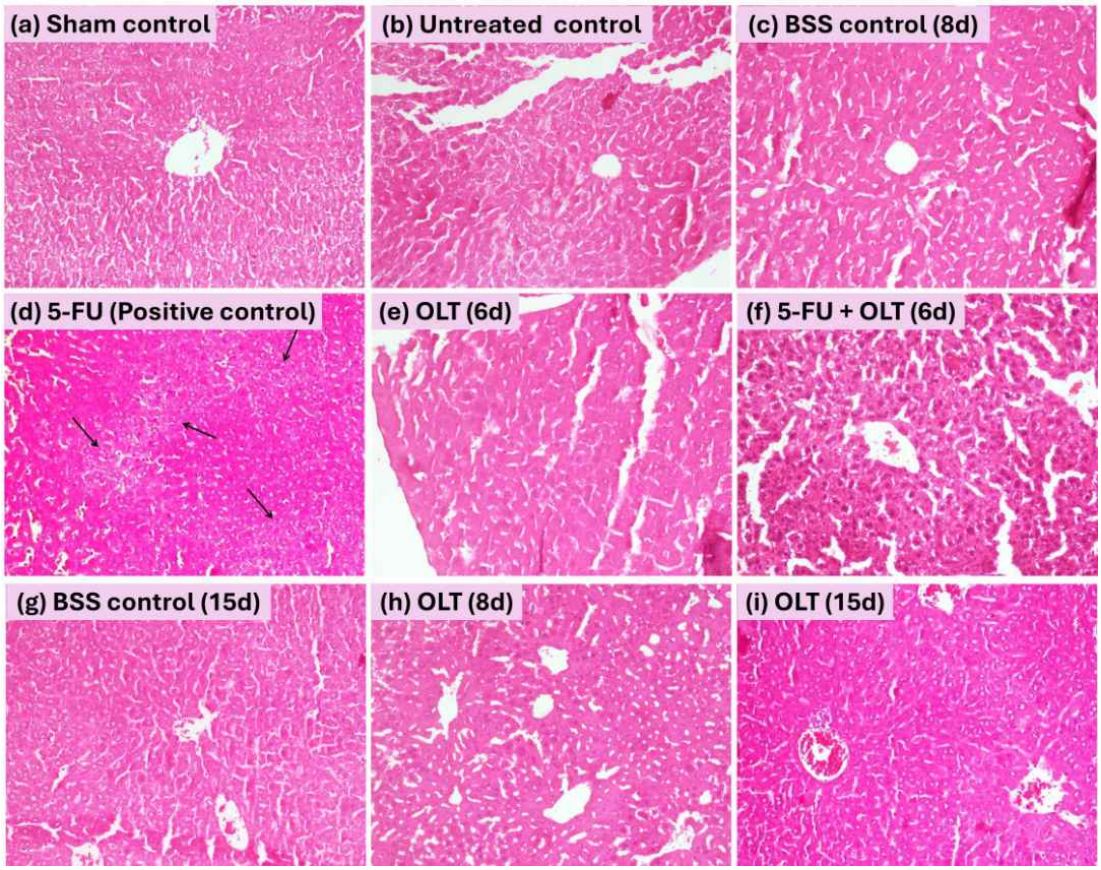

Figure 4.131. Histopathological appearance of liver tissues from experimental groups (H&E staining) (Magnification: 200x).

(a) Sham control, (b) Untreated tumor control, (c) BSS control (8 d), (d) 5-FU–treated positive control, (e) OLT (6 d), (f) 5-FU + OLT (6 d), (g) BSS control (15 d), (h) OLT (8 d), and (i) OLT (15 d).

All groups except 5-FU exhibited normal hepatic architecture with well-preserved hepatocyte cords and sinusoidal organization. In the 5-FU group (d), arrows indicate characteristic hepatotoxic changes including cytoplasmic vacuolization and nuclear pyknosis. Co-administration of OLT (f) prevented these lesions, maintaining normal morphology.

