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Study the Potential Role of *Tribulus terrestris* Extract on Cancer Treatment

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Running Title: *Tribulus terrestris* and Cancer

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Abstract

Background: In recent years, phytochemicals have gained international scientific recognition for their ability to prevent major health problems. Research on the chemical structures and pharmacological mechanisms of plant extracts with anticancer effects has attracted a lot of attention. Work's objective: The work of the current study aimed to assess the chemotherapeutic anti-tumor ability of *Tribulus terrestris* extract against mammary carcinogenesis in Swiss albino mice produced by Ehrlich ascites carcinoma (EAC) implantation. **Materials and methods:** *Tribulus terrestris* extracts were evaluated for their effectiveness against EAC by tracking tumor weight, volume, and occurrence. Lipid peroxidation (MDA) and the biochemical oxidative stress-related profile were studied, as were the antioxidants-related 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 the hepatic and renal toxicity markers (creatinine, urea, and transaminases) and histopathological changes after treatment. The **Results**: showed that oxidative stress was reduced, antioxidant levels were raised, liver and kidney functions were restored, and tumor and neovascularisation were inhibited. **Conclusion**: Based on all obtained data, our research suggests that *Tribulus terrestris* extract treatment offered robust chemotherapeutic action as well as antioxidant defense against EAC-implanted mammary tumors.

Keywords: *Tribulus terrestris*; Ehrlich Ascites Carcinoma; Oxidative Stress; Antioxidants

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Introduction

Breast cancer is the most common cancer in women worldwide, accounting for 22.9% of all female cancer cases. Breast cancer in Egypt has a terrible prognosis, with a death rate of 29% and an incidence-to-mortality ratio of 1:3.7. [1]. According to a World Health Organization (WHO) study, breast cancer accounts for 16% of all cancer-related deaths in women globally. It is the most common solid tumor diagnosed in women. Age is a risk factor for

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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 20 species in the genus Tribulus, *Tribulus terrestris* (also known as puncture vine or Gokharu) is a significant member of the Zygophyllaceae family. It is frequently grown on pasture areas, cropland, and the sides of roads in arid and hot desert environments. Although it originated in the Mediterranean, *Tribulus terrestris* is now widely cultivated in warm climates in Asia, Africa, America, and Australia. Chinese and Indian traditional medical systems have been using it for thousands of years. Additionally, this plant is listed in several official pharmacopeias. Its roots and fruit, either separately or in combination, are used to treat many conditions related to the genitourinary tract, including ocular diseases, male infertility, sperm motility, kidney and bladder stones, urinary tract infections, and libido loss in both men and women. *Tribulus terrestris* has also been shown in some investigations to have cytotoxic and antibacterial effects on the cardiovascular system. According to reports, the tribal people in the southwestern city of Jazan Province, Saudi Arabia, employ *Tribulus terrestris* to treat vitiligo, kidney stones, and other skin conditions. There are several pharmaceutical preparations and dietary supplements on the market that contain *Tribulus terrestris*, and they are used for various medicinal purposes. According to a review of the literature, *Tribulus terrestris* functions as an antidiabetic, cardiotonic, hypolipidemic, hepatoprotective, immunomodulatory, analgesic, antispasmodic, diuretic, antiurolithic, anti-inflammatory, anti-cancer, anthelmintic, antibacterial, and larvicidal [9].

The purpose of this study was to examine the efficacy of *Tribulus terrestris* 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 20±5 grams and aged 8±2 weeks, were created. Regarding the LD50, various *Tribulus terrestris* concentrations were given to the experimental groups. ***Tribulus terrestris*** 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 10^6 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 1000 mg/kg/day of *Tribulus terrestris* following EAC implantation; ten mice in subgroup B-3 received 750 mg/kg/day of *Tribulus terrestris* following EAC implantation; ten mice in subgroup B-4 received 500 mg/kg/day of *Tribulus terrestris* following EAC implantation; and ten mice in

subgroup B-5 received 250 mg/kg/day of *Tribulus terrestris* following EAC implantation.

Methods

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

3.1. Assessment of tumor growth and inhibition

Every day over the course of treatment, the tumor's growth was monitored. A slide caliper was used to measure the tumors' length and width, and the following formula was used to determine the tumor volume (in millimeters). TV (mm3) equals (length/2) x (width/2) / 22/7x4/3. The mice were slaughtered two weeks following the therapy, and the tumors were excised 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 *Tribulus terrestris* at different concentrations (1000 mg, 750 mg, 500 mg, and 250 mg), **Fig. (1)** illustrates the correlations between tumor diameters and treatment duration. The findings indicate that a 250 mg dose of *Tribulus terrestris* has no discernible effect on the tumor volume. Tumor cells and volume have been observed to be more affected by *Tribulus terrestris* doses of 500 and 750 mg. *Tribulus terrestris* treatment at 1000 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 *Tribulus terrestris*.

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 *Tribulus terrestris* **Fig. (2)** exhibit a significant increase in both enzymatic and non-enzymatic antioxidant defense in comparison to mice who only received EAC.

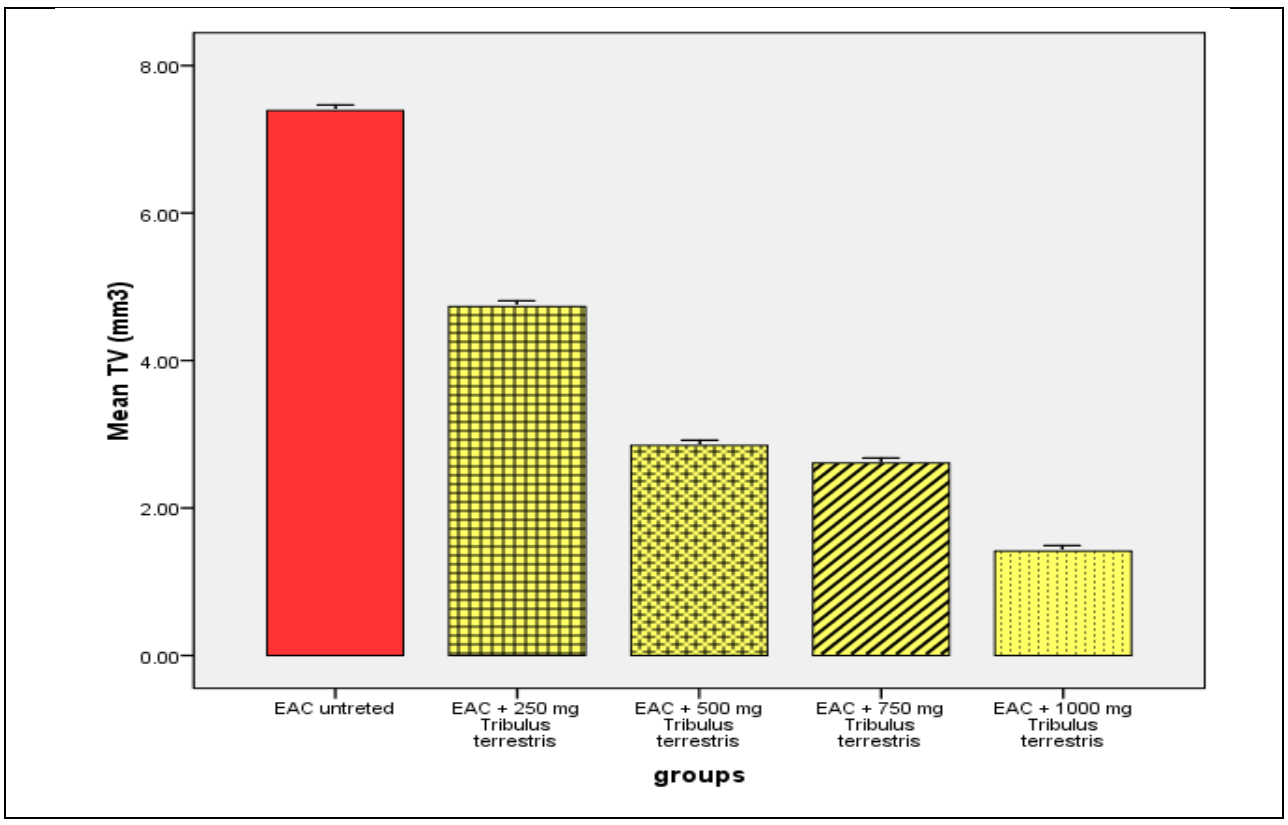
Treatment's effects on liver and kidney function tests

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 *Tribulus terrestris* supplementation raised blood levels of urea and creatinine, which are indicators of renal protection. This lends more credence to *Tribulus terrestris*'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, *Tribulus terrestris* treatment stopped blood AST and ALT levels from rising, suggesting that *Tribulus terrestris* 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 500 mg/kg body weight (77%), animals receiving *Tribulus terrestris* extract (750 and 1000 mg/kg body weight) had notable necrosis regions in their excised tumors, at 88 and 92%, respectively. On the other hand, tumors treated with 250 mg/kg body weight (67%) showed distinct necrosis foci areas **(Fig. 5)**.



