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The Possible Benefits of Tongkat Ali (*Eurycoma longifolia*) Extract for the Treatment of Cancer

Miras Hasan Madhloom[1*]
[1]Environment Department, College of Science, University of Al-Qadisiyah, Iraq

Abstract: ***Background***: In recent years, phytochemicals have gained international scientific recognition for their potential to prevent major health problems. Research on the chemical structures and pharmacological mechanisms of plant extracts with anticancer effects has received considerable attention. ***Work's objective***: The present study aimed to evaluate the antitumor chemotherapeutic potential of Tongkat Ali (*Eurycoma longifolia*) extract against breast carcinogenesis in Swiss albino mice resulting from Ehrlich's ascites carcinoma (EAC) transplantation. ***Materials and Methods****:* The efficacy of Tongkat Ali (*Eurycoma longifolia*) extract against Ehrlich's ascites carcinoma was evaluated by monitoring tumor weight, size, and recurrence. Lipid peroxidation (MDA) and the biochemical profile associated with oxidative stress were studied, as well as the antioxidant profile, which included the activity of superoxide dismutase (SOD), glutathione reductase (GR), glutathione-s-transferase (GST), total antioxidant capacity (TAC), catalase, and CAT, as well as markers of hepatotoxicity and renal toxicity (creatinine, urea, and aminotransferases), and histopathological changes after treatment. ***The Results***: Reduced oxidative stress, increased antioxidant levels, restored liver and kidney function, and inhibited tumor growth and neovascularization. ***Conclusion***: Based on all the data obtained, our research indicates that treatment with *Eurycoma longifolia* extract provided strong chemotherapeutic efficacy and antioxidant defense against breast tumors implanted in EAC.

Keywords: *Eurycoma Longifolia*, Ehrlich Ascites Carcinoma, Oxidative Stress, Antioxidants.

RESEARCH PAPER

***Corresponding Author:**
Miras Hasan Madhloom
Environment Department, College of Science, University of Al-Qadisiyah, Iraq

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INTRODUCTION

Breast cancer is the most common cancer among women worldwide, accounting for 22.9% of all female cancer cases [1]. According to a study by the World Health Organization (WHO), breast cancer accounts for 16% of all cancer-related deaths among women globally. It is the most common solid tumor among women. Age is a risk factor for breast cancer, but lifestyle and environmental factors also have a significant impact [2].

Radiation therapy, chemotherapy, surgery, and/or any combination of these are available as treatments for breast cancer and associated disorders. The death rate from cancer is still high despite these treatment choices. The primary reasons for this include the challenges associated with early detection of breast cancer, the high cost of treatment, and the fact that breast cancer often appears later in women than other types of cancer [3–5]. These numerous drawbacks need the development of novel therapeutic alternatives that would improve the prognosis of patients with breast cancer while posing minimal to no adverse effects [6].

Phytochemical therapy for severe health conditions has recently received international scientific approbation. There is a lot of interest in studying the pharmacological mechanisms and chemical composition of herbal extracts that give them their anticancer effects [7, 8]. Among the various species of the genus *Eurycoma*, *Eurycoma longifolia* (also known as Tongkat Ali) is the most well-known and widely used medicinally and as a dietary supplement. Although native to some East Asian countries, it is now cultivated in other environments outside its native habitat, but its cultivation success remains somewhat limited due to its specific climatic requirements, slow growth, and the sensitivity of its roots to climate and soil. It has been used in traditional Chinese and Indian medicine for thousands of years. In addition, this plant is listed in several official pharmacopoeias. Its roots and fruits, either alone or in combination, are used to treat numerous conditions

related to increased testosterone, improved sexual performance, stress reduction, and antioxidant properties. *Eurycoma longifolia*, also known as Tongkat Ali in Malaysia, is a widely grown, well-known tree throughout Southeast Asia. Practitioners of traditional medicine are familiar with it as a plant that can treat a wide range of illnesses. The plant's main quassinoid, eurycomanone, has a number of notable impacts on different cancer cell lines. In vitro and in vivo tests have demonstrated that eurycomanone promotes cell death and suppresses the proliferation of malignant cells. Although safety and toxicity investigations have been carried out, a significant knowledge gap remains in establishing a scientific basis for the molecular mechanism and intervention method in real human cancer cells [9]. The purpose of this study was to examine the efficacy of *Eurycoma longifolia* as a chemotherapeutic treatment for Ehrlich ascites cancer. The following actions were taken to accomplish this goal:

MATERIALS

Animals

Two groups of female Swiss albino mice, weighing 25±5 grams and aged 12±2 weeks, were created. Regarding the LD50, various ***Eurycoma longifolia*** concentrations were given to the experimental groups. ***Eurycoma longifolia*** was given to mice in escalating dosages. Appendix 2, Guiding Principles for Biomedical Research Involving Animals (2011), which outlines the ethical standards of Alexandria University's Medical Research Institute was followed when using experimental animals in the study methodology.

Group A: 10 mice that received solely PBS treatment as a control group.

Group B: To implant EAC breast cancer, 50 mice underwent a single subcutaneous injection of 2 x 106 EAC cells. Five subgroups were created from this group: subgroup B-1 consisted of ten mice with EAC alone who received no therapy. Ten mice in subgroup B-2 received 20 mg/kg/day of ***Eurycoma longifolia*** following EAC implantation; ten mice in subgroup B-3 received 15 mg/kg/day of ***Eurycoma longifolia*** following EAC implantation; ten mice in subgroup.