4.2.2. Stomach

The gastric mucosa in the sham, untreated tumor control, BSS control, and OLT-only groups appeared normal, with intact glandular organization and no signs of mucosal injury or inflammatory infiltration (Figure 4.11.). However, the 5-FU group showed focal lymphocyte infiltration in the gastric mucosa, as indicated by the arrows in the histological images (Figure 4.11.d). No erosions, ulceration, or glandular destruction were noted, but the presence of localized lymphocytic aggregates reflects chemotherapy-associated mild gastric irritation. In the 5-FU +

OLT group, these inflammatory foci were absent, and overall gastric architecture remained normal (Figure 4.11.f).

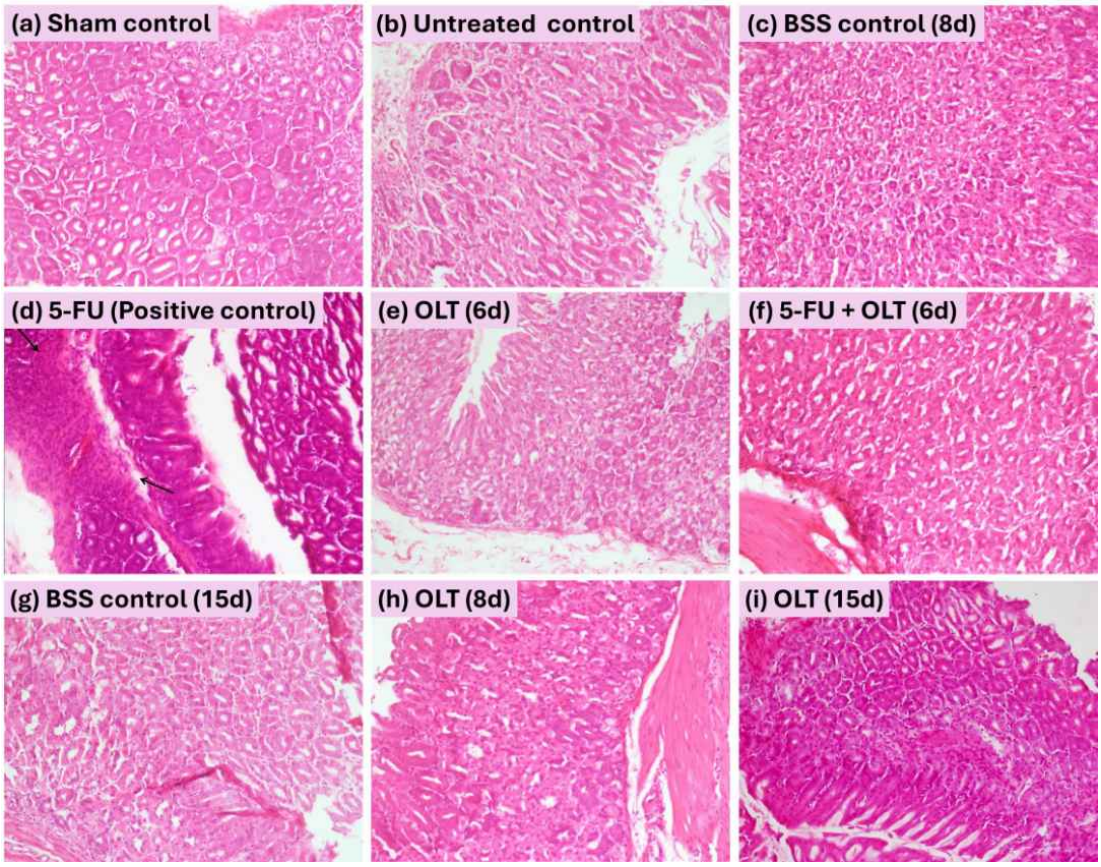

Figure 4.132. Histopathological appearance of stomach tissues from experimental groups (H&E staining) (Magnification: 200x).

(a) Sham control, (b) Untreated tumor control, (c) BSS control (8 d), (d) 5-FU–treated positive control, (e) OLT (6 d), (f) 5-FU + OLT (6 d), (g) BSS control (15 d), (h) OLT (8 d), and (i) OLT (15 d).

Gastric mucosa remained structurally normal in all groups except 5-FU. The 5-FU group (d) showed focal lymphocyte infiltration (arrows) within the mucosa, consistent with mild chemotherapy-induced irritation. OLT exposure, alone or combined with 5-FU, preserved normal mucosal architecture.

