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Biochemical and Histoarchitectural Evaluation of 4-Vinylcyclohexane Induced Ovarian Cancer Against Alpinia Purpurata (Vieill). K. Schum

ARUL RAJ CHITHAMONY
GKNM Hospital: G Kuppuswamy Naidu Memorial Hospital
ANUSOORIYA PALNIRAJAN
Tamil Nadu Agricultural University Agricultural College and Research Institute
MANIKANDAN VANI RAJU
Karpagam University: Karpagam Academy of Higher Education
MEENAKSHI KANIYUR CHANDRASEKARAN
Karpagam University: Karpagam Academy of Higher Education
RATHI MUTHAIYAN AHALLIYA
Karpagam University: Karpagam Academy of Higher Education
GURUKUMAR DUGGANABOYANA
JSS University: JSS Academy of Higher Education and Research
GOPALAKRISHNAN VELLIYUR KANNIAPPAN (vkgopalakrishnan@gmail.com)
Karpagam University: Karpagam Academy of Higher Education https://orcid.org/0000-0002-2097-1424

Research Article

Keywords: Ovarian cancer, Alpinia purpurata, Ethyl acetate, 4-vinylcyclohexane, Wistar albino rats

Posted Date: October 12th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3391077/v1

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Abstract

Objective

Alpinia purpurata is being studied for its potential to treat various conditions, including diabetes, arthritis, and tuberculosis. This research explores the impact of *Alpinia purpurata* (Vieill). K. Schum on ovarian cancer induced by 4-vinyl cyclohexane in Wistar albino rats.

Materials and methods

Five sets of 100-120g Wistar albino rats were assembled. Group 1 was the control group. Group 2 received intraperitoneal 4-vinyl cyclohexane (80 mg/kg) for one month. For two months, Group 3 was given *A. purpurata* leaf extract (200 mg/kg) orally. Cisplatin (5 mg/kg) intraperitoneal twice per week for two months was given to Group 4 as a standard drug. For two months, Group 5 acquired daily oral *A. purpurata* leaf extract (200 mg/kg). The rats were euthanized after the experiment under light chloroform anesthesia. Ovary and liver samples were obtained for lipid peroxidation, anti-oxidants, membrane-bound enzymes, tumor indicators, and histological investigation.

Results

Over a 60-day period, rats were given an ethyl acetate extract of *A. purpurata* at a dose of 200 mg/kg, which lead to in a substantial ($p < 0.05$) increase in body protein content, as well as enzyme levels. Furthermore, the use of the ethyl acetate extract significantly ($p < 0.05$) recovered the altered lipid peroxidation activities in the ovarian tissues of both control and experimental rats to near-normal levels. These data imply that the extract has the capacity to quench free radicals, indicating possible anticancer effects.

Conclusion

The results suggested that, the ethyl acetate extract of *A. purpurata* exhibited significant antitumor activity on ovarian cancer bearing rats.

1. INTRODUCTION

Ovarian cancer, which can arise from germ cells, granulosa cells, or stromal cells, is the most lethal gynaecological cancer. The surface epithelium, a thin layer of cells covering the ovary, is where the majority of ovarian cancers originate. In the year 2020, there were 313,959 new cancer cases diagnosed globally, accounting for a percentage of all cancer cases. Regrettably, ovarian cancer stands as the sixth most common cancer among women on a global scale, resulting in 207,252 cancer-related fatalities [1]. When compared to other gynaecological cancers, ovarian cancer affects the human population the most globally [2]. Ovarian cancer is diagnosed in 225,000 new cases every year, and 140,000 of those cases lead to mortality [3]. When ovarian cancer is in its early stages, a unilateral salpingo-oophorectomy can be used to treat it, whereas a bilateral salpingo-oophorectomy can be used to treat it when it is in its

advanced stages (BSO) [4]. Utilizing animal models that accurately replicate the cellular and molecular irregularities linked to the initiation and progression of ovarian cancer could significantly contribute to the advancement of improved approaches for early detection and treatment of this disease [5].

In the process of making synthetic rubber, the reaction known as dimerization of 1, 3-butadiene produces vinyl cyclohexane (VCH). Several studies have shown that the liver is where 4-vinylcyclohexane depoxide (VCD), an ovo-toxic variant of VCH, can be bio activated in mice [6]. The potential function of the ovary in the metabolism of VCH and /or VCD has not yet been fully analyzed. Several xenobiotics can be bio transformed and detoxicated by enzymes found in the ovary. Cytochrome P-450, epoxide hydrolase and glutathione S-transferases are enzymes responsible for metabolizing known ovarian toxins like VCH and VCD in the ovaries of rats and mice [7]. There has not yet been research done to determine whether these phase II metabolic enzymes are more frequently expressed in the small prenatal follicles.

Even though the precise role of free radicals in the development and progression of cancer is still being studied, mounting evidence suggests that particular antioxidants are linked to a decreased risk of developing certain malignancies. Despite studies on plants and other products to prevent cancer, there are few information on the function of *A. purpurata*. Cancer-fighting properties of *A. purpurata* have been reported *in vitro* [8] and *in vivo* research [9] as well as antioxidant and radical scavenging characteristics, hepatoprotective [10], antibacterial [11], and vasodilator properties. The goal of the current study is to evaluate the anticancer potential of the ethyl acetate extract from *A. purpurata* against 4-vinyl cyclohexane induced ovarian cancer in Wistar albino rats.

2. MATERIALS AND METHODS

2.1 Plant collection and extract preparation

A. purpurata was collected from Kanyakumari (Tamil Nadu, India). The plant specimen was verified and authenticated (in Coimbatore, India) by Dr. G.V.S. Murthy, Director of the Botanical Survey of India. In the lab, a voucher specimen (BSI/SC/5/23/10–11/Tech) has been stored for potential future use. Leaf sample from *A. purpurata* was collected, dried in sun shade, and ground. A 500 g sample of powder was extracted with ethyl acetate using a Soxhlet apparatus over a 48-hour period in a 1:5 (weight to volume) ratio. After that, a rotary flash evaporator of the Buchi type was used to carefully dry the extract for use in future studies.

2.2 Experimental design

Wistar albino rats which weigh 100–120 g was purchased from the Karpagam University's Animal House in Coimbatore. Five groups of six rats each were selected for the study. This research work was approved (KU / IAEC / Ph.D / 087/ 26.07.2012)by the Institutional Animal Ethical Committee (IEAC), which was established in accordance with the CPCSEA, Government of India.

Group 1: Control rats

Group 2: 4-Vinyl cyclohexane-induced (80 mg/kg body weight) for one month

intraperitoneally

Group 3: Treated similarly to Group 2 and then given daily oral administration of *A. purpurata*

ethyl acetate leaf extract (200 mg/kg body weight) for a duration of two months

Group 4: Treated similarly to Group 2 and then treated with Standard Drug Cisplatin (5 mg/kg

body weight) weekly twice

Group 5: Two months of daily oral administration of 200 mg of ethyl acetate leaf extract of *A.*

purpurata per kg of body weight.

