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## Study the Potential Role of *Ginko Biloba* Extract on Cancer Treatment

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### Abstract

The present study aimed to evaluate the antitumor chemotherapeutic potential of *Ginkgo biloba* extract against breast carcinogenesis by Ehrlich ascites carcinoma implanted in Swiss albino mice. Chemotherapy was evaluated by monitoring tumor incidence and size, as well as analyzing the status of (a) the biochemical profile related to oxidative stress, including; total antioxidant capacity (TAC), glutathione reductase (GR) activity, glutathione-S-transferase (GST) activity, superoxide dismutase (SOD) activity, catalase (CAT) activity and lipid peroxidation (MDA), markers of renal and hepatic toxicity (urea, creatinine, alanine transaminase). Histopathological changes indicated tumor inhibition and neovascularization after treatment. Overall, these results suggest that treatment with *Ginkgo* extract provides antioxidant protection as well as high chemotherapeutic efficacy against breast tumors implanted with stem cells.

**Keywords:** Biloba, Ginko, carcinoma, chemotherapy, Breast

### 1. Introduction

Breast cancer is the most common cancer among women, accounting for 22.9% of all malignancies among women worldwide. The prognosis for breast cancer in Egypt is poor, with a mortality rate of 29% and an incidence-to-fatality ratio of 1:3.7. [1] According to a report by the World Health Organization (WHO), breast cancer accounts for 16% of all cancer deaths among women worldwide. It is the most commonly diagnosed solid tumor in women. The risk of breast cancer increases with age, and lifestyle and environmental factors contribute to the development of the disease. [2]

Breast cancer and related diseases are treated with surgery, chemotherapy, radiation therapy, or a combination of these treatments. Despite these treatment options, the cancer is still associated with a high mortality rate. This is mainly due to problems with early detection, high treatment costs, and the often late onset of breast cancer, which indicates a diagnosis of cancer in women. [3-5] As a result of these various shortcomings, we need new treatment options that can increase the chances of survival of breast cancer patients with few or no side effects. [6]

Phytochemical treatments for serious health problems have recently gained worldwide scientific recognition. Studies on the pharmacological mechanisms and chemical components of plant extracts responsible for anticancer activity have attracted widespread attention. [7-8]

*Ginkgo biloba* extract (GbE) is a well-known medicinal plant that is widely used as complementary and alternative medicine (CAM) for various diseases, including breast cancer. GbE is a complex mixture of over 300 chemicals, mainly composed of flavonoid glycosides and terpenoids such as ginkgolide and bilobalide. GbE is used for the prevention and treatment of neurological disorders, circulatory problems, memory loss, and Alzheimer's disease. In fact, several GbE compounds have been shown to possess pharmacological properties such as cell cycle regulation, antioxidant, antiproliferative, antiangiogenic, and antiestrogenic properties. [9]

The aim of this study was to investigate the chemotherapy efficacy of *Ginkgo biloba* for the treatment of Ehrlichial ascites carcinoma. To achieve this aim, the following were performed:

## 2. Materials

### 2.1. Animals

Female Swiss albino mice weighing 20-25 grams and 8-10 weeks old were divided into two groups. There are experimental groups that received different concentrations of *Ginkgo* relative to DL50. The mice are treated with increasing doses of *Ginkgo*. Uses of experimental animals in the study protocol were carried out in accordance with the ethical guidelines of the Medical Research Institute, Alexandria University (April 2, 2011). Group A: 10 mice receive the same phosphate buffered saline (PBS) as a control group. Group B: 50 mice received 2 This group will be divided into different groups; Subgroup B-1: 10 mice with EAC safely and without treatment. Subgroup B-2: 10 rats with 1000 mg/kg/day of ginkgo leaves from EAC implant, Subgroup B-3: 10 rats with 750 mg/kg/day of ginkgo leaves from EAC implant, Subgroup B-4: 10 rats with 750 mg/kg/day of ginkgo leaves from EAC implant 500 mg/kg/day of ginkgo/Ginkgo Subgroup B-5.

## 3. Methods

For evaluation of the treatment effects to all studied groups the following investigations were done:

### 3.1. Tumor growth/inhibition evaluation

During the treatment session, tumor growth was checked regularly every day. The length and width of the tumor were measured using calipers and the tumor volume (mm<sup>3</sup>) was calculated using the following equation:  $TV (mm^3) = \frac{22}{7} \times \frac{4}{3} \times (\text{length}/2) \times (\text{width}/2)^2$ . After 2 weeks of treatment, the mice were sacrificed and the tumors were dissected and weighed (in grams).

### 3.2. Biochemical investigations

Blood samples (2.5 ml of venous blood) were drawn from all groups of mice. These blood samples were allowed to clot completely for 20 min and then centrifuged at  $3000 \times g$  for 20 min

to separate serum for biochemical assays. All biochemical analyses were performed on an automatic analyzer.

### 3.3. Oxidative stress and antioxidant status

Lipid peroxidation (MDA) assay kit (BioVision Catalog #K739-100), total antioxidant capacity (TAC) assay kit (BioVision Catalog #K274-100), glutathione reductase (GR) activity assay kit (BioVision Catalog #K761-100), glutathione-s-transferase (GST) activity assay kit (BioVision Catalog #K263-100), superoxide dismutase (SOD) activity assay kit (BioVision Catalog #K335-100), Catalase (CAT) activity assay kit (BioVision Catalog #K773-100), were used according to the manufacturer's instructions.

### 3.4. Kidney and liver function tests

Urea (Sigma Catalog # MAK179), creatinine (Sigma Catalog # MAK080), Alanine Transaminase (ALT) Activity Assay Kit (Sigma Catalog # MAK052), Aspartate Aminotransferase (AST) Activity Assay Kit (Sigma Catalog #MAK055) were used according to the manufacturer's instructions.

### 3.5. Histopathological examination

Small pieces of Ehrlich body tumor tissue were obtained from the experimental groups and processed and examined with hematoxylin and eosin (H&E) as follows: small pieces of Ehrlich body tumor tissue were fixed with 10% formaldehyde, dehydrated in increasing amounts of alcohol, embedded in paraffin to produce paraffin blocks, the paraffin blocks were cut into 3- to 4- $\mu$ m-thick sections, floated in a water bath, cleared in xylene, rehydrated in decreasing amounts of alcohol, stained with hematoxylin and eosin, cleared again in ethylene and coverslipped, and the slides were prepared for light microscopy

## 4. Results

### 4.1. Effects of treatment on tumor volume and mass

The relationship between the treatment period of Ginkgo and tumor volumes at different concentrations (250 mg, 500 mg, 750 mg, 1000 mg Ginkgo) was shown in Figure (1). The results obtained indicated that Ginkgo treatment at a dose of 250 mg had little effect on tumor volume. The increasing dose of Ginkgo 500 and 750 mg became more effective on tumor cells and tumor volume. While the dose of 1000 mg Ginkgo treatment showed the highest effect on reducing tumor volume and cells.

### 4.2. Effects of treatment on oxidative stress related parameters

This study reported that lipid peroxidation increased during EAC implantation. Compared with the normal group of animals, MDA levels were significantly increased in all EAC-implanted groups. The Ginkgo biloba-implanted animal group had significantly lower MDA levels than animals implanted with EAC alone.

In the current study, antioxidant activities (SOD, CAT, GR, GST, and TAC) were reduced in mice with cancer compared to healthy animals. On the other hand, animals treated with Ginkgo biloba had significantly increased enzyme and non-enzyme antioxidant protection compared to animals receiving EAC alone (Figure (2)).

#### 4.3. Effects of treatment on renal and liver function tests

In this study, the biomarkers of renal function, creatinine and urea, were considered. In this study, EAC caused a significant increase in serum urea and creatinine levels. In addition, Ginkgo treatment improved serum creatinine and urea levels, which are indicators of renal protection. This also confirmed the protective effect of Ginkgo against EAC-induced nephrotoxicity. The present study also considered the biomarkers of liver function, ALT and AST. In this study, EAC caused a significant increase in serum ALT and AST activities. Whereas Ginkgo treatment prevented the increase in serum ALT and AST levels, which are indicators of Ginkgo protecting the liver from EAC-induced hepatotoxicity (Figures (3, 4)).