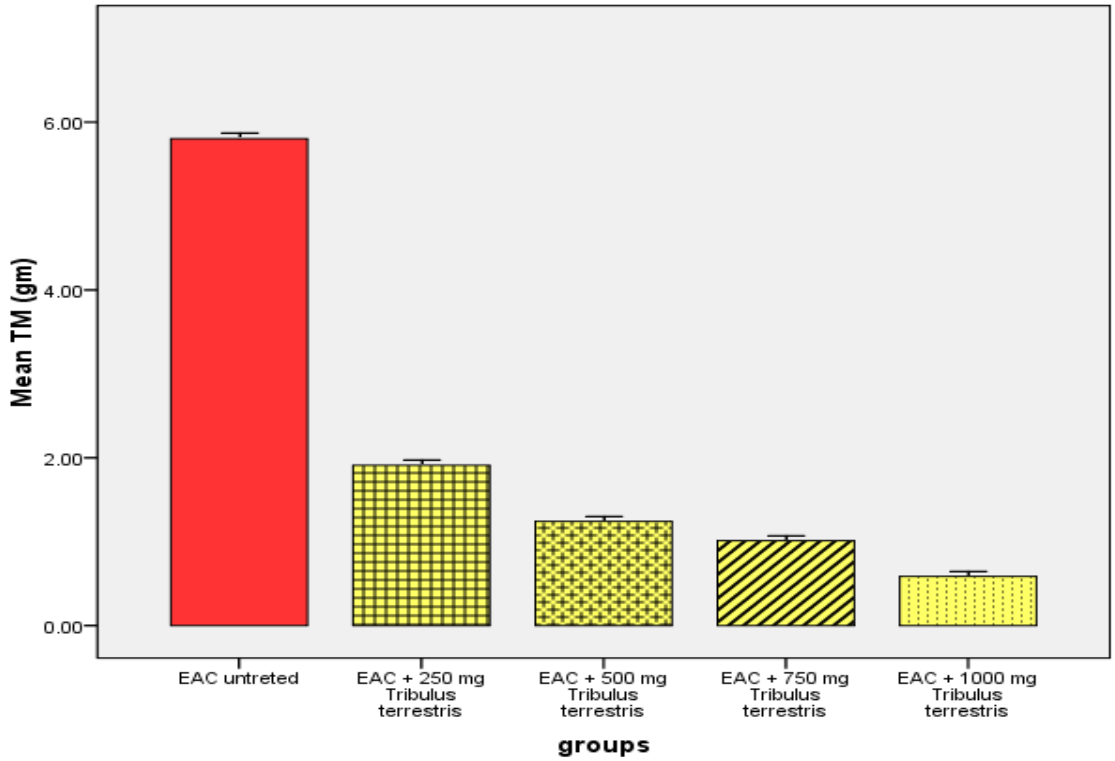
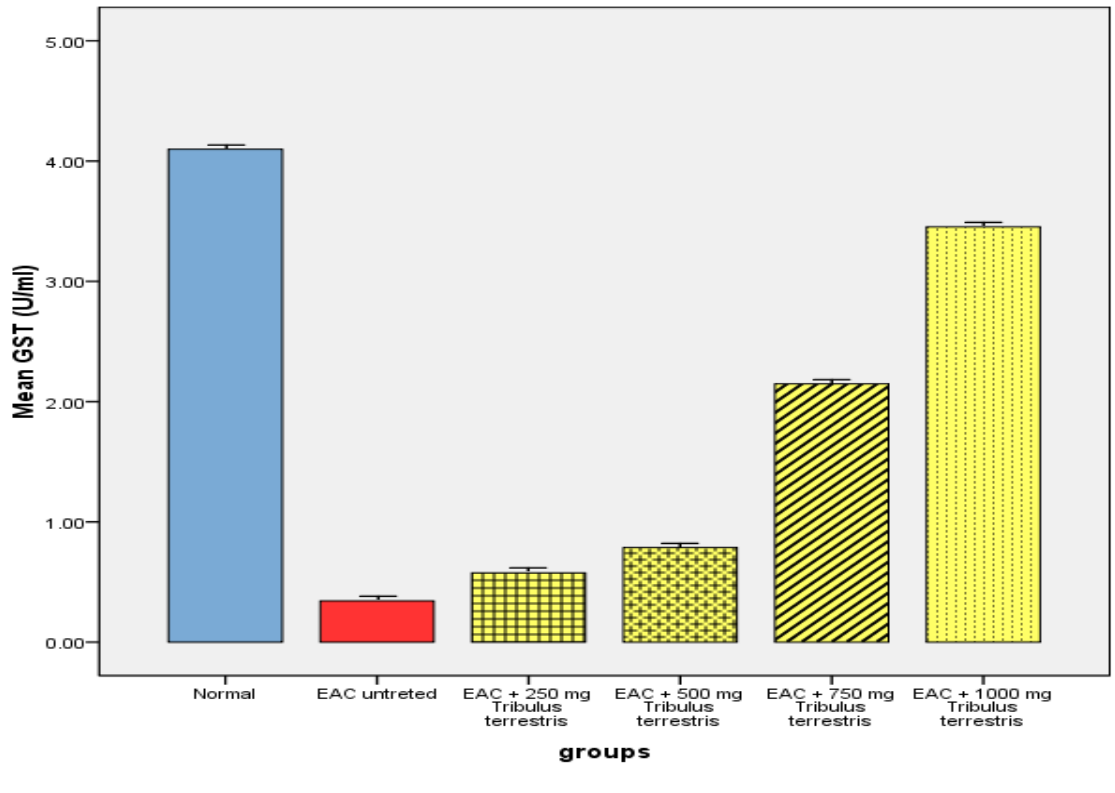
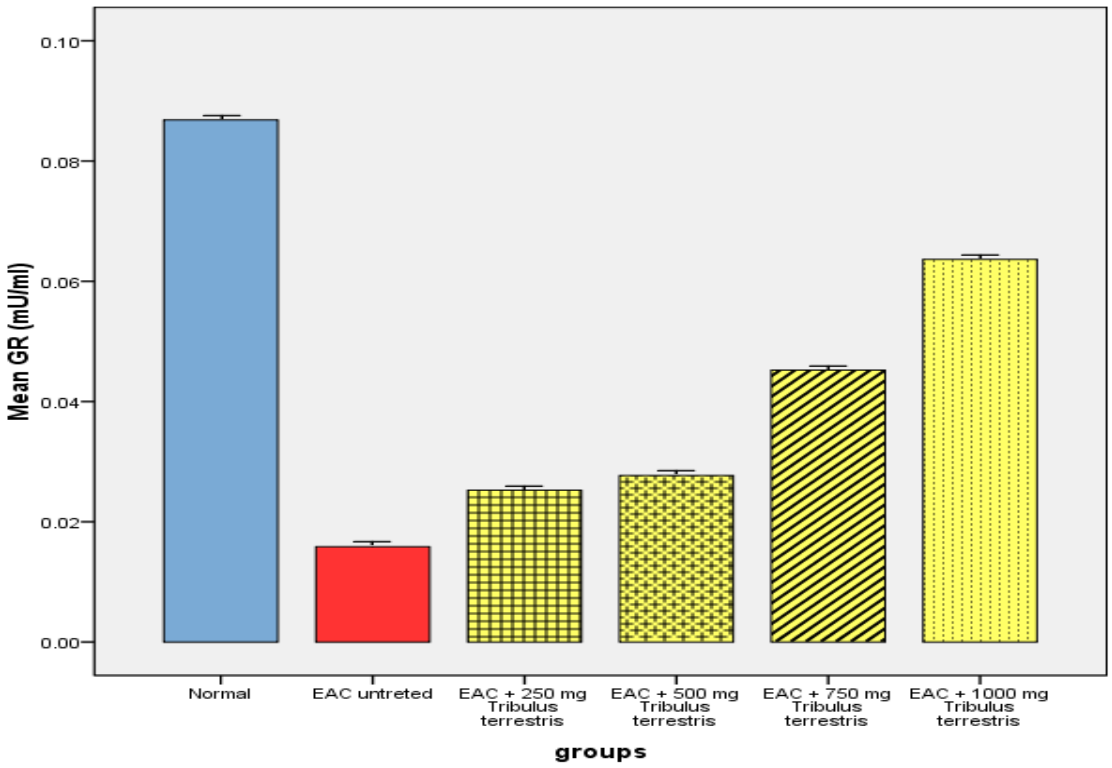
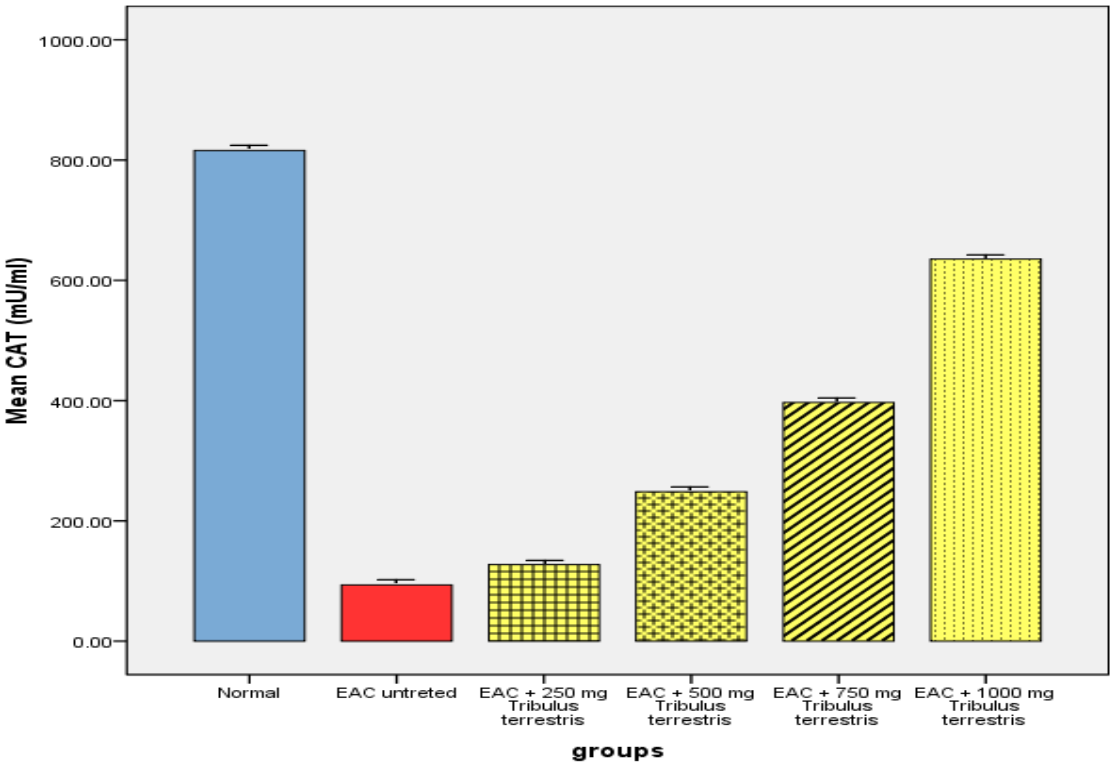
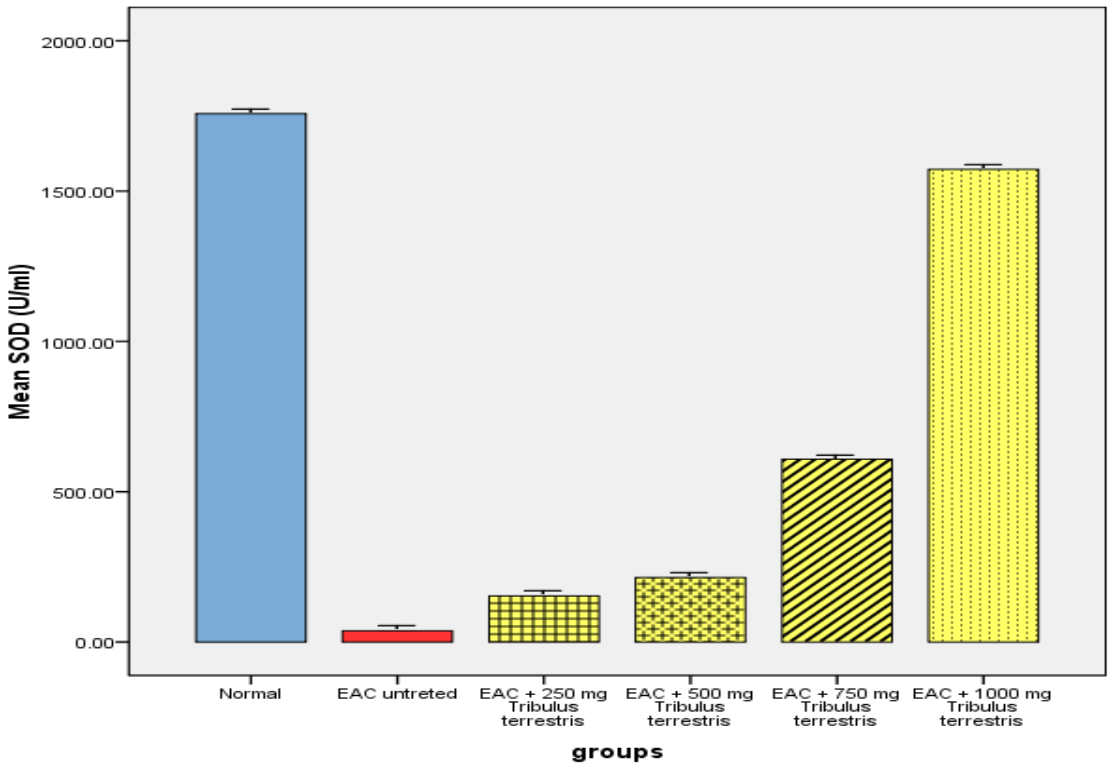


