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Biochemical Effects of *Olea europaea* L. and *Zingiber officinale* on Ehrlich Ascites Carcinoma in Mice

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Abstract: Cancer is an important health problem worldwide, killing 10 million people each year. Antioxidant and anticancer medicinal plants are widely used in traditional medicine to treat a wide range of diseases. The objective of this study was to evaluate the efficacy of olive leaf extract and ginger extract in treating mice with Ehrlich ascites carcinoma (EAC). 48 mice were split into eight groups (n = 6) after tumor induction, and for 14 days, daily doses of 150 mg/kg of the extracts were administered to each group. Mice were sacrificed after the experiment, serum and tissues were obtained, and biochemical and molecular parameters were measured. Oral administration of OLE and ginger extracts leads to a substantial reduction in tumor volume, MDA activities, and liver enzyme activity, as well as an increase in SOD and GPx levels in the treated groups. The present research shows that olive leaf and ginger extracts have antitumor activity in vivo, which results in biochemical parameter normalization when compared to EAC bearing mice.

keywords: Ehrlich ascites carcinoma; Antioxidants; Antitumor; Flavonoids

1.Introduction

Different cultures use plants as medicine, and the pharmaceutical industry uses them as an origin of many effective medications since they consist of certain bioactive compounds [1]. Plant extract screening is a novel approach to discovering therapeutically active compounds in various plant species [2-4]. Cancer is a significant global health problem; 10 million people died from it in 2020 [5]. Low- and middle-income countries (LMICs) endure an excessive burden, with 65% of all cancer deaths predicted to happen there by 2023. This trend is expected to get worse, with 16 million cancer deaths worldwide expected in 2040 [6].

The Ehrlich ascites carcinoma (EAC) is a popular experimental tumor model due to its rapid growth, malignancy, high transplant ability, and brief lifespan. [7]. Because of its utility in testing anticancer drugs, this model is widely used in studies on cancer [7]. EAC cells are a good choice for studying a variety of animal hosts due to their special properties.

Traditional medicine frequently uses medicinal plants with antioxidant and anticancer properties to treat a variety of diseases [8, 9]. These herbal remedies work by several mechanisms, including cytoprotection and antioxidant effects [10]. Medicinal plants have been shown in studies to be a rich source of antioxidants such as flavonoids, phenolics, carotenoids, and vitamins, and administration of these plants, whether in the form of fresh extracts or chemical components, is often associated with a lower risk of degenerative diseases such as cancer [10]. As a result, for cancer treatment, researchers have focused on biologically active secondary metabolites from plants such as polyphenols, flavonoids, steroids, and alkaloids [11]. Plant-derived substances have been shown in previous studies to have antineoplastic effects on mice with EAC, increasing their mean survival time and lifespan [12].

Olea europaea L. is a Mediterranean evergreen tree in the Oleaceae family. [13].

Olive leaves are a significant origin of bioactive components such as hydroxycinnamic acid derivatives, hydroxytyrosol, triterpenes, secoiridoids, and flavonoids, which are a byproduct of the olive oil industry during trimming [14, 15]. Traditional medicine has used olive leaves to treat surgical infections, influenza, diarrhea, and malaria [16]. Olive leaves contain flavonoids, which have anti-inflammatory, antimicrobial, antihypertensive, cardioprotective, and antioxidant properties [17–19].

The anticancer properties of olive leaf extract were studied by Milanizadeh [20], who discovered that the extract decreased the size and weight of cancer cells in mice. Because of their several biological properties, olive leaves are widely used in the food, pharmaceutical, and cosmetic industries [21]. Ginger (*Zingiber officinale*), a Zingiberaceae family member, is frequently used as a spice or in traditional medicine, especially in Southeast Asia. Because of their medicinal properties, its rhizomes are frequently used in traditional medicine [22]. Ginger extract has been shown in studies to be effective against a variety of cancers, and gingerols and shogaols found in gingerol and shogaol extracts have anti-inflammatory, antioxidant, and anticarcinogenic properties [23]. Studies conducted in vitro and in vivo have shown that ginger extract has anticancer potential because of its flavonoid and polyphenolic chemical components [24, 25]. Ginger extract has been shown to inhibit cancer growth, cause cell death, and contain antioxidant characteristics that neutralize free radicals and inhibit peroxidation [22, 26].