#### 4.4. Effects of treatment on histological structural changes

Histological examination showed that all tumors in the control group were highly malignant cells and the tumors exhibited 5-10% necrosis. Tumors removed from animals receiving ginkgo extract (750 and 1000 mg/kg body weight) showed large necrotic areas (85 and 90%, respectively) compared with the treated group (500 mg/kg body weight) (75%), while in the treated tumors (250 mg/kg body weight), foci of necrotic areas (65%) were clearly visible (Figure (5)).

### 5. Discussion

Carcinogenesis is a multistep process induced by physical, chemical, or viral processes that involves the coordinated acquisition of beneficial genetic defects and complex interactions between the tumor and host tissue, resulting in an aggressive metastatic phenotype. [10]

Research has shown that the unlimited generation of free radicals and reactive oxygen species (ROS) is responsible for changing the antioxidant status, which ultimately leads to oxidative stress and carcinogenesis. [11] Oxidative stress can damage a variety of macromolecules, including lipids, proteins, and nucleic acids, leading to key changes in cellular metabolism, such as lipid peroxidation [12]. Carcinogenesis is associated with lipid peroxidation [13], which can produce harmful chemicals such as MDA and 4-hydroxynonenal. These compounds have the potential to cause cancer by attacking cellular targets. [14]

Breast tissue can be the primary target of toxicological effects of various lipophilic carcinogens and EAC if these substances cannot be biotransformed into hydrophilic metabolites that are easily excreted. [15]

Numerous studies have shown that EAC can be used to generate experimental breast cancer in mice and that this process alters the redox balance of the tissue, which means that oxidative damage can lead to biochemical and pathophysiological changes. [16,17] Under normal conditions, free radicals in subcellular compartments are eliminated by antioxidant defense

mechanisms in the corresponding cells. [18] EAC can easily disrupt protective mechanisms, alter the balance of prooxidants and antioxidants, and cause cellular abnormalities. The high concentration of polyunsaturated fatty acids in cell membranes makes them susceptible to lipid peroxidation, which can have serious consequences. [19]

EAC generates free radicals and oxidative radicals. [20] In turn, the resulting oxidative stress negatively affects the initiation of lipid peroxidation. [21] Overall, EAC can cause severe oxidative damage to many organs in the body, especially the liver and breast, making it a viable and useful tool for developing in vivo models of breast cancer. [22,23]

As a tumor grows, many biochemical changes occur. [24] The pathological growth of human tumors takes a long time to progress from a precancerous stage to a malignant tumor. Therefore, there is still a chance to reverse the progression of the tumor. As a result, in recent years, intensive cancer research has decreased and prevention has increased. Chemotherapy techniques involve the use of chemicals with specific effects to reduce the cancer process.

In recent years, there has been growing interest in understanding the role of lipid peroxidation in cancer growth, using MDA as a measure of oxidative stress. MDA is a low molecular weight aldehyde that is formed when free radicals attack polyunsaturated fatty acids. [25, 26]

Elevated serum lipid peroxides in breast cancer may be due to a defect in the antioxidant system, which causes lipid peroxides to accumulate in cancer tissues and be released into the blood. [27] MDA is a major aldehyde with high cytotoxicity and the end product of the peroxide radical of lipid peroxidation. It is considered an inhibitor of protective enzymes. Therefore, it has the potential to induce mutations and carcinogenesis. [28]

Our study showed that lipid peroxidation increased with EAC implantation. MDA levels were significantly increased in all EAC-treated groups compared with normal animals. The ability of ginkgo to reduce malondialdehyde (MDA) is primarily attributed to its scavenging of reactive free radicals involved in peroxidation reactions. [29] MDA levels of animals in the ginkgo-treated group were significantly lower than those in the EAC-only group. This confirms the lipid antiperoxidative activity of Ginkgo biloba as evidenced by its ability to reduce MDA by scavenging free radicals.

The antioxidant defense system protects cells from damage caused by ROS. The antioxidant defense system can scavenge ROS, which play a key role in initiating lipid peroxidation, thereby preventing cancer [30]. This defense system utilizes both enzymatic (SOD, GPx, GST, and CAT) and nonenzymatic (mainly GSH) components. [30,31] SOD is the main defense mechanism of the antioxidant system against oxidative stress, which decomposes harmful superoxide anions ( $O_2^-$ ) into  $O_2$  and  $H_2O_2$ . GPx and catalase can remove  $H_2O_2$  and convert it into harmless metabolites, thereby preventing ROS. [32]

Furthermore, GPx is highly effective in scavenging reactive free radicals generated by oxidative stress and in detoxifying peroxides and hydroperoxides that lead to the oxidation of GSH.[33] Furthermore, GST catalyzes the conjugation of the thiol functional group of GSH to electrophilic xenobiotics, resulting in the removal or conversion of xenobiotic-GSH conjugates.[34] In this process, GSH is oxidized to GSSG, which GR can convert to GSH by consuming NADPH.[35] GSH is the major non-enzymatic antioxidant in mammalian cells.[36] GSH is said to be involved in many physiological functions, including the detoxification of endogenous and xenobiotic

chemicals. It effectively protects cells from the harmful effects of oxidative stress by scavenging free radicals, removing H<sub>2</sub>O<sub>2</sub>, and inhibiting lipid peroxidation. [37]

In this study, cancer-infected mice were found to exhibit lower antioxidant activities (SOD, CAT, GR, GST, and TAC) compared to normal animals. Our results are consistent with previous studies. [38,39] According to Pradeep et al. [40], the decreased antioxidant defense capacity is due to the decreased expression of these antioxidants after mammary gland injury. In contrast, when animals received ginkgo and EAC together, both enzymatic and non-enzymatic antioxidant defenses were significantly enhanced compared to animals receiving EAC alone. This increase was attributed to the ability of ginkgo to inhibit free radical production, enhance endogenous antioxidant activity, neutralize its anti-radical properties, and reduce the formation of mammary lipid peroxides. [41]

Antioxidant enzyme activities were increased in mice treated with Ginkgo biloba compared with those treated with EAC alone, indicating that Ginkgo biloba extract possesses potent antioxidant activity due to the presence of alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, tannins and triterpenoids. [42–47] All the above data indicate that the protective effect of Ginkgo biloba extract can be attributed to the antioxidant activity of flavonoids from this plant [42–51], which act as potent quenchers of superoxide radicals and singlet oxygen.

In the present study, a significant negative correlation was found between mean plasma MDA levels and antioxidant activity. Kumaraguruparan et al. suggested that elevated MDA levels might be due to the failure of antioxidant system leading to accumulation of lipid peroxides in cancer tissues. [52] Furthermore, Sener et al. [53] found that total antioxidant capacity of breast cancer group was significantly decreased and serum MDA level was significantly increased compared with treatment group and control group. The results of this study are also consistent with those of other studies. [54-69]

Creatinine and urea are metabolic products that are removed from the circulation by the kidneys to prevent their accumulation. Elevated serum levels of these compounds are considered to indicate a decline in renal function. [70, 71] The results of this study showed that alkylating drugs can cause a decline in renal function, which is consistent with previous observations. [72,73] This study took into account markers of renal function such as creatinine and urea. We found that Ginkgo biloba could improve serum levels of creatinine and urea, indicating a renal protective effect. This confirms that Ginkgo biloba has a preventive effect on CAD-induced renal damage. The liver is an organ involved in the biotransformation of drugs and other hepatotoxic substances. Serum bilirubin levels and the activities of the liver enzymes ALT, AST, ALP, and GGT are considered useful indicators of hepatotoxicity. [74,75] Elevated serum ALT and AST levels may be due to leakage of damaged hepatocytes (hepatocellular injury). [76] Bilirubin is located in the reticuloendothelial cells of the liver, bile, intestine, and spleen, while ALP and GGT are associated with the cell membrane. [77] Serum bilirubin, ALP, and GGT levels are elevated in cases of hepatobiliary injury, decreased hepatic clearance, and overproduction or leakage of these enzymes. [77] This study observed liver function indicators such as ALT and AST. In the present study, EAC significantly increased the activities of serum ALT and AST. ALT and AST are mainly present in the cytoplasm and mitochondria of hepatocytes. [77] In the present study, pretreatment and concomitant treatment with Ginkgo prevented the increase in serum ALT and AST levels, indicating that Ginkgo has a hepatoprotective effect. This confirms

that Ginkgo has a preventive effect on EAC-induced hepatotoxicity. The results of this study are also consistent with other studies. [54-69]

