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In vivo Evaluation of the Anticancer Efficacy of Polysaccharide Extracted from the Fruiting Bodies of Pleurotus Ostreatus

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the effect of *Pleurotus ostreatus* polysaccharide (POP) on Ehrlich Ascites Carcinoma (EAC) cells both *In vitro* and In vivo. *In vitro* analysis revealed a dose-dependent decrease in EAC cell viability with increasing concentrations of POP, with pronounced cytotoxic effects observed at concentrations exceeding 150 µg/mL. In vivo studies demonstrated that POP

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treatment significantly extended lifespan and mitigated tumor-induced weight loss compared to untreated EAC groups. Furthermore, POP treatment exhibited a substantial inhibitory effect on tumor growth, as evidenced by reductions in tumor weight and volume. Hematological and biochemical analyses indicated that POP treatment reversed tumor-induced alterations in hematological parameters and ameliorated biochemical markers associated with cellular damage. EAC and EAC+Bleomycin decreased hemoglobin, red blood cell count, and platelet count, while EAC increased white blood cell count. Conversely, POP(4xIC50) caused slight increases in these parameters, suggesting milder cellular damage. On the other hand, the elevated levels of CK-MB, LDH, AST, ALT, ALP, Creatinine, and BUN indicated cellular damage in groups treated with EAC, EAC+Bleomycin, and POP(4xIC50) compared to normal. These markers suggested myocardial, tissue, liver, bone, and kidney damage, highlighting the adverse effects of these treatments on cellular health and organ function. Histological examination revealed a protective effect of POP on cardiac, hepatic, and renal tissues, suggesting its potential in preserving organ function. These findings underscored the therapeutic potential of POP in targeting cancer cells and mitigating tumor-induced physiological and pathological changes.

Keywords: Pleurotus ostreatus polysaccharide; Ehrlich Ascites Carcinoma; anti-tumor effects; cytotoxicity; tumor growth inhibition.

1. INTRODUCTION

In recent times, natural products have gained significant importance for their roles in bolstering the immune system and serving as anti-cancer agents [1,2]. These naturally derived substances are increasingly recognized for their ability to enhance immune responses, which is critical for protecting the body against infections and diseases. Moreover, many of these products possess bioactive compounds that can directly inhibit the growth of cancer cells and induce apoptosis, or programmed cell death, thereby preventing the progression and spread of tumors [3]. A multitude of pharmacological studies have been dedicated to exploring potential anticancer agents, with a particular focus on polysaccharides extracted from various herbaceous plants [4]. These polysaccharides, natural compounds commonly found in plants, have garnered attention for their promising anti-cancer properties. Research has revealed that these polysaccharides possess the ability to inhibit the growth and proliferation of cancer cells, making them potential candidates for cancer therapy [5]. Mushrooms are a vital and nutrient-rich food source, notable for their low calorie and cholesterol content, as well as minimal sodium levels, which vary by species [6]. Among the diverse range of edible mushrooms, *Pleurotus ostreatus*, commonly known as the oyster mushroom, stands out due to its widespread consumption and commercial availability around the world. This mushroom is particularly valued for its robust nutritional profile, which includes essential vitamins, minerals, proteins, and dietary fiber, making it a beneficial addition to a balanced diet [7]. A key component of *Pleurotus ostreatus* that has garnered significant attention is its β-glucans, a type of polysaccharide found in the cell walls of fungi. β-Glucans are known for their immune-modulating properties, which contribute to the mushroom's anti-cancer effects [8]. Research has shown that these compounds can enhance the immune system by activating various immune cells, such as macrophages, natural killer cells, and T-cells [9]. For instance, the polysaccharide fraction of *Pleurotus ostreatus* has been demonstrated to increase cytokine secretion in animal models, indicating a strong immunostimulatory effect [10]. This enhancement of the immune response is crucial in combating cancer cells and preventing tumor growth. The anti-cancer potential of *Pleurotus ostreatus* β-glucans has been specifically observed in the context of liver and colon cancers. Studies have indicated that β-glucans can induce apoptosis, or programmed cell death, in liver cancer cells, thereby inhibiting tumor growth and reducing metastasis [11]. Additionally, β-glucans have been utilized in the treatment of colon cancer, where they help modulate the gut microbiota, bolster the immune response in the colon, and directly inhibit the proliferation of colon cancer cells [12]. These findings highlight the therapeutic potential of Pleurotus ostreatus β-glucans, making them a promising natural option for cancer prevention and treatment, and underscoring the mushroom's significant health benefits beyond its nutritional value [13-15].