The in vivo effects of combining olive leaf and ginger extracts on EAC have not been fully investigated. The aim of the study was to establish the antitumor and antioxidant effects of olive leaf extract and ginger extract, as well as their combination, in female Swiss albino mice with EAC. The study also aimed to identify the underlying molecular mechanisms involved in tumorigenesis by investigating vital biological parameters, such as tumor volume, antioxidants levels and liver functions.

2. Materials and methods

Chemicals and kits

All chemicals were purchased from Sigma-Aldrich, and all chemicals and reagents were of analytical grade.

Extraction of plant materials

Preparation of olive leaf extract

Olive leaves were obtained from a herbalist in Mansoura, Egypt. The extraction was conducted

as described by Syed Salleh, Mohd Hanapiah et al. [27].

Preparation of ginger extract

Fresh ginger rhizomes were obtained from a herbalist in Mansoura, Egypt. The extraction was carried out in the manner described by Goyal [28].

In vivo studies

Animals and experimental protocol

Forty-eight adult female Swiss albino mice weighing 22 to 26 g were obtained from the animal Farm of Vacsera in Helwan, Egypt. They were kept in clean polypropylene cages with a 12-hour light/dark cycle, controlled temperature and humidity, standard rodent chow, and unlimited access to water. Before the experiment, the mice were acclimated for ten days. The experiments were carried out in accordance with all applicable guidelines and regulations. They were conducted following the regulations of the Institutional Animal Ethics Committee of Mansoura University in Mansoura, Egypt.

Induction of EAC cells

On the tenth day of the experiment, mice were given an intraperitoneal injection of 200 µl of a solution containing 2.5 x 10^6 cells. The cell lines were kept alive by performing intraperitoneal transplants of 2.5 x 10^6 EAC cells in 0.2 mL of ascitic fluid per mouse on a regular basis [29]. Tumors were developed in the peritoneal cavity of mice 10–14 days after induction.

Experimental design

48 adult Swiss Albino mice were divided into eight equal groups of six mice each. 42 Swiss female mice were injected intraperitoneally with 200 microliters of a

$2.5x10^6$ cells/mouse solution to induce EAC cells. On the 10^{th} day after the EAC cell transplant, the treatments started, and they continued every day for 14 days. The mice were separated into eight groups, as follows: Group I consisted of healthy mice and served as a normal control; Group II included EAC-bearing mice which were not given any treatment; Group III mice were administered the standard drug Cytoplatin-10 orally at a dose of 150 mg/kg body weight; Group IV mice were given orally a dose of 150 mg/kg body weight of ethanol extract (OLET) of olive leaves; Group V mice were given orally a dose of 150 mg/kg body weight of aqueous extract (OLAQ) of olive leaves; Group VI mice were given orally a dose of 150 mg/kg body weight of ginger extract (GE); Group VII mice were given orally a dose of 150 mg/kg body weight of a mixture of OLAQ and GE (1:1); and Group VIII mice were given orally a dose of 150 mg/kg body weight of a combination of OLET and GE (1:1). Until the end of the experiment, all mice received their respective dose. The doses of Ginger administrated to animals were chosen based on Rong et al [30] and Tajaddini Mahani et al [31] studies, respectively. The selected dose of Olive leaves was based on previous studies [32].

Blood and tissue collection

Until the end of the experiment, all mice received their respective dose. Mice's ascitic fluid was collected for EAC volume determination. Blood samples were collected in dry tubes, centrifuged for 20 minutes at 2000 rpm, and stored until analysis. The serum was used to assess liver function.

The liver tissues were carefully removed and examined for antioxidant status. Liver specimens were homogenized in an ice-cold phosphate buffer (50 mM, pH 7.4) to give 10% homogenate (w/v). The homogenate was centrifuged at 4000 rpm for 15 min to remove the nuclear and mitochondrial fractions, and the supernatant was stored at -4 °C until the biochemical measurements.