B-4 received 10 mg/kg/day of *Eurycoma longifolia* following EAC implantation; and ten mice in subgroup B-5 received 5 mg/kg/day of *Eurycoma longifolia* following EAC implantation.

METHODS

The following investigations were carried out to assess the treatment effects for each of the groups under study:

Assessment of Tumor Growth and Inhibition

The tumor's growth was tracked daily during the course of treatment. The tumors' length and width were measured with a slide caliper, and their volume (in millimeters) was calculated using the formula below. TV (mm3) is equivalent to 22/7x4/3 (length/2) x (width/2). Two weeks after the treatment, the mice were killed, and the tumors were removed and weighed (in grams).

Biochemical Analysis

A 2.5 ml sample of venous blood was extracted from every mouse group. After allowing the blood samples to clot completely for 20 minutes, the serum was separated for biochemical analyses by centrifuging them at 3000×g for 20 minutes. Using Auto-analyzer, all biochemical analysis was completed.

State of Antioxidants and Oxidative Stress

The assay kits (BioVision Catalogue #K274-100, #K739-100, #K263-100, #K761-100, #K773-100, #K335-100) for total antioxidant capacity (TAC), lipid peroxidation (MDA), glutathione-s-transferase (GST), glutathione reductase (GR), catalase (CAT), and superoxide dismutase (SOD)activities were used in compliance with the manufacturer's instructions.

Tests for Liver and Kidney Function

The aspartate aminotransferase (AST), alanine transaminase (ALT), creatinine, and urea assay Kits (Sigma Catalogue #MAK055, #MAK080, #MAK179, #MAK052) were used in accordance with the manufacturer's instructions.

Histopathological Analysis

The slides for light microscopy analysis were prepared by fixing small pieces of Ehrlich tumor tissue from the experimental groups in 10% formaldehyde, dehydrating them in increasing alcohol grades, embedding them in paraffin to create paraffin blocks, and cutting them into 3.4 µm thick sections that floated in a water bath. Before being coated with covering slides, the blocks were cleansed with xylene, rehydrated in decreasing alcohol grades, stained with hematoxylin and eosin stain, and then cleaned with ethylene once more.

RESULTS

Treatment's Effects on the Mass and Volume of the Tumor

For *Eurycoma longifolia* at different concentrations (20 mg, 15 mg, 10 mg, and 5 mg), Fig. (1) illustrates the correlations between tumor diameters and treatment duration. The findings indicate that a 5 mg dose of *Eurycoma longifolia* has no discernible effect on the tumor volume. Tumor cells and volume have been observed to be more affected by *Eurycoma longifolia* doses of 10and 15 mg. *Eurycoma longifolia* treatment at 20 mg had the biggest effect on tumor cells and tumor volume reduction.

Treatment's Effects on Factors Related to Oxidative Stress

Our results showed an increase in lipid peroxidation during EAC implantation. All EAC-implanted groups have MDA levels that are noticeably greater than those of the animals in the control group. In contrast, MDA levels were much greater in rats implanted with EAC alone than in groups receiving *Eurycoma longifolia*. The antioxidant (GR, GST, SOD, CAT, and TAC) activities of the cancer-bearing mice in the current study were lower than those of the healthy animals. On the other hand, the experimental animals administered *Eurycoma longifolia* Fig. (2) exhibit a significant increase in both enzymatic and non enzymatic antioxidant defense in comparison to mice who only received EAC.

Treatment's effects on Liver and Kidney Function Tests

Urea and creatinine, two indicators of renal function, were considered in this investigation. EAC considerably increased the levels of urea and creatinine in the serum during this study. Nonetheless, it was demonstrated that *Eurycoma longifolia* supplementation raised blood levels of urea and creatinine, which are indicators of renal protection. This lends more credence to *Eurycoma longifolia's* ability to protect against kidney damage caused by EAC. The liver function indicators ALT and AST were also considered in this investigation. EAC considerably raised the serum levels of AST and ALT in this study. Nevertheless, *Eurycoma longifolia* treatment stopped blood AST and ALT levels from rising, suggesting that *Eurycoma longifolia* is hepatoprotective against EAC-induced hepatotoxicity Fig. (3,4).

Treatment's Impact on Histological Structural Alterations

A histological analysis revealed that every tumor in the cancerous control group was made up of highly malignant cells and had 5–10% necrosis. Compared to the group treated with 10 mg/kg body weight (77%), animals receiving *Eurycoma longifolia* extract (15 and 20 mg/kg body weight) had notable necrosis regions in their excised tumors, at 88 and 92%, respectively. On the other hand, tumors treated with 5 mg/kg body weight (67%) showed distinct necrosis foci areas (Fig).