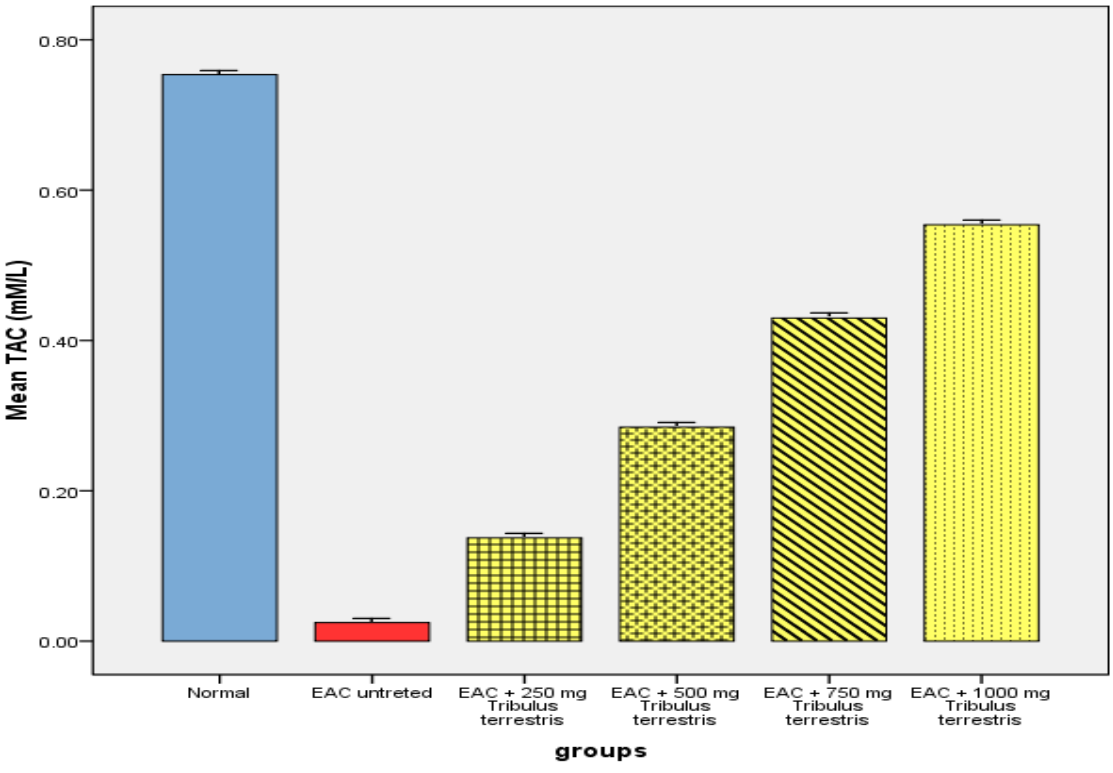
Figure (1): TV and TM of EAC treated groups with *Tribulus terrestris* with different conc.




Mean GR (mU/ml)
0.10
0.08
0.06
0.04
0.02
0.00
Normal
EAC untreted
EAC + 250 mg Tribulus terrestris
EAC + 500 mg Tribulus terrestris
EAC + 750 mg Tribulus terrestris
EAC + 1000 mg Tribulus terrestris
groups


Mean CAT (mU/ml)
1000.00
800.00
600.00
400.00
200.00
0.00
Normal
EAC untreted
EAC + 250 mg Tribulus terrestris
EAC + 500 mg Tribulus terrestris
EAC + 750 mg Tribulus terrestris
EAC + 1000 mg Tribulus terrestris
groups


2000.00
1500.00
1000.00
500.00
0.00
Mean SOD (U/ml)
Normal
EAC untreted
EAC + 250 mg Tribulus terrestris
EAC + 500 mg Tribulus terrestris
EAC + 750 mg Tribulus terrestris
EAC + 1000 mg Tribulus terrestris
groups


0.80
0.60
0.40
0.20
0.00
Mean TAC (mM/L)
Normal
EAC untreted
EAC + 250 mg Tribulus terrestris
EAC + 500 mg Tribulus terrestris
EAC + 750 mg Tribulus terrestris
EAC + 1000 mg Tribulus terrestris
groups

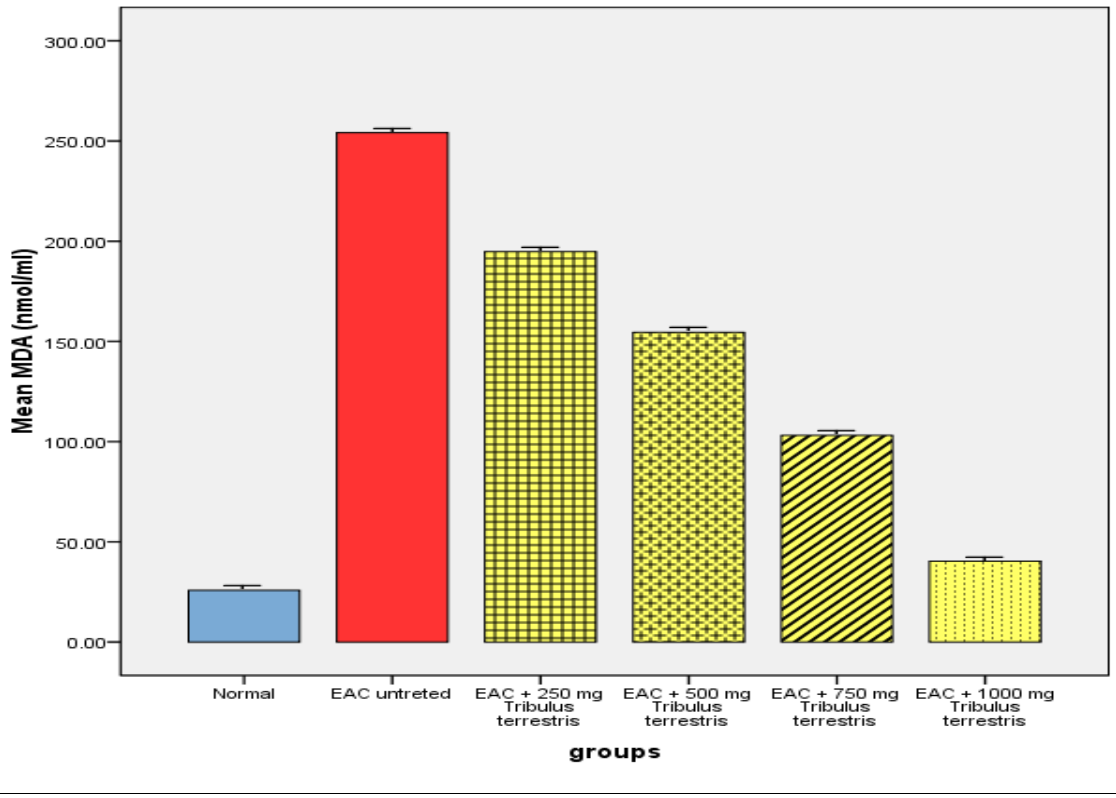
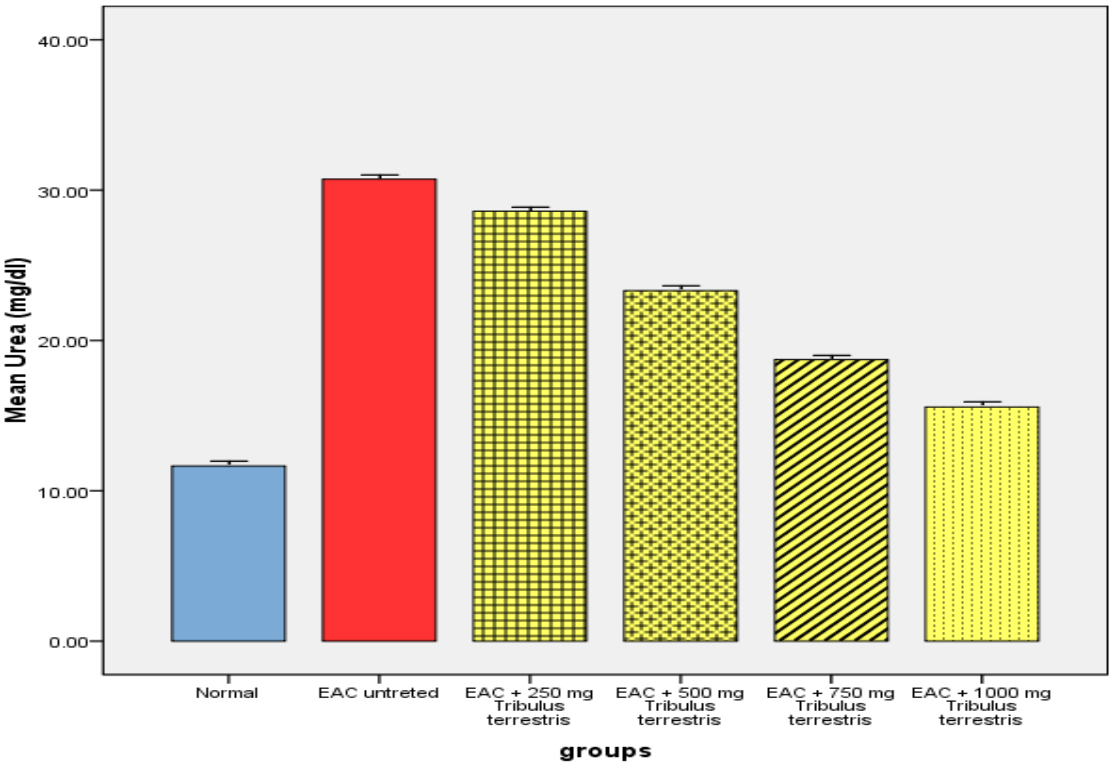