The rats were gently sedated using chloroform after the experiment was completed. The ovarian and liver tissues were quickly dissected out, thoroughly washed to remove any foreign matter, treated with a cold 0.1 M Tris-HCl solution (pH 7.4), then patted dry and weighed. To examine membrane-bound enzymes, non-enzymatic and enzymatic antioxidants, tumor markers and lipid peroxidation, a 10% w/v homogenate in 0.1 M Tris-HCl buffer was prepared. For a histological analysis, ovarian tissue was cut into 5 mm thick pieces, embedded in paraffin, fixed in 10% formalin, and stained with hematoxylin and eosin.

2.3 Enzymatic and non-enzymatic antioxidant assays

Using the methods for superoxide dismutase (SOD) [12], catalase [13], glutathione reductase (GR) [14], Glutathione-S-transferase (GST), total reduced glutathione (TRG), vitamin C, and vitamin E, respectively [15], the impact of an ethyl acetate extract of *A. purpurata* on the activity of enzymatic and non-enzymatic antioxidants in the ovaries of rats in the control and experimental group was examined.

2.4 Activity estimation for the ATPase enzymes and tumor markers

The estimation of Mg^{2+} ATPase and the Ca^{2+} ATPase were carried out by the method of [16] and the estimation of for 5’- Nucleotidase and γ-glutamyl transferase was carried out by the method of [17, 18].

2.5 Estimation of nucleic acid constituents, renal markers and liver enzymes

According to the manufacturer's instructions, kit protocols were followed for nucleic acid constituents, renal markers and liver enzymes assays (Diatek, India).

2.6 Histopathological examination

Each experimental group's ovarian and liver tissue samples underwent an immediate saline wash before being fixed in a 10% buffered neutral formalin solution. The tissues were processed by embedding them in paraffin after fixation. Following Hematoxylin and Eosin (HE) staining, these tissue blocks were divided into sections, which were then patiently studied and captured photograph using a powerful light microscope [19].

2.7 Statistical analysis

Mean is used to describe the results as standard deviation (SD). The statistical significance was assessed using a One-way Analysis of Variance (ANOVA) in GraphPad Prism version 10.0. The following pairwise comparisons were performed using the Dunnet Multiple Range Test (DMRT) [20]. If the p-value was less than 0.05, a difference between groups was deemed significant. Probability level $p < 0.0001$(****) and $p = 0.01$ to 0.05(*).

3. RESULTS AND DISCUSSION

The medicinal plant *A. purpurata* is used to cure a variety of illnesses, such as diabetes mellitus, arthritis, and pulmonary tuberculosis. The aim of this study was to investigate the anti-cancer properties of oral *A. purpurata* against ovarian cancer in rats that was generated by 4-vinyl cyclohexane.

3.1 Effect of enzymatic and non - enzymatic antioxidants

The impact of *A. purpurata* on enzymatic antioxidants in the ovaries of both control and experimental animals is shown in Fig. 1. Rats in group II that were exposed to 4-vinyl cyclohexane had considerably lower levels of enzymatic antioxidants. However, compared to group II, animals who had received medication treatment (groups III and IV) experienced a return to normal levels of enzymatic activity. Between Group V and the control group (Group I), that just received the plant extract, there was no discernible difference.

Free radicals have the potential to induce lipid peroxidation and harm the macromolecules and cellular structures within an organism, such as the endothelium and erythrocytes. This process of lipid peroxidation can alter membrane permeability and lead to tissue damage. An essential protective mechanism against free radical-induced damage is provided by the antioxidant system [21]. The cell membrane is easily penetrated by hydrogen peroxide, which then attacks various places and damages DNA by breaking both single and double strands. The levels of antioxidant status in the ethyl acetate extract of *A. purpurata* were examined in this study in relation to ovarian tissue.

SOD, functioning to dismutase two superoxide radicals into H_2O_2 and O_2, plays a pivotal role as the body's initial defense against superoxide radicals. Furthermore, CAT and GPx, by converting H_2O_2 into H_2O, serve as complementary antioxidant enzymes, safeguarding cells against reactive oxygen species (ROS) damage. The endogenous antioxidant selenoprotein GPx actively participates in this protective mechanism and is located within both the cytosol and the mitochondrial matrix [22]. A rise in radical

generation during DMBA metabolism may be to blame for the decline in enzymatic activity. According to the findings of the current investigation, the rise in MDA formation is probably due to an increase in reactive oxygen species (ROS), which is consistent with the finding that these free radicals tend to decrease SOD function. Based on the biochemical data, it was found that 4-vinyl cyclohexane-exposed rats' SOD, CAT, and GPx activity was lower than that of control rats. This reduction in enzymatic activity may be attributed to the excessive production of radicals, which could be a consequence of ovarian cancer [23].

In the investigation of 4-vinylcyclohexane-induced ovarian cancer, Fig. 1 shows the effect of *A. purpurata* on non-enzymatic antioxidant levels. When compared to the control group, group II mice's ovarian tissue showed a considerable decline in non-enzymatic antioxidant activity. But when compared to mice induced with 4-vinyl cyclohexane, the *A. purpurata* and cisplatin therapy increased GSH, vitamin C, and vitamin E levels ($p < 0.05$) in these groups. Following treatment, animals in group V that had only been exposed to the plant extract did not vary significantly from those in group I ($p < 0.05$) in any way.

When ascorbic acid and carotenoids are present, two other cooperative antioxidants, vitamin E functions well as a lipid-soluble, chain-breaking antioxidant. This cooperative effect strengthens its capacity to guard against oxidative damage. It has been demonstrated that altering non-enzymatic antioxidants including total reduced glutathione (GSH), vitamin C, and vitamin E during cancer therapy can lower the risk of cancer recurrence and lessen side effects linked to elevated free radical levels [24].

Vitamins E and C, which are non-enzymatic antioxidants, are found in healthy cells and can be consumed through diet. In addition to reducing H_2O_2 and hydro peroxide levels, antioxidants can also trap metal ions, halt chain reactions by scavenging reactive free radicals, and even encourage the repair and/or removal of cell damage. Additionally, some antioxidants promote the manufacture of defense enzymes or additional antioxidants [25].

Various amounts of these antioxidants were found, but they were not enough to offset the rise in reactive oxygen stress, which damages cells and molecules and encourages the cancer cell cycle [26]. The finding that GSH, vitamin C, and vitamin E levels were decreased in cancer-bearing mice was another indication of oxidative stress, which contributes to damage to cells and a breakdown of cell membrane working stability.

3.2 Effect of membrane bound enzymes and tumor markers

The enzyme activity of membrane-bound ATPase enzymes in the ovarian tissue of experimental and non-experimental mice is shown in Fig. 2. When 4-vinylcyclohexane was used to stimulate mice, it was shown that these enzymes' activity considerably decreased ($p < 0.05$) when compared to control animals. However, during pharmaceutical therapy, their levels showed a noticeable dose-dependent rise. Comparing the group receiving only the plant extract and the group receiving a healthy control, there were no noticeable distinction.