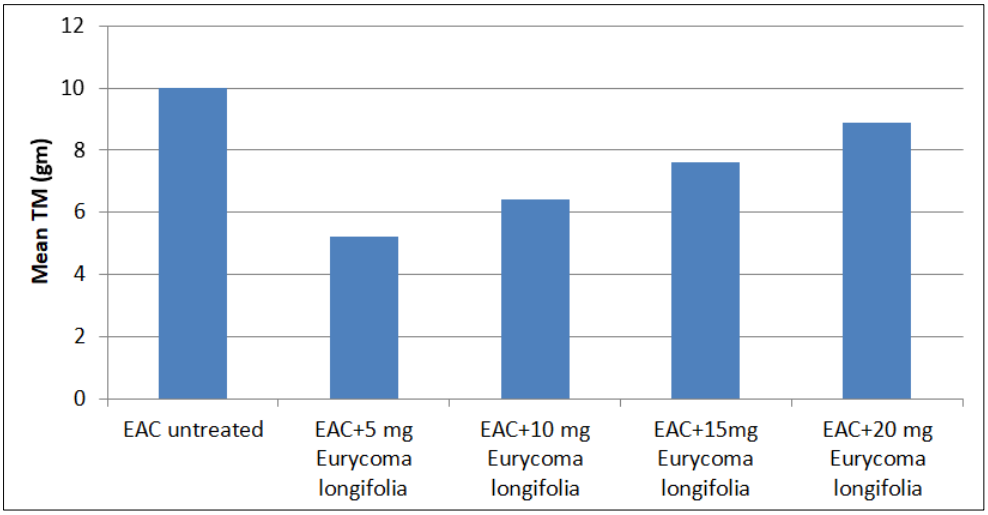
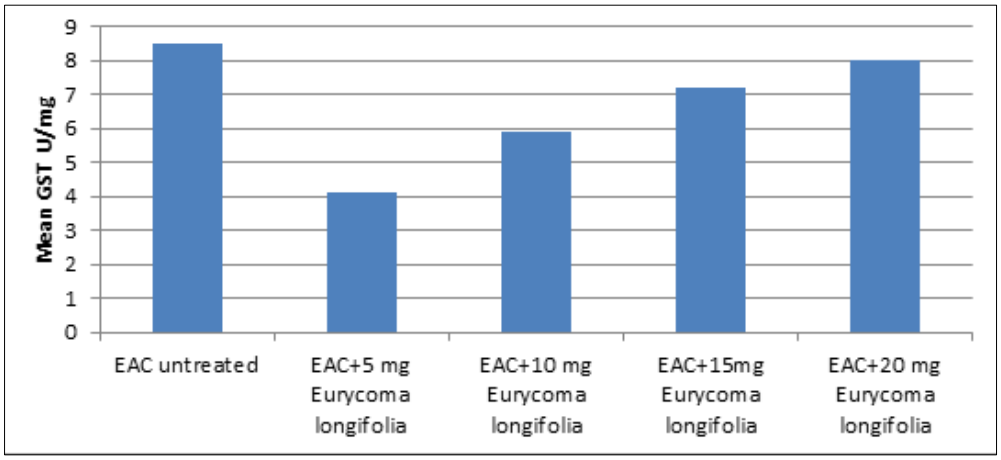
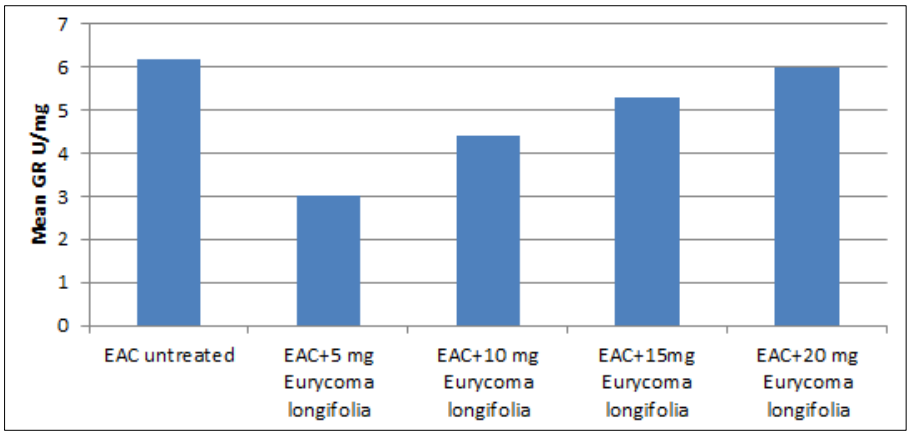
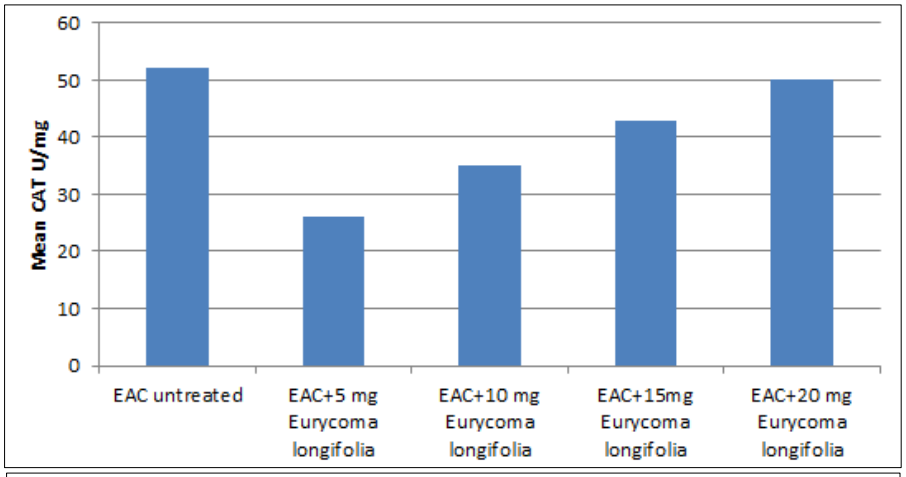
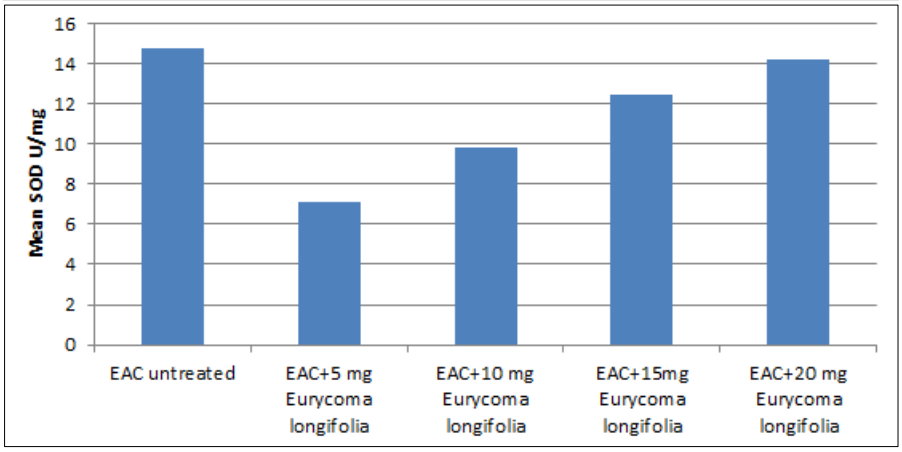


