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Study the Potential Role of *Black cohosh* Extract on Cancer Treatment

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****Running Title**: Black cohosh and Cancer

Abstract

Background: International scientific recognition has recently been given to the use of phytochemicals to avoid major health problems. Research on pharmacological processes and the search for chemical structures of plant extracts with anticancer activities aroused interest. The **Aim of work:** This study was to assess the chemotherapeutic anti-tumor ability of *Black cohosh* extract against mammary carcinogenesis in Swiss albino mice that was generated by the implantation of Ehrlich ascites carcinoma (EAC). **Materials and techniques:** *Black cohosh* 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:** The results showed that oxidative stress was reduced, antioxidant levels were raised, liver and kidney functions were restored, and tumor and neovascularization were inhibited. **Conclusion:** Based on all obtained data, our research suggests that treatment with *Black cohosh* extract offered robust antioxidant protection and chemotherapeutic efficacy against mammary tumors implanted with EAC.

Keywords: *Black cohosh*; Ehrlich Ascites Carcinoma; Oxidative Stress; Antioxidants

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Introduction

Breast cancer is the most common cancer in women, accounting for 22.9% of all female cancer cases globally. Egypt's breast cancer prognosis is bleak, with a 29% death rate and an incidence-to-mortality ratio of 1:3.7. [1] Breast cancer accounts for 16% of cancer-related deaths among women worldwide, according to data from the World Health Organization (WHO). It is the most often diagnosed solid tumor in women. Although aging is a risk factor for breast cancer, lifestyle decisions, and environmental variables also have a big influence on the disease. In [2].

Treatment options for conditions linked to breast cancer include radiation therapy, chemotherapy, surgery, and/or a combination of these treatments.

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Despite the abundance of therapeutic options, cancer continues to take a significant toll on lives. This is mostly because breast cancer diagnoses in women often occur with delayed presentation, challenges in early detection, and the high cost of treatment [3-5]. Novel therapy techniques that improve prognoses while causing minimum to no adverse effects are necessary for patients with breast cancer because of these many disadvantages [6].

Phytochemical therapy has just been approved by international scientific bodies to treat serious medical diseases. A lot of individuals want to know more about the chemical makeup and pharmacological processes of herbal extracts that give them their anticancer properties [7–8].

A herbal treatment called *black cohosh* is used by women going through menopause to treat hot flashes, mood swings, insomnia, and other menopausal symptoms. *Cimicifuga racemosa* is a North American natural plant whose rhizomes are used to isolate *black cohosh*. Astringent, diuretic, antidiarrheal, and antioxidant qualities have all been reported for it. *black cohosh* has been used for ages by people from many cultures to treat a range of illnesses, including Native Americans who utilize it as an anti-inflammatory. Although it has only been studied in the People's Republic of China, its demonstrated *black cohosh* anticancer action has been proven. The precise mechanism by which *Black cohosh* stops the proliferation of cancer cells is still unknown [9].

The purpose of this study was to examine the efficacy of *Black cohosh* 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 *Black cohosh* concentrations were given to the experimental groups. *Black cohosh* 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 *Black cohosh* following EAC implantation; ten mice in subgroup B-3 received 750 mg/kg/day of *Black cohosh* following EAC implantation; ten mice in subgroup B-4 received 500 mg/kg/day of *Black cohosh* following EAC implantation; and ten mice in subgroup B-5 received 250 mg/kg/day of *Black cohosh* 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

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

Fig. (1) illustrates the correlations between tumor sizes and treatment duration for *Black cohosh* at different concentrations (1000 mg, 750 mg, 500 mg, and 250 mg). It was shown that a 250 mg dose of *Black cohosh* had no discernible effect on the tumor volume. Tumor cells and volume have been observed to be more affected by *Black cohosh* doses of 500 and 750 mg. *Black cohosh* 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 to those receiving *Black cohosh*, animals implanted with EAC alone displayed significantly greater levels of MDA.

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

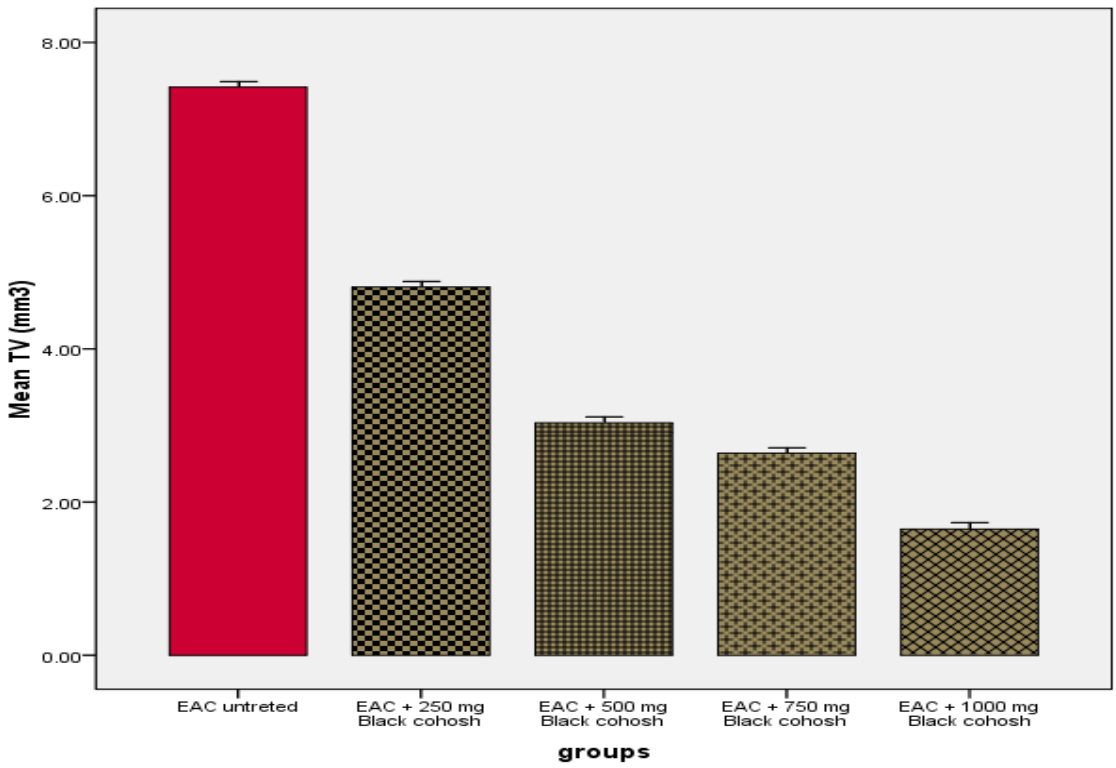
Treatment's effects on liver and kidney function tests

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 consuming *Black cohosh* raised blood levels of urea and creatinine, which are indicators of renal protection. This lends more credence to *Black cohosh*'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. However, AST and ALT blood levels were not elevated after *Black cohosh* administration, suggesting that *Black cohosh* 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 (69%), animals receiving *Black cohosh* extract (750

and 1000 mg/kg body weight) had notable necrosis regions in their excised tumors, at 78 and 86%, respectively. In contrast, tumors treated with 250 mg/kg body weight (59%) showed distinct necrosis foci areas **Fig. (5)**.