4.2.3. Duodenum

As seen on Figure 4.12., all experimental groups, including sham, tumor control, BSS, OLT-only, 5-FU, and 5-FU + OLT, showed entirely normal duodenal morphology. Villus height, epithelial integrity, crypt architecture, and lamina propria were preserved without evidence of inflammatory infiltration, epithelial

degeneration, or mucosal injury. These findings indicate that neither the tumor burden, OLT administration, nor 5-FU treatment produced detectable duodenal toxicity.

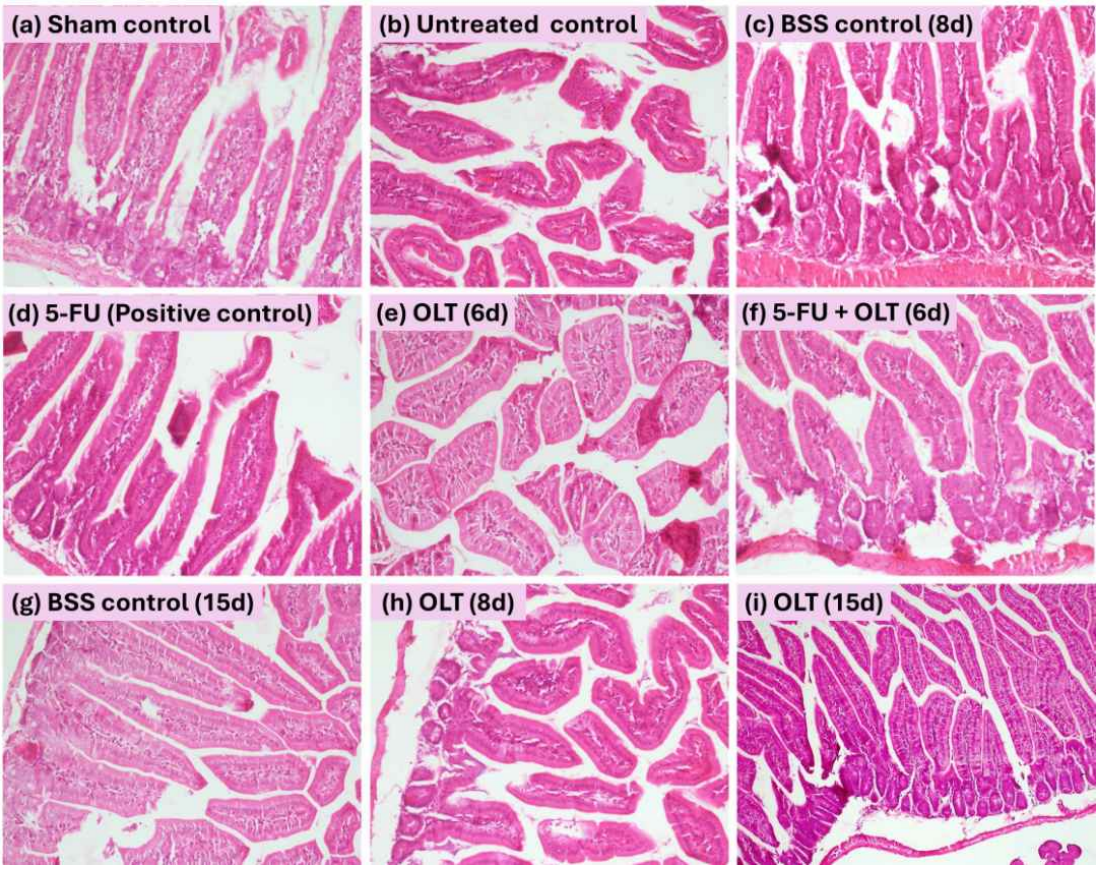

Figure 4.133. Histopathological appearance of duodenum tissues from experimental groups (H&E staining) (Magnification: 200x).

(a) Sham control, (b) Untreated tumor control, (c) BSS control (8 d), (d) 5-FU-treated positive control, (e) OLT (6 d), (f) 5-FU + OLT (6 d), (g) BSS control (15 d), (h) OLT (8 d), and (i) OLT (15 d).

All groups demonstrated completely normal duodenal structure, with intact villus height, crypt organization, epithelial integrity, and lamina propria features. No pathological alterations were identified in any group, including 5-FU, indicating that duodenal tissue was unaffected by the treatments or tumor burden.