Figure 1: TV and TM of EAC treated groups with *Eurycoma longifolia* with different conc.









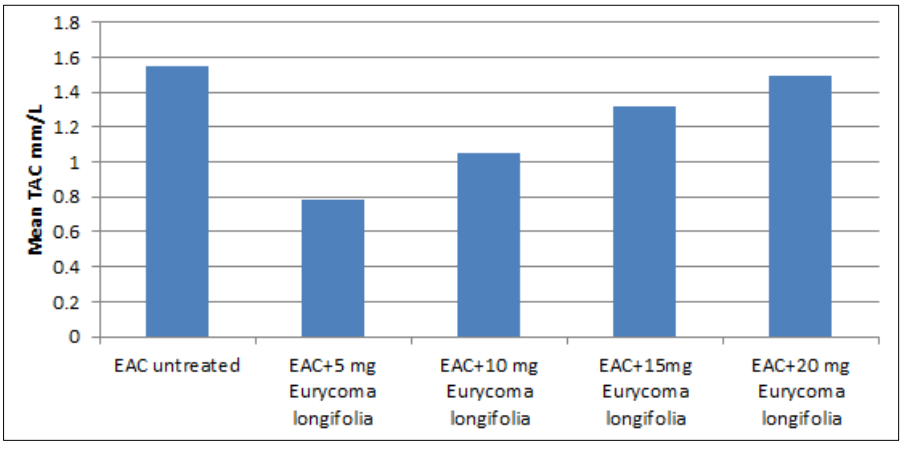


Figure 2: GST, GR CAT, SOD TAC and MDA of EAC treated groups with *Eurycoma longifolia* with different conc

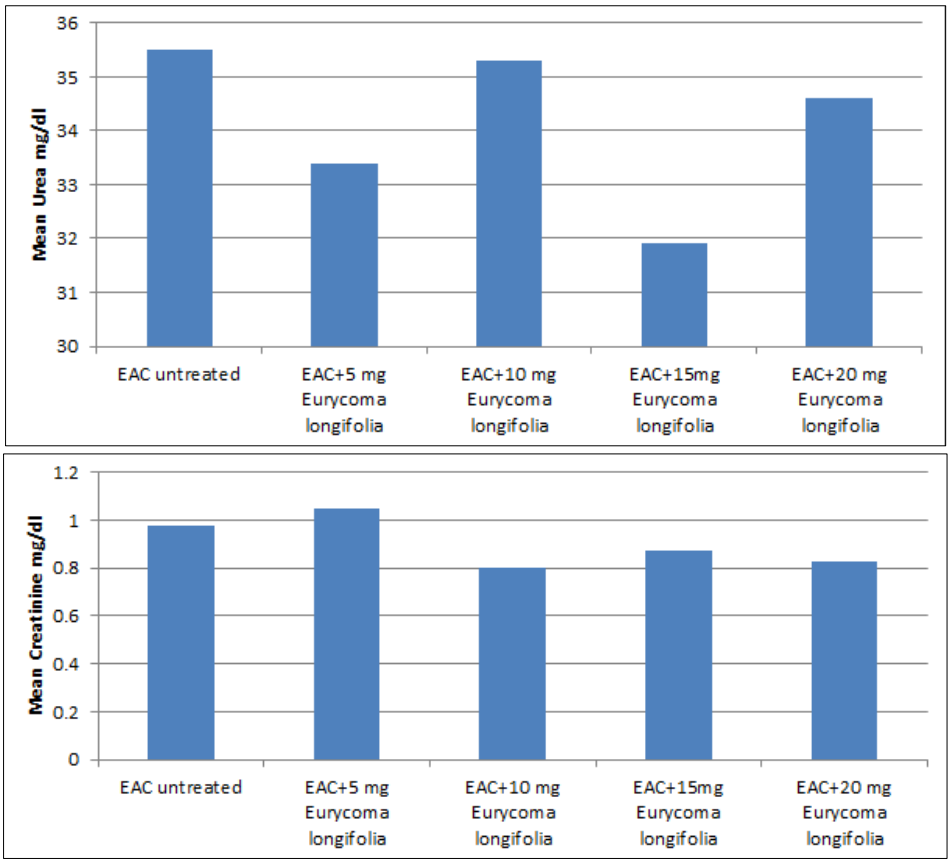


Figure 3: Urea and creatinine of EAC treated groups with *Eurycoma longifolia* with different conc