Among the many variables that can affect ATPase levels are peroxidation of lipids and modifications in membrane fluidity. A decrease in ATPase activity under ischemia conditions can cause irreversible necrotic alterations in the myocardial cells that are damaged, as well as a loss of function. The oxidation of "SH" groups inside the binding sites of Na^+/K^+ ATPase and Ca^{2+} ATPase, that is caused by lipid peroxidation in the cellular membrane, is the main cause of this drop-in ATPase activity. This shift in enzyme conformation is frequently linked to this decrease in ATPase activity [27]. In fact, an increase in intracellular levels of calcium ions that are free (Ca^{2+}) can be brought on by a reduction in Ca^{2+} ATPase activity. This can have a variety of effects, such as alterations in signal transduction systems and adjustments to cellular fluidity. Numerous cellular activities depend on the control of intracellular calcium levels, and any disturbance in this equilibrium can have a major influence on cell functioning and communication [28]. The functionality of different cell types, particularly platelets and the nuclear cells in peripheral blood, can be affected by changes in Na^+/K^+-ATPase activity. The rate of signal transmission is impacted by this enzyme's critical involvement in catalyzing the Ca^{2+}-dependent interchange of hydride ions with calcium ions. This procedure plays a key role in the rapid dissolution of secretory granules and the creation of actino-myosin compounds in platelet surfaces during adhesion, which regulate cellular activities and reactions.

According to reports, ATPase activity is inhibited in cancerous situations. As these enzymes are membrane-bound, any disruption with the cell membrane, especially adjustments to the solubility and ionic concentration of the membrane, can in fact affect how they function. Because ATPase plays a part in malignant conditions, its decreased activity may also be linked to cellular harm. As a result, any changes to the cell membrane may result in changes to the functions of membrane-bound ATPase, which may indicate how serious the underlying disease is. These findings highlight the complex interplay between ATPase activity, membrane integrity, and the health or illness of cells [29].

The plant medicine's bioactive ingredients are known for their capacity to affect the permeability of cellular membranes. They can interact with animal cell Na^+/K-ATPase pumps and may even have the ability to promote electron transport. Due to these characteristics, ATPase activity may be brought back to normal levels, which would then allow the cell to function normally. These bioactive components are essential for controlling cellular processes and may be able to reverse cellular malfunction [30].

Following administration of *A. purpurata* extract, experimental and control rats, ovarian tissue range of 5'-nucleotidase and γ-glutamyl transpeptidase altered, as indicated in Fig. 2. The ovarian tissues of group II mice treated with 4-vinylcyclohexane showed a significant ($p < 0.05$) increase in the quantity of a cancer marker compared to the control animals in group I. These marker enzyme levels were dramatically lowered and matched those in the cancer-bearing animals in group II in group III and IV, when mice were given *A. purpurata* and cisplatin ($p < 0.05$). Notably, the marker activity of the enzyme in group V, which animals received only the plant extract as a treatment, did not differ significantly from that of the group I control animals.

At the sinusoidal and bile canalicular sides of hepatocytes' plasma membranes, there is an enzyme called 5'-nucleotidase (5'NT). It works by hydrolyzing nucleotides with phosphate groups linked to the carbon atoms of the ribose sugar. It's interesting that mice with cancer were shown to have increased activity. 5'NT is also used as an indicator to evaluate liver damage. The presence and activity of this enzyme can be used to monitor liver health and function as a marker for specific liver diseases [31]. In the current study, rats that were carrying ovarian cancer showed increased 5'NT activity. The emergence of the marker enzyme may be connected to the growth of the tumor and hepatic cell damage, which may cause 5'-nucleotidase spillage into the bloodstream. In this sense, treatment with *A. purpurata* significantly decreased the activity of the enzyme 5'NT. This might be connected to the plant extract's ability to quench free radicals, which would compensate for the hepatic cell damage.

Strongly secretory or detoxifying cells have gamma glutamyl transferase (γ-GT), a membrane-bound enzyme, on their surface. Because it has been demonstrated that disorders such bile duct necrosis and cholestasis cause the enzyme level to be raised in serum and the liver, it is one of the important indicators of liver damage. There have been reports of a variety of substances, notably xenobiotic, forming a substrate of γ-GT following their attachment to GSH, which mainly occurs in the liver. The loss of GSH may also result in a rise in hepatic γ-GT activity through an elevation in the synthesis of its mRNA. It was found in the present study that rats with cancer had increased levels of γ-GT activity. This is consistent with the earlier observation that [32]. However, ethyl acetate extract restores the level of the γ-GT enzyme throughout treatment. This might be as a result of the extract's cytoprotective properties, which might have assisted in stabilizing the cellular membranes of the hepatocytes and other bodily tissues and preventing the loss of the functional properties of the cell membrane.

3.3 Effect of nucleic acid constituents in ovary, serum liver marker enzymes and renal markers of control and experimental animals

In a variety of tumor settings, DNA content is an important growth indicator that is more relevant to the pharmacological and functional aspects of the tumor. According to [33], the amount of DNA is a trustworthy predictor and frequently coincides with the DNA composition of the tumor. The effects of *A. purpurata* ethyl acetate extract on the levels of nucleic acids (DNA and RNA) in the ovarian tissues of experimental and control mice are shown in Fig. 3. Rats treated with 4-vinylcyclohexane in group II showed noticeably larger amounts of nucleic acid constituents than the control animals in group I ($p < 0.05$). When contrasted to the cancer-bearing rats in group II, however, these elevated levels were noticeably decreased in group III and group IV animals administered *A. purpurata* along with cisplatin ($p < 0.05$).

In contrast, when comparing the group V animals administered exclusively using the plant extract to the group I control animals, no appreciable variations in nucleic acid quantities were found.

In the current study, there was an observed increase in the amount of RNA in animals afflicted with ovarian cancer. This increase could be attributed to the heightened DNA content associated with cancerous conditions, potentially leading to increased transcription and, consequently, elevated RNA

levels. Conversely, the rats treated with *A. purpurata*, both DNA and RNA levels returned to normal. Consequently, the application of the ethyl acetate extract from *A. purpurata* not only inhibited tumor growth but also regulated nucleic acid biosynthesis.

These findings align with earlier research studies on the subject [34]. Marker enzyme analysis is a useful tool for locating tumors and directing medical decisions. The effect of *A. purpurata* extract on transaminases and alkaline phosphatase blood levels in both experimental and control animals is shown in Fig. 3. Marker enzyme levels were significantly ($p < 0.05$) lower in the 4-vinylcyclohexane-induced animals in group II compared to the control animals in group I, indicating the impact of this induction. On the other hand, these enzyme levels were markedly decreased in the *A. purpurata* and cisplatin-treated animals in groups III and IV and were comparable to the levels seen in the cancer-bearing animals in group II ($p < 0.05$).