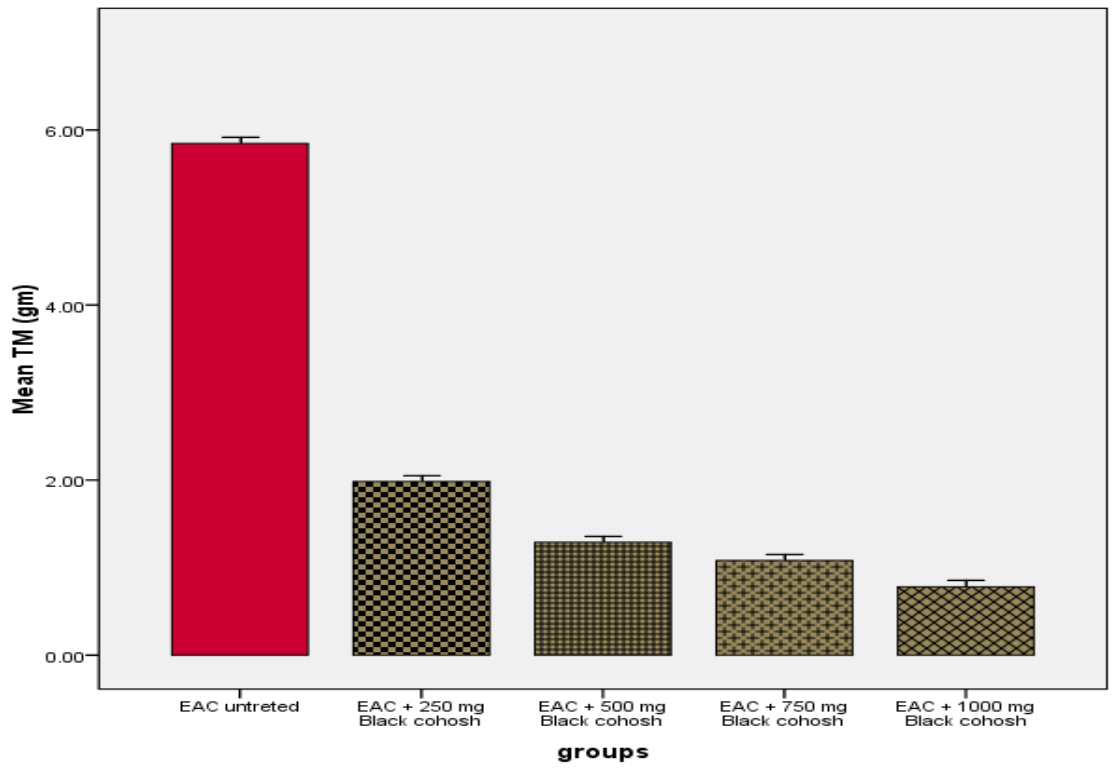
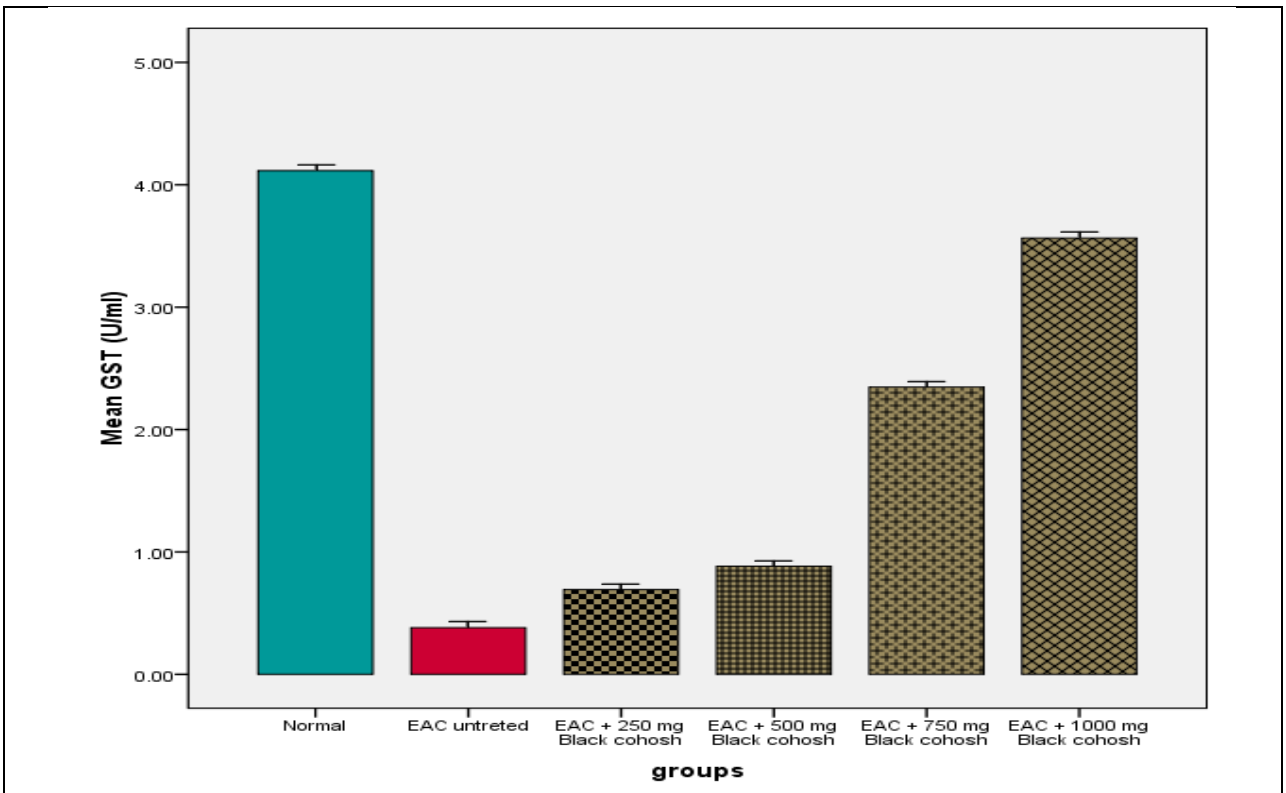
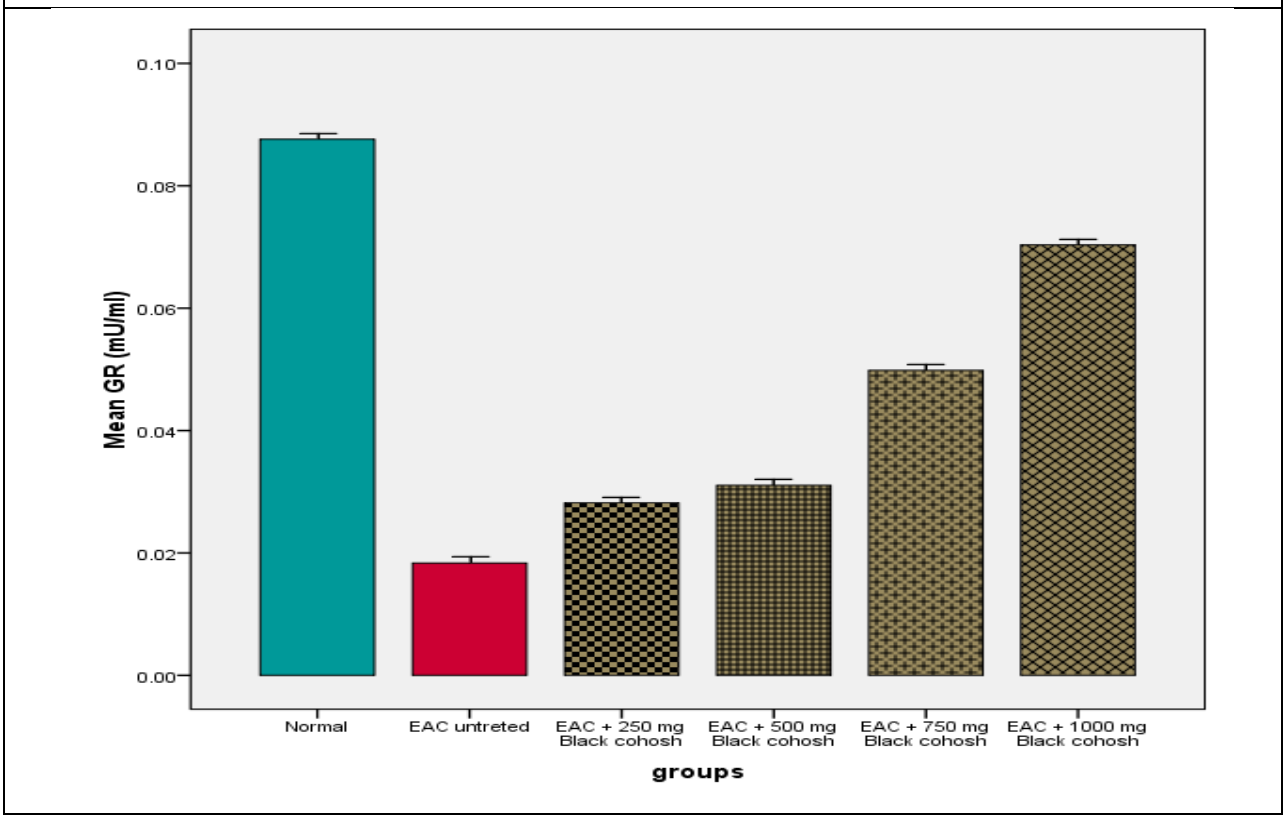
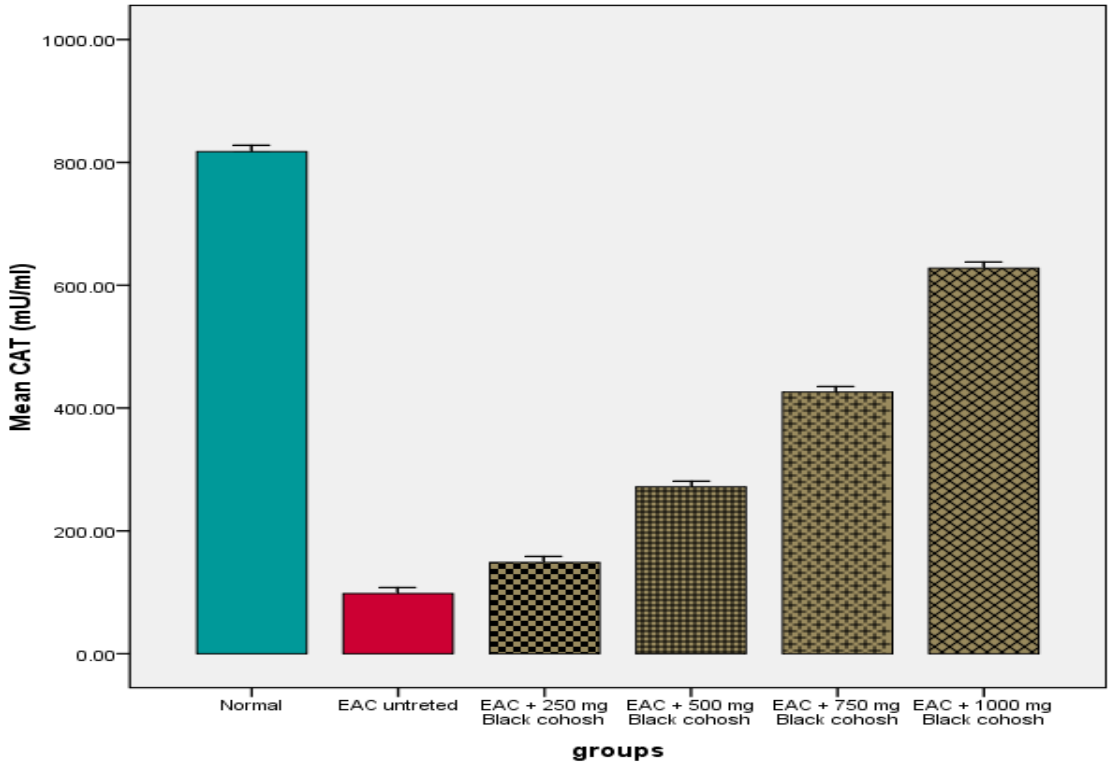
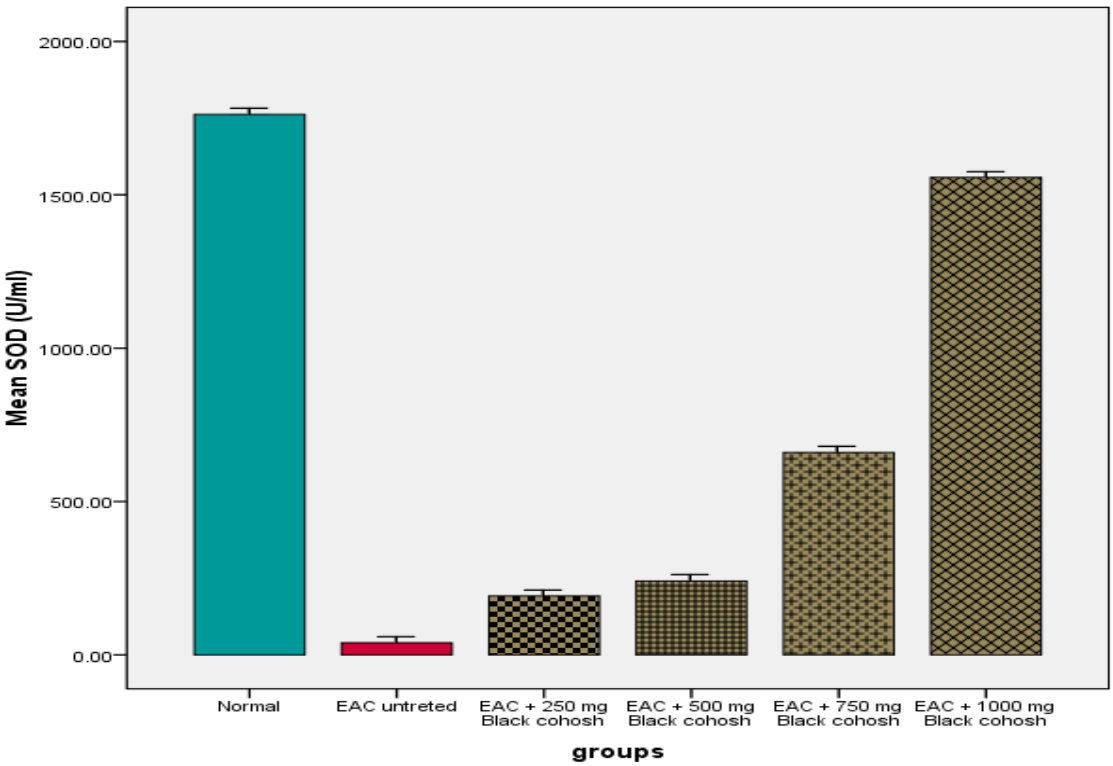