4.2.4. Kidney

Renal tissues from all groups showed normal histological appearance, including well-defined renal corpuscles and intact proximal and distal tubular epithelium (Figure 4.13.). No tubular degeneration, vacuolization, necrosis, or

interstitial inflammation was observed in any of the groups. Thus, both tumor induction and experimental treatments, including 5-FU and OLT, did not induce nephrotoxicity under the conditions of this study.

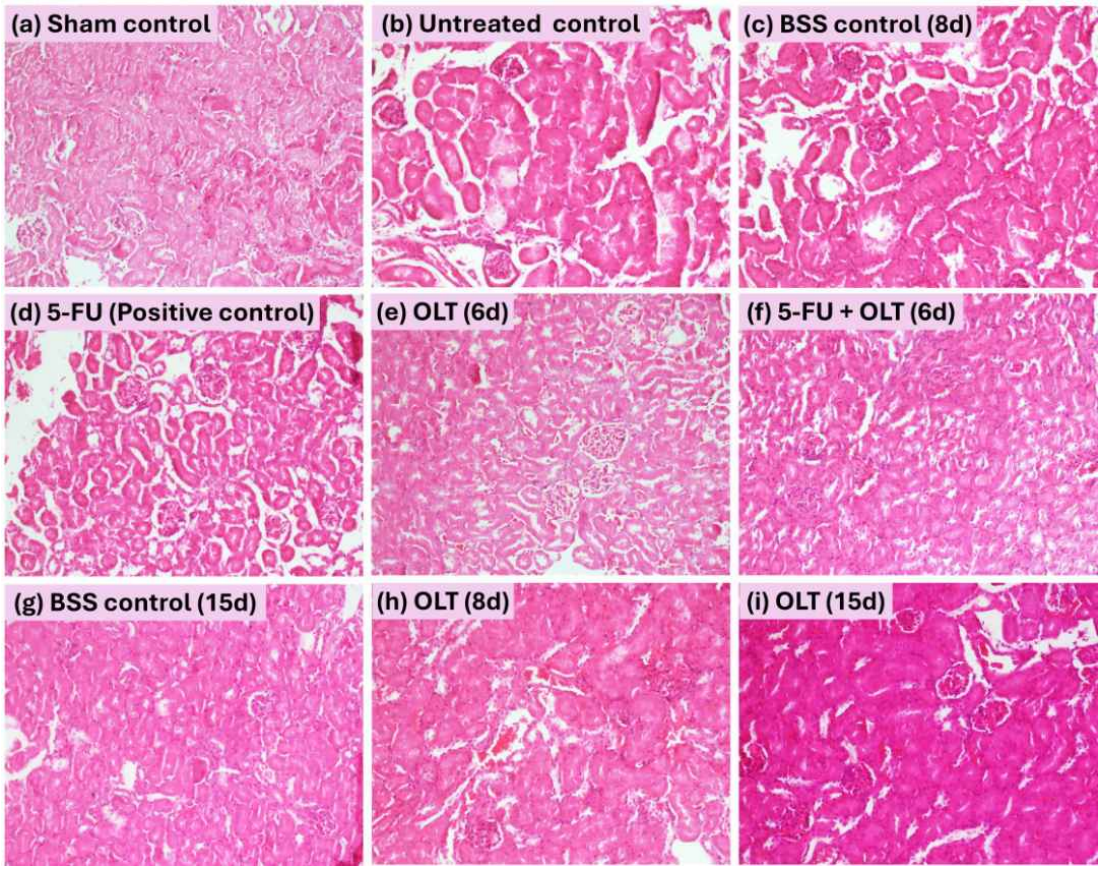

Figure 4.134. Histopathological appearance of kidney tissues from experimental groups (H&E staining) (Magnification: 200x).

(a) Sham control, (b) Untreated tumor control, (c) BSS control (8 d), (d) 5-FU–treated positive control, (e) OLT (6 d), (f) 5-FU + OLT (6 d), (g) BSS control (15 d), (h) OLT (8 d), and (i) OLT (15 d).

Renal corpuscles and tubular structures appeared normal in all groups, with no signs of tubular degeneration, necrosis, interstitial inflammation, or glomerular abnormality. Even 5-FU administration did not produce detectable nephrotoxicity, and OLT treatment did not induce any renal alterations.