However, there were no discernible variations in the marker enzyme activity in animals that were treated exclusively using the plant extract in group V as compared to the untreated animals in group I. In particular, biochemical marker enzymes are used to screen for cancer disorders to aid in differential diagnosis, tracking of advancement, and therapy response [35]. These enzymes have become more distinct, and changes in their activity reveal the effects of metabolic cycle and cell division on proliferating cells. The amount of changed cells present in malignant conditions has been discovered to be highly correlated with their greater activity. The transport processes of cell organelles, such as hepatocytes, are disturbed in malignant situations. Due to modifications in the plasma membrane's permeability, this disruption results in the release of enzymes. As a result, the quantities of these enzyme markers in the serum or blood increase while their concentration within the cells declines. According to reports, when animals are exposed to toxicity, the skeletal framework of their cells is weakened, which causes enzymes to diffuse from the cell cytoplasmic into the blood stream [36]. It is thought that the intracellular enzyme concentrations are over three orders of magnitude greater than external fluid concentrations. As a result, it is a sensitive indication since even a small amount of cellular damage can cause a considerable increase in enzyme activity in the extracellular fluids or plasma. Due to its clinical importance, measuring enzyme activity in both serum and tissues is a popular diagnostic tool in human medicine. Due to the major clinical consequences of both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, research on serum transaminase activities as a measure of tissue injury has mostly concentrated on human subjects. In the current study, ALT levels showed the opposite tendency to AST levels, which were shown to be high and dropping in the blood and tissues of mice with cancer. The increased enzyme activity in the serum may be caused by a number of things, including the release of enzymes into the bloodstream from cancerous cells, the presence of tumors causing the emit of enzymes from normal tissue, or a possible impact of a tumor on distant tissue that results in a breakdown of an enzyme and its discharge into the bloodstream [37].

Figure 3 displays the concentrations of uric acid, creatinine and urea. The animals in group II that had been exposed to 4-vinylcyclohexane had considerably higher levels of urea and creatinine. Animals treated with extract and cisplatin had lower levels (group III and IV). The above amounts of treatment for

rats using the group V medication alone were comparable to the control groups. However, group II cancer-induced animals had lower uric acid levels. Animals treated with extract and cisplatin had considerably higher levels (group III and IV). The above amounts of treatment for rats using the group V medication alone were comparable to the control groups.

Urea, also known as carbamide, serves as the primary nitrogen-containing compound in the urine of mammals. It is an organic chemical with a crucial role in the metabolism of nitrogen-containing substances in animals. The rate of glomerular filtration determines how much urea is eliminated, and as production outpaces excretion, plasma levels rise. Renal function is measured by serum creatinine. It is created endogenously by tissue creatinine breakdown and an increase in serum creatinine may be attributable to the tissue damage. The glomerular filtration rate determines how much creatinine is expelled; when this excretion does not equal the production, serum creatinine increases [38].

The enhanced usage of uric acid against lipid peroxide, a hallmark of cancer conditions, may be the cause of the lower level of uric acid in ovarian cancer. Higher uric acid levels seen following therapy for *A. purpurata* could be caused by a lower tumor burden, which is consistent with earlier observations of [39].

3.4 Estimation the level of tissue protein and Lipid peroxidation

The levels of protein activity in the ovarian tissue of experimental and control mice are shown in Fig. 4 (B). Induced with 4-vinylcyclohexane, the ovarian tissues of Group II mice displayed a conspicuous drop in protein levels when compared to the control group (Group I) mice ($p < 0.05$). The protein levels in Group III and IV animals were, however, noticeably higher than in Group II rats ($p < 0.05$). The protein levels in the control and drug-treated animals (Group V) did not differ noticeably from one another.

Because they are macromolecular structures, proteins are essential for preserving the structural integrity of cells. Any protein level alterations can cause metabolic imbalances, especially in cancer situations, which can result in severe cellular abnormalities. The greatest amount of protein synthesis occurs in tissues, wherein the metabolism of proteins is a critical activity. Protein wastage is characterized by an apparent rise in the rate of protein breakdown while the visible rate of protein production appears to be relatively steady. This indicates an underlying metabolic imbalance. A drop in ovarian protein levels in 4-vinylcyclohexane-induced cancer-bearing mice can be related to an increased efflux of amino acids from tissue and a reduction in the recycling of amino acids, both of which happen under tumor conditions [40]. Degradation of tissue proteins results from the host's reaction to the tumor load. However, therapy with *A. purpurata* may have stopped this protein deterioration, returning protein levels to a more typical range. The modulation of protein synthesis during this therapy may have also affected the overall protein level, indicating that the plant extract significantly regulates both protein production and maintenance. These results are consistent with past studies from the reference [41].

Figure 4 (A) shows changes in the amount of lipid peroxidation (LPO) in the ovarian tissue of experimental and control rats. The 4-vinylcyclohexane-induced rats (Group II) showed a noticeable rise in

LPO levels. On the other hand, animals receiving the plant extract plus cisplatin (Group III and Group IV) as compared to those with cancer showed a substantial ($p < 0.05$) reduction in malondialdehyde, employed as an indicator of peroxidation of lipids levels. This implies that using these medications to correct the aberrant variations in LPO levels was successful.

When compared to the control group, animals administered only with *A. purpurata* did not show any statistically significant variations ($p < 0.05$) in LPO levels, showing that the plant extract did not significantly affect LPO levels on its own.

Free radicals can react with polyunsaturated fatty acids to create malondialdehyde. It is well known for its effect on lipid peroxidation as well as its impact on the control of radicals during the development of cancer. Naturally, there is a delicate balance between the body's creation of free radicals and the antioxidant defense system's defensive mechanisms, which defend the body from disease [42]. It has been proposed that elevated lipid peroxidation seen in erythrocytes may be caused by a compromised antioxidant system. As a result, the amount of MDA is frequently employed as a meter to evaluate the severity of harm brought on by lipid peroxidation triggered by free radicals.

3.5 Histopathological observation

3.5.1 Histopathology of ovary

Figure 5 shows the outcomes of histological examinations on samples of ovarian tissue taken from experimental and control rats.

The histopathological changes in the ovarian tissues of the experimental as well as control animals are shown in Fig. 5. The ovarian tissue in the control group underwent a histological test, and the results showed normal ovarian tissue architecture and more follicles (Fig. 5B). Rats with ovarian cancer exhibited cell proliferation, a lack of follicles, and lymphocyte aggregation. It shows little lymphocyte clumping and scant evidence of tumor regression when *A. purpurata* extract is utilized (Fig. 5C). Multiple areas of tumor regression were visible in mice given the conventional drug cisplatin (Fig. 5D). Rats given ethyl acetate extract alone (Fig. 5E) showed normal morphological morphologies of ovarian tissues and normal follicles (Fig. 5A), in contrast to the healthy control group.

3.5.2 Histopathology of liver

Histological examinations were conducted on liver tissue samples from both control and experimental rats, as depicted in Fig. 6.

Figure 6 displays the histological alterations in the liver tissues of the experimental and control mice. The histological evaluation of the liver tissue from the control group (Fig. 6A) revealed a healthy liver architecture with hepatocytes encircling the periportal lobules. In (Fig. 6B) ovarian cancer bearing rats exhibits periportal inflammatory cell infiltrate and central necrosis. Animals subjected to an extract of ethyl acetate of *A. purpurata* display minor necrosis as well as hepatocyte inflammation (Fig. 6C).