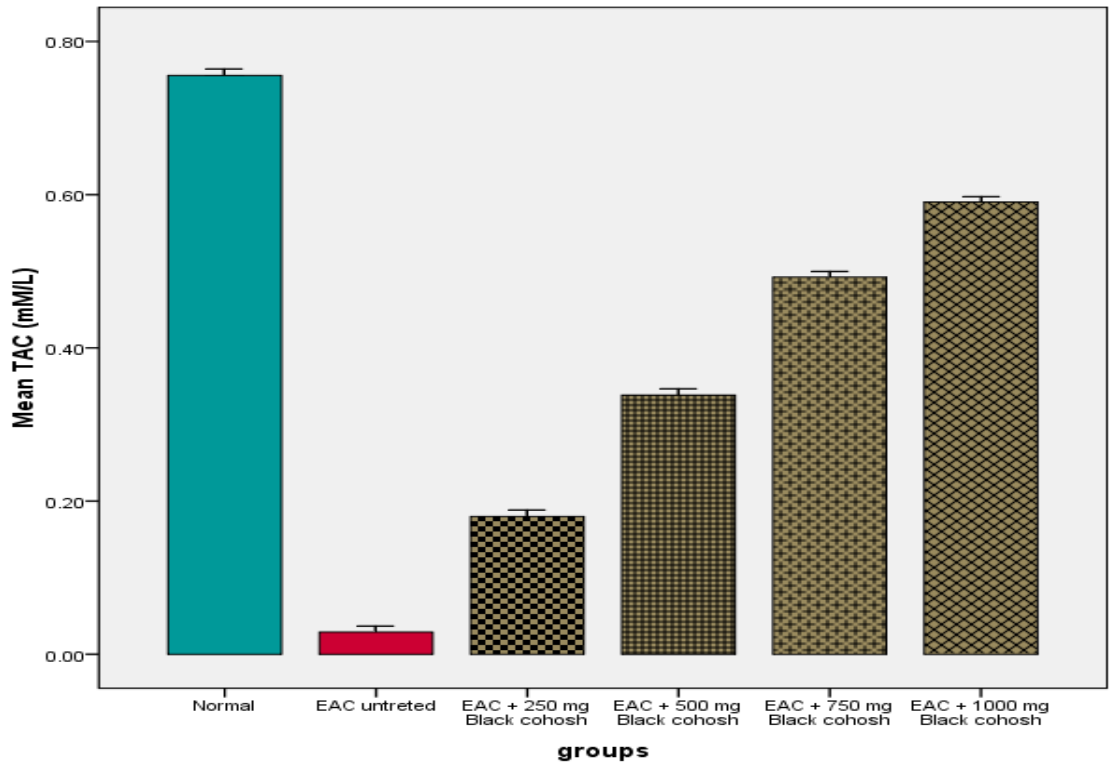
Figure (1): TV and TM of EAC treated groups with *Black cohosh* with different conc.