Figure (2): GST, GR CAT, SOD TAC and MDA of EAC treated groups with *Tribulus terrestris* with different conc.



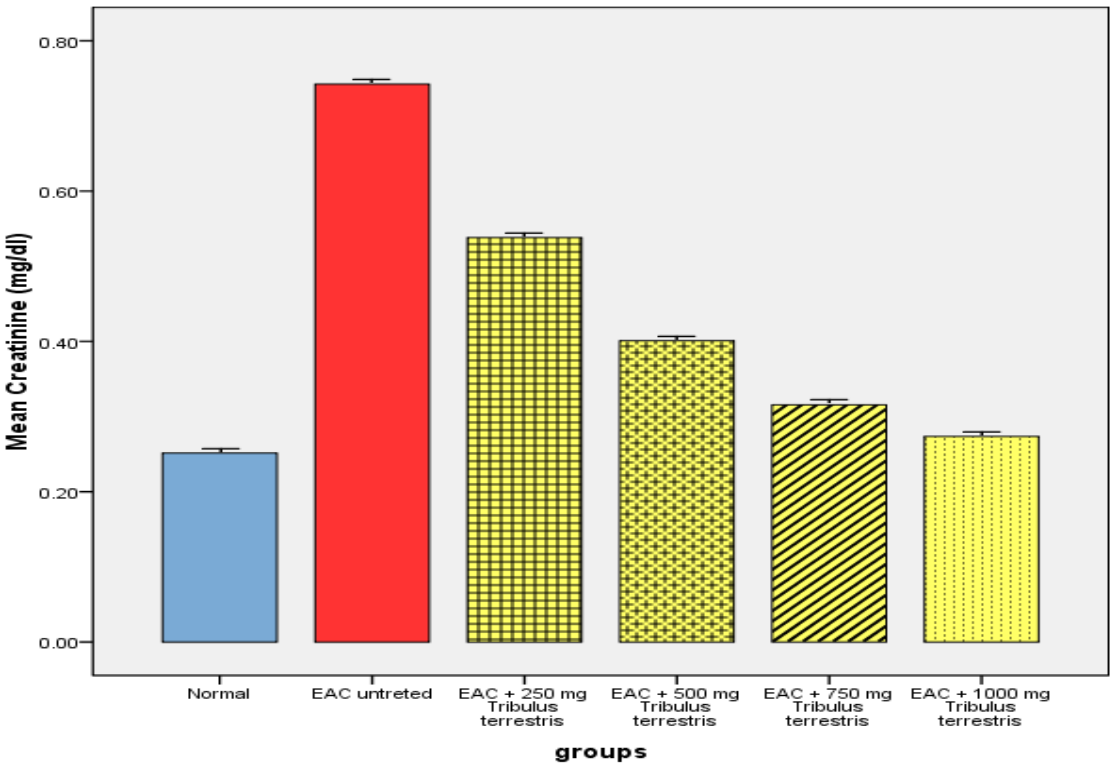
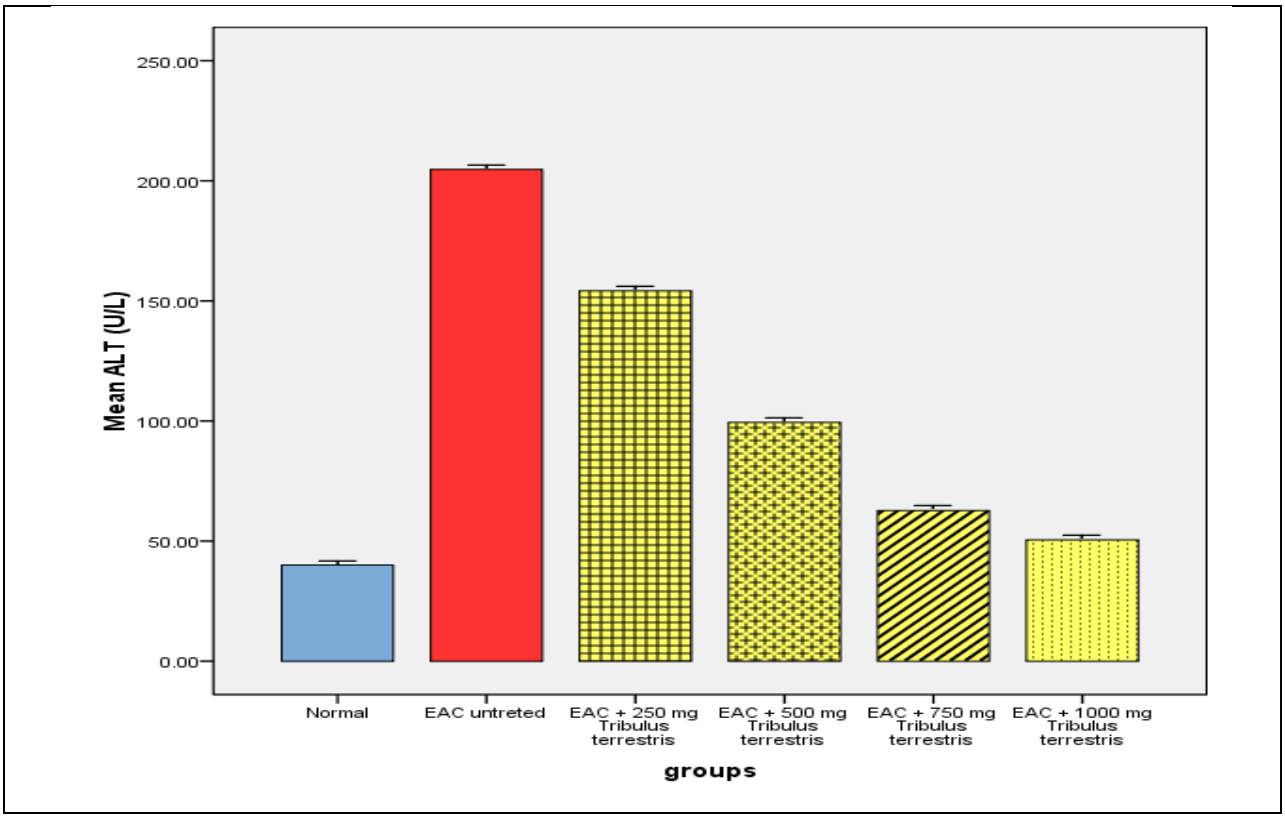


Figure (3): urea and creatinine of EAC treated groups with *Tribulus terrestris* with different conc.