Cisplatin, a common drug, caused minor hemorrhagic necrosis of the hepatocytes in animals (Fig. 6D). In contrast to the normal group, rodents treated with ethyl acetate extract alone (Fig. 6E) animals displayed normal morphological appearances of hepatocytes (Fig: 6 A).

4. CONCLUSION

The method by which *A. purpurata* leaf extract exerts its chemo preventive effects may involve blocking the carcinogen (4-vinylcyclohexane). The abundance of phytochemicals and antioxidants in *A. purpurata* may be responsible for its effects. The above findings support the idea that the ethyl acetate leaf extract of *A. purpurata* may have substantial anti carcinogenic and anti-oxidative effects in an experimental animal model, as well as the potential to function as a powerful chemo preventive agent.

Declarations

ACKNOWLEDGEMENTS

We, the authors are thankful to our Chancellor, Chief Executive officer, Vice Chancellor and Registrar of Karpagam Academy of Higher Education for providing facilities and encouragement.

Fundings

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

- *Arul Raj C, Rathi MA, Gurukumar D and Gopalakrishnan VK contributed to the study conception and design.*
- *Plant collection was done by Arul Raj C, Manikandan VR and Meenakshi KC.*
- *Material preparation, and Biochemical parameters were performed by Arul Raj C and Anusooriya P.*
- *The first draft of the manuscript was written by Arul Raj C and Anusooriya P.*
- *All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.*

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Figures

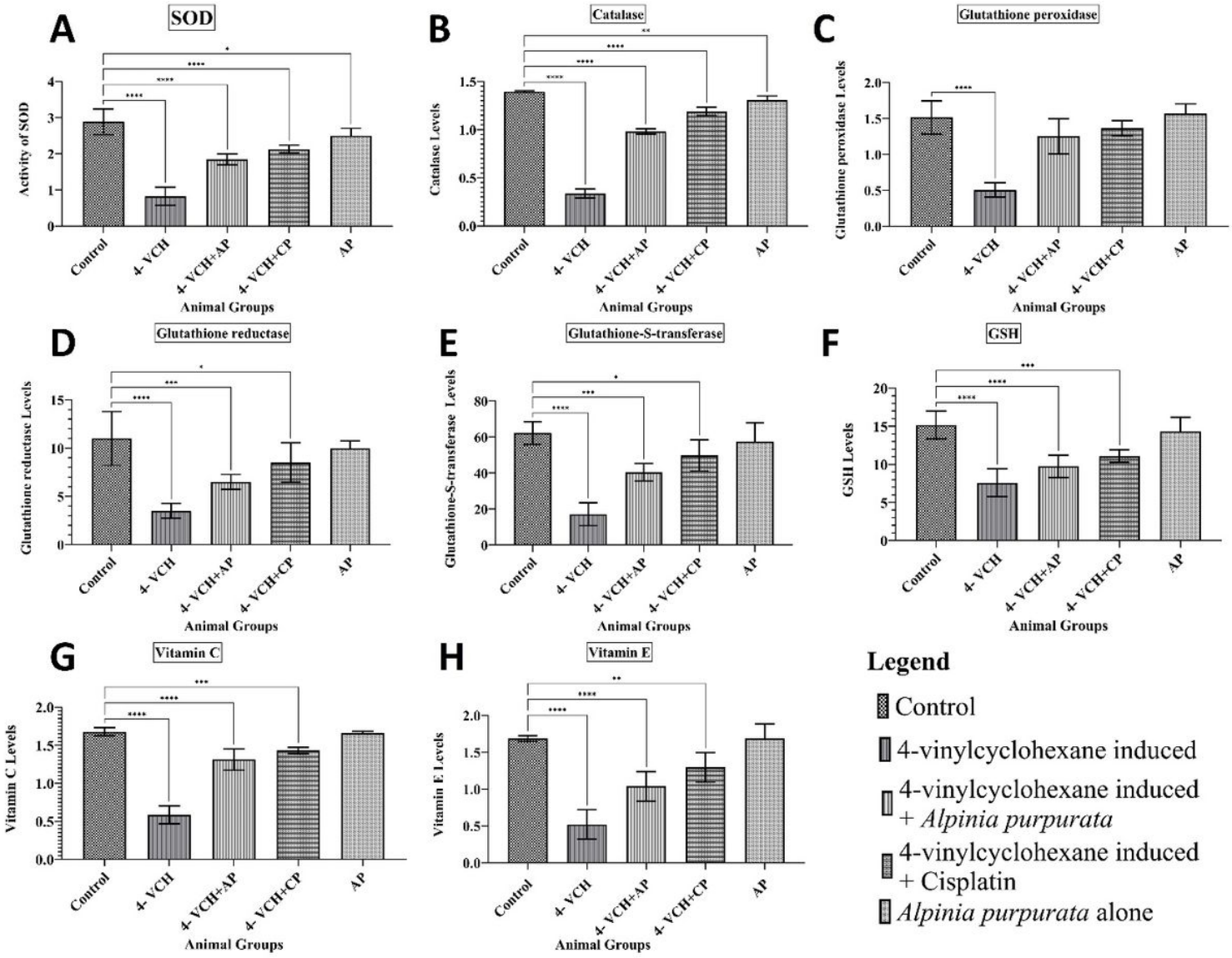


Figure 1

Effect of ethyl acetate extract of *A. purpurata* on the activities of enzymatic and non-enzymatic antioxidants in ovary of control and experimental groups. Values for six animals are shown as mean ± SD. **(A)** SOD: Formation of 50% nitrite inhibited/min/mg of protein. **(B)** CAT: Consumption of mole of H_2O_2 /min/mg of protein. **(C)** GPx: Glutathione oxidized in g/min/mg of protein. **(D)** GR: Utilization of glutathione in mol/min/mg of protein. **(E)** GST: Formation of GSH conjugate in mol/min/mg of protein. **(F)** GSH: g/mg protein. **(G)** Vitamin C: g/mg protein and **(H)** Vitamin E: g/mg protein.