The aim of the present study was to analyze the anti-tumor effects of *Pleurotus ostreatus* polysaccharide (POP) on EAC cells in vivo. This

study seeks to provide a scientific foundation for the research and practical application of polysaccharides in cancer therapy.

2. MATERIALS AND METHODS

2.1 Mushroom Collection, POP Extraction, Purification and Identification

In meticulous detail, *Pleurotus ostreatus* mushrooms were systematically collected from their natural habitat, ensuring sample integrity and noting pertinent environmental factors. Subsequently, Pleurotus ostreatus polysaccharide (POP) was extracted with precision, employing suitable solvents and methods to maintain its structural integrity. The extracted POP underwent thorough purification to eliminate impurities, employing techniques like filtration and chromatography. Rigorous identification procedures, including spectroscopy and mass spectrometry, were then employed to confirm the molecular structure and composition of the purified polysaccharide. Throughout these processes, strict adherence to methodologies outlined in our prior publication was maintained to ensure the reliability and reproducibility of results [16].

2.2 Experimental Animals

Swiss albino mice, aged two months and weighing 30±2g, were obtained from the animal research division of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICCDRB). These mice were housed under controlled conditions in an air-conditioned room, maintaining a temperature range of 22-25˚C and a humidity level of 55±1%, with a 12-hour light/dark cycle. They were provided with a standard commercial rodent pellet diet and had unrestricted access to water. Prior to the commencement of the experimental session, a seven-day acclimatization period was allowed for the mice. All animal procedures strictly adhered to the guidelines set forth by the Institutional Animal Ethics Committee at Primeasia University in Dhaka, Bangladesh.

2.3 EAC Cell Line: Collection and Maintenance

Thanks to the Department of Pharmacy at Jahangirnagar University in Savar, Bangladesh, we acquired Ehrlich ascites carcinoma (EAC) cells. These cells were proliferated by injecting 1 × 10^6 cells into the peritoneal cavity of a mouse. After ten days, cell count and viability assessment were conducted using a hemocytometer and the trypan blue dye exclusion method, respectively, to prepare for in vivo tests. To maintain the EAC cells in vivo, 2x10^6 cells suspended in PBS were intraperitoneally transplanted into Swiss albino mice every 10 days. Ascitic fluid was collected from mice with EAC cells during the logarithmic phase of tumor growth (days 7-8). Each experimental subject received 0.1 ml of cancer cell suspension, containing 2x10^6 cells, via intraperitoneal injection. These undifferentiated carcinoma (EAC) cells exhibit hyperdiploidy and demonstrate notable transplantability, resistance to regression, rapid proliferation, short lifespan, 100% malignancy, and the absence of tumor-specific transplantation antigen.

2.4 *In Vitro* Anticancer Activity

2.4.1 MTT assay

The experiment aimed to assess the cytotoxic effects of a polysaccharide (POP) using a colorimetric assay called the 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. This assay is based on the reduction of MTT to formazan crystals by active mitochondrial reductase enzymes, which are predominantly present in viable cells. Therefore, the amount of formazan produced is directly proportional to the number of living cells, enabling the evaluation of the polysaccharide's cytotoxicity through the construction of a dose-response curve. The resultant purple solution, indicative of cell viability, is quantified using spectrophotometry. Ehrlich ascites carcinoma (EAC) cells were cultured in complete Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution. Cells were seeded at a density of 5000 cells per well in a 96-well plate and allowed to attach for 24 hours under a 5% CO2 atmosphere. Subsequently, wells were treated with increasing concentrations of the polysaccharide, ranging from 25 ųg mL−1 to 800 ųg mL−1, and further incubated for 24 hours. To serve as controls, blank wells containing only DMEM and wells with cells but no polysaccharide were included on each plate. Following the incubation period, MTT reagent was added to each well at a concentration of 0.5 mg mL−1 and incubated for an additional 3 hours to allow for the formation of formazan crystals. The formazan crystals were then dissolved in dimethyl sulfoxide