Mean GST (U/ml)
5.00
4.00
3.00
2.00
1.00
0.00
Normal
EAC untreted
EAC + 250 mg Black cohosh
EAC + 500 mg Black cohosh
EAC + 750 mg Black cohosh
EAC + 1000 mg Black cohosh
groups


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


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


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



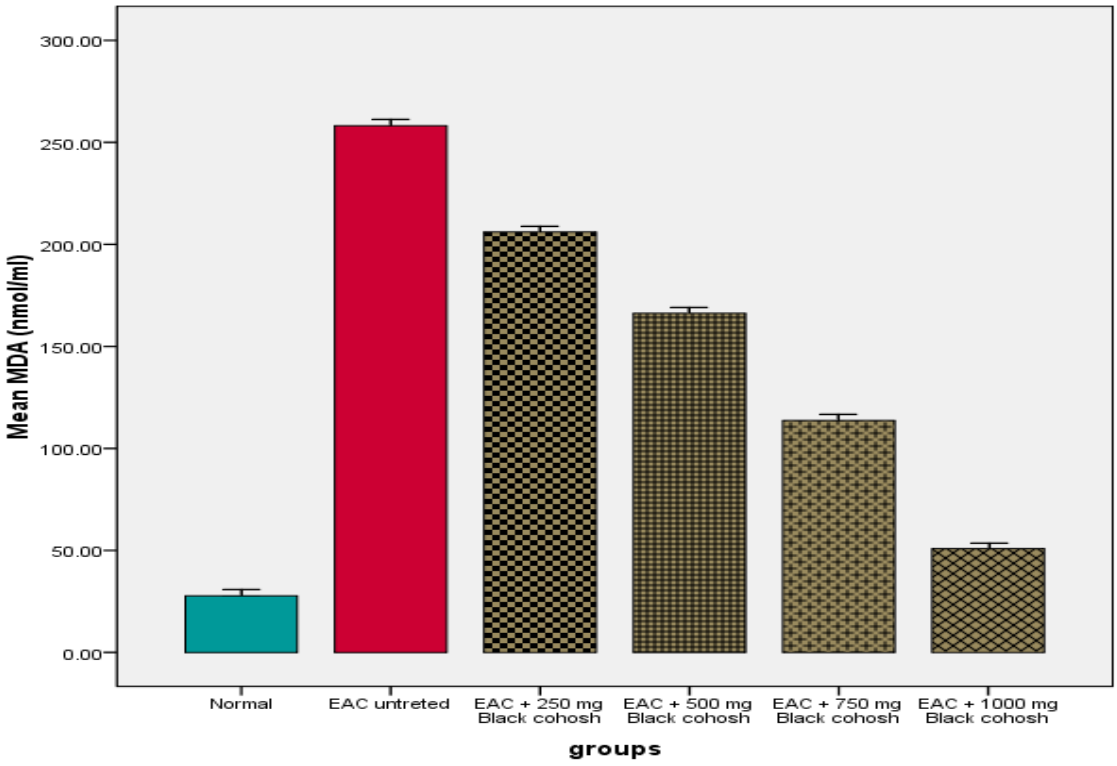
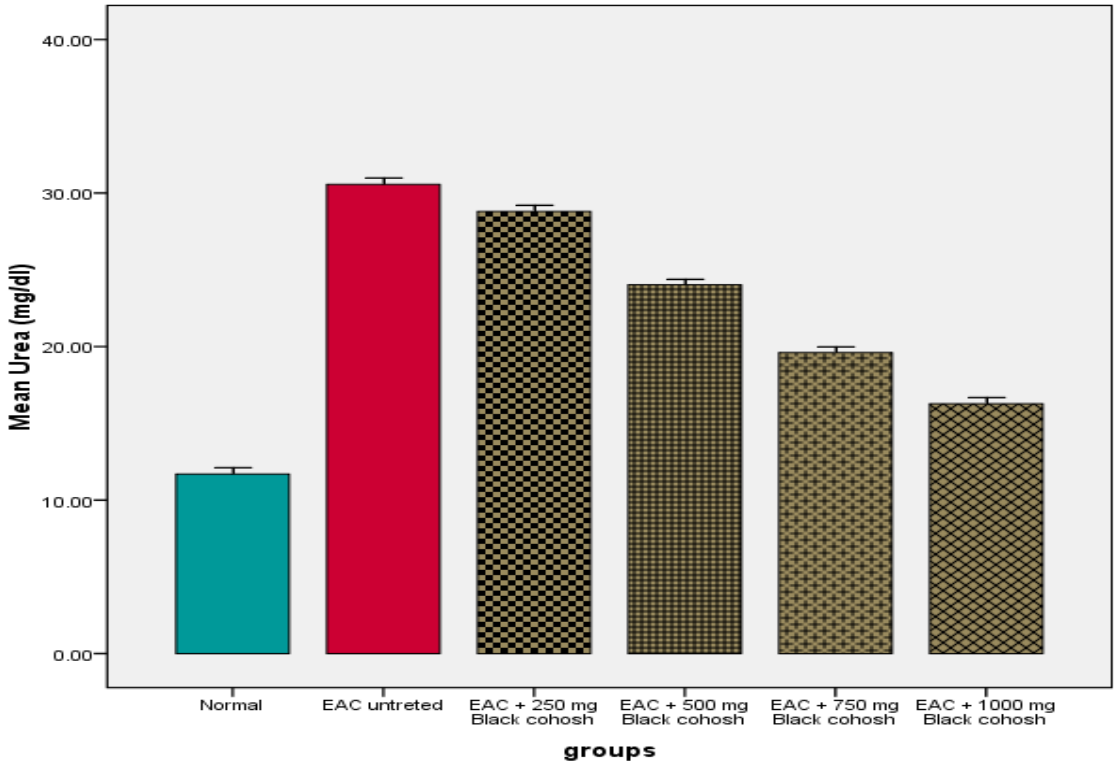


Figure (2): GST, GR CAT, SOD TAC and MDA of EAC treated groups with *Black cohosh* with different conc.