Note: * p-value <0.01 to 0.05; ** p- value <0.001 to 0.01; *** p- value< 0.0001 to 0.001 and **** p- value <0.000

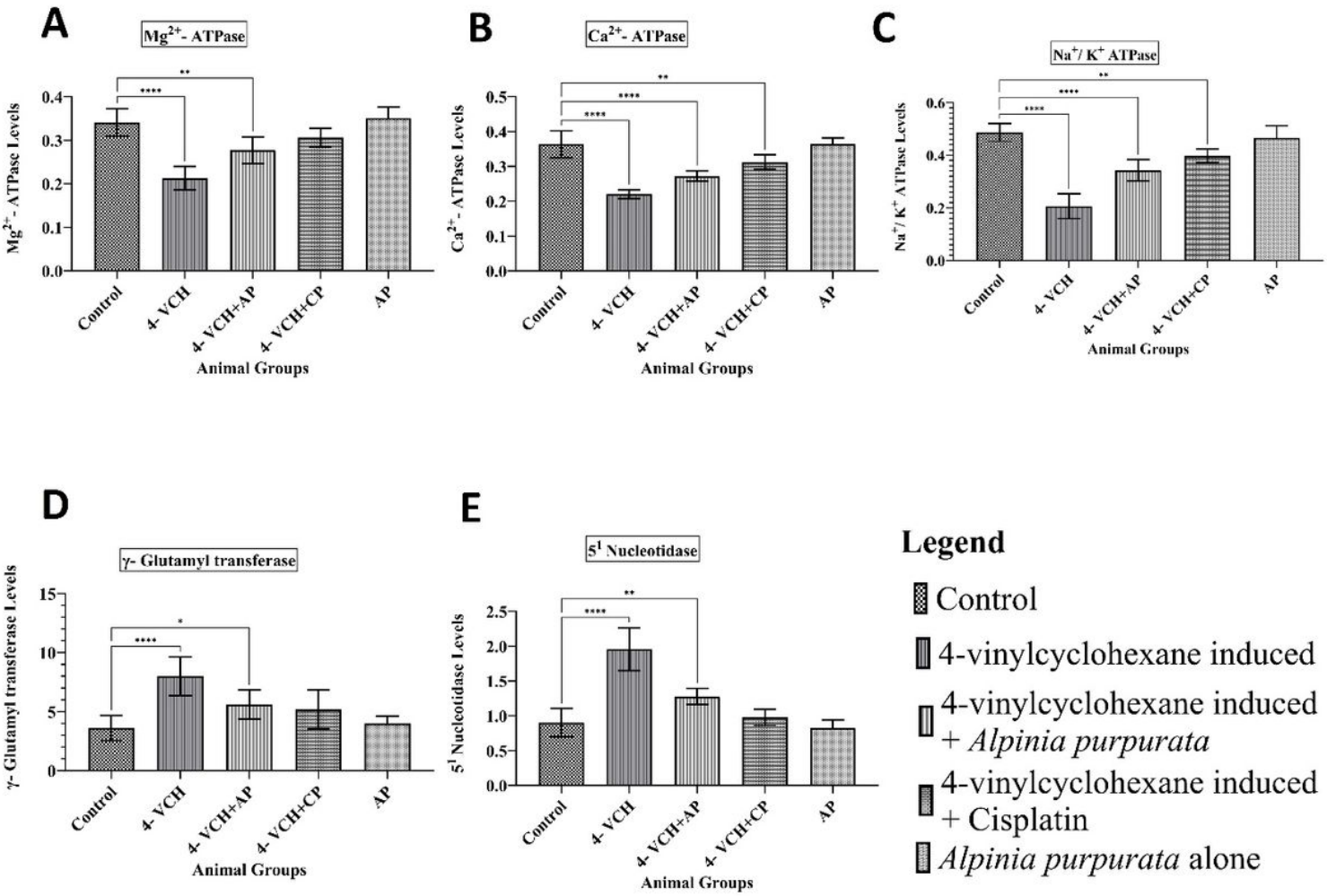


Figure 2

Effect of *A. purpurata* on the activities of tumour markers and the impact of *A. purpurata* in the membrane bound enzymes in ovaries of both control and experimental animals. Data for six animals are shown as mean ±SD. **(A)** Levels of Mg^{2+}ATPase, **(B)** Levels of Ca^{2+}ATPase, **(C)** Levels of Na^{+} / K^{+} ATPase, **(D)** 5'NT - Liberation of µmol of phosphorus / min/L and **(E)** γ-GT – Liberation of µmoles of p-nitroanilide /min/L.

Note: * p-value <0.01 to 0.05; ** p- value <0.001 to 0.01; *** p- value< 0.0001 to 0.001 and **** p- value <0.000

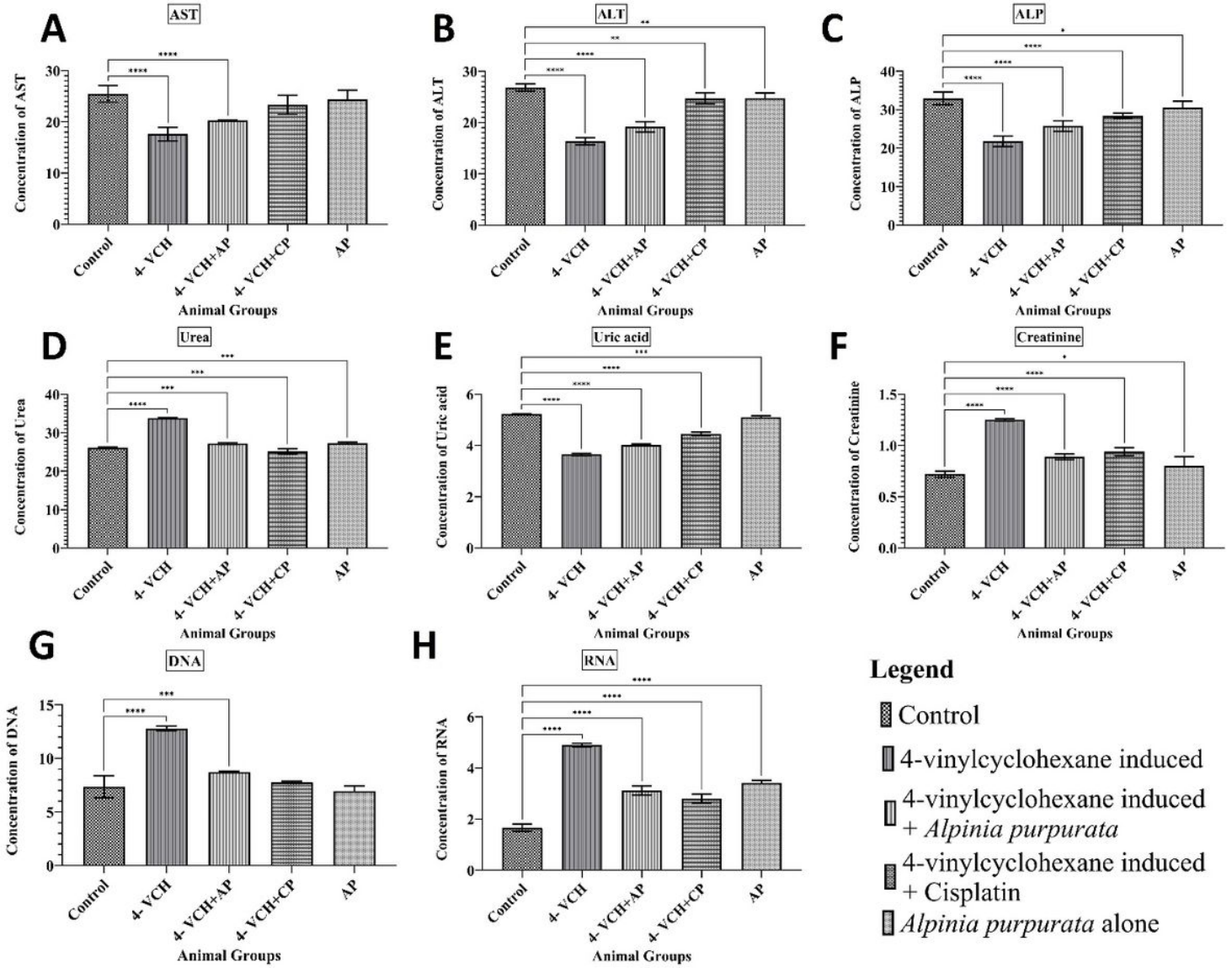
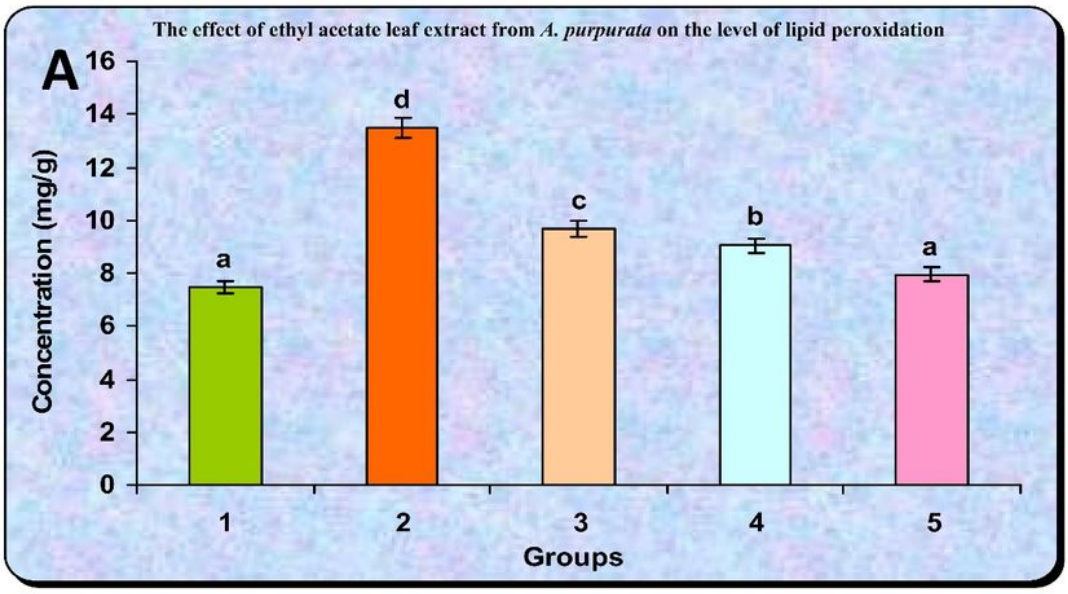