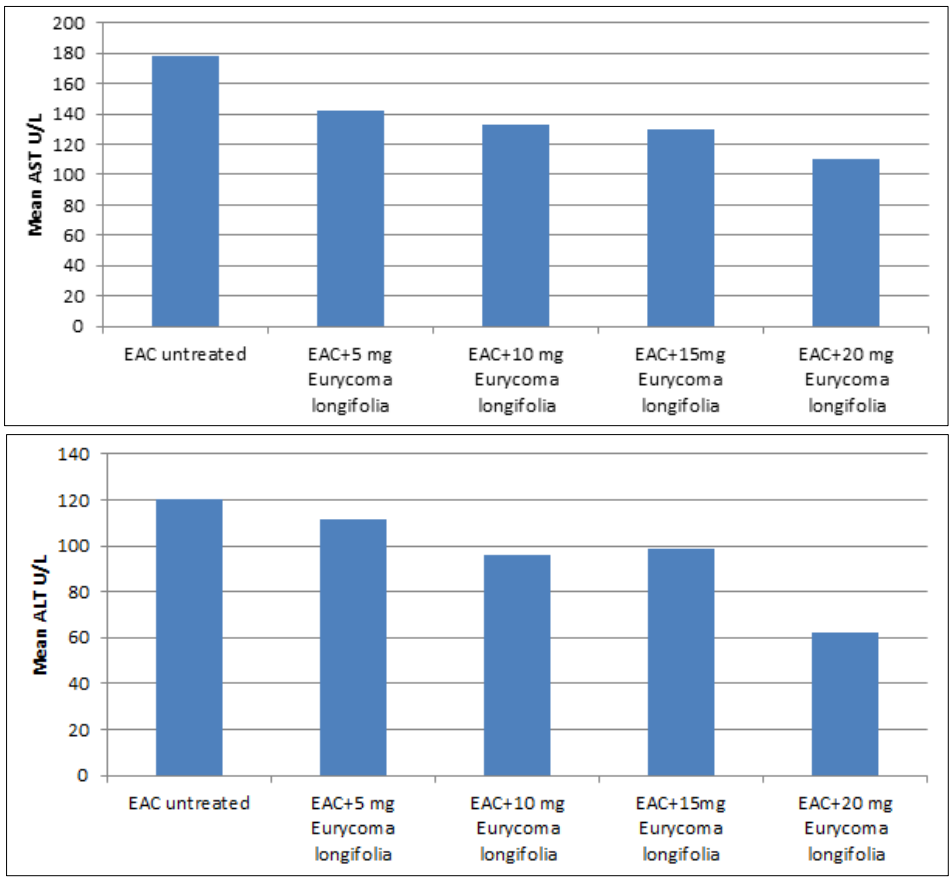
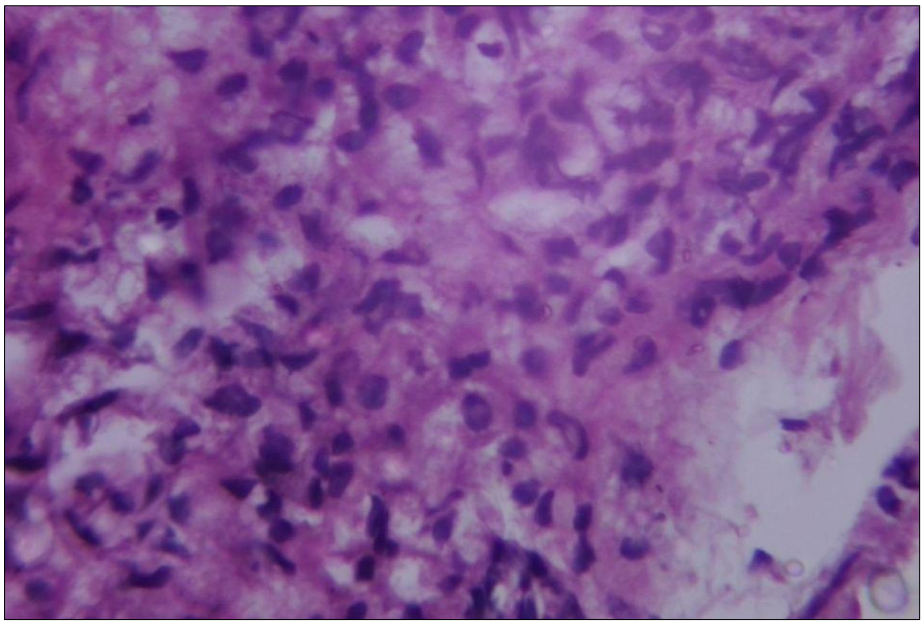
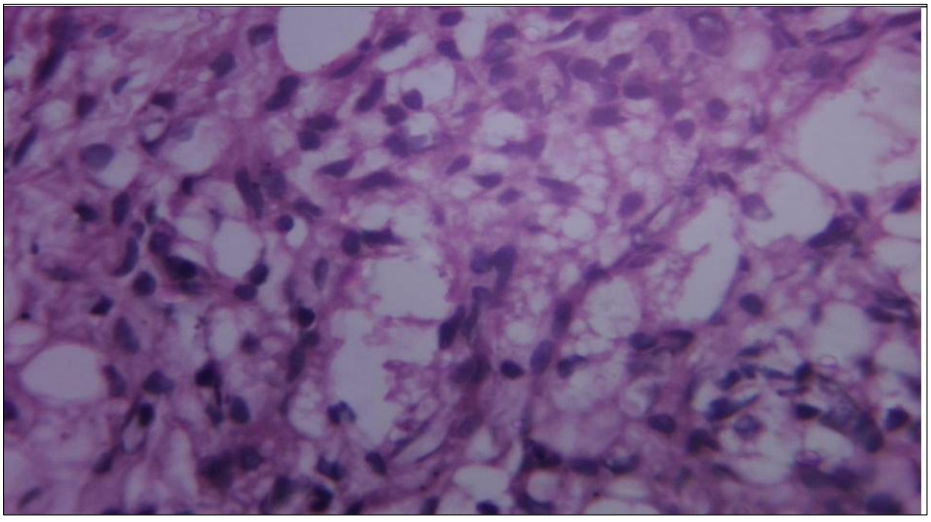