(DMSO), and the absorbance of the resulting solution was measured at 570 nm using a microplate reader. A reference wavelength of 630 nm was used to correct for background absorbance. The absorbance of the treated sample is then compared to the absorbance of the untreated control sample (cells without any treatment) to calculate cell viability. Typically expressed as a percentage, cell viability is determined using the formula: Cell Viability (%) = (Absorbance of Treated Sample / Absorbance of Control Sample) × 100%. This quantitative analysis enables researchers to assess the cytotoxic effects of the tested compound, such as the polysaccharide, on the cultured cells, providing valuable insights into its potential therapeutic or toxicological properties.

2.4.2 Grouping animals

In the present study, a total of 60 animals were randomly divided into four groups (n = 15 per group). Group 1 served as the normal control group (n = 15), while the remaining groups were induced with tumors by subcutaneously injecting EAC cells (2×10^6 cells suspended in 0.2 mL saline per mouse). Tumor size in all EAC-inoculated mice was monitored, and when it crossed the 50 mm^3 limit (considered day 0, which was 11 days after implantation), treatment was initiated. Tumor size was measured weekly using a Vernier caliper, and tumor volume was calculated using the following equation as described by Schirner et al. (1998):

Tumor volume = Longest diameter x Shortest width x 0.5

The study included the following animal groupings:

1. Normal Group:Vehicle (2% gum acacia, p.o., OD)
2. EAC Group: Vehicle (2% gum acacia, p.o., OD)
3. Bleomycin Group: EAC + Bleomycin (0.3 mg/kg, i.p.)
4. POP Group: EAC + 4xIC_{50}/kg, p.o.

The treatment was administered for 21 days. Five animals from each group were randomly selected for detailed analysis. Blood was collected via the retro-orbital route for hematological and biochemical analysis. These animals were then euthanized using an overdose of ketamine to collect tumor masses and vital organs (heart, liver, and kidneys) for histopathological studies. The remaining animals (n = 10 per group) were kept for survival analysis, monitored for up to 60 days. The mean survival time (MST) was calculated as follows:

Mean Survival Time (MST):

$$\mathrm{MST} = \frac{\sum_{i=1}^{n} t_i}{n}$$

where t_i represents the survival time of each individual animal, and n is the total number of animals in the group.

Increased Lifespan:

To determine the increased lifespan as a percentage compared to the control group, the following formula was used:

$$\text{Increased Lifespan } (\%) = \left(\frac{\mathrm{MST}_{\text{treated}} - \mathrm{MST}_{\text{control}}}{\mathrm{MST}_{\text{control}}} \right) \times 100$$

where:

- $\mathrm{MST}_{\text{treated}}$ is the mean survival time of the treated group,
- $\mathrm{MST}_{\text{control}}$ is the mean survival time of the control group.

These calculations allow for the quantification of the treatment's effect on survival and provide a basis for comparing the efficacy of different treatments in extending lifespan.

2.5 Measurements

As previously mentioned, throughout the duration of the study, both body weight and tumor size were meticulously assessed on a weekly basis to track any changes over time. To ensure a comprehensive analysis, five animals from each experimental group were chosen at random for in-depth examination. For sample collection, blood samples were carefully drawn via the retro-orbital route, a method designed to minimize discomfort and ensure accurate results. Additionally, vital organs including the heart, liver, and kidneys, along with tumor masses, were carefully excised and prepared for further analysis. The evaluation of biochemical parameters, crucial for understanding physiological changes, involved the utilization of Biosystems kits specifically designed for

assessing markers such as creatine kinase-MB, lactate dehydrogenase, AST, ALT, ALP, creatinine, and urea. These assessments were conducted using a semi-automated analyzer, the A15 Biosystems, ensuring precise and reproducible measurements. Concurrently, hematological parameters were analyzed using an automatic hematology analyzer, the Erba H560, allowing for the measurement of key indicators such as red blood cell count (RBC), white blood cell count (WBC), platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and packed cell volume (PCV). Lastly, for histopathological analysis, tissue samples extracted from the heart, liver, kidneys, and tumors were meticulously sectioned, carefully fixed in 10% formalin to preserve cellular structures, stained with hematoxylin and eosin, and then meticulously examined under a light microscope, specifically an Olympus BH-2, to discern any histological alterations or abnormalities. This rigorous and detailed approach to measurements ensured thorough assessment of both physiological and pathological aspects, providing valuable insights into the effects of the experimental treatments on the animals under study.