Figure 3

Effect of ethyl acetate extract of *A. purpurata* on the activities of liver marker enzymes, renal marker and nucleic acid concentration of control and experimental groups. Values are expressed as Mean ±SD for six animals. **(A)**AST, **(B)** ALT - µmoles of pyruvate liberated/L, **(C)** ALP - µmoles of phenol liberated/L, **(D)** Urea concentration in mg/dl (E) Uric acid concentration in mg/dl, **(F)** Creatinine concentration in mg/dl, **(G)**DNA concentration in mg/g tissue and **(H)** RNA concentration in mg/g tissue.

Note: * p-value <0.01 to 0.05; ** p- value <0.001 to 0.01; *** p- value< 0.0001 to 0.001 and **** p- value <0.000



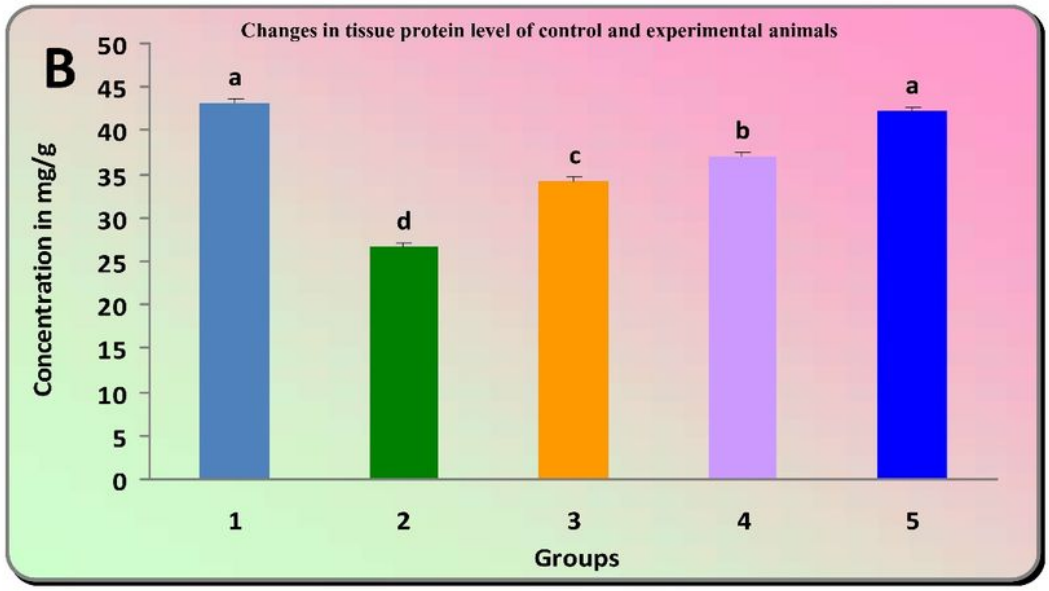


Figure 4

Changes in **(A)** lipid peroxidation and **(B)** tissue protein level of control and experimental animals. Group I - Control, Group II – Cancer Induced, Group III – Cancer induced +Plant treated, Group IV – Cancer induced +Standard drug treated, Group V – Plant alone. Values are expressed as Mean ± SD for six animals. Values not sharing common superscript letters (a-d) differ significantly at $p< 0.05$ (DMRT). Units: LPO – nM of MDA formed / min/ mg protein, Protein concentration in mg/g tissue.