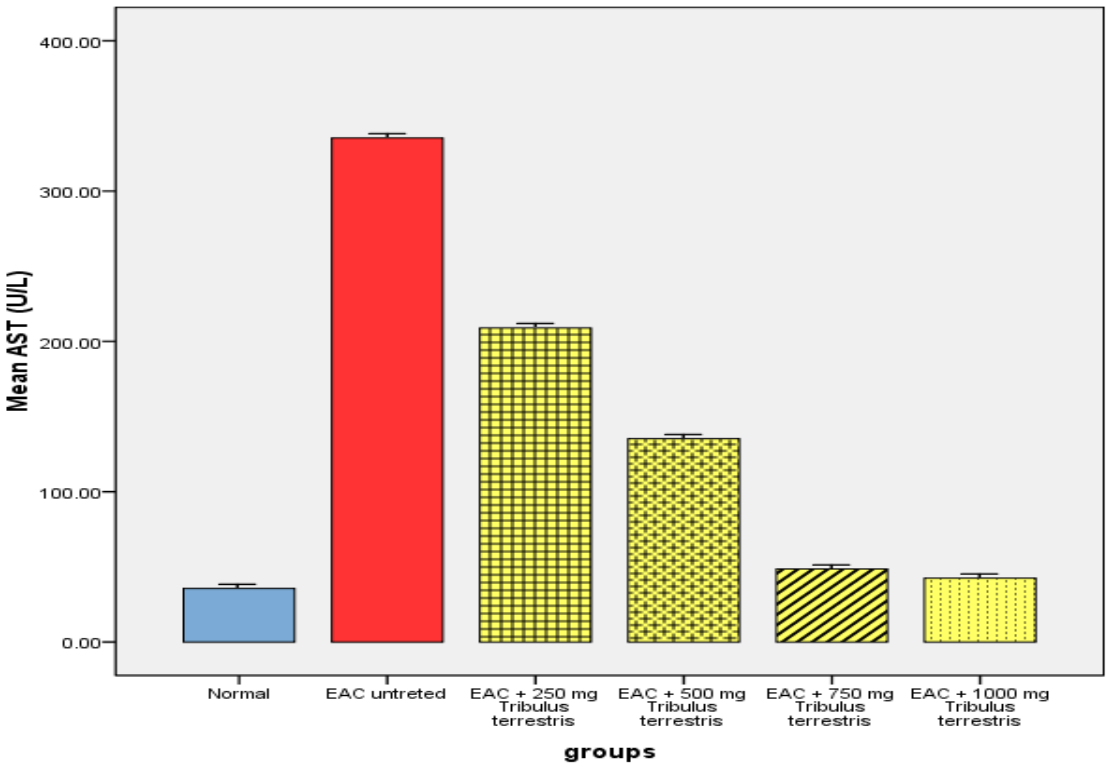
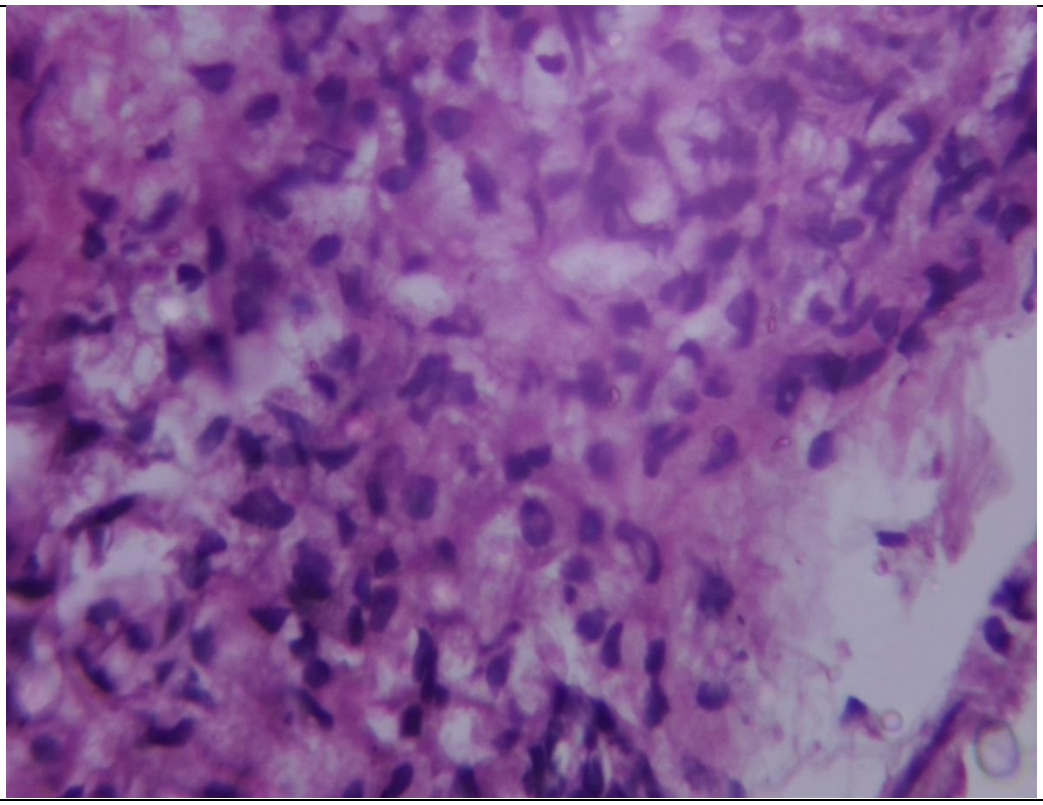
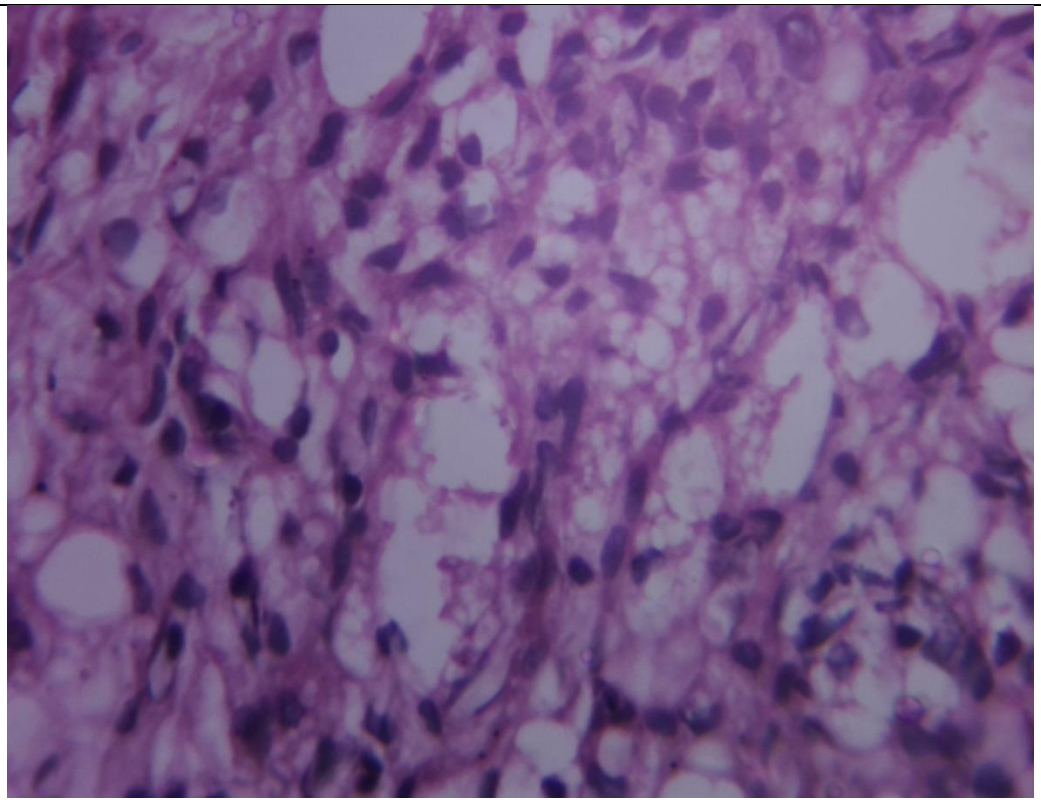


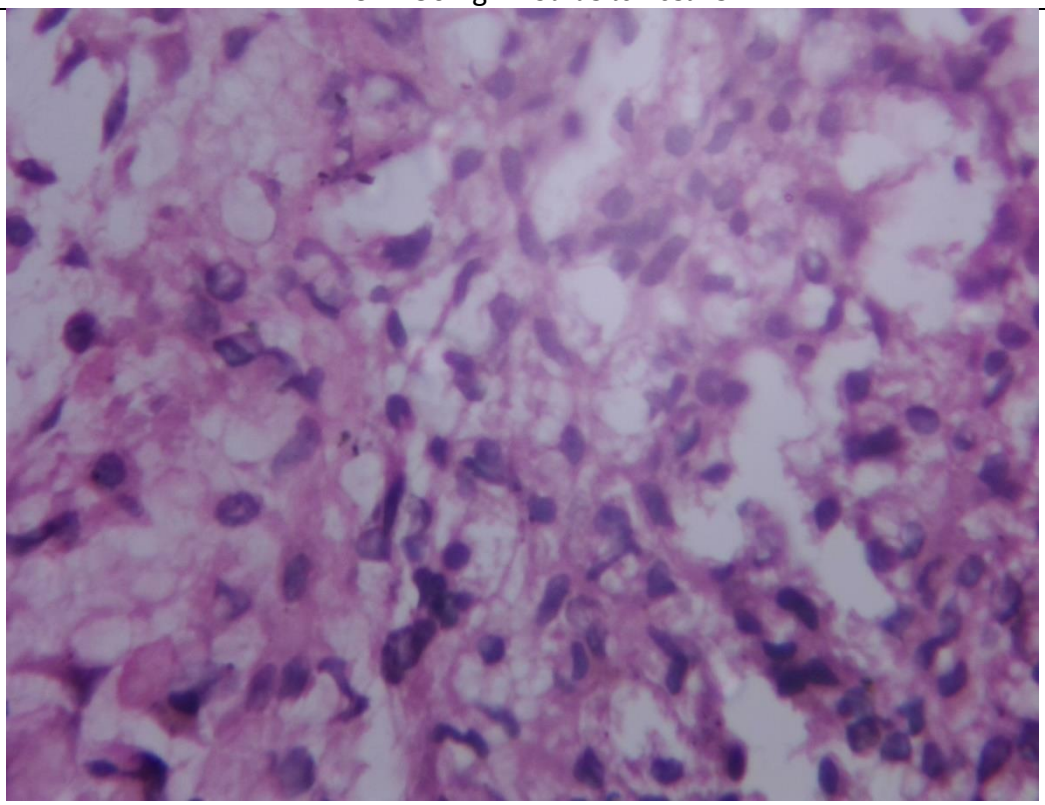
Figure (4): ALT and AST of EAC treated groups with *Tribulus terrestris* with different conc.