2.6 Statistical Analysis

In the statistical analysis, the data were expressed in terms of mean values along with their corresponding standard error of the mean (SEM), providing insight into the central tendency and variability within each group. To assess the differences in means between the various experimental groups, a one-way analysis of variance (ANOVA) was employed. This method enabled the comparison of means across multiple groups simultaneously, allowing for the detection of any significant differences in the measured parameters. Subsequently, Tukey's post-hoc test was applied to further scrutinize specific group differences, facilitating pairwise comparisons while controlling for type I error. Utilizing GraphPad Prism version 5, a widely used statistical software, ensured accurate and efficient analysis of the data. A difference in mean values was deemed statistically significant if the calculated p-value was less than 0.05, signifying a low probability of obtaining the observed results by chance alone. This rigorous analytical approach enabled robust interpretation of the data, providing valuable insights into the experimental outcomes and supporting informed conclusions regarding the efficacy or impact of the treatments under investigation.

3. RESULTS

3.1 Effect of POP on EAC Cell Viability *In vitro*

The provided data represents, Fig. 1, the effect of different concentrations of POP on the viability of EAC (Ehrlich Ascites Carcinoma) cells. The concentrations of POP range from 25 µg/mL to 800 µg/mL, and for each concentration, the percentage of cell viability is measured. At lower concentrations of POP (25 µg/mL to 150 µg/mL), there is a gradual decrease in EAC cell viability as the concentration of POP increases. For example, at 25 µg/mL of POP, the cell viability is 98.1%, which decreases to 69.4% at 150 µg/mL. This suggests that POP has a dose-dependent effect on reducing EAC cell viability within this concentration range. As the concentration of POP exceeds 150 µg/mL, the decrease in EAC cell viability becomes more pronounced and accelerates. At concentrations of 300 µg/mL and above, the decrease in cell viability is more substantial, with values dropping below 50% at 300 µg/mL and below 30% at 600 µg/mL and 800 µg/mL. Overall, the data suggests that POP has a dose-dependent cytotoxic effect on EAC cells, with higher concentrations leading to a more significant reduction in cell viability. This information could be valuable for further research into the potential therapeutic use of POP in targeting cancer cells such as EAC.

3.2 Effect of POP Treatment on Survival

Throughout the duration of the study, no deaths were observed among the animals assigned to the normal group, indicating their sustained survival without any adverse events (Fig. 2). However, a notable contrast was observed in the EAC group, where mortality occurred progressively from the 24th to the 41st day post-treatment initiation. This pattern of mortality suggests a significant impact of the induced tumors on the survival of the animals within this group. Furthermore, upon administration of treatments, both POP and bleomycin demonstrated a notable extension of lifespan compared to the untreated EAC group. Particularly striking was the maximal increase in lifespan observed within the POP-treated group, indicating a potentially promising therapeutic effect. This finding was further supported by statistical analysis using the log-rank (Mantel–

Cox) test, which confirmed a significant increase in lifespan among animals treated with POP compared to those receiving other interventions or no treatment. This detailed observation underscores the potential efficacy of POP treatment in prolonging survival and highlights its significance in the context of the experimental study.

3.3 Effect of POP on Body Weight, Tumor Weight, Tumor Volume

A considerable increase in the percentage change in body weight ($p < 0.001$) was observed within the EAC group compared to the normal group, indicating a notable physiological impact of the induced tumors on the animals' weight (Fig. 3). However, this trend was consistently reversed across all treatment groups, suggesting an effective mitigation of the weight loss associated with EAC induction. Furthermore, a significant reduction ($p < 0.001$) in tumor weight was noted with both bleomycin and POP treatments, indicating a substantial inhibitory effect on tumor growth regardless of the treatment type. Similarly, a significant decrease ($p < 0.001$) in tumor size progression was observed from the 14th day onwards in the POP treatment groups, indicating an early and sustained suppression of tumor growth with POP administration. Additionally, by the 21st day, a significant decrease ($p < 0.001$) in tumor size was evident in all treatment groups compared to the untreated EAC group, underscoring the effectiveness of both bleomycin