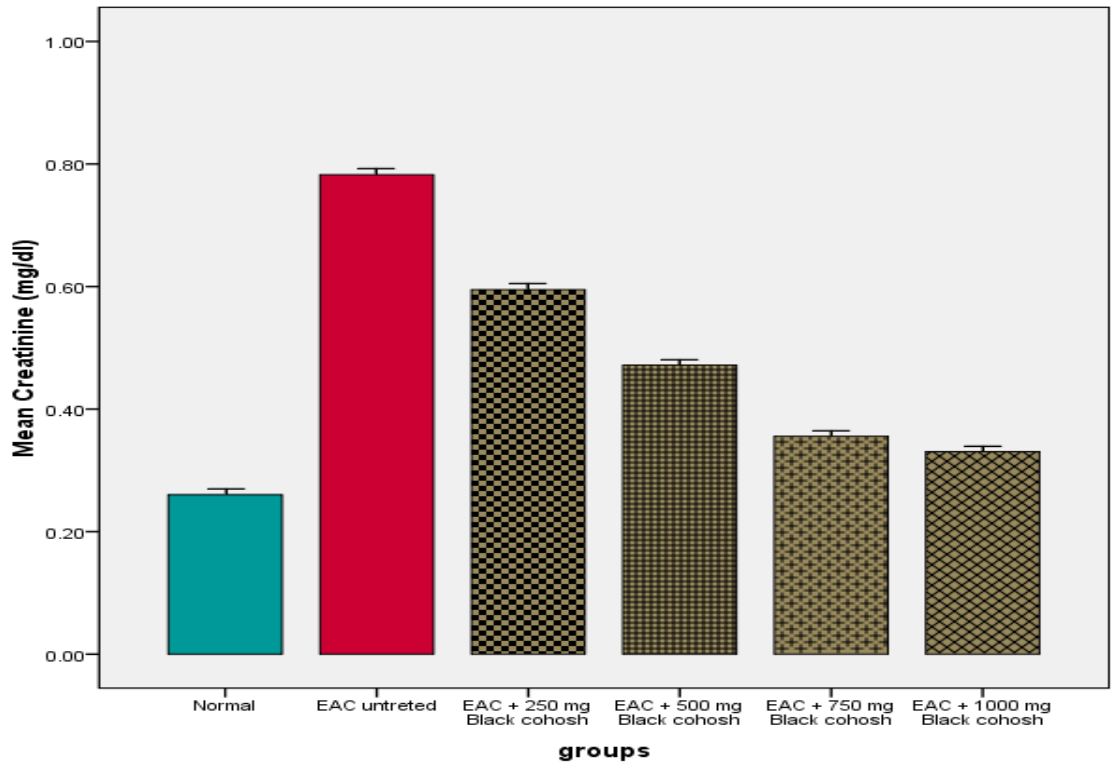
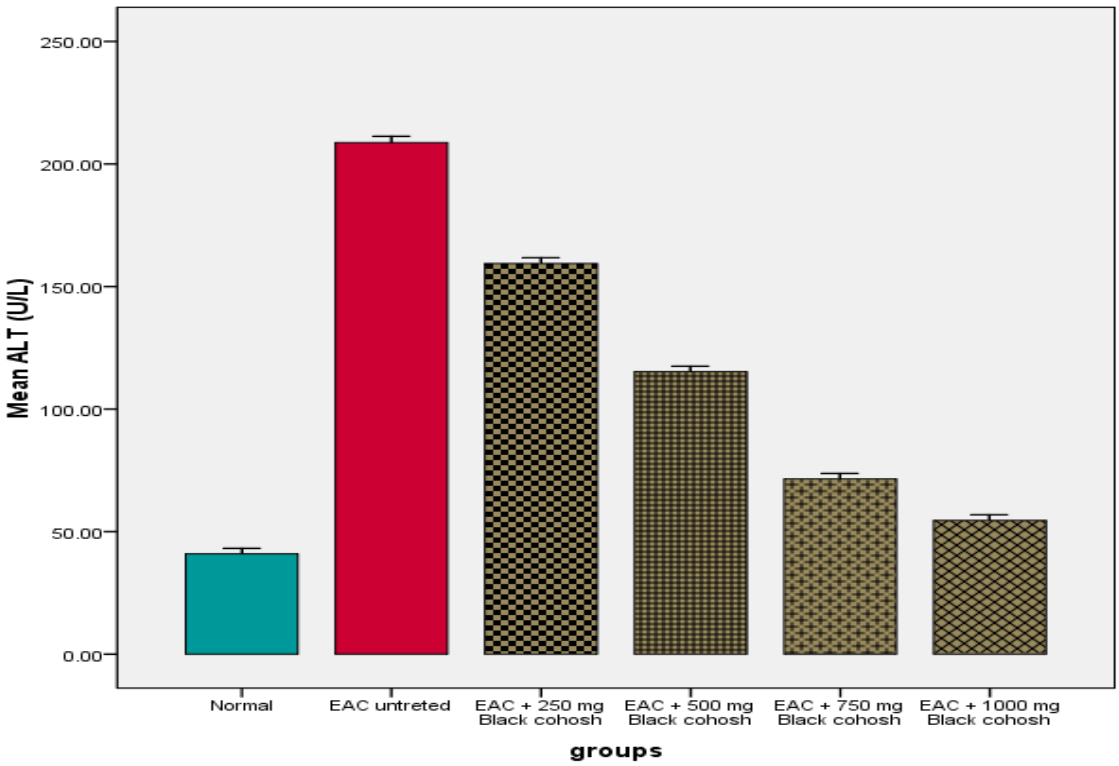


Figure (3): urea and creatinine of EAC treated groups with *Black cohosh* with different conc.