The results of this study showed that the histopathological changes were synchronized with the metabolic changes throughout the experiment. Histological examination showed that the tumors in the cancer control group were all high-grade malignant cells without necrosis. The tumors removed from the animals treated with Ginkgo extract (750 and 1000 mg/kg body weight) showed significant necrotic areas (85% and 90%, respectively) compared with those in the treated group (500 mg/kg body weight) (75%), while necrotic areas were visible in the tumors treated with Ginkgo extract (250 mg/kg body weight) (65%). According to the present results, it is consistent with previous studies conducted by other authors. [17, 21-22, 53]

## 6. Conclusion

Current research shows that Ginkgo has good chemotherapeutic properties in the treatment of cancer.

## 7. Recommendations

Based on the results of this study, the use of Ginkgo biloba can be recommended as an alternative therapy for the treatment of cancer with an expanding treatment course.

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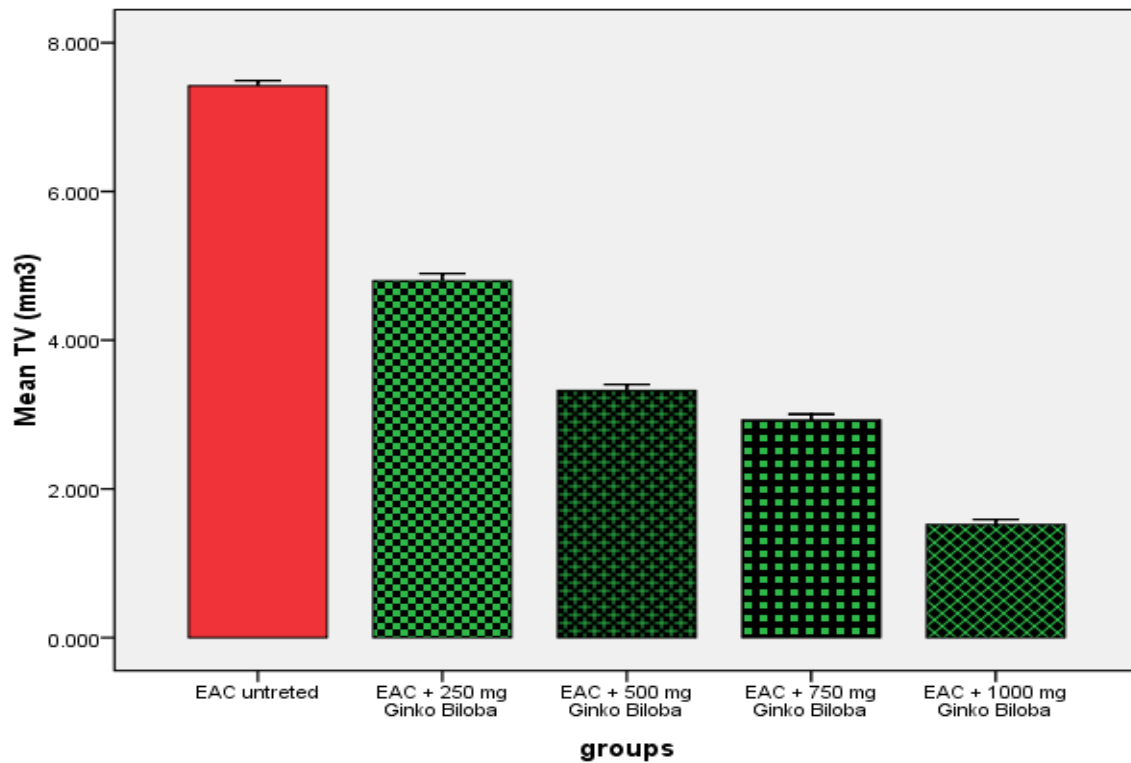
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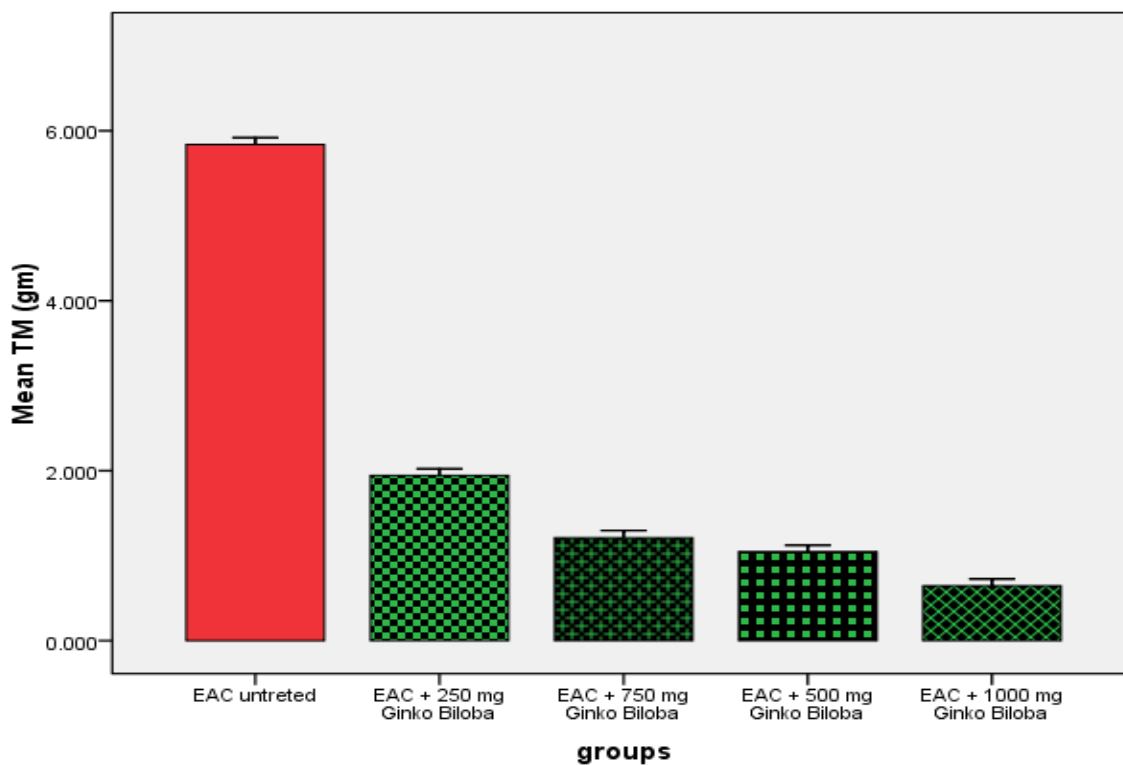
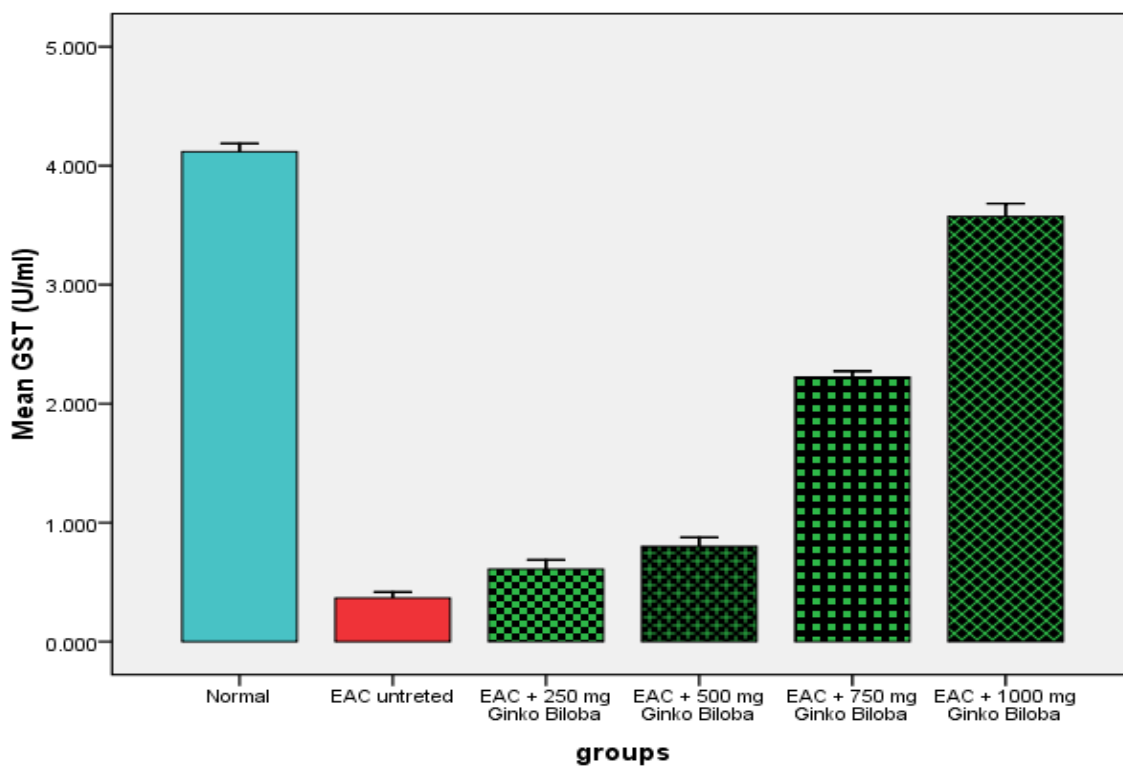
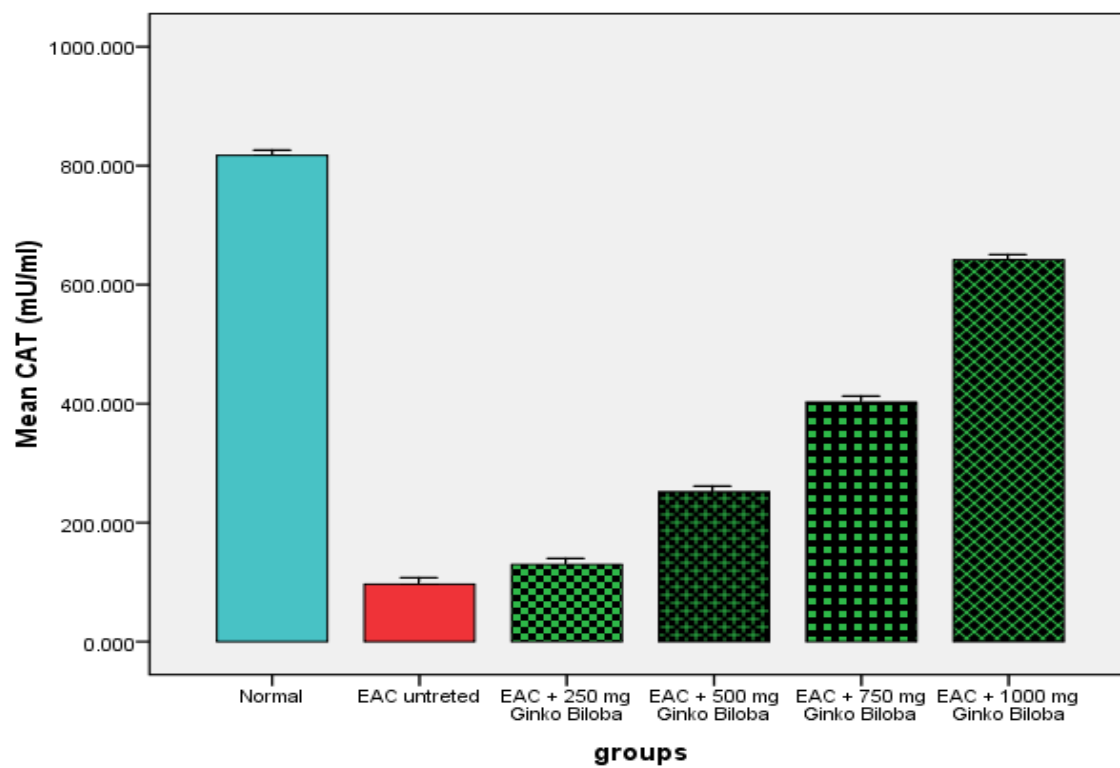
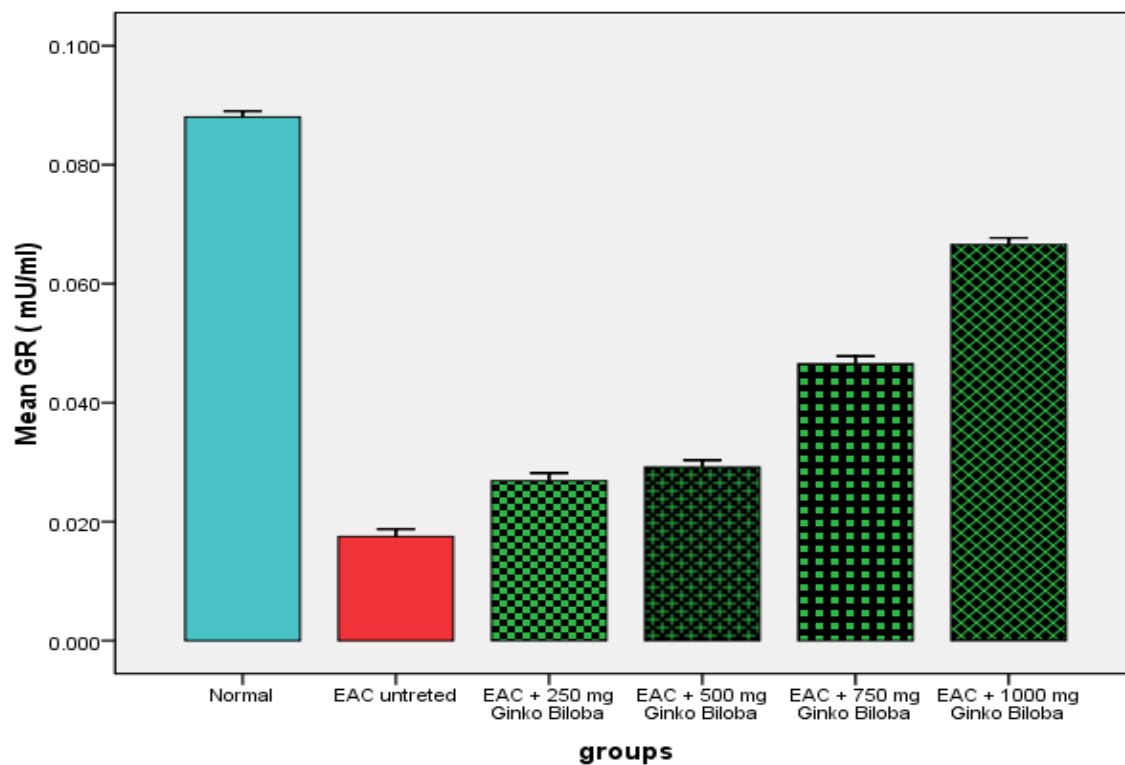
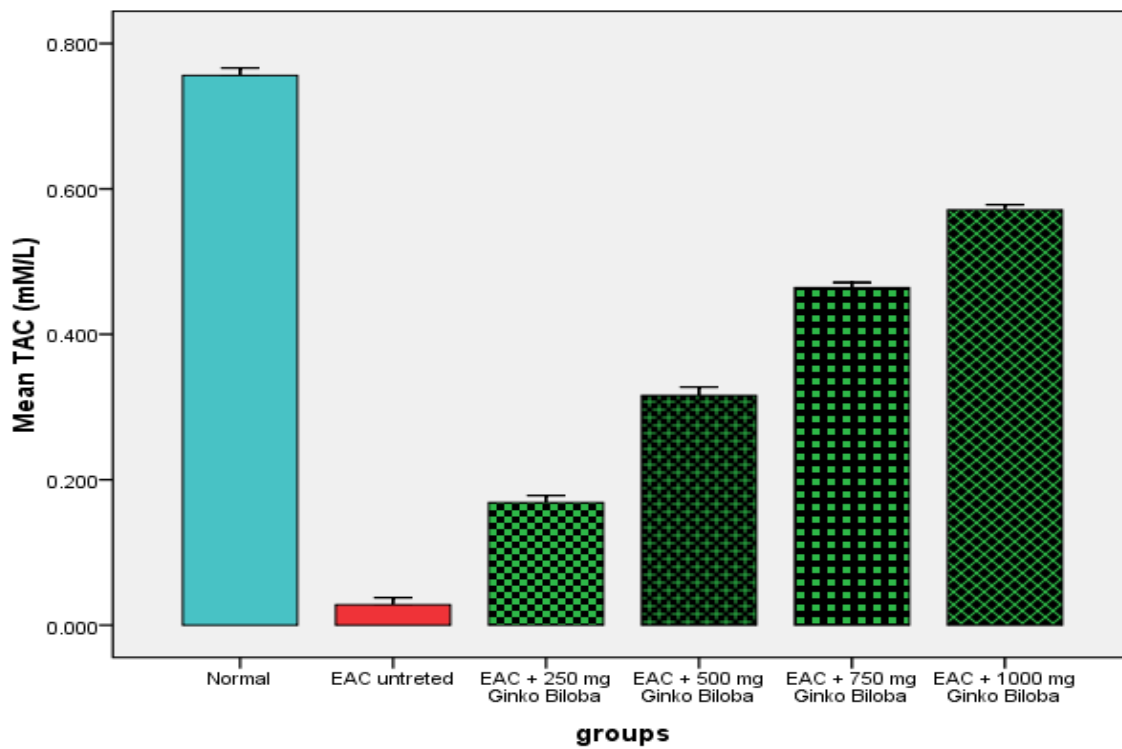
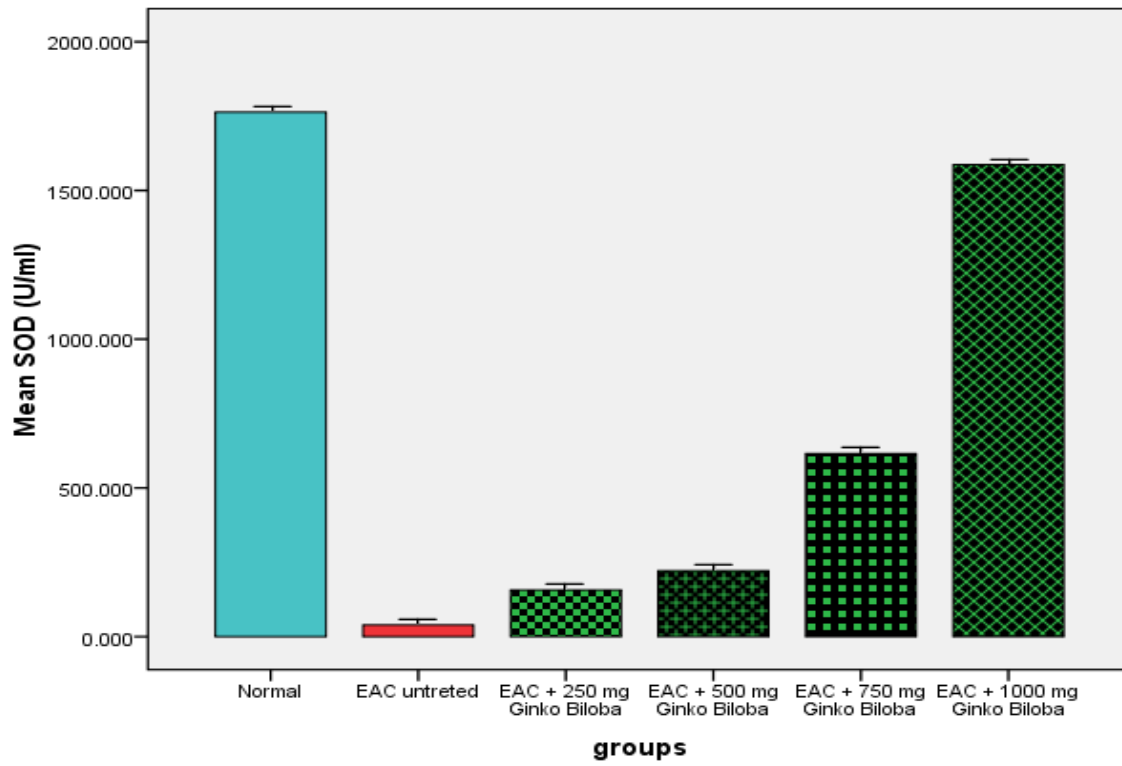