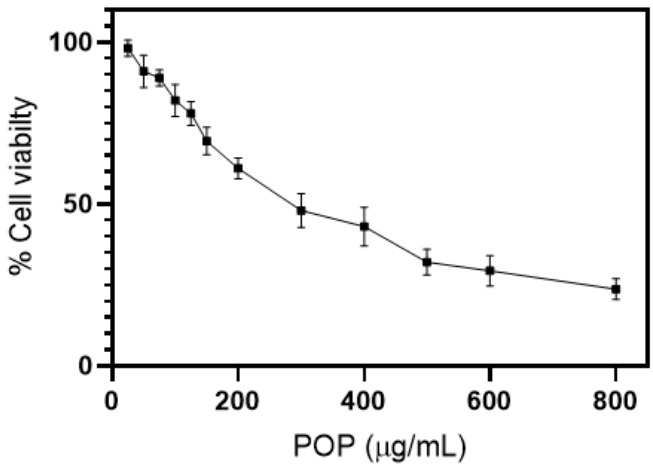

Fig. 1. The impact of POP on EAC cell viability was assessed, revealing a dose-dependent effect of POP. The results are depicted as the mean ± 95% confidence interval (CI) for each administered dose

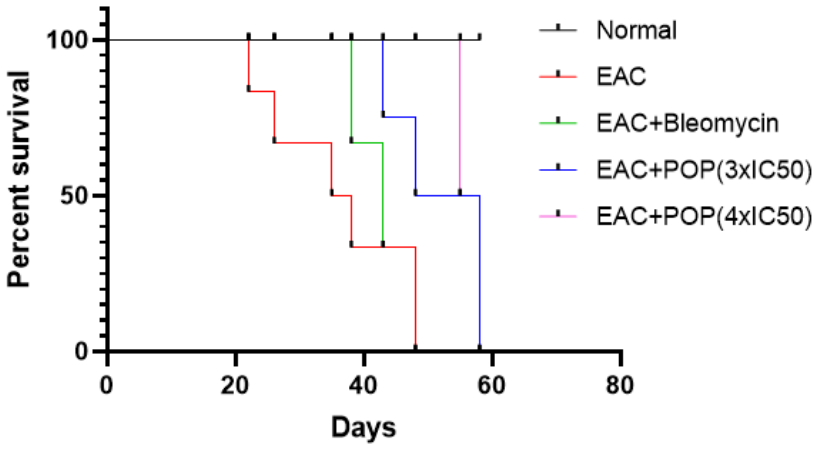

Fig. 2. The Kaplan-Meier survival curve depicted the survival outcomes for both the POP- and bleomycin-treated groups. A significant disparity ($p < 0.001$) was evident in the Mantel-Cox log-rank analysis when comparing these groups