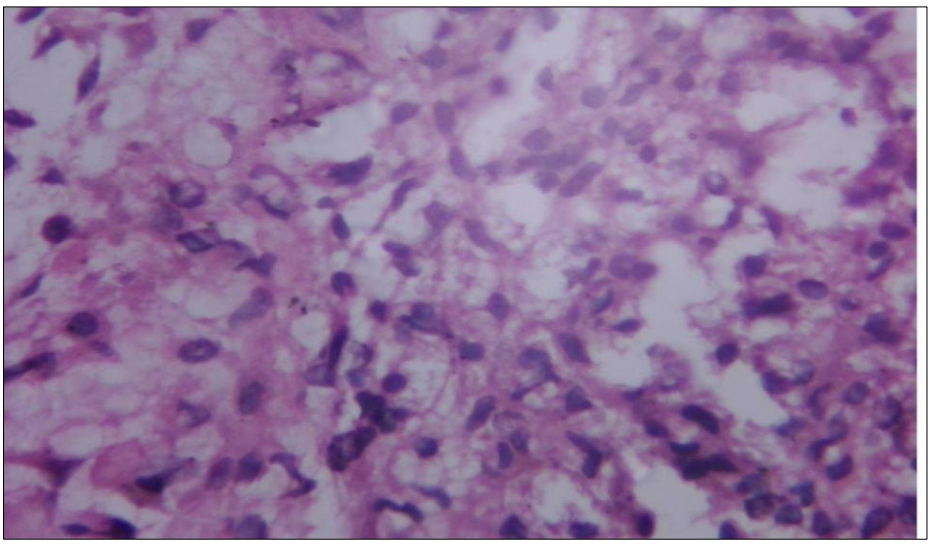
Figure 4: ALT and AST of EAC treated groups with *Eurycoma longifolia* with different conc



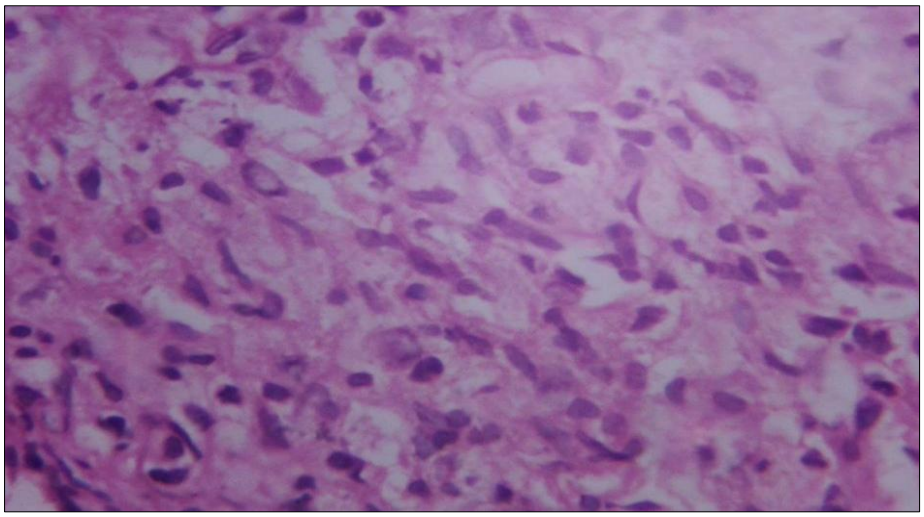
EAC Untreated



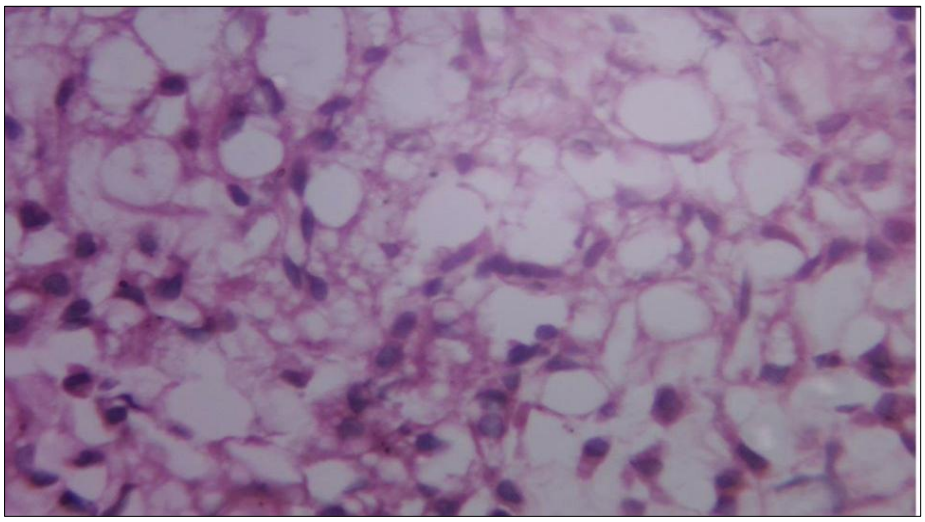
EAC + 5 mg *Eurycoma longifolia*



EAC + 10 mg *Eurycoma longifolia*



EAC + 15 mg *Eurycoma longifolia*



EAC + 20 mg *Eurycoma longifolia*

Figure 5: H&E of EAC treated groups with *Eurycoma longifolia* with different conc.