Figure (1): TV and TM of EAC treated groups with Ginkgo with different conc.







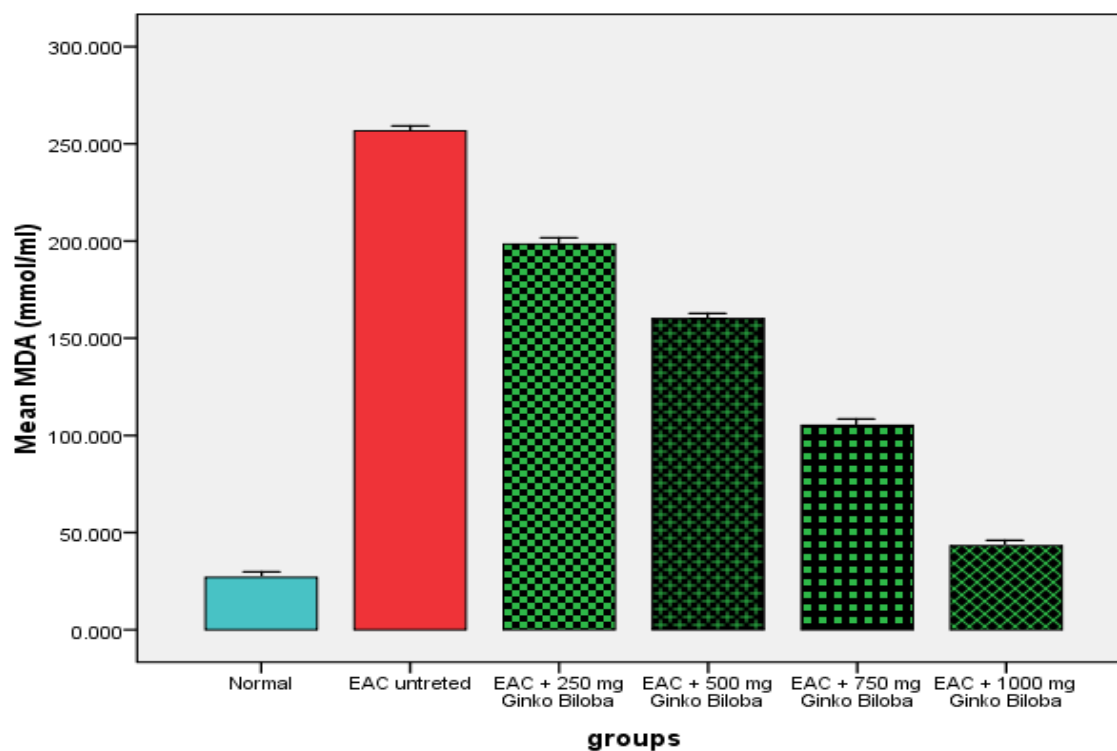
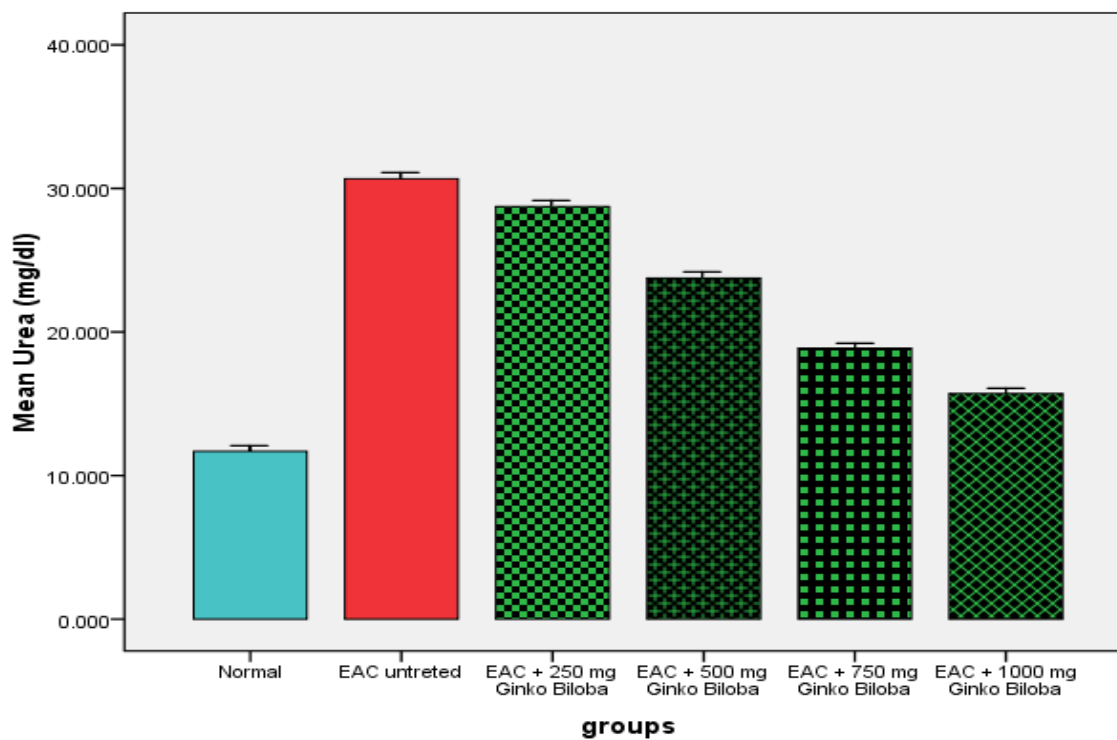