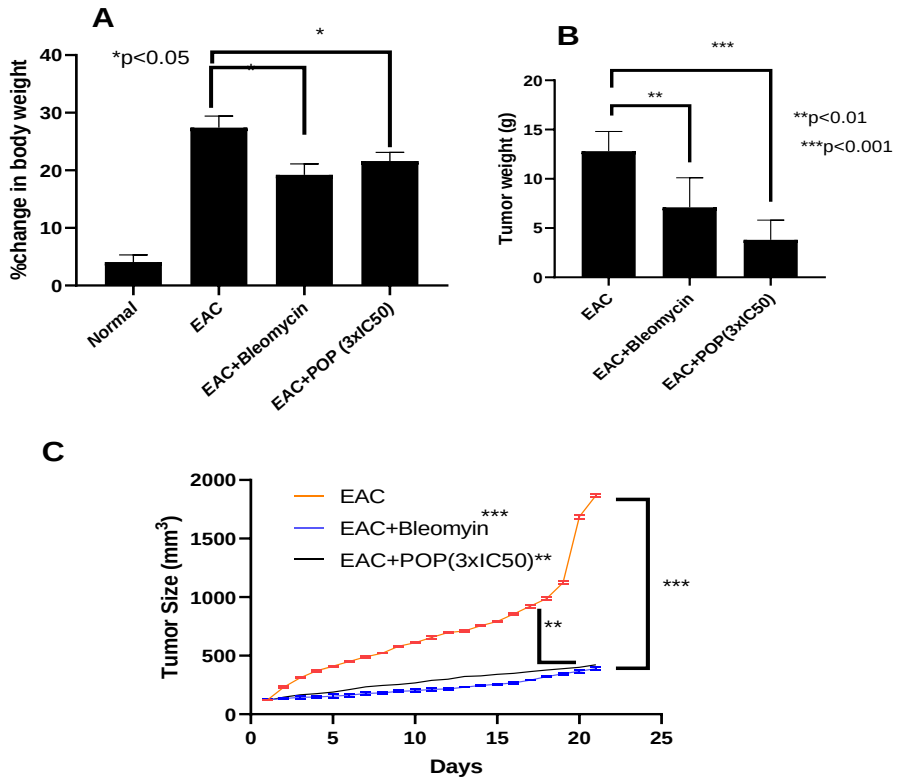

Fig. 3. The impact of POP on body weight, tumor weight, and tumor volume was assessed. (A) Percentage change of body weight revealed an increase within the EAC group, while treatment groups exhibited significantly reduced body weight compared to the EAC group ($p < 0.05$). (B) Analysis of tumor weight change demonstrated a decrease in tumor size within the treatment groups compared to the EAC group ($p < 0.05$). (C) Evaluation of tumor volume indicated a statistically significant reduction in tumor size within the treatment groups compared to the EAC group ($p < 0.05$)

and POP treatments in reducing tumor burden. These detailed observations highlight the therapeutic potential of both treatments in mitigating tumor-induced weight loss and suppressing tumor growth in this experimental setting.

3.4 Effect of POP on Hematological Parameters

A noteworthy decrease in hemoglobin levels ($p < 0.001$) was evident in the EAC group (8.9±1.2%) compared to the normal group (12.67±0.3), a pattern that was significantly reversed ($p < 0.01$) with independent POP treatment (Table 1). Furthermore, a further decline in hemoglobin levels was observed in the bleomycin group (7.56±1.0%), which saw a significant increase in the POP group (11.39±1.1%) compared to the bleomycin group. Additionally, a significant increase ($p < 0.001$) in white blood cell (WBC) count was noted in the EAC group compared to the normal group, a finding that was significantly mitigated ($p < 0.001$) in all treatment groups compared to the EAC group (Table 1). Moreover, there was a significant decrease in red blood cell (RBC) and platelet count observed in the EAC group ($p < 0.001$), with these counts further declining following bleomycin treatment, but were restored in the POP treatment groups. Furthermore, a significant reduction ($p < 0.001$) in packed cell volume (PCV), mean corpuscular hemoglobin concentration (MCHC), and lymphocytes was noted compared to normal levels, with substantial improvements ($p < 0.005–0.001$) observed with POP treatment. Conversely, there was a significant increase ($p < 0.001$) in neutrophils, eosinophils, monocytes, and mean corpuscular hemoglobin (MCH) ($p < 0.01$) observed within the EAC group compared to the normal group, a trend that was attenuated with POP treatment (Table 1).

Table 1. Effect of POP treatment on hematological parameters

	Normal Mean±SEM	EAC Mean±SEM	EAC+Bleomycin Mean±SEM	POP(4xIC50) Mean±SEM
Hb (%)	12.67±0.3	8.9±1.2	7.56±1.0	11.39±1.1$^{##}$
RBS million (cell/cmm)	9.1±0.6	5.4±1.3	4.67±1.4	8.76±1.9$^{##}$
WBCs (cell/cmm)	27.09±0.9	56.98±1.7	21.89±1.2	31.77±1.4$^{###}$
Platelet (cell/cmm)	1187±1.3	678.34±1.9	609.45±1.8	1134±1.3$^{##}$
PCV (%)	53.66±1.2	28.96±0.4	34.21±1.6	41.22±1.7$^{#}$
MCV(fL)	50.23±1.6	51.11±0.1	50.91±0.5	51.07±1.2
MCHC (gm/dl)	31.03±0.2	28.34±1.3	30.87±0.6	31.61±1.8
Lymphocytes (%)	75.12±0.1	18.54±1.6	23.98±0.3	46.37±0.2$^{##}$
Neutrophils (%)	22.34±0.5	76.33±1.9	69.79±0.2	50.84±0.7$^{###}$
Eosinophils (%)	1.62±0.8	3.02±1.3	3.27±1.6	1.76±0.5$^{#}$
Monocytes (%)	0.92±0.9	2.11±1.7	2.98±1.9	1.09±0.3
MCH (pg)	18.33±1.1	21.05±1.4	17.87±1.0	17.09±0.9