Evaluation of EAC volume

The volume of EAC cells was measured by obtaining ascitic fluid from sacrificed mice. The fluid was extracted from the mouse's abdomen and centrifuged at 3000 rpm for 10 minutes at 4°C. To determine the volume of the cells, the total volume in the graduated centrifuge tube was subtracted from the volume of the supernatant.

Biochemical parameters and antioxidant assays

Several assays were carried out to assess the antioxidant enzyme activity. The activity of superoxide dismutase (SOD) was measured using the method stated in [33]. Catalase activity (CAT) was determined using the methods described in [34, 35]. Glutathione peroxidase (GSH) activity was measured using the protocol stated in [36]. The level of lipid peroxide, or malondialdehyde, was determined using the method described in [37, 38]. Furthermore, as described in [39], serum levels of the enzymes alanine transaminase (ALT) and aspartate transaminase (AST) were measured using a colorimetric method.

Statistical analysis

The data analysis was performed using a one-way analysis of variance (ANOVA) with groups as the sole factor, as reported in [40]. The results were displayed as the mean ± standard deviation of each group. Pairwise comparisons of means were performed using the Tukey–Kramer multiple comparison tests, as described in Steel and Torrie [41]. The statistical significance was established based on the following cut-off values: a p-value of less than 0.05 was considered statistically significant, a p-value of less than 0.001 was considered highly significant, and a p-value of less than 0.0001 was considered very highly significant.

3. Results

Volume of Ehrlich ascites tumor

The ascitic fluid volume was measured to assess the inhibitory effect of OLE and ginger on EAC cells in vivo. Figure 1 shows that after intraperitoneal transplantation of EAC cells, daily treatment with OLE and ginger for 14 days resulted in a significant reduction (p 0.0001) in ascitic fluid volume.

Effect of olive leaf extract and ginger extract on liver tissue oxidative markers in EAC-induced mice

Figure 2 shows the effects of OLE and ginger on liver oxidative markers in normal and

EAC-induced mice. The oxidative marker SOD significantly changed after OLE and ginger were administered to mice with EAC. When compared to the other groups, the SOD level in the EAC control group was significantly lower. On the contrary, the normal group showed a significant rise in SOD activity when compared with the other groups. MDA levels in the EAC control group were significantly higher than in the treated and normal groups ($p < 0.05$), respectively.

In contrast, the GPx level in the EAC control group was significantly lower than in the treated and normal groups. When compared to the treated groups, CAT activity was significantly higher in the normal and EAC control groups. Simultaneously, it diminished in the EAC group when compared to the normal group ($p < 0.05$).

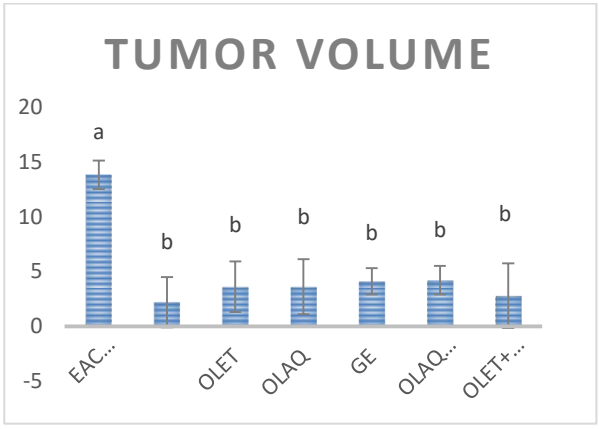
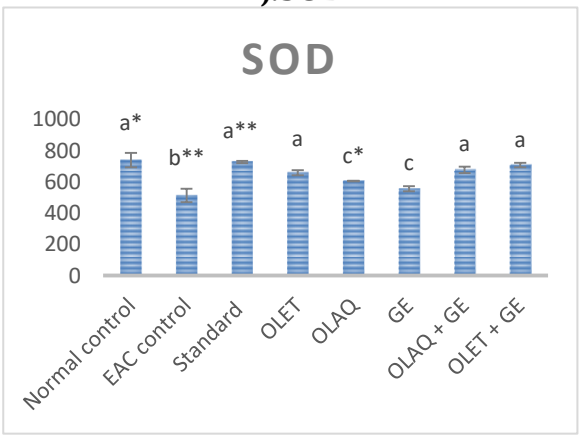