Figure (2): GST, GR CAT, SOD TAC and MDA of EAC treated groups with Ginko with different conc.



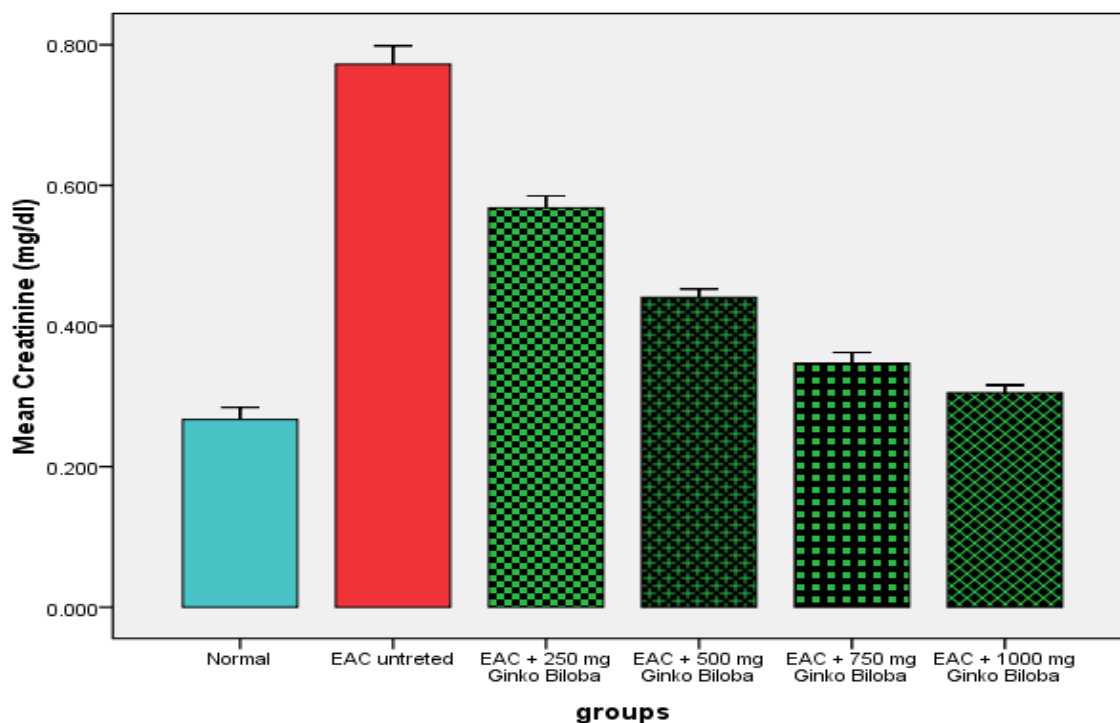
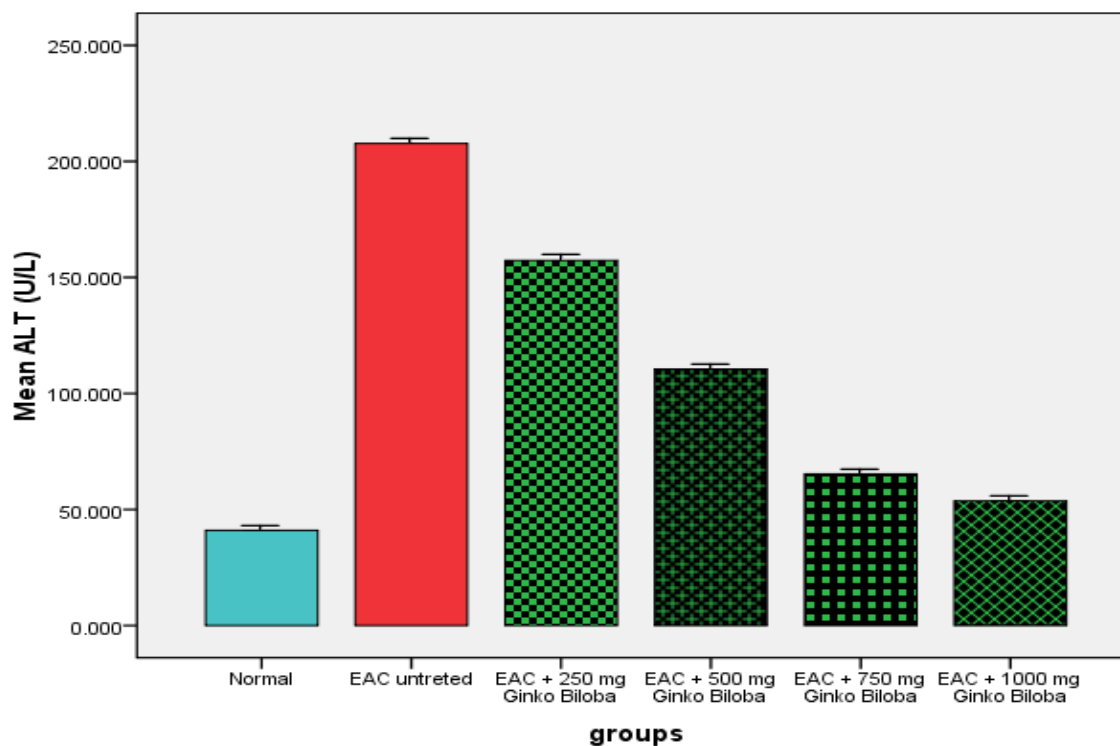


Figure (3): urea and creatinine of EAC treated groups with Ginkgo with different conc.