4.2.5. Bladder

Bladder sections from all experimental groups exhibited normal urothelial layering, intact lamina propria, and well-organized muscularis (Figure 4.14.). No edema, inflammation, epithelial thinning, or architectural disruption was observed in

any group, including the 5-FU-treated animals. These observations demonstrate that the bladder was not adversely affected by either tumor presence or the treatments administered.

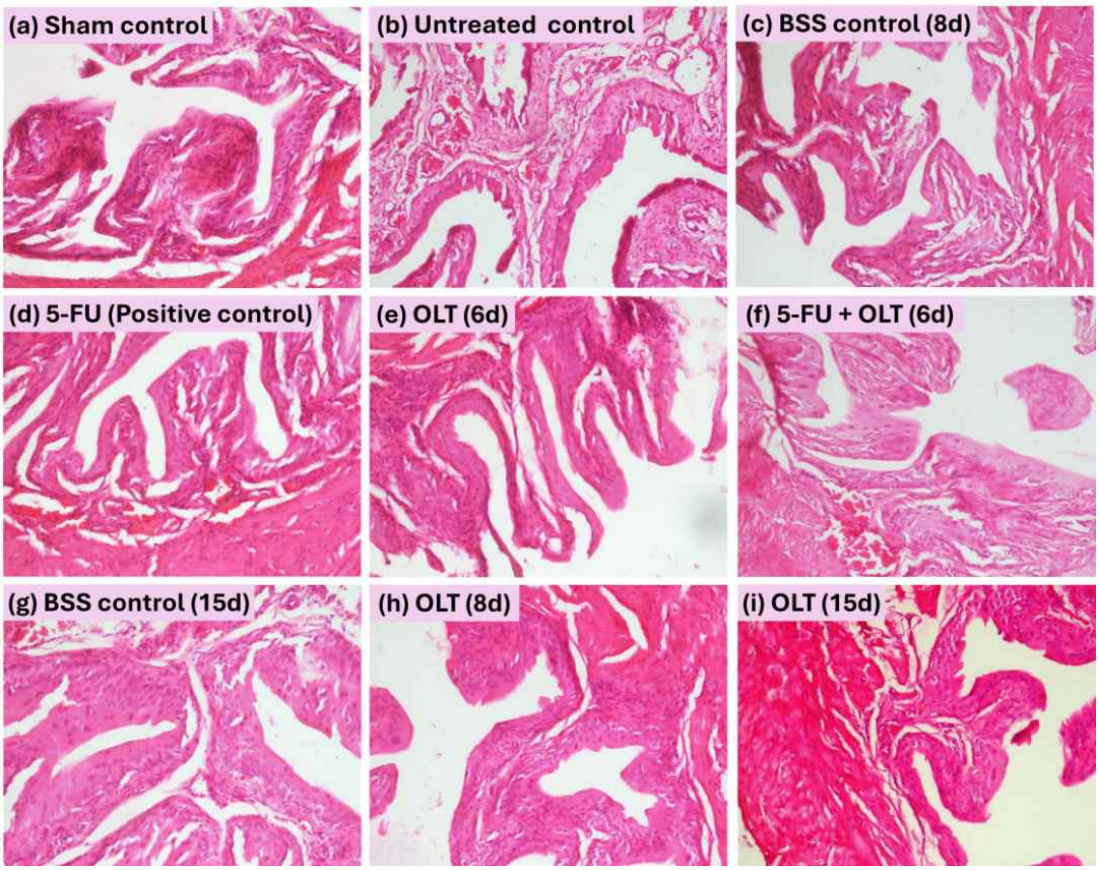

Figure 4.149. Histopathological appearance of bladder tissues from experimental groups (H&E staining) (Magnification: 200x).

(a) Sham control, (b) Untreated tumor control, (c) BSS control (8 d), (d) 5-FU–treated positive control, (e) OLT (6 d), (f) 5-FU + OLT (6 d), (g) BSS control (15 d), (h) OLT (8 d), and (i) OLT (15 d).

Across all treatment groups, including 5-FU, urothelial layering, lamina propria integrity, and muscular wall morphology remained normal. No inflammatory infiltration, edema, or epithelial damage was observed, indicating absence of bladder toxicity in all experimental conditions.