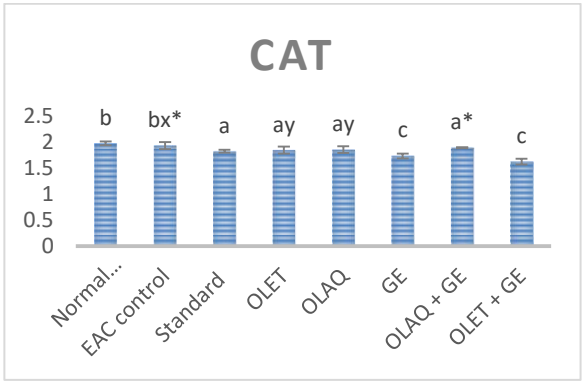
Fig 1: Effect of OLE and ginger extracts on tumor volume, of EAC tumor-induced mice.

a-b different letters between groups are very high significant (P<0.0001)

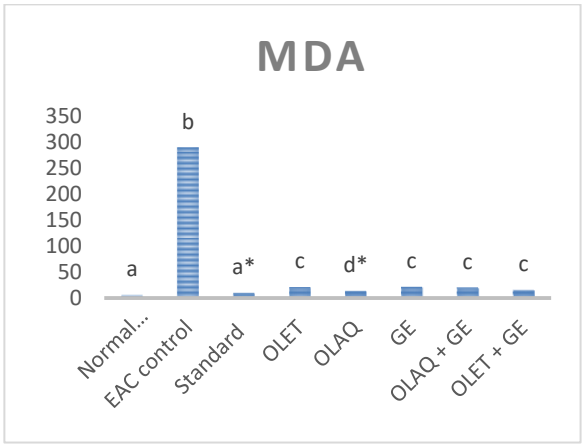
A).SOD



B) CAT



C) MDA



D) GPx

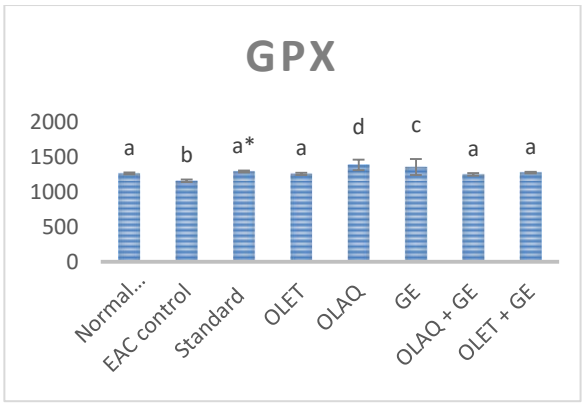


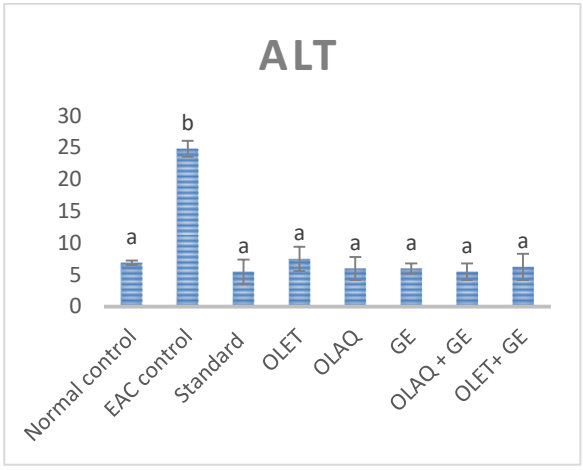
Fig 2 (a, b, c, d): Effect of OLE and ginger extracts on the liver oxidative markers in normal and EAC-induced in mice**.**

a,b.c significant letter between groups when $p \leq 0.05$

Effect of olive leaf extract and Ginger on liver function

The levels of ALT and AST in the control and treated groups are shown in figure 3. In comparison to the EAC group, the levels of the hepatic enzymes ALT and AST were significantly reduced in the normal and treated groups (p 0.05).