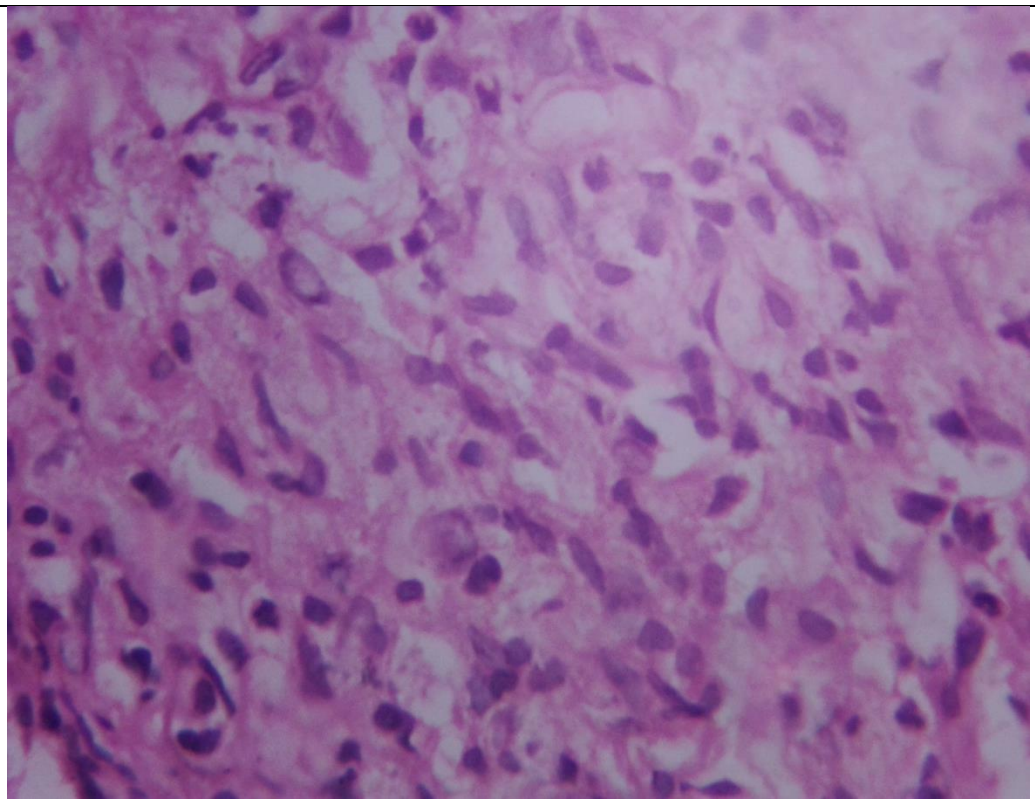
EAC untreated



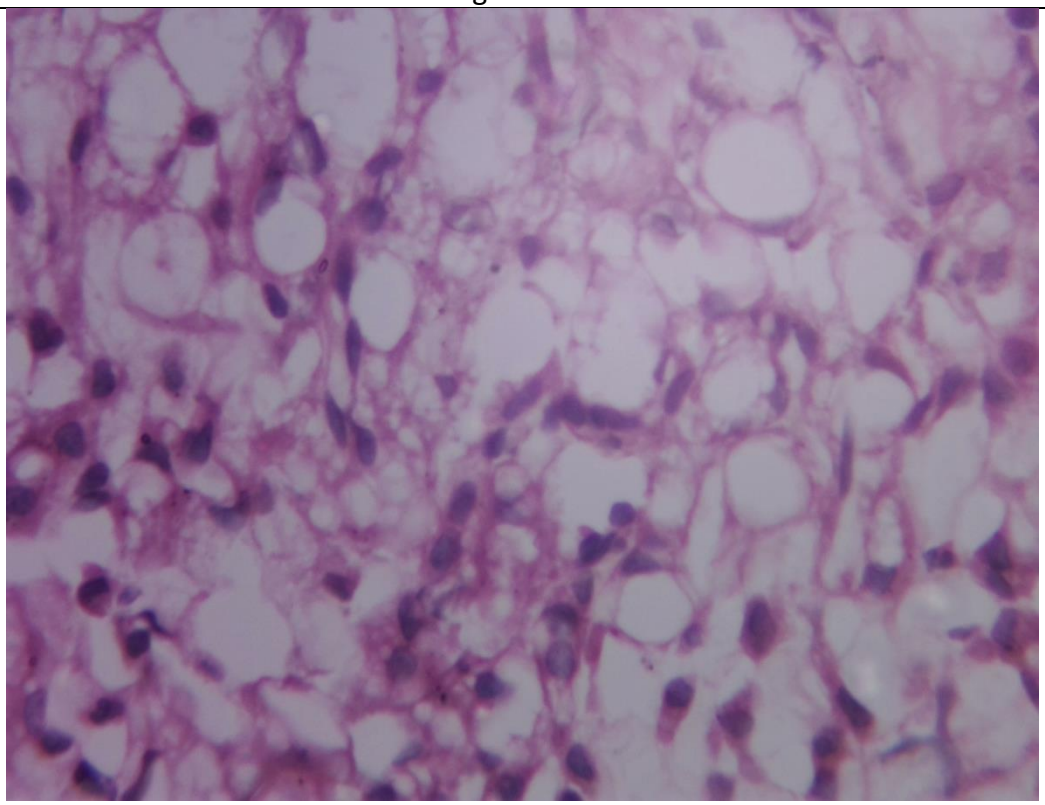
EAC + 250mg *Tribulus terrestris*



EAC + 500mg *Tribulus terrestris*



EAC + 750mg *Tribulus terrestris*



EAC + 1000mg *Tribulus terrestris*

Figure (5): H&E of EAC treated groups with *Tribulus terrestris* with different conc.

Discussion

The multistage process of carcinogenesis ends with an aggressive metastatic phenotype. Viral, chemical, or physical factors might all play a role. Intricate interactions between the tumor and the host tissues are required, as is the coordinated accumulation of beneficial genetic abnormalities [10].

It has been proposed that the disturbance of antioxidant status, which leads to oxidative stress and carcinogenesis, is caused by the unchecked production of free radicals and reactive oxygen species (ROS) [11]. Oxidative stress damages several macromolecular species, including proteins, lipids, and nucleic acids, and causes significant, interrelated disruptions in cellular metabolism, including lipid peroxidation [12]. Carcinogenesis has been linked to lipid peroxidation, which can produce harmful substances such as MDA and 4-hydroxynonenal [13]. Cancer may emerge as a result of these drugs' potential to target particular cellular targets [14].

The toxicological effects of EAC and some other lipophilic carcinogens may preferentially harm breast tissue if they are not biotransformed into hydrophilic metabolites that are easily removed [15].

Numerous studies have demonstrated that EAC can be used to create a model of breast cancer in mice. This process upsets the tissue's redox balance, indicating that oxidative damage may result in pathological and metabolic changes [16,17]. Free radicals in subcellular compartments are normally scavenged by antioxidant defense mechanisms in the relevant cells [18]. EAC is easily able to get past defenses, disrupting the equilibrium between pro- and antioxidants and causing abnormal cell activity. Cellular membranes that contain high levels of polyunsaturated fatty acids are susceptible to lipid peroxidation, which can be harmful [19].

EAC produces radicals, free radicals, and oxygenated metabolites [20]. This leads to the initiation of lipid peroxidation, which is harmful since it causes oxidative stress [21]. Considering that EAC may induce severe oxidative damage to a number of body organs, including the liver and breast, it is a useful and practical tool for developing in vivo breast cancer models [22, 23].

Numerous metabolic changes accompany the promotion of tumors [24]. Because the pathological progression of human tumors from preneoplastic to malignant is slow. As a result, there is always a possibility to prevent tumor growth. As a result, as prevention has increased, intensive cancer research has decreased in recent years. In order to slow down the carcinogenesis process, chemotherapeutic techniques apply chemicals with specific properties.

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].

A malfunctioning antioxidant system that releases lipid peroxides into the bloodstream after they build up in cancerous tissue is most likely the cause of the elevated serum lipid peroxide level in breast cancer [27]. MDA and a highly dangerous major aldehyde are the final peroxyl radical byproducts of lipid peroxidation. It is believed to act as an inhibitor of protective enzymes. Thus, it may lead to both mutagenesis and carcinogenesis [28].

Our findings indicate that lipid peroxidation increased as a result of EAC implantation. All EAC-treated groups' MDA levels are noticeably greater than those of the control group's animals. *Tribulus terrestris* primarily lowers MDA by scavenging reactive free radicals involved in peroxidation [29]. MDA levels were considerably lower in animals administered *Tribulus terrestris* than in mice given EAC alone. *Tribulus terrestris*'s ability to scavenge free radicals and reduce MDA indicates that it has antilipid peroxidative activity.

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. It effectively shields cells from oxidative stress by inhibiting lipid peroxidation, scavenging free radicals, and removing H2O2 [37].

In the current study, cancer-bearing mice exhibited lower antioxidant activity (GR, GST, SOD, CAT, and TAC) than normal animals. Our findings are consistent with earlier studies [38, 39]. According to Pradeep et al. [40], the subsequent drop in antioxidant defense is caused by reduced expression of these antioxidants after mammary gland injury. Animals administered *Tribulus terrestris* + EAC demonstrated a significant increase in both non-enzymatic and enzymatic antioxidant defense when compared to those given EAC alone. This rise is explained by *Tribulus terrestris*'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 *Tribulus terrestris* as opposed to those given EAC alone indicates that *Tribulus terrestris* 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, *Tribulus terrestris* 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. *Tribulus terrestris*, we found, increased serum levels of urea and creatinine, indicating kidney preservation. This illustrates the preventive effect of *Tribulus terrestris* 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 overproduction 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]. This study suggests that *Tribulus terrestris* has hepatoprotective properties since it prevented rises in serum ALT and AST levels both before and after therapy. This indicates that *Tribulus terrestris* has a protective effect against EAC-induced hepatotoxicity. The results of this analysis are consistent with those of other studies [54–70].

The results of the present study demonstrated that histological changes coincided with metabolic changes throughout the experiment. Histological examination revealed that every tumor in the cancerous control group included highly malignant cells and lacked necrosis. Tumors removed from animals given 750 and 1000 mg/kg body weight exhibited significant regions of necrosis (88% and 92%, respectively), in contrast to the group given 500 mg/kg body weight (77%), even though foci of necrosis (67%) were visible in the tumors administered with 250 mg/kg body weight. The current findings were consistent with previous studies conducted by various authors [17, 21–22, 53].

Conclusion

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

Recommendations

The results of this study show that it is possible to support *Tribulus terrestris* 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.

Conflict of interest

The authors declare that they have no competing interests.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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References

[1] King R.J., Natural history: the life of a cancer, in: R.J. King, M.W. Robins (Eds.), Cancer Biology, third ed., Longman Essex (pub.), 2006, pp. 9–31 (chapter 1).

[2] Abeloff M.D., Wolff A.C., Weber B.L., Abeloff M.D., Cancer of the breast, in: J.O. Armitage, A.S. Lichter, et al. (Eds.), Clinical Oncology, fourth ed., Elsevier (pub., Philadelphia, Pa, 2008, pp. 1875–1943.