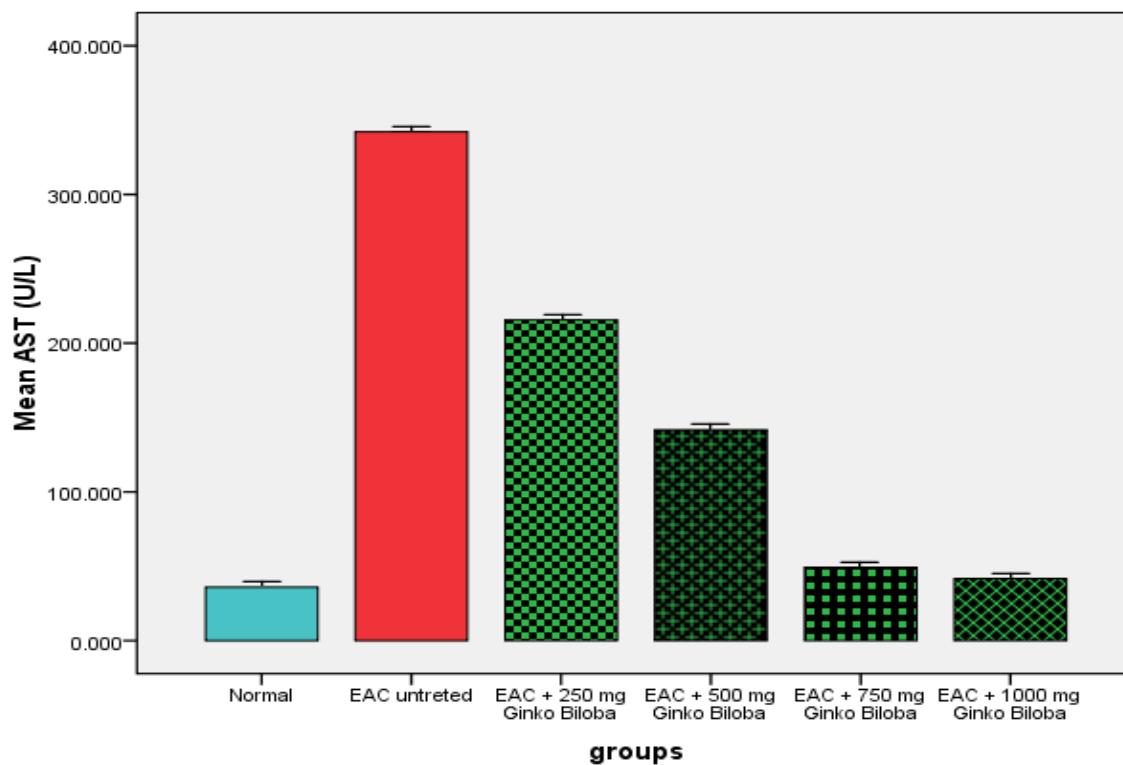
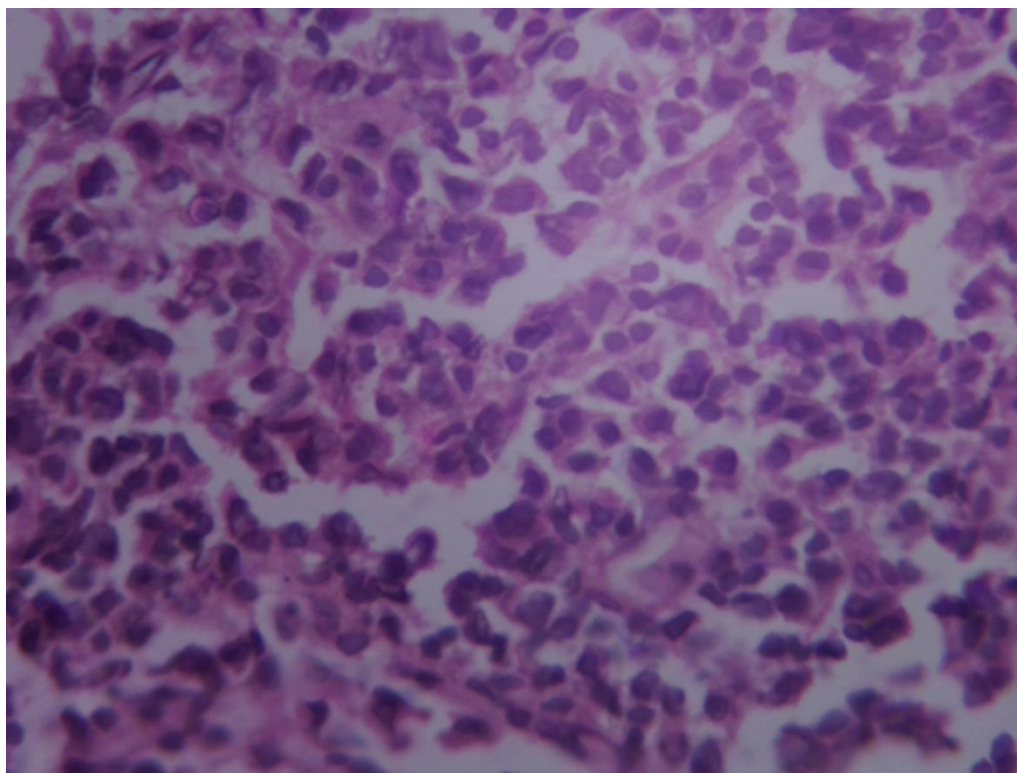
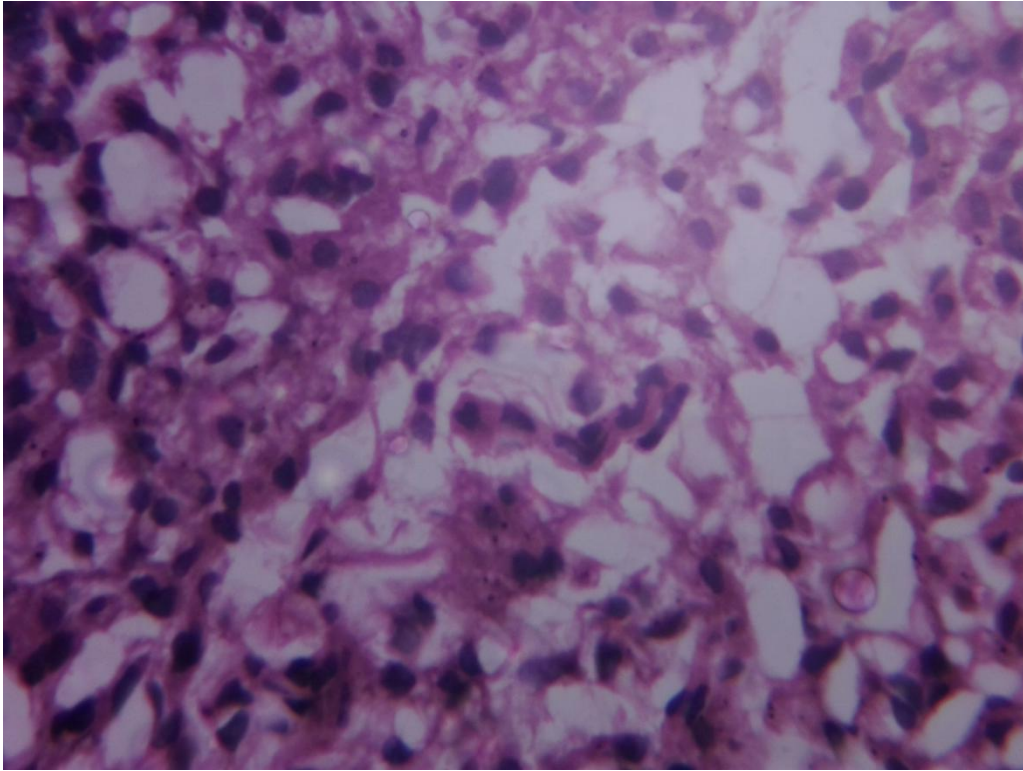