A).ALT



B).AST

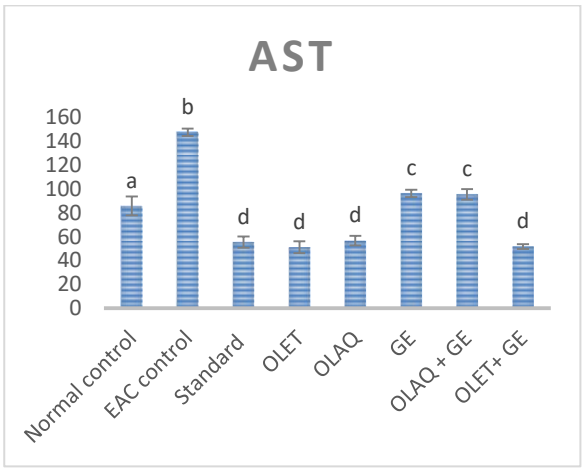


Fig 3.a, b: ALT and AST levels in control and treated groups.

a,b.c significant letter between groups when $p \leq 0.05$

4. Discussion

The purpose of this study was to assess the antitumor and antioxidant effects of olive leaf extract and ginger extract, as well as their combination, in the treatment of EAC-induced tumors in female Swiss albino mice. Cancer is defined by uncontrolled growth of cells, which results in tumor development, which then spreads to other parts of the body via the blood and lymphatic systems, leading to tissue proliferation and death of cells [7]. Chemotherapy is one of the most used cancer treatments. Cancer cells, on the other hand, frequently develop resistance to certain chemotherapy medications, decreasing their efficacy and causing harm to normal cells [42]. Consequently, there has been an increase in interest in using natural products as a substitute for or in addition to traditional chemotherapy [43]. Anticancer agents' primary goal is to prevent tumor cell growth or damage them while causing no harm to normal cells. Natural products are thought to be a safer and effective alternative to chemotherapy and radiotherapy for cancer treatment [22]. The anticancer effects of numerous natural products have been investigated using various experimental models [44].

Ability to decrease tumor volume and prolong life is one of the primary criteria for assessing the efficacy of an anticancer or antitumor compound [45]. Our research discovered a statistically significant variance in tumor volume between the EAC-induced control and treated groups. According to the findings, ginger extract significantly reduced tumor volume growth, and this is consistent with prior studies [46]. Lowering oxidative stress is essential in preventing liver cell damage or injury because tumors can affect how vital organs function, particularly the liver [45]. According to the current study, the treated groups had significantly lower MDA levels than the EAC control group and significantly higher levels of the antioxidant enzymes SOD, CAT, and GPx than the EAC control group. These results are consistent with previous research [47]. According to Gholampour's research, decreased levels of MDA, that can be associated with ginger extract's antioxidant activity, efficiently prevented peroxidative liver damage caused by ferrous sulfate [48]. In addition to lowering toxicity, ginger extract, a powerful antioxidant, is also believed to have anti-tumor activity and to improve the therapeutic effects of various anticancer drugs [49].

OLE improved both enzymatic and nonenzymatic antioxidant defenses. SOD, CAT, and GPx are the most effective antioxidant defenses in cells for controlling or stopping the production of free radicals and reactive species. Flavonoids have been shown to have antioxidant and radical scavenging characteristics in in vivo animal models of diabetes, cancer, and drug-induced toxicity [50–52].

In our study, we found that tumor-induced mice had elevated levels of hepatic ALT and

AST activities because the toxic activity of hepatic tumor cells increases cell permeability or liver damage [45]. In EAC-induced mice that received extract treatment, their ALT and AST levels were significantly reduced. Additionally, this research found that tumor mice had elevated liver enzymes related to cell damage. On the contrary, after receiving treatment with the extract shown to exhibit a hepatoprotective effect, these above-mentioned markers returned to near normal levels.

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