An aggressive metastatic phenotype is the final stage of the multistage carcinogenesis process. Physical, chemical, or viral elements could all be involved. Complex In addition to the coordinated buildup of advantageous genetic defects, interactions between the tumor and the host tissues are necessary [10].

It has been suggested that the unrestrained generation of free radicals and reactive oxygen species (ROS) is the source of the disruption of antioxidant state, which results in oxidative stress and carcinogenesis [11]. In addition to causing major, interconnected changes in cellular metabolism, such as lipid peroxidation, oxidative stress affects a number of macromolecular species, such as proteins, lipids, and nucleic acids [12]. Lipid peroxidation, which can result in the production of dangerous compounds including MDA and 4-hydroxynonenal, has been connected to carcinogenesis [13]. The ability of these medications to target specific cellular targets may lead to the development of cancer [14].

If EAC and some other lipophilic carcinogens are not biotransformed into other substances, their toxicological effects may preferentially damage breast tissue. Metabolites that are hydrophilic and readily eliminated [15]. EAC has been shown in numerous studies to be effective in producing a mouse model of breast cancer. The redox balance of the tissue is altered by this process, suggesting that oxidative damage may lead to pathogenic and metabolic alterations [16, 17].

The relevant cells' antioxidant defense mechanisms typically scavenge free radicals in subcellular compartments [18]. Due to its ease of evading defenses, EAC causes aberrant cell activity and upsets the balance between pro- and antioxidants.

High concentrations of polyunsaturated fatty acids in cellular membranes make them vulnerable to lipid peroxidation, which can be detrimental [19].

Oxygenated metabolites, free radicals, and radicals are all produced by EAC [20]. Lipid peroxidation is triggered as a result, which is detrimental. As a result of oxidative stress [21]. EAC is a helpful and practical technique for creating in vivo breast cancer models since it may cause severe oxidative damage to several human organs, including the liver and breast [22, 23].

The growth of tumors is accompanied by a variety of metabolic alterations [24]. Because human cancers have a delayed pathogenic development from preneoplastic to malignant. Consequently, there is always a chance to stop tumor growth. Consequently, rigorous cancer research has declined in recent years while prevention has expanded. Chemotherapeutic approaches use substances with certain characteristics to slow down the carcinogenesis process.

Since MDA has emerged as a valuable marker of oxidative stress, there has been an increase in interest in understanding the function of lipid peroxidation in the development of cancer in recent years. When free radicals attack polyunsaturated fatty acids, they can create low molecular weight aldehydes like MDA [25, 26]. Lipid peroxides accumulate in malignant tissue and are released into the bloodstream by a failing antioxidant system, which is most likely the cause of the increased level of serum lipid peroxide in cases of breast cancer [27]. Lipid peroxidation's last peroxyl radical byproducts are MDA and a very hazardous major aldehyde. It is thought to function as a protective enzyme inhibitor. As a result, it could cause carcinogenesis as well as mutagenesis [28].

Our results show that EAC implantation led to an increase in lipid peroxidation. The MDA levels in all EAC-treated groups are notably higher. Compared to the animals in the control group. By scavenging reactive free radicals involved in peroxidation, *Eurycoma longifolia* mainly reduces MDA [29]. Mice fed EAC alone had significantly higher MDA levels than animals given *Eurycoma longifolia*. *Eurycoma longifolia* exhibits antilipid peroxidative activity based on its capacity to scavenge free radicals and lower MDA.

Defense mechanisms against damage from free radicals shield cells from harm. By scavenging reactive oxygen species (ROS), which are required for the initiation of lipid peroxidation, the defensive anti-oxidative system may prevent the development of cancer [30]. Both enzymatic (GPx, GST, SOD, and CAT) and nonenzymatic (mostly GSH) components are used in this defense mechanism [30, 31]. The primary defense mechanism of the antioxidant system against oxidative stress is SOD, which converts toxic superoxide anions (O2−) into O2 and H2O2.

Tension to defend against ROS, Gpx and catalase can scavenge H2O2 and convert it into innocuous metabolites [32]. Cells are protected from damage by defense mechanisms against free radical damage. The defensive anti-oxidative system may stop cancer from developing by scavenging reactive oxygen species (ROS), which are necessary for the start of lipid peroxidation [30]. This defense mechanism uses both nonenzymatic (primarily GSH) and enzymatic (GPx, GST, SOD, and CAT) components [30, 31]. SOD, which transforms harmful superoxide anions (O2−) into O2 and H2O2, is the antioxidant system's main defense mechanism against oxidative stress. Catalase and Gpx can scavenge H2O2 and transform it into harmless metabolites to protect against ROS [32].