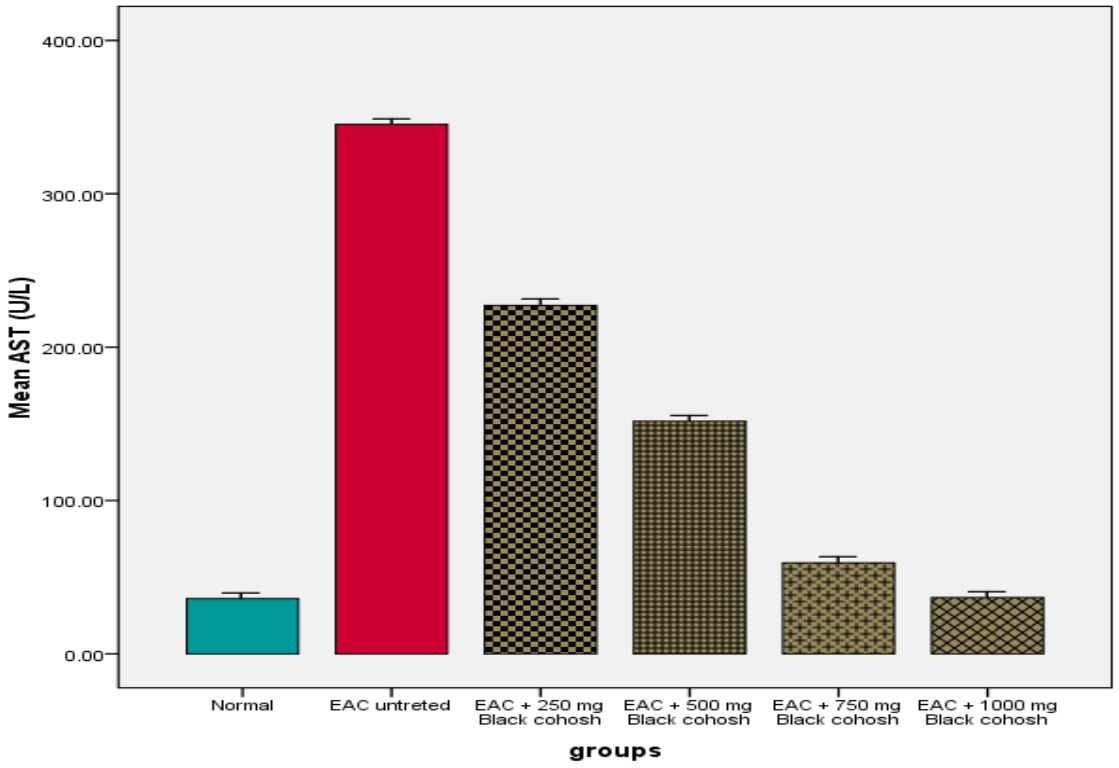
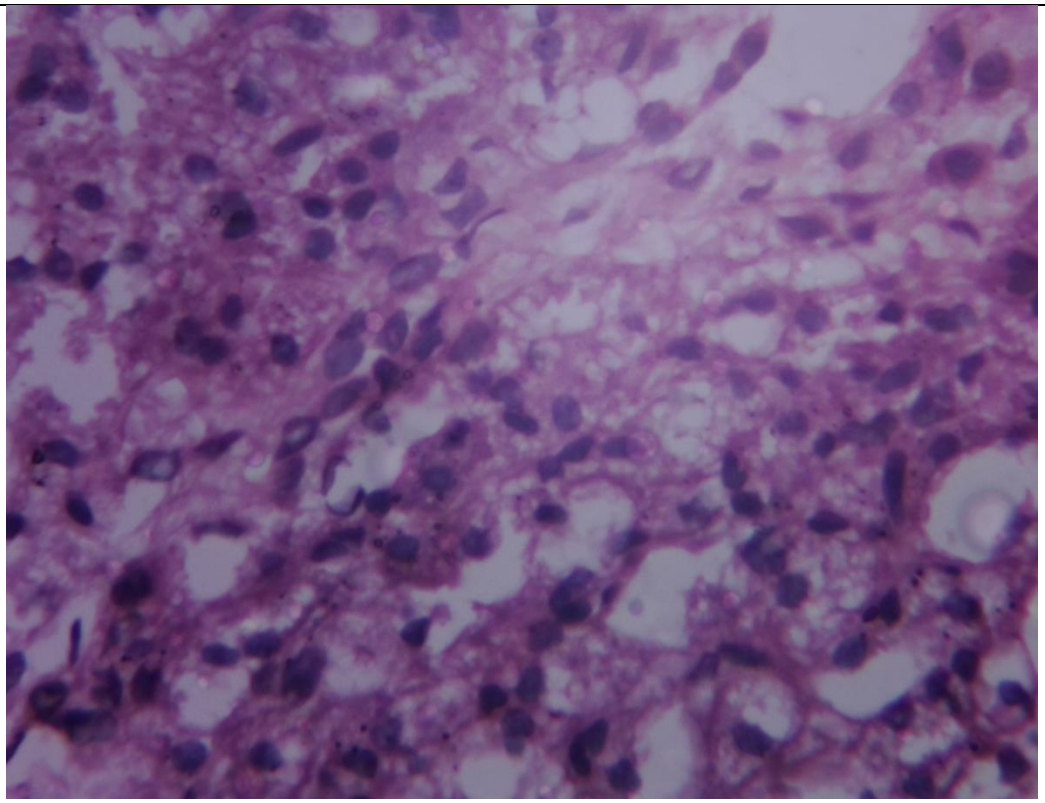
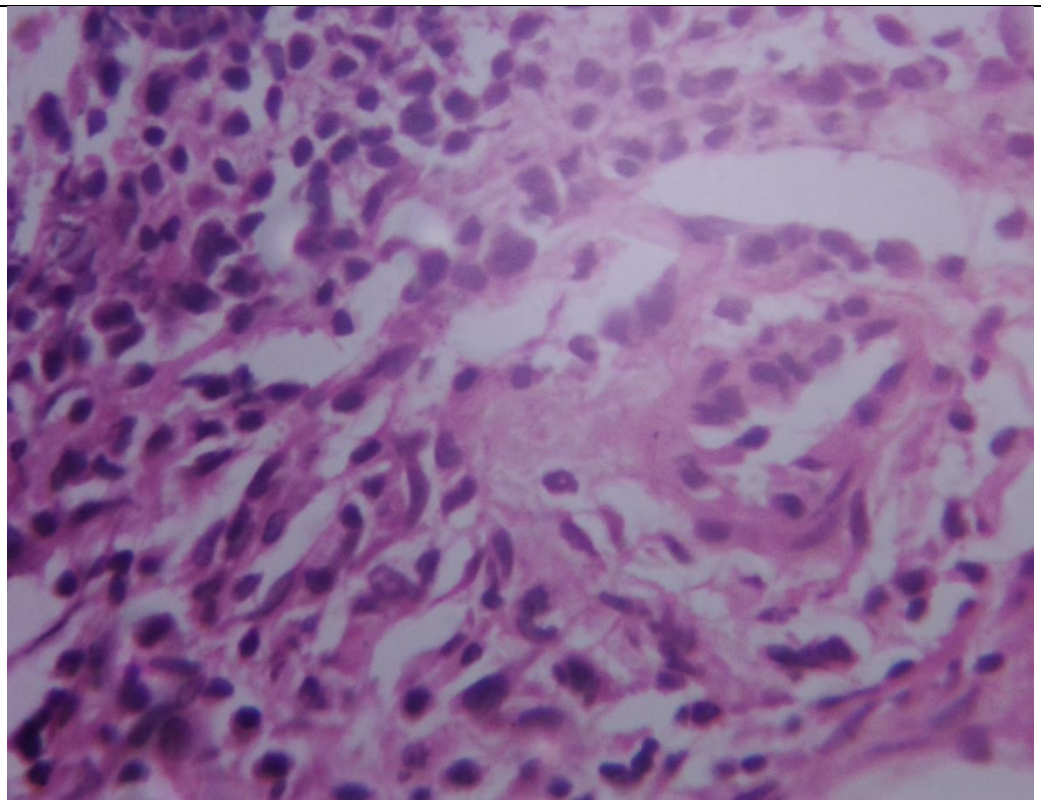