5. DISCUSSION

5.1. Liver and Gastrointestinal System

The antioxidant response observed in the OLT groups was further supported by elevated glutathione (GSH) levels and increased activity of glutathione S-transferase (GST), both key components of the cellular redox defense system. GSH acts as a primary non-enzymatic antioxidant, while GST catalyzes the conjugation of GSH to xenobiotic substrates, facilitating detoxification. In the untreated control group, a clear depletion in GSH was detected, indicating oxidative imbalance. Conversely, GSH and GST levels were significantly restored in the OLT-treated groups, especially in the 15-day treatment group. This demonstrated that OLT not only prevents oxidative damage but also restores antioxidant capacity. In a study, it was reported that olive leaf extract reversed oxidative damage in rats exposed to lead acetate or ethanol by restoring antioxidant enzymes and lipid peroxidation levels and improved damaged testicular tissues (Ahmed et al., 2021).

Another enzyme investigated in this study was carboxylesterase (CaE), a member of the esterase family involved in the detoxification of xenobiotics. This enzyme is predominantly found in the liver of mammals (Ozkaya & Turkan, 2021). An unanticipated elevation in esterase activity (CaE), which is typically expected to be inhibited under toxic conditions (e.g., the enzyme activity level of the Untreated control group being higher than that of the Sham control group), may suggest the involvement of apoptosis-related pathways. This phenomenon has been previously reported, with studies demonstrating a concurrent increase in both apoptosis and CaE activity following exposure to gold nanorods and ethanol (Sun et al., 2017; Costa et al., 2020). It is postulated that the induction of GST and CaE enzymes plays a crucial role in mitigating oxidative stress and counteracting toxicity. Similar results were obtained for GST and CaE in mice EAC inoculated and OLT-treated for 6, 8, and 15 days. These findings support this hypothesis and indicate that the use of OLT has a potentially improving effect on health. Moreover, the normalization of CaE and GST activities during the recovery phase may be interpreted as a biomarker of recovery, reflecting the attenuation of carcinogen-induced stress (Turhan & Gungordu, 2022).

Nutritional factors are crucial in the development, advancement, and prevention of cancer. Olive leaves contain several bioactive compounds such as oleuropein, hydroxytyrosol, and oleic acid that play essential roles in enhancing the body's antioxidant defense mechanisms and maintaining cellular membrane integrity (Colomer & Menéndez, 2006; Cicerale et al., 2010). These phytochemicals,

particularly polyphenols and monounsaturated fatty acids (MUFAs, e.g., oleic acid), are known to reduce oxidative stress by inhibiting DNA oxidation and promoting the activity of endogenous antioxidants like glutathione (GSH) (Bello et al., 2006; Gur et al., 2020). Based on our findings, it is probable that these compounds likely contributed to the observed elevation in GSH levels, the normalization of detoxifying enzyme activities, and the reduction in tumor burden, thus suggesting their potential role in modulating carcinogenesis and supporting hepatic function.

In addition to biochemical parameters, histological analysis provided morphological confirmation of OLT's hepatoprotective effect. While no pathological alterations were noted in the livers of mice treated solely with OLT (400 mg/kg/day), the 5-FU (chemotherapy agent) group exhibited mild hepatocellular degeneration, such as cytoplasmic vacuolization and nuclear pyknosis which hallmarks of drug-induced hepatotoxicity (Lo et al., 2023; Barakat et al., 2024). Additionally, no pathological findings were observed in the stomach and duodenum tissues of animals administered OLT orally. However, in animals administered 5FU alone, mild focal lymphocyte infiltration was observed, consistent with the side effects of chemotherapeutic agents (Madisch et al., 2002). Especially, animals receiving both 5-FU and OLT exhibited mitigated histopathological alterations. This suggested a protective synergy.

In summary, the data collectively indicate that olive leaf tea, particularly when administered before tumor initiation, offers substantial protective effects against hepatic oxidative damage and tumor progression. These findings highlight the therapeutic potential of OLT in liver-related pathologies and warrant further investigation in mechanistic and clinical studies.