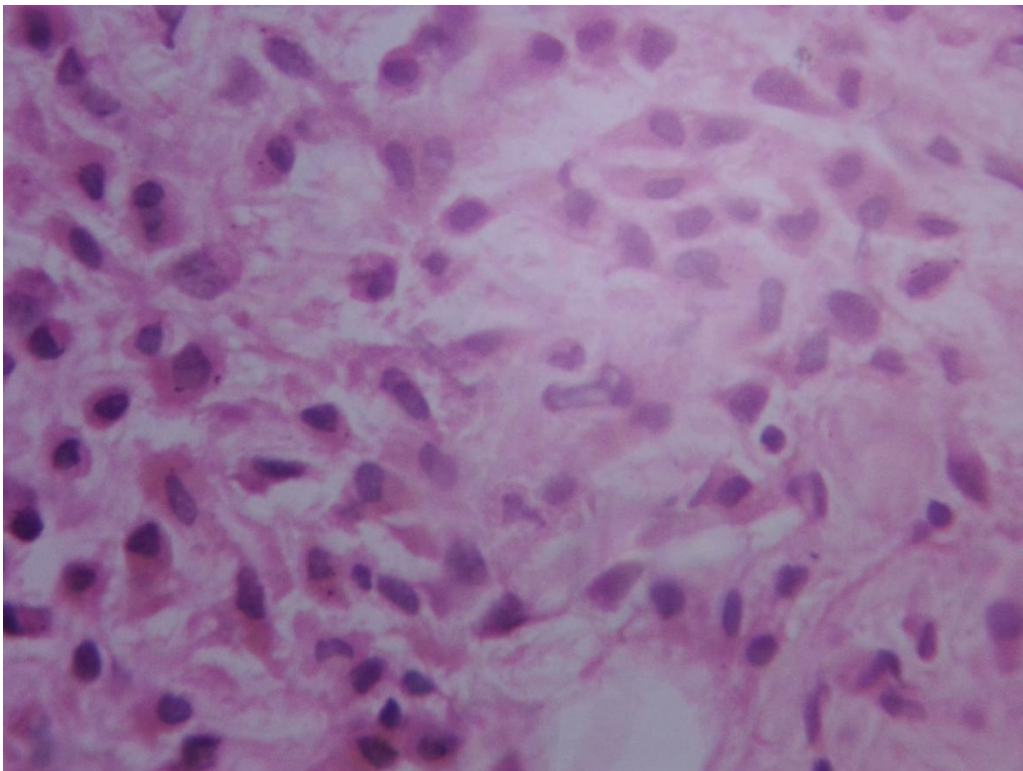
Figure (4): ALT and AST of EAC treated groups with Ginko with different conc.



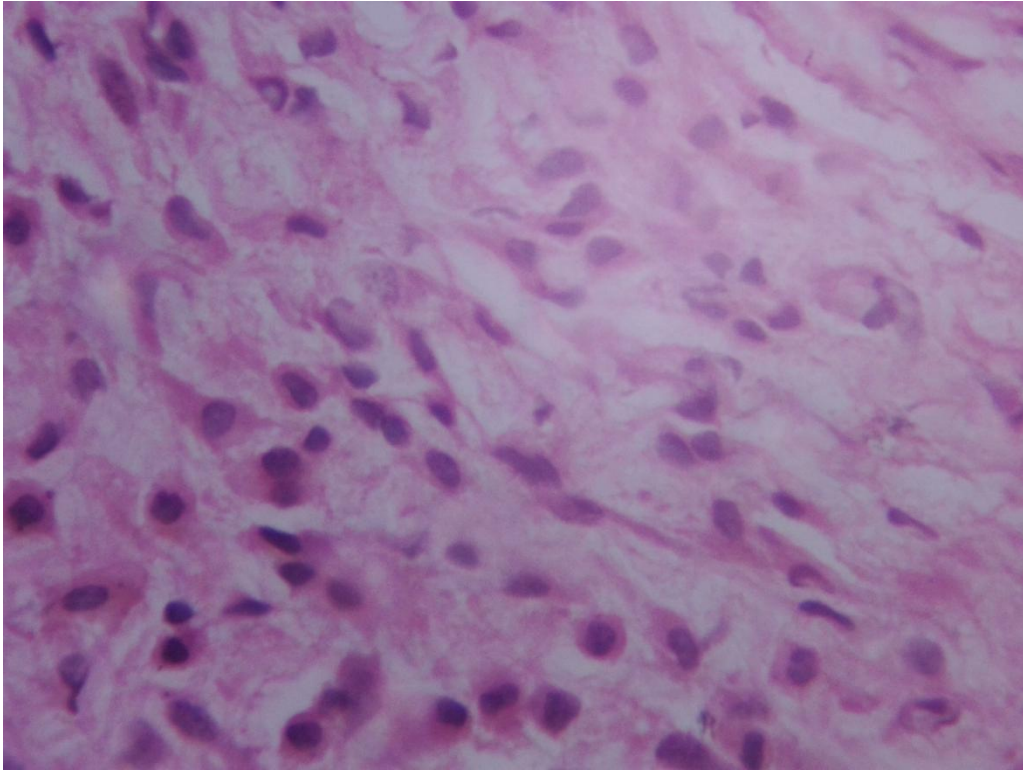
EAC untreated



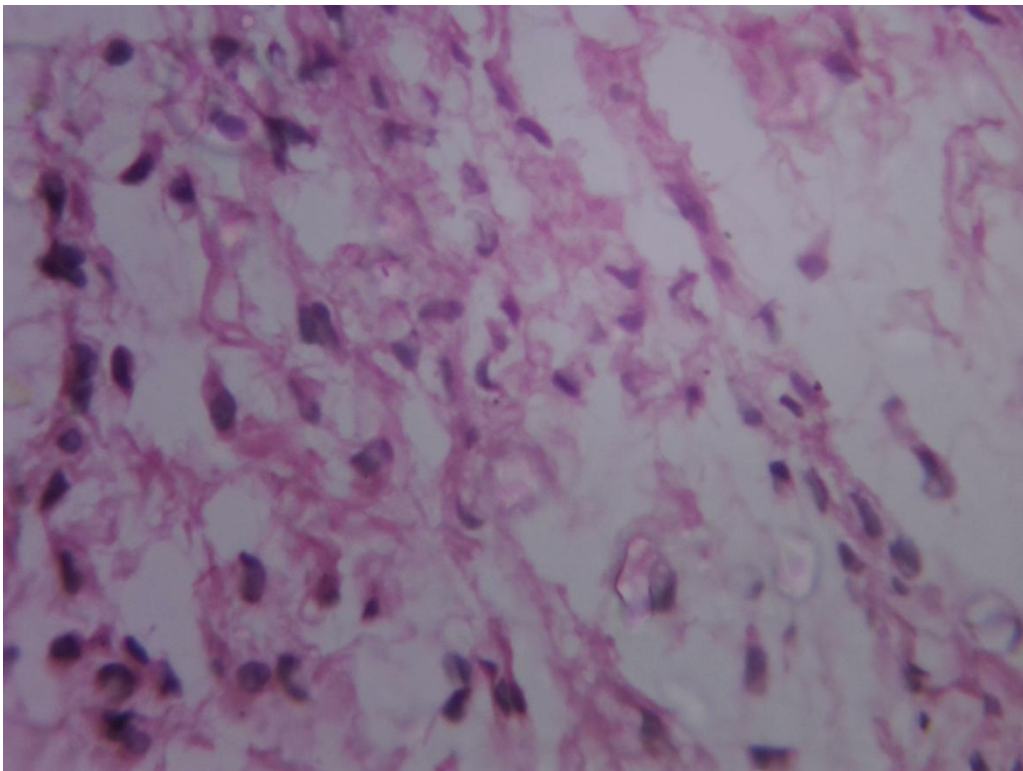
EAC + 250mg Ginko Biloba



EAC + 500mg Ginko Biloba



EAC + 750mg Ginko Biloba



### EAC + 1000mg Ginko Biloba

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

A.B., B.C. and C.D. designed the study.

A.B., D.E. and E.F. performed the xyz experiments.

F.G. and G.H. performed XYZ simulations.

I.H. and M.C. expressed and purified all proteins.

A.B., H.J., B.C. and C.D. analyzed the data.

A.B., B.C. and C.D. wrote the paper with input from all authors.

Or A.B., B.C., C.D. and D.E. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.