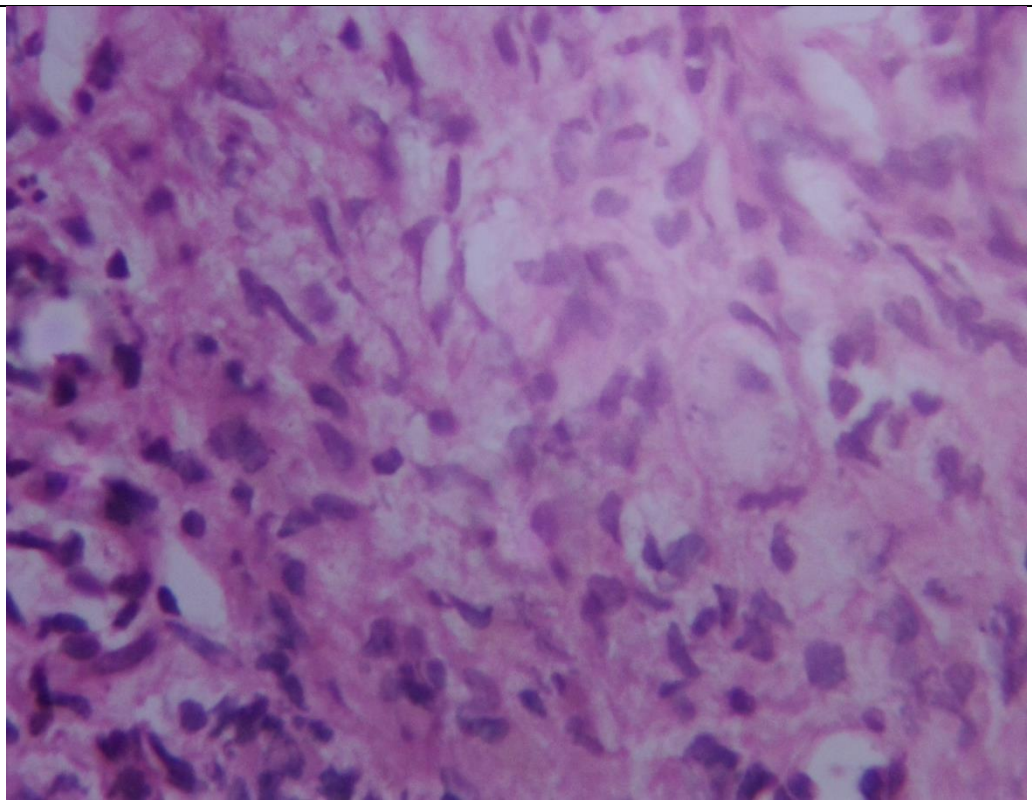
Figure (4): ALT and AST of EAC treated groups with *Black cohosh* with different conc.



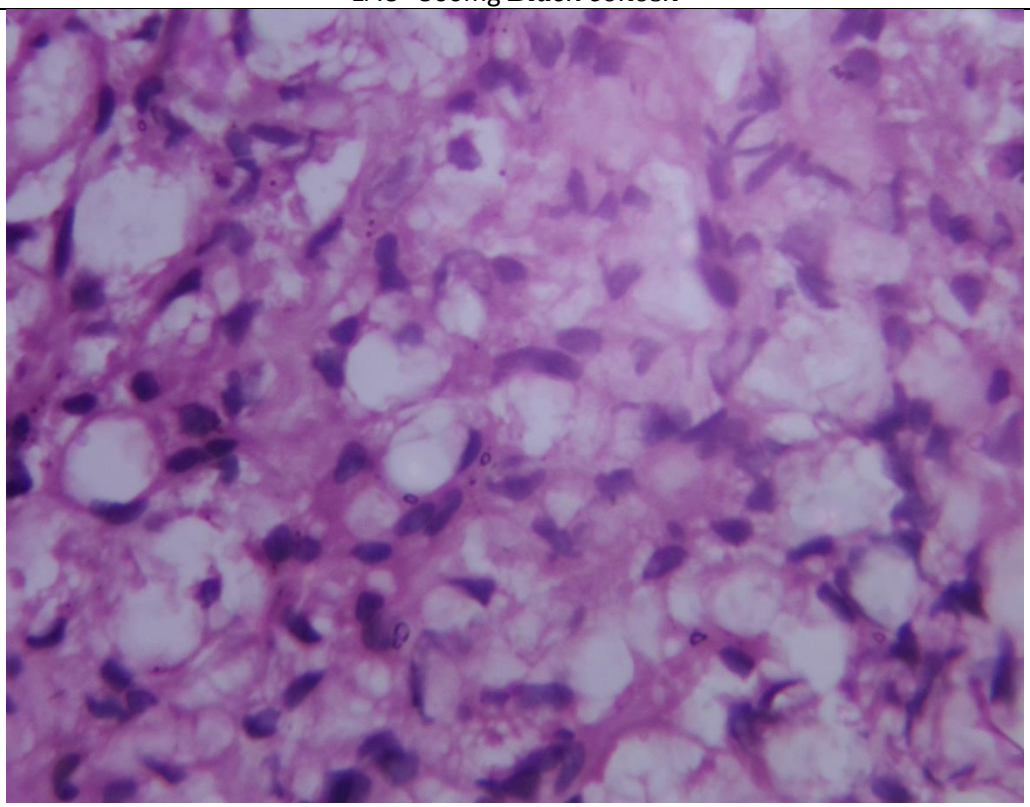
EAC untreated



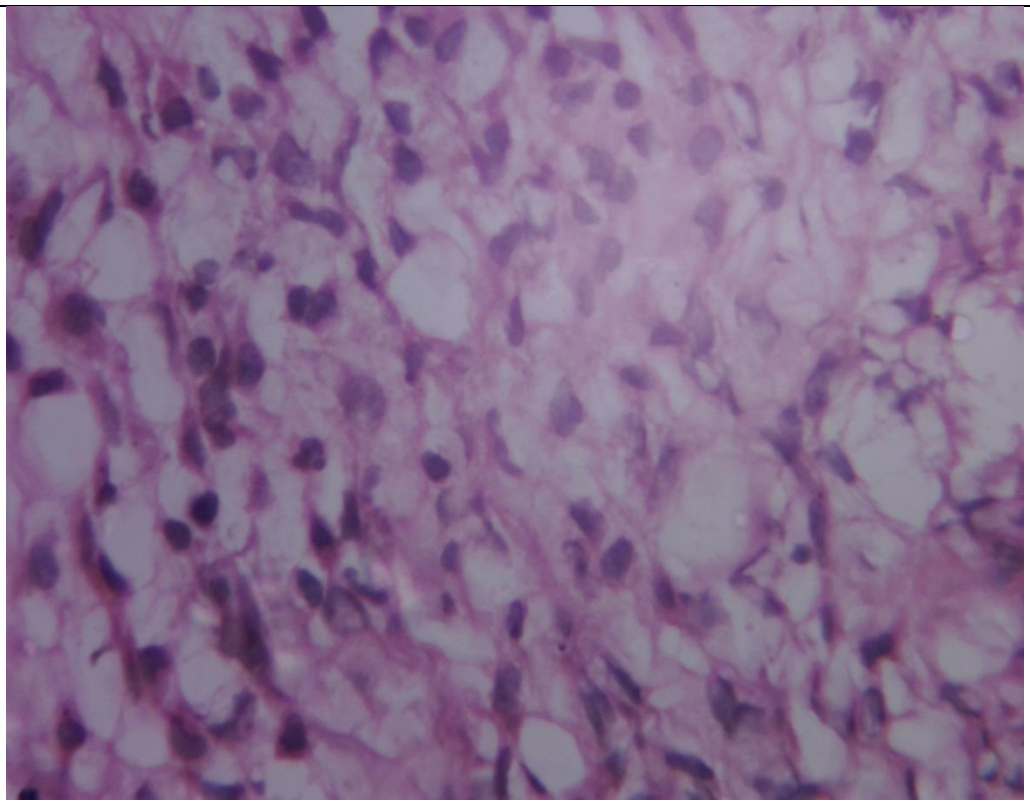
EAC+ 250mmg *Black cohosh*



EAC+ 500mg *Black cohosh*



EAC+ 750mg *Black cohosh*



EAC+ 1000mg *Black cohosh*

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

Discussion

The multistage process of carcinogenesis leads to an aggressive metastatic phenotype. This disorder may be caused by physical, chemical, or viral factors. It requires complex interactions between the tumor and host organs, as well as the coordinated accumulation of favorable genetic abnormalities [10].