All values are expressed as a mean±SEM. One-way analysis of variance (ANOVA) followed by Tukey's test.
**p<0.05; **p<0.01; ***p<0.001, Compared with normal*
#p<0.05; ##p<0.01; ###p<0.001, Compared with EAC

Table 2. Effect of POP on biochemical parameters

	Normal Mean±SEM	EAC Mean±SEM	EAC+Bleomycin Mean±SEM	POP(4xIC50) Mean±SEM
CK-MB (U/L)	180.4±1.2	268.1±1.3	365.3±1.6	234.9±1.1$^{#}$
LDH (U/L)	2007.3±1.7	4377.4±1.5	5166.3±1.8	2176.3±1.6$^{###}$
AST (U/L)	213.2±1.4	722.4±1.0	983.3±1.5	402.4±1.5$^{###}$
ALT (U/L)	68.6±1.3	201.1±1.7	287.4±1.2	89.2±1.3$^{##}$
ALP (U/L)	23.2±0.9	45.2±0.9	56.1±0.9	18.09±1.9$^{###}$
Creatinine (mg%)	0.123±0.7	0.31±0.5	0.39±0.5	0.19±2.0$^{#}$
BUN (mg%)	32.1±0.5	82.6±1.2	98.1±0.2	54.2±0.9$^{##}$

All values are expressed as a mean±SEM. One-way analysis of variance (ANOVA) followed by Tukey's test.
**p<0.05; **p<0.01; ***p<0.001, Compared with normal*
#p<0.05; ##p<0.01; ###p<0.001, Compared with EAC

3.5 Effect of POP on Biochemical Parameters

In our current investigation, we observed a significant elevation ($p < 0.001$) in the levels of CK-MB, LDH, ALT, AST, ALP, creatinine, and BUN within the EAC groups. This finding indicates a substantial disruption in various biochemical markers associated with cellular damage and organ function. Furthermore, these biomarkers exhibited notable increases ($p < 0.05$–0.001) in the bleomycin group, suggesting an exacerbation of the pathological processes induced by the treatment. In contrast, significant improvements ($p < 0.05$–0.001) were noted in these markers in the POP group, indicating a beneficial effect of POP treatment in mitigating the adverse biochemical changes associated with the experimental condition. This detailed observation underscores the potential therapeutic efficacy of POP in ameliorating the dysregulation of these crucial biomarkers.

3.6 Effect of POP on Histology of Heart, Liver, Kidney, and Tumor

In the normal group of animals, thorough histological examination revealed that the heart, liver, and kidney tissues maintained their typical and healthy architectural characteristics. Specifically, the myocardium of the heart displayed well-organized cardiomyocytes with clear intercalated discs, ensuring efficient contraction and conduction of electrical signals (Fig. 4). In the liver, hepatocytes were arranged in lobules, with central veins and radiating hepatic cords, facilitating metabolic processes and detoxification functions (Fig. 5). Similarly, the kidney tissue exhibited intact nephrons, including glomeruli, proximal and distal tubules, and collecting ducts, supporting filtration, reabsorption, and secretion processes essential for maintaining fluid and electrolyte balance (Fig. 6).