5.2. Excretory System: Kidneys and Bladder

CaE activity, elevated in tumor-bearing mice, was effectively reduced by OLT, particularly in the 8- and 15-day treatment groups, suggesting normalization of detoxification pathways. GST activity increased in both untreated and BSS groups, indicating a stress response. Interestingly, long-term OLT treatment (15d) led to the highest GST activity, while 5-FU + OLT co-treatment reduced it, possibly reflecting a balanced redox state. The most significant increase in GSH was observed in the OLT (6d) group, suggesting improved antioxidant capacity. This is in agreement with prior studies showing polyphenols enhance GSH synthesis and recycling (Di Giacomo et al., 2023).

Histological analyses confirmed that neither OLT nor 5-FU induced structural damage in kidney or bladder tissues, further supporting the safety and efficacy of OLT. Overall, these findings suggest that OLT can attenuate tumor- and chemotherapy-induced renal oxidative stress by modulating key detoxification enzymes and restoring redox balance, offering promise as an adjunctive therapy.

5.3. Brain

In terms of detoxification enzymes, brain carboxylesterase (CaE) activity significantly increased in all groups compared to the sham control, with the highest activity observed in BSS controls. Interestingly, OLT treatment in tumor-bearing groups slightly mitigated this elevation. In the literature, it has been recorded that esterases can up- or down-regulate in stress as a systemic xenobiotic response (Stocker et al., 2004; Djeridane et al., 2006). Glutathione S-transferase (GST), a critical phase II enzyme in detoxification and oxidative stress defense, was markedly elevated in BSS and long-term OLT groups. OLT (15d) significantly increased GST activity relative to sham. However, GST activity was lowest in the 5-FU group and was partially restored by co-treatment with OLT. Dzah et al. (2024) reported that the polyphenols can restore GST activity under drug-induced stress conditions. Moreover, olive leaf extract may help counteract chemotherapy-associated oxidative enzyme suppression (Geyikoglu et al., 2017). Glutathione (GSH) levels in brain tissue were relatively preserved across groups, though OLT treatment, especially in the 6-day group, led to significant increases. GSH plays a fundamental role in maintaining redox homeostasis in the central nervous system (Goldstein et al., 2022), and its elevation supports the neuroprotective potential of olive leaf compounds (Gonçalves et al., 2024). Interestingly, no significant differences in GSH were observed between tumor-bearing and sham animals. This suggests that the brain may activate compensatory mechanisms to maintain basal antioxidant status under moderate systemic stress.

Taken together, these results indicate that OLT exerts subtle but beneficial effects on brain oxidative metabolism, particularly by modulating GST and GSH levels and maintaining enzyme activity profiles under tumor or chemotherapy stress. While the 5-FU + OLT combination showed unexpected elevations in aminotransferase levels, likely reflecting complex drug-nutrient interactions, OLT alone demonstrated a favorable profile. This supports the growing evidence that olive-derived polyphenols may serve as neuroprotective agents through antioxidant and

anti-inflammatory mechanisms (Gorzynik-Debicka et al., 2018; Gonçalves et al., 2024).

6. CONCLUSION

Based on the biochemical outcomes, olive leaf tea (OLT) markedly improved redox / detoxification status in tumor-bearing mice. In liver tissue, reduced glutathione (GSH) levels, which dropped drastically from 0.121 nmol GSH mg protein^{-1} in sham to 0.067 nmol GSH mg protein^{-1} in the untreated EAC control, increased significantly when OLT was administered prophylactically for 15 days (0.099 nmol GSH mg protein^{-1}), indicating partial restoration of antioxidant capacity. A similar pattern was observed for glutathione-S-transferase (GST): while the untreated tumor control showed 105.58 nmol min^{-1} mg protein^{-1}, OLT (15 days) caused a marked elevation (142.82 nmol min^{-1} mg protein^{-1}), demonstrating improved phase-II detoxification. Carboxylesterase (CaE) activity also increased from 1580-1630 nmol min^{-1} mg protein^{-1} (sham vs. untreated) to 1798-1898 nmol min^{-1} mg protein^{-1} in OLT-treated groups, reflecting normalization and enhancement of xenobiotic metabolism under OLT supplementation.

Histopathological evaluation strongly supported these biochemical findings. In all organs examined (liver, kidney, stomach, intestine, bladder), OLT alone did not induce any pathological alterations. In contrast, 5-fluorouracil (5-FU) produced mild hepatocellular injury (cytoplasmic vacuolization and nuclear pyknosis) and focal lymphocytic infiltration in the gastric mucosa. Remarkably, these lesions were clearly attenuated in the 5-FU + OLT group, confirming that OLT co-administration reduced chemotherapy-associated tissue injury. No nephrotoxicity or urothelial damage was detected in any OLT-treated conditions.