It has been suggested that the unchecked production of reactive oxygen species (ROS) and free radicals is the cause of the change in antioxidant status, which ultimately results in oxidative stress and carcinogenesis [11]. Numerous macromolecular species, such as proteins, lipids, and nucleic acids, are harmed by oxidative stress, which leads to serious, interconnected disorders in cellular metabolism, like lipid peroxidation [12]. Cancer has been connected to lipid peroxidation, which can generate dangerous compounds like MDA and 4-hydroxynonenal [13]. These substances can target certain cells, which can lead to the development of cancer [14].

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

According to a number of studies, EAC can be used to create mouse models of breast tumors. This procedure upsets the tissue's redox balance, suggesting that oxidative damage could result in pathological and metabolic alterations [16,17]. Free radicals are normally scavenged in subcellular compartments by the antioxidant defense mechanisms of the relevant cells [18]. EAC can readily evade defenses, upsetting the equilibrium of pro- and antioxidants and causing aberrant cellular

activity. The high concentration of polyunsaturated fatty acids in cellular membranes makes them susceptible to the potentially dangerous process of lipid peroxidation [19].

Radicals, free radicals, and oxygenated metabolites are all produced by EAC [20]. After that, lipid peroxidation starts, which has a detrimental effect because of the oxidative stress that happens [21]. Since EAC can significantly oxidatively damage a variety of bodily tissues, especially the liver and breast, it is a useful and practical agent for creating in vivo breast cancer models [22, 23].

As a tumor grows, it undergoes many metabolic changes [24]. Due to the gradual progression of human tumors from preneoplastic to malignant. Therefore, there is always a chance to halt the growth of the tumor. As a result, prevention measures have expanded while intensive cancer research has reduced in recent years. Chemotherapeutic approaches use chemicals with particular functions to stop the development of cancer.

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 results showed that EAC implantation enhanced lipid peroxidation. MDA levels are significantly higher in all EAC-treated groups than in the control group. *Black cohosh* primarily lowers MDA by scavenging reactive free radicals implicated in peroxidation [29]. MDA levels were considerably lower in animals administered *Black cohosh* than in mice given EAC alone. *Black cohosh*'s capacity to scavenge free radicals and lower MDA indicates that it has anti-lipid peroxidative activity.

Defenses against free radicals prevent harm to cells caused by ROS. By scavenging reactive oxygen species (ROS), which are necessary for the initiation of lipid peroxidation, the anti-oxidative defense system may offer protection against cancer [30]. Both enzymatic (GPx, GST, SOD, and CAT) and non-enzymatic (mostly GSH) components are used in this defense mechanism [30, 31]. The main defense mechanism of the antioxidant system against oxidative stress is SOD, which transforms toxic superoxide anions (O2−) into O2 and H2O2. To guard against ROS, Gpx and catalase can scavenge H2O2 and convert it into innocuous metabolites [32].

Additionally, GPx works well to detoxify reactive free radicals that scavenge in response to oxidative stress and peroxides and hydroperoxides that cause GSH oxidation [33]. Additionally, by conjugating the thiol functional groups of GSH with electrophilic xenobiotics, GST transforms or eliminates the xenobiotic-GSH complex [34]. GSH undergoes oxidation during this process to GSSG, which GR can then transform back into GSH by utilizing NADPH [35]. The main nonenzymatic antioxidant in mammalian cells is GSH [36]. Among its many physiological functions, GSH is in charge of both internal and exterior drug detoxification. By removing H2O2, scavenging free radicals, and preventing lipid peroxidation, it successfully shields cells from oxidative stress [37].

According to the current study, mice with cancer have reduced antioxidant activity compared to healthy animals (GR, GST, SOD, CAT, and TAC). 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, according to Pradeep et al. [40]. Animals given *Black cohosh* + EAC showed a significant improvement in both enzymatic and non-enzymatic antioxidant defense when compared to those given EAC alone. *Black cohosh*'s capacity to minimize the creation of free radicals, increase endogenous antioxidant activity in addition to scavenging free radicals, and lessen the synthesis of breast lipoperoxides may be the cause of this increase [41].

The increased activity of antioxidant enzymes in mice treated with *Black cohosh* compared to animals given EAC alone indicates that *Black cohosh* extract has effective antioxidant activity because it contains flavonoids, alkaloids, phytosterols, tannins, amino acids, glycosides, saponins, and triterpinoids [42–47]. According to the previously given data, *Black cohosh* extract offers a protective 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].

Antioxidant activity and plasma mean MDA levels were found to be significantly statistically inversely related in this experiment. A malfunction in the antioxidant system that results in the buildup of lipid peroxides in cancerous tissue may be the source of the elevated MDA level, according to Kumaraguruparan et al. [52]. Additionally, Sener et al. [53]. found that compared to the treated and control groups, the breast cancer group had significantly higher blood MDA levels and a statistically significant decrease in total antioxidant capacity. This study's results are consistent with those of prior investigations [54–70].

The kidney prevents buildup by filtering metabolic products from the bloodstream, such as creatinine and urea. An increase in these compounds' serum levels is thought to indicate deterioration in renal function [71, 72]. Consistent with previous research, the outcomes of this study suggested that alkylating drugs caused a decrease in renal function [73, 74]. This study includes renal function markers such as creatinine and urea. *Black cohosh*, we found, increased serum levels of urea and creatinine, indicating kidney preservation. This indicates that *Black cohosh* protects against renal 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) [77,78]. Bilirubin is present in the bile, liver, intestines, and reticuloendothelial cells of the spleen, whereas GGT and ALP are affixed to the cell membrane [79]. Serum levels of bilirubin, GGT, and ALP rise in hepatobiliary damage, poor hepatic clearance, and overproduction or leakage of these enzymes [79-81]. 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 [79]. This study suggests that *Black cohosh* has hepatoprotective properties since it prevented rises in serum ALT and AST levels both before and after therapy. This indicates that *Black cohosh* has a preventive effect against EAC-induced hepatotoxicity. The results of this study are consistent with those of other studies [54–70].

The current investigation discovered that histological changes correlated with metabolic changes throughout the experiment. Histological analysis showed that every tumor in the cancerous control group had highly malignant cells and no necrosis. Tumors removed from animals given 750 and 1000 mg/kg body weight exhibited significant regions of necrosis (78% and 86%, respectively), in contrast to the group given 500 mg/kg body weight (69%), even though necrosis foci (59%) were visible in tumors administered with 250 mg/kg body weight.

The present results were in line with other research projects carried out by other authors [17, 21, 22, 53].

Conclusion

According to the current study, *Black cohosh* may have promising chemotherapeutic qualities for the treatment of cancer.

Recommendations

The results of this study show that it is possible to *Black cohosh* 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.

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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Conflict of interest

The author declares that he has no competing interests.

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