[3] Fleming I.D., Breast, in: S.B. Edge, D.R. Byrd, C.C. Compton, A.G. Fritz, F.L. Greene, A. Trotti, et al. (Eds.), AJCC Cancer Staging Manual, seventh ed., vol.7, Springer (pub., New York, 2010, pp. 347–369.

[4] Alexandria Cancer Registry Annual Report, Alexandria, Egypt, Medical Research Institute, Alexandria University, 2010.

[5] Beral V. Breast cancer and hormone-replacement therapy in the million women study, Lancet 2003;362, 419–427.

[6] Burstein H.J., Harris J.R., Morrow M., Malignant tumors of the breast, in: V.T. DeVita, T.S. Lawrence, S.A. Rosenberg (Eds.), DeVita, Hellman, and Rosenberg's Cancer: Principles and Practice of Oncology, eighth ed., Lippincott Williams & Wilkins (pub., Philadelphia, Pa, 2008, pp. 1606–1654.

[7] Ojeswi B.K., Khoobchandani M., Hazra D.K., Srivastava M.M. In vitro anti-breast cancer efficacy of two indigenous plants on human cancer cell line MCF-7, Natl. Acad. Sci. Lett. 2009;32,105–109.

[8] Arthur F.K.N., Woode E., Terlabi E.O., Larbie C., Evaluation of acute and subchronic toxicity of Annona muricata (Linn.) aqueous extract in animals, Eur. J. Exp. Biol. 2011; 1, 115–124.

[9] Alshabi A.M., Alkahtani S.A., Shaikh I.A., Orabi M.A.A., Abdel-Wahab B.A., Walbi I.A., et al. Tribulus terrestris Cytotoxicity against Breast Cancer MCF-7 and Lung Cancer A549 Cell Lines Is Mediated via Activation of Apoptosis, Caspase-3, DNA Degradation, and Suppressing Bcl-2 Activity. Separations. 2022; 9,383.

[10] Hanahan D., Weinberg R.A., The hallmarks of cancer, Cell 2000;100, 57–70.

[11] Gey K.F., Prospects for the prevention of free radical disease, regarding cancer and cardiovascular disease, Br. Med. Bull. 1993; 49, 679–699.

[12] Revathi R., Manju V., The effects of Umbelliferone on lipid peroxidation and antioxidant status in diethylnitrosamine induced hepatocellular carcinoma, Journal of Acute Medicine 2013; 3, 73–82.

[13] Banakar M.C., Paramasivan S.K., Chattopadhyay M.B., Datta S., Chakraborty P., Chatterjee M., et al., 1alpha, 25-dihydroxyvitamin D3 prevents DNA damage and restores antioxidant enzymes in rat hepatocarcinogenesis induced by diethylnitrosamine and promoted by phenobarbital, World J. Gastroenterol. 2004; 10, 1268–1275.

[14] de Zwart L.L., Meerman J.H., Commandeur J.N., Vermeulen N.P. Biomarkers of free radical damage applications in experimental animals and in humans, Free Radic. Biol. Med. 1999; 26 202–226.

[15] Rieder C.R., Parsons R.B., Fitch N.J.S. Williams A.C., Human brain cytochrome P4501B1: immunohistochemical

localization in human temporal lobe and induction by dimethylbenz(a)anthracene in astrocytoma cell line (MOG-G-CCM), Neurosci. Lett. 2000; 278, 177–180.

[16] Giri U., Sharma S.D. Abdulla M., Athar M., Evidence that in situ generated reactive oxygen species act as a potent stage I tumor promoter in mouse skin, Biochem. Biophys. Res. Commun. 1995; 209, 698–705.

[17] Henry L., Narendra P.S. Oral artemisinin prevents and delays the development of 7,12-dimethylbenz[a]anthracene (DMBA)-induced breast cancer in the rat, Cancer Lett. 2006; 231, 43–44.

[18] Salet C., Moreno G. Photosensitization of mitochondria. Molecular and cellular aspects, J. Photochem. Photobiol., B 1990; 5, 133–150.

[19] Arulkumaran S., Ramprasath V.R., Shanthi P., Sachdanandam P. Restorative effect of Kalpaamruthaa, an indigenous preparation, on oxidative damage in breast gland mitochondrial fraction in experimental mammary carcinoma, Mol. Cell. Biochem. 2006; 291, 77–82.

[20] Cavalieri E., Roth R., Rogan E. Mechanisms of tumor initiation by polycyclic aromatic hydrocarbons, Carcinogenesis 1978; 3, 273–287.

[21] Choi E.J. Antioxidative effects of hesperetin against 7,12 dimethylbenz(a)anthracene-induced oxidative stress in mice, Life Sci. 2008; 82, 1059–1064.

[22] Benakanakere I., Besch-Williford C., Carroll C.E., Hyder S.M. Synthetic progestins differentially promote or prevent 7,12-DMBAinduced mammary tumors in Sprague-Dawley rats, Cancer Prev. Res. 2010; 3, 1157–1167.

[23] Vinothini G., Murugan R.S., Nagini S. Evaluation of molecular markers in a rat model of mammary carcinogenesis, Oncol. Res. 2009; 17, 483–493.

[24] Boutwell R.K. The function and mechanism of promoters of carcinogenesis, CRC Crit. Rev. Toxicol. 1974; 2, 419–443.

[25] Gupta R.K., Patel A.K., Kumari R., Chugh S., Shrivastav C., Mehra S., et al. Interactions between oxidative stress, lipid profile and antioxidants in breast cancer: a case control study, Asian Pac. J. Cancer Prev. APJCP 2012; 13, 6295–6298.

[26] Rao C.S.S., Kumari D.S. Changes in plasma lipid peroxidation and the antioxidant system in women with breast cancer, Int. J. Basic Appl. Sci. 2012; 1, 429–438.

[27] Kumaraguruparan R., Subapriya R., Viswanathan P., Nagini S. Tissue lipid peroxidation and antioxidant status in patients with adenocarcinoma of the breast, Clin. Chim. Acta 2002; 325, 165–170.

[28] Ziech D., Franco R., Georgakilas A.G., Georgakila S., Malamou-Mitsi V., Schoneveld O., et al. The role of reactive oxygen species and oxidative stress in environmental carcinogenesis and biomarker development, Chem. Biol. Interact. 2010;188, 334–339.

[29] Arthur F.K.N., Woode E., Terlabi E.O., Larbie C. Evaluation of acute and subchronic toxicity of Annona muricata (Linn.) aqueous extract in animals, Eur. J. Exp. Biol. 2011; 1, 115–124.

[30] LÓpez-Lázaro M. Anticancer and carcinogenic properties of curcumin: considerations for its clinical development as a cancer chemo preventive and chemotherapeutic agent, Mol. Nutr. Food Res. 2008; 52, 103–127.

[31] Zhang C., Zeng T., Zhao X., Yu L., Zhu Z., Xie K. Protective effects of garlic oil on hepatocarcinoma induced by Nnitrosodiethylamine in rats, Int. J. Biol. Sci. 2012; 8, 363–374.

[32] Chen B., Ning M., Yang G. Effect of paeonol on antioxidant and immune regulatory

activity in hepatocellular carcinoma rats, Molecules 2012; 17, 4672–4683.

[33] Vásquez-Garzón V.R., Arellanes-Robledo J., García-Román R., Aparicio- Rautista D.I., Villa-Treviño S. Inhibition of reactive oxygen species and pre-neoplastic lesions by quercetin through an antioxidant defense mechanism, Free Radic. Res. 2009; 43, 128–137.

[34] Usunomena U., Ademuyiwa A.J., Tinuade O.O., Uduenevwo F.E., Martin O., Okolie N.P. N-nitrosodimethylamine (NDMA), liver function enzymes, renal function parameters and oxidative stress parameters: a Review, Br. J. Pharmacol. Toxicol. 2012; 3, 165–176.

[35] Rao G.M., Rao C.V., Pushpangadan P., Shirwaikar A. Hepatoprotective effects of rubiadin, a major constituent of Rubia cordifolia Linn, J. Ethnopharmacol. 2006;103, 484–490.

[36] Revathi R., Manju V. The effects of Umbelliferone on lipid peroxidation and antioxidant status in diethylnitrosamine induced hepatocellular carcinoma, Journal of Acute Medicine 2013; 3, 73–82.

[37] Wu G., Fang Y.Z., Yang S., Lupton J.R., Turner N.D. Glutathione metabolism and its implications for health, J. Nutr. 2004; 134, 489–492.

[38] Blair I.A. Endogenous glutathione adducts, Curr. Drug Metabol. 2006; 7, 853–872.

[39] Ghosh D., Choudhury S.T., Ghosh S., Mandal A.K., Sarkar S., Ghosh A., et al. Nano capsulated curcumin: oral chemopreventive formulation against diethylnitrosamine induced hepatocellular carcinoma in rat, Chem. Biol. Interact. 2012; 195, 206–214.

[40] Rajeshkumar N.V., Kuttan R. Inhibition of N-nitroso diethylamine induced hepatocarcinogenesis by Picroliv, J. Exp. Clin. Cancer Res. 2000; 19, 459–465.

[41] Pradeep K., Mohen C.V., Gobian K., Karthikeyan S. Silymarin modulates the oxidant-antioxidant imbalance during diethylnitrosamine induced oxidative stress in rats, Eur. J. Pharmacol. 2007; 560, 110–116.

[42] Bruck R., Ashkenazi M., Weiss S., Goldiner I., Shapiro H., Aeed H., et al. Prevention of liver cirrhosis in rats by curcumin, Liver Int. 2007; 27, 373–383.

[43] Ren W., Qiao Z., Wang H., Zhu L., Zhang L. Flavonoids: promising anticancer agents, Med. Res. Rev. 2003;23, 519–534.

[43] Bemis D.L., Capodice J.L., Gorroochurn P., Katz A.E., Buttyananti R. Anti-prostate cancer activity of a s-carboline alkaloid enriched extract from Rauwolfia vomitoria, Int. J. Oncol. 2006; 29, 1065–1073.