Additionally, in response to oxidative stress, GPx is highly effective at scavenging reactive free radicals and detoxifying peroxides and hydroperoxides that cause GSH oxidation [33]. Furthermore, by conjugating the GSH thiol functional groups with electrophilic xenobiotics, GST catalyzes the conversion or elimination of the xenobiotic-GSH complex [34]. During this process, GSH is oxidized to GSSG, which GR can then convert back into GSH by consuming NADPH [35]. GSH is the primary nonenzyme antioxidant in mammalian cells [36]. GSH is responsible for several physiological functions, such as the detoxification of internal and external toxins.

y preventing lipid peroxidation, scavenging free radicals, and eliminating H2O2, it efficiently protects cells from oxidative stress [37]. The antioxidant activity (GR, GST, SOD, CAT, and TAC) of cancer-bearing mice was lower than that of healthy animals in the current study. Our results are in line with previous research [38, 39]. Reduced expression of these antioxidants following mammary gland injury is the reason for the subsequent decline in antioxidant defense, claim Pradeep *et al*., [40]. Animals given EAC and *Eurycoma longifolia* showed a notable improvement in antioxidant defense, both enzymatic and non-enzymatic, in comparison to those treated with EAC alone. This rise is explained by *Eurycoma longifolia*'s ability to reduce the development of breast lipoperoxides, boost endogenous antioxidant activity above and beyond its ability to scavenge free radicals, and prevent the generation of free radicals [41]. The higher activity of antioxidant enzymes in mice treated with *Eurycoma longifolia* as opposed to those given EAC alone indicates that *Eurycoma longifolia* extract has effective antioxidant activity because it contains flavonoids, alkaloids, phytosterols, tannins, amino acids, glycosides, saponins, and triterpenoids [42–47]. According to the information above, *Eurycoma longifolia* extract has a preventive effect. The plant's flavonoids, which have potent antioxidative qualities and function as potent singlet and superoxide radical quenchers, may be responsible for this action [42–51].

The results of the investigation demonstrated a statistically significant inverse connection between

plasma mean MDA levels and antioxidant activity. According to Kumaraguruparan *et al*., the high MDA level could be the result of an antioxidant system breakdown that causes lipid peroxides to accumulate in cancer tissue [52]. Furthermore, Sener *et al*., [53], found that, as compared to the treated and control groups, the breast cancer group exhibited statistically significant decreases in total antioxidant capacity and considerably higher blood MDA levels.

The results of this investigation align with those of other studies [54–70]. Urea and creatinine are examples of metabolic products that the kidney removes from the bloodstream to prevent buildup. Serum levels of these substances are thought to increase when renal function declines [71, 72]. The investigation's findings suggested that alkylating drugs caused a decrease in renal function, which is consistent with previous research [73, 74]. This study includes renal function indicators such as urea and creatinine. *Eurycoma longifolia*, we found, increased serum levels of urea and creatinine, indicating kidney preservation. This illustrates the preventive effect of *Eurycoma longifolia* against kidney damage caused by EAC. The liver is one organ that aids in the biotransformation of drugs and other hepatotoxicants. The blood bilirubin level and the activity of the liver enzymes AST, ALT, GGT, and ALP are reliable markers of hepatotoxicity [75, 76].

Elevated blood ALT and AST levels could be the result of hepatocyte damage (hepatocellular injury) [77-81]. Bilirubin is present in the bile, liver, intestines, and reticuloendothelial cells of the spleen, whereas GGT and ALP are affixed to the cell membrane [78]. Serum levels of bilirubin, ALP, and GGT rise in hepatobiliary damage, poor hepatic clearance, and 0verproduction or leakage of these enzymes [78]. This study looked at hepatic function markers like ALT and AST. EAC significantly increased the levels of ALT and AST serum activity in this investigation. AST and ALT are mostly found in the mitochondria and cytoplasm of hepatocytes [78].

Because *Eurycoma longifolia* inhibited increases in blood ALT and AST levels prior to and following therapy, this study implies that it possesses hepatoprotective qualities. This suggests that *Eurycoma longifolia* protects against hepatotoxicity brought on by EAC. This analysis's findings align with those of previous research [54–70]. The current study's findings showed that during the trial, metabolic and histological alterations occurred simultaneously. Histological examination revealed that every tumor in the cancerous control group included highly malignant cells and lacked necrosis. Tumors removed from animals given 15 and 20 mg/kg body weight exhibited significant regions of necrosis (88% and 92%, respectively), in contrast to the group given 10 mg/kg body weight (77%), even though foci of necrosis (67%) were visible in the tumors administered with 5mg/kg body weight. The current findings were consistent with previous studies conducted by various authors [17–53].

CONCLUSION

According to the present research, *Eurycoma longifolia* may have promising chemotherapeutic qualities for the treatment of cancer.

Recommendations

The results of this study show that it is possible to support *Eurycoma longifolia* as a therapeutic substitute for cancer treatment with a longer course of treatment.

List of Abbreviations

ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
CAT: Catalase
DMBA: 7,10-Dimethyl-1,2-Benzanthracene
GPx: Glutathione peroxidase
GR: Glutathione reductase
GSH: Reduced glutathione
GST: Glutathione-S-transferase
MDA: Malondialdehyde
ROS: Reactive oxygen species
SOD: Superoxide dismutase
TAC Total antioxidant capacity

Ethics Approval

The requirements of the Institutional Committee for the Care and Use of Animals (IACUC) under the Institute of Medical Research Institute of Alexandria University, Alexandria, Egypt, as well as the policies of the European Convention for the Protection of Vertebrates Used for Experimental and Scientific Purposes regarding animal care and use in research and teaching are followed in all animal experiments described in this study. Every attempt was made to lessen the animals' suffering, and when necessary, authorized anesthetic techniques were used.

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