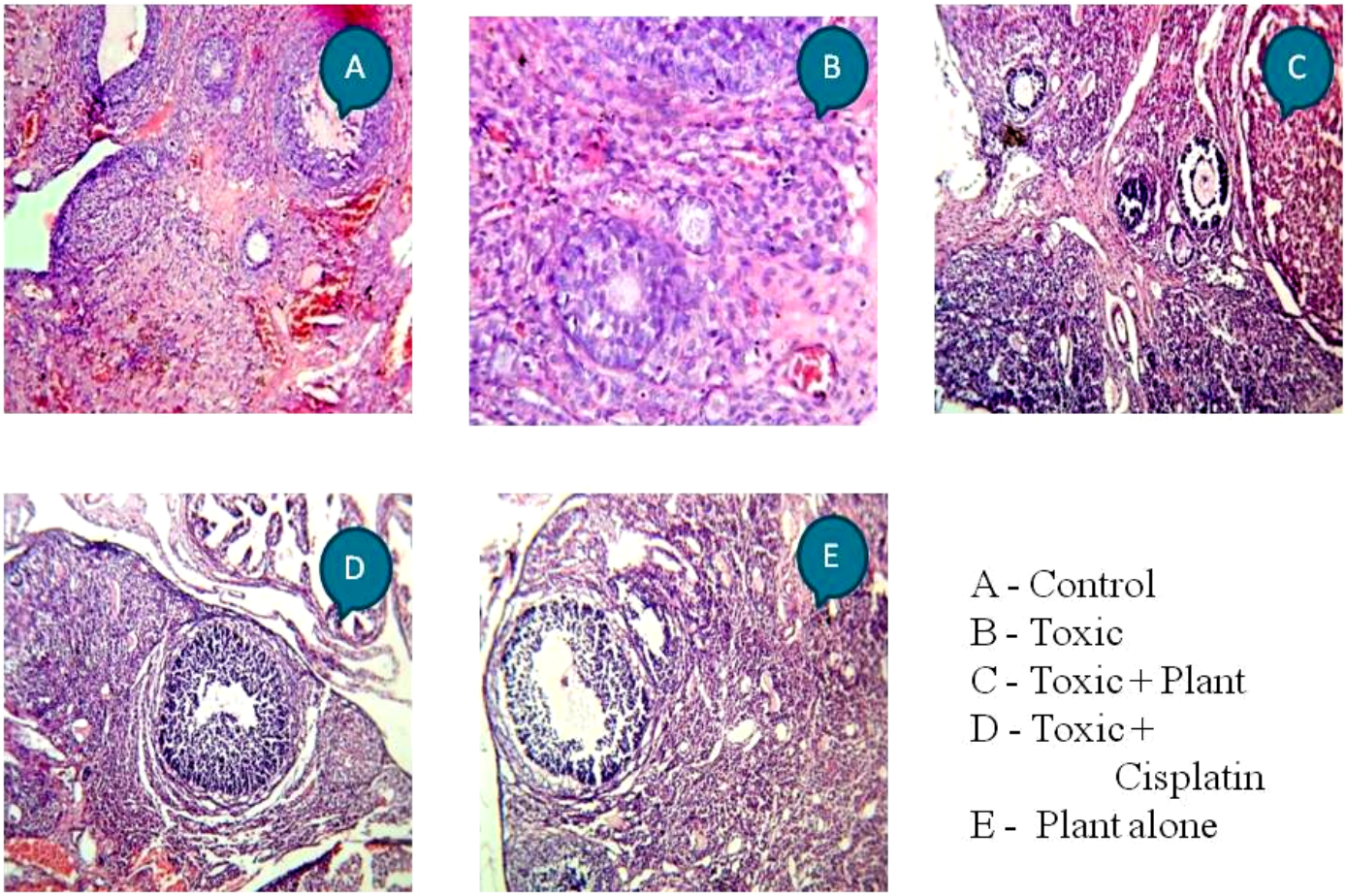


Figure 5

Histopathology of ovary and liver (x100 magnification)

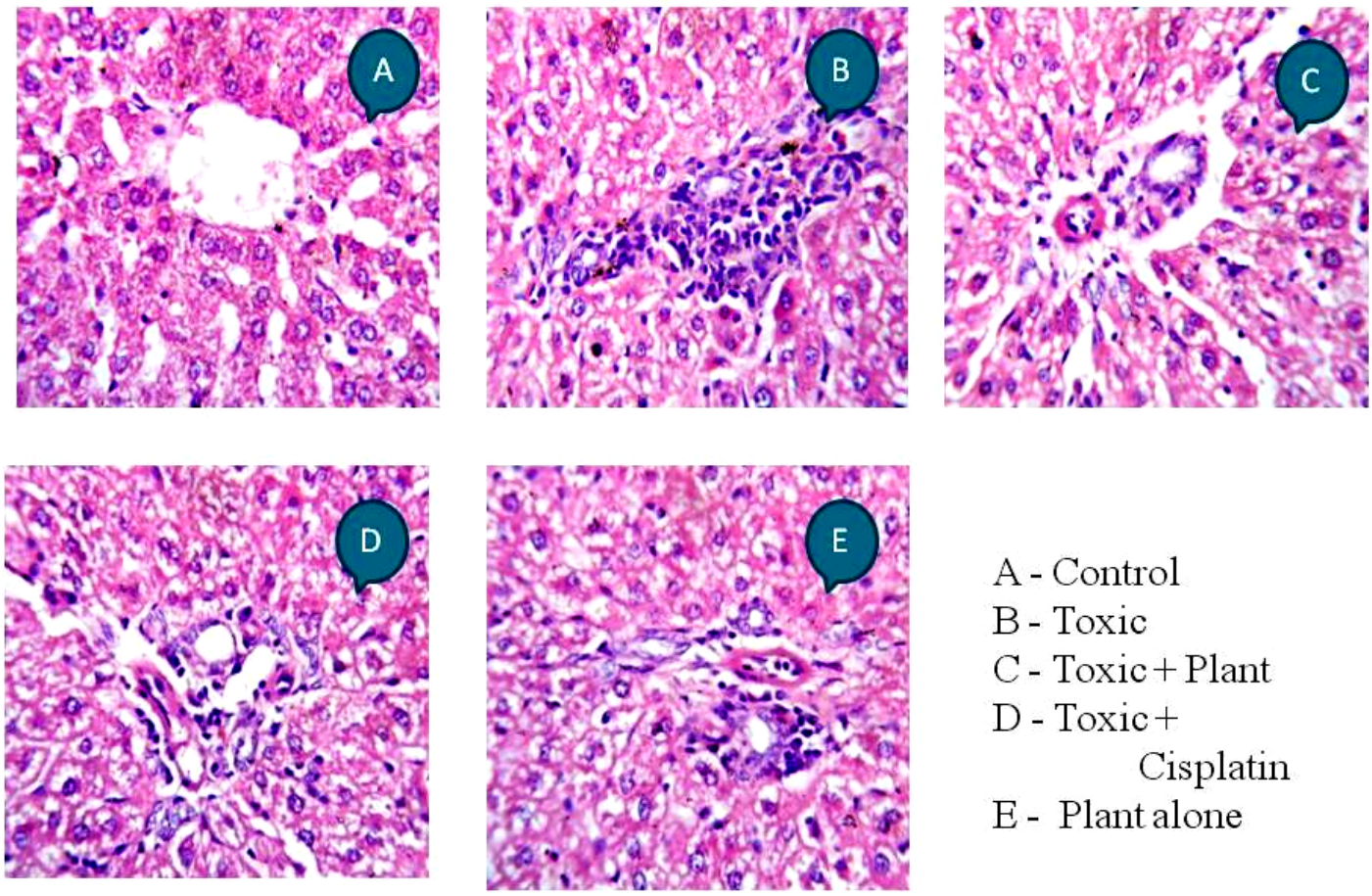


Figure 6

Histopathology of liver (x100 magnification)

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