[44] Anne A., Grippo K.C., Ben R., Bill J., Gurley C. Analysis of flavonoid phytoestrogens in botanical and ephedra-containing dietary supplements, Ann. Pharmacother. 2007; 41, 1375–1382.

[45] Jiang J., Evodiamine H.C. A novel anti-cancer alkaloid from Evodia rutaecarpa, Molecules 2009; 14, 1852–1859.

[46] Kabashima H., Miura N., Shimizu M., Shinoda W., Wang X., Wang Z., et al. Preventive impact of alkaloids with anti-cancer effect extracted from natural herb and the derivatives, WebmedCentral 2010; 1, 1–19.

[47] Thoppil R.J., Bishayee A. Terpenoids as potential chemopreventive and therapeutic agents in liver cancer, World J. Hepatol. 2011; 3, 228–249.

[48] Kuno T., Tsukamoto T., Hara A., Tanaka T. Cancer chemoprevention through the induction of apoptosis by natural compounds, J. Biophys. Chem. 2012; 3, 156–173.

[49] Haghiac M., Walle T. Quercetin induces necrosis and apoptosis in SCC-9 oral cancer cells, Nutr. Canc. 2005; 53, 220–231.

[50] Priyadarsini R.V., Murugan R.S., Maitreyi S., Ramalingam K., Karunagaran D., Nagini S. The flavonoid quercetin induces cell cycle arrest and mitochondriamediated apoptosis in human cervical cancer (HeLa) cells through p53 induction and NF-jB inhibition, Eur. J. Pharmacol. 2010;649, 84–91.

[51] Bishayee K., Ghosh S., Mukherjee A., Sadhukhan R., Mondal J.K., Bukhsh A.R. Quercetin induces cytochrome-c release and ROS accumulation to promote apoptosis and arrest the cell cycle in G2/M, in cervical carcinoma: signal cascade and drug–DNA interaction, Cell Prolif 2013; 46, 153–163.

[52] Kumaraguruparan R., Subapriya R., Kabalimoorthy J., Nagini S. Antioxidant profile in the circulation of patients with fibroadenoma and adenocarcinoma of the breast, Clin. Biochem. 2002; 35, 275–279.

[53] Sener D.E., Gönenç A., Akinci M., Torun M. Lipid peroxidation and total antioxidant status in patients with breast cancer, Cell Biochem. Funct. 2007; 25, 377–382.

[54] Abu Rakhey M.M. M., Abd El-Kareem S.A., Daoxin Ma. Folic Acid Conjugated Graphene Oxide GraviolaNanoparticle for Sono-Photodynamic Leukemia Treatment: Up-To-Date Cancer Treatment Modality. J. Biosci. Appl. Res. 2022; 8, 28-45.

[55] Abd El-Kareem S.A. Biochemical and biophysical study of chemopreventive and chemotherapeutic anti-tumor potential of some Egyptian plant extracts, Biochem. Biophys. Rep. 2019; 18, 100637.

[56] Wanas W., Abd El-Kareem S.A., Ebrahim S., Soliman M., Karim M. Cancer bioimaging using dual mode luminescence of graphene/FA-ZnO nanocomposite based on novel green technique, Sci. Rep. 2023; 13, 13-27.

[57] Abd El-Kareem S.A., Abd Elsamie G.H., Abd-Alkareem A.S. Sono-photodynamic modality for cancer treatment using bio-degradable bio-conjugated sonnelux nanocomposite in tumor-bearing mice: Activated cancer therapy using light and ultrasound, Biochem. Biophys. Res. Commun. 2018; 503, 1075.

[58] Abd El-Kareem S.A., Mohamed H.A., El-Kholey S.M., Abu Rakhey M.M.M., ELkallaf A.M.S., Soliman A.S.M., Khodary M.A. Ultrasound Allied Laser Sono-Photobiomodulation Activated Nano-Curcumin: Up-and-Coming Selective Cancer Cell Killing Modality, BioNanoSci. 2023; 13, 49-65.

[59] Dardeer A.G.E., Hussein N.G.,. Abd El-Kareem S.A. New Impacts of Garcinia Cambogia as Sono/photosensitiser on Ehrlich Ascites Carcinoma Bearing Mice, Int. J. Inf. Res. Rev. 2021;8, 7182.

[60] Abdulrahman J.M., Abd Elsamie G.H., Al-rawi R.A.A., Abd El-kareem S.A. Anti-tumor synergistic activity of nano-chlorophyll with sonophotodynamic on Ehrlich ascites carcinoma in mice, Zanco J. Med. Sci. 2020;24, 132.

[61] Abd El-Kareem S.A., Abd Elsamie G.H., Metwally M.A., Almamoori A.Y.K. Sono and photostimulated chlorine e6 nanocomposite in tumor-bearing mice: upcoming cancer treatment, Radiol. Med. Diag. Imag. 2019; 2, 1.

[62] Jasim A.A., Abdulrahman J.M., Abd El-kareem S.A., Hosny G. Biochemical and pathological evaluation of the effectiveness of nano-targeted sono-photodynamic therapy in breast cancer, J. Biosci. Appl. Res. 2019; 5, 18.

[63] Abd El-Kareem S.A., Abd Elsamie G.H., Isewid G.A. Laser and Ultrasound Activated Photolon Nanocomposite in Tumor-Bearing Mice: New Cancer Fighting Drug-Technique, Arch. Oncol. Cancer Ther. 2019; 2, 1.

[64] Dardeer A.G.E., Hussein N.G., Abd El-Kareem S.A. New Garcinia Cambodia as Sono/Photosensitizer on Ehrlich Ascites Carcinoma Bearing Mice in Presence of Dieldrin Pesticide as an Environmental Pollutant, Int. J. Innov. Sci. Res. 2021; 10, 1583.

[65] Abd El-Kareem S.A., Abd Elsamie G.H., Abbas A.J. Sono and photo sensitized gallium-porphyrin nanocomposite in tumor-bearing mice: new concept of cancer treatment, Am. J. Nanotechnol. Nanomed. 2019; 2, 5.

[66] Abd El-Kareem S.A., Hussein N.G.A., El-Kholey S.M., Elhelbawy A.M.A.E.I. Microneedle combined with iontophoresis and electroporation for assisted transdermal delivery of *goniothalamus macrophyllus* for enhancement sonophotodynamic activated cancer therapy. Sci. Rep. 2024;14, 7962.

[67] El Dine R. S. S., Abd El-kareem S. A. Therapeutic effects of laser on the stiffness of the Achilles tendon mammalian muscles. Rom. Biophys. J. 2015; 25, 47–62.

[68] El Dine R.S.S., Abd El-kareem S.A., Elkholy S.M., Abd El maniem N.A. Comparative study for the therapeutic effect of shark care and 5-fluorouracil drugs in mice with hepatocellular carcinoma in the presence of electric field. Amer. J. Biomed. Sci. 2015; 7, 76–97.

[69] Han W.K., Bonventre J.V. Biologic markers for the early detection of acute kidney injury, Curr. Opin. Crit. Care 2004;10, 476–482.

[70] Abd El-Kareem S.A., Zedan D.F.M., Mohamed H.M., Soliman A.S.M., El-Kholey S.M., Nasra M.K.E. High-frequency ultrasound-assisted drug delivery of chia, cress, and flax conjugated hematite iron oxide nanoparticle for sono-photodynamic lung cancer treatment in vitro and in vivo. Cancer Nanotechnol 2024. 15: 46.

[71] George G.S., Wakasi M.E., Egoro E. Creatinine and urea levels as critical markers in end-stage renal failure, Res. Rev.: Journal of Medical and Health Sciences 2014; 3, 41–44.

[72] Paliwal R., Sharma V., Pracheta, Sharma S., Yadav S., Sharma S.H. Antinephrotoxic effect of administration of Moringa oleifera Lam. in amelioration of DMBA-induced renal carcinogenesis in Swiss albino mice, Biol. Med. 2011; 3, 27–35.

[73] Sharma V., Paliwal R., Janmeda P., Sharma S.H. The reno-protective efficacy of Moringa oleifera pods on xenobiotic enzymes and antioxidant status against 7,12 dimethylbenz[a]anthracene exposed mice, J. Chin. Integr. Med. 2012; 10, 1171–1178.

[74] Boone L., Meyer D., Cusick P., Ennulat D., Bolliger A.P., Everds N. Selection and interpretation of clinical pathology indicators of hepatic injury in preclinical studies, Vet. Clin. Pathol. 2005; 34, 182–188.

[75] El-Kareem, S.A.A., Ebied, S.A.EM., Sadek, N.A., Saad D.M., Nadwan E.T. Serum estrogen and its soluble receptor levels in Egyptian patients with acute leukemia: case-control study. Egypt J Med Hum Genet 2021;22, 68.

[76] El-Kareem, S.A.A., Ebied, S.A.EM., Sadek, N.A., Attia K.A.H., Eman Attia Nadwanet al. Serum Estrogen and its Soluble Receptor Levels in Egyptian Patients with Chronic Myeloid Leukemia: A Case–Control Study. Indian J Hematol Blood Transfus 2022;38, 246–254.

[77] El Kashif H.K.A. , Ebied, S.A.EM., Sadek, N.A., Zaki N.E., El-Kareem S.A.A. Prognostic value of soluble angiotensin II receptor 1 and soluble angiotensin converting enzyme (CD 143) in patients with acute leukemia. Acta Haematologica Polonica 2018; 49,240-250. [75] Singh A.,

Bhat T.K., Sharma O.M. Clinical biochemistry of hepatotoxicity, J. Clin. Toxicol. 2011; 4, 1–19.

[78] Ozer J., Ratner M., Shaw M., Bailey W., Schomaker S. The current state of serum biomarkers of hepatotoxicity, Toxicology 2008; 245, 194–205.

[79] Yameny, A., Alabd, S., Mansor, M. MiRNA-122 association with TNF-α in some liver diseases of Egyptian patients. *Journal of Bioscience and Applied Research*, 2023; 9(4): 212-230. doi: 10.21608/jbaar.2023.329927

[80] Ramaiah S.K. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters, Food Chem. Toxicol. 2007; 45, 1551–1557.

[81] Amacher D.E. A toxicologist's guide to biomarkers of hepatic response, Hum. Exp. Toxicol. 2002; 21, 253–262.