Taken together, OLT strengthened antioxidant defense (increased GSH and GST), supported detoxification (increased CaE), and preserved histological integrity. These effects were most pronounced when OLT was administered before tumor induction, highlighting its preventative value and its potential utility as a safe, natural co-therapy to mitigate chemotherapy-related oxidative and histological damage.

7. RECOMMENDATIONS

Olive leaf tea, when given before tumor development, helped restore antioxidant balance (increased GSH, and GST) and supported detoxification capacity (increased CaE), while also preventing visible tissue damage — and even reduced the histological toxicity of 5-FU. These findings suggest that OLT has real potential as a safe, natural supportive nutraceutical — especially in the preventive / adjuvant setting — and may help protect organs against oxidative and chemotherapy-related injury.

Considering all the results together, we suggest the following:

1. Since the most pronounced improvements in GSH restoration, GST induction and CaE normalization were observed when olive leaf tea (OLT) was administered before tumor induction, future studies should prioritize prophylactic / pre-treatment designs rather than post-tumor therapeutic applications.

2. GSH depletion clearly accompanies tumor burden, and OLT has been found to partially improve this parameter. Therefore, future studies could investigate which molecular pathways of the glutathione system (e.g., Nrf2 signaling, GSR/GPx interactions, cysteine supply) primarily contribute to this improvement.

3. The increase in GST activity following OLT suggests that olive leaf polyphenols may affect phase II detoxification. Therefore, examining isolated phenolic fractions (such as oleuropein or hydroxytyrosol) separately could help determine which compounds play the dominant role.

4. Partial normalization of CaE activity by OLT suggests a potential protective contribution to esterase-related metabolism. Therefore, in vitro CaE kinetic analyses with purified OLT polyphenols may provide clearer insight into this mechanism.

5. Since the histopathological evaluation in the current study relied solely on H&E light microscopy, the inclusion of additional confirmatory techniques such as immunohistochemistry (NF-κB, caspase-3, Ki-67) or oxidative stress markers (8-OHdG, MDA) could support a more in-depth mechanistic interpretation at the tissue level.

6. Given that OLT alone did not cause any tissue lesions and that its coadministration with OLT reduced 5-FU-associated damage, OLT may be considered a promising nutraceutical supplement option to help reduce chemotherapy-associated toxicity. Future studies could also investigate other chemotherapy agents, different dose escalation scenarios, and different treatment timing strategies.

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RESUME

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APPENDICES

APPENDIX 1

Evrak Tarih ve Sayısı: 23/05/2019-E.22314

T.C.
HARRAN ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURUL BAŞKANLIĞI
(HRÜ-HADYEK)

Oturum No	Karar	Tarih / Saati	Yeri
2019/002	01-02	29.04.2019/ 15:00	HADYEK Toplantı Salonu

KARAR 2019/002/02: 08/03/2019 tarih ve 6064 sayılı başvuru dosyası incelendi. İnceleme sonucunda; Yürütücülüğünü Dr. Öğr. Üyesi Hatice AKTAŞ'ın yapacağı "***Zeytin yaprağının Ehrlich Ascites Tümörü taşıyan fareler üzerindeki etkileri***" isimli çalışmaya, Etik Kurul izni verilmesinin uygun olduğuna;

Oy çokluğu/birliğiyle karar verilmiştir.

Prof. Dr. Hisamettin DURMAZ
Başkan

Doç. Dr. Fatih SOKMEN
Üye

Dr. Öğr. Üyesi Sibel TÜREDİ
Başkan V.

Doç. Dr. Füsun TEMAMOĞULLARI
Raportör

Doç. Dr. Sabri YURTSEVEN
Üye

Dr.Öğr. Üyesi Mücahit KAHRAMAN
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Dr. Öğr. Üyesi Mustafa Ü. BOYRAZ
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Dr. Öğr. Üyesi Tuba ÖZGÖÇER
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Arş. Gör. Gülşah GÜNGÖREN
Üye

Ahmet Mevlüt BALIKÇI
STK-Üye

3

Local Ethics Committee Approval document