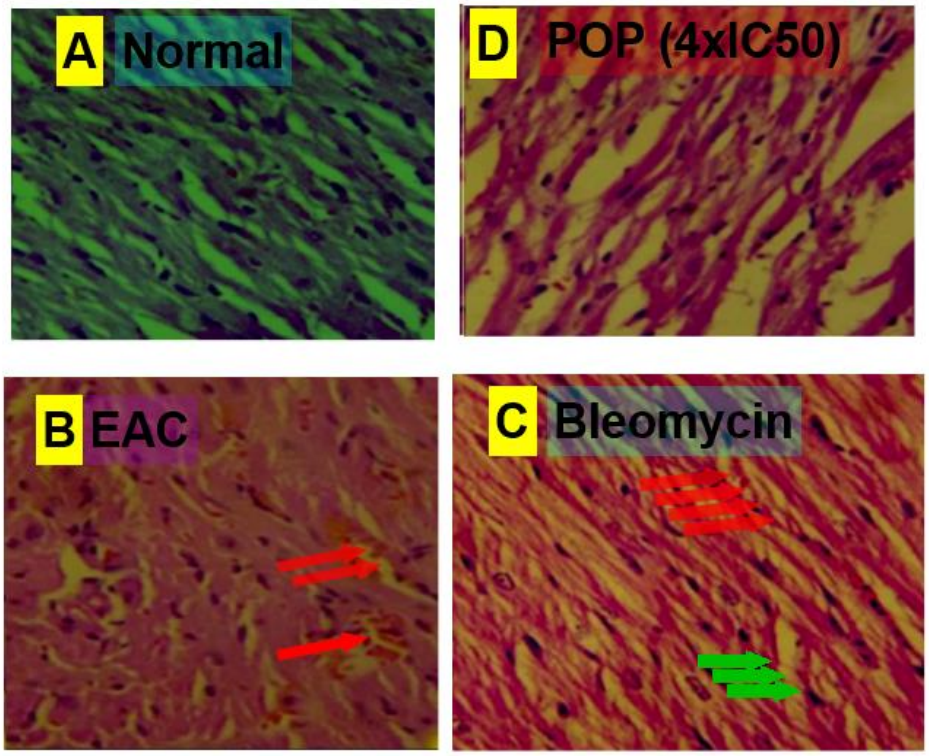

Fig. 4. Heart sections from different treatment groups stained with hematoxylin and eosin at ×40 magnification reveal distinct histological features. The normal group (A) exhibits typical cardiac architecture with well-organized cardiomyocytes and clear intercalated discs. In contrast, both the EAC (Ehrlich Ascites Carcinoma) and Bleomycin-treated groups (B and C, respectively) display pronounced congestion and myofibrillar myopathy, characterized by disrupted myofibrils and irregular cell structure. Conversely, the heart tissue from the POP (4xIC50) treated group (D) shows no signs of congestion or myofibrillar damage, maintaining a histological profile comparable to the untreated normal group. This suggests that POP treatment at 4xIC50 does not adversely affect cardiac histology, highlighting its potential cardioprotective effects

4. DISCUSSION

The outcomes derived from our study provide compelling evidence that POP demonstrated superior antitumor activity in comparison to bleomycin alone. This assertion is supported by several key observations. Firstly, we observed an extended survival time among the subjects treated with POP, indicating a potent inhibitory effect on tumor progression and overall disease burden. Additionally, significant regression in tumor size was noted in the POP-treated group, suggesting a robust suppression of tumor growth. These detailed observations underscore the efficacy of POP as a promising therapeutic intervention for combating tumor development and progression. Such findings hold significant implications for the development of novel treatment strategies aimed at improving patient outcomes in cancer therapy. In cancer chemotherapy, organ toxicity is a significant complication that disrupts homeostatic functions, particularly in treatments involving bleomycin [17,18]. To thoroughly investigate this issue, we quantified several key biomarkers, including CK-MB, LDH, AST, ALT, ALP, creatinine, and BUN across different treatment groups. Our results indicated that in animals treated with bleomycin alone, these biomarkers were significantly elevated compared to the EAC (Ehrlich Ascites Carcinoma) group, highlighting the presence of chemotherapy-induced organ toxicity. Specifically, bleomycin therapy is known to generate free radicals, which cause significant damage to the myocardium. This damage increases cell membrane permeability, resulting in the release of cardiac-specific enzymes such as CPK-MB and LDH into the bloodstream. Our data showed that bleomycin treatment led to a two-fold increase in CPK-MB levels and a 1.2-

fold increase in LDH levels compared to the EAC group. Furthermore, liver and kidney function markers also indicated significant toxicity. The levels of AST and ALT, enzymes indicative of liver function, were elevated by 1.3-fold and 1.4-fold respectively, compared to the EAC group. Similarly, kidney function markers, creatinine and BUN, showed a 1.1-fold increase in both markers compared to the EAC group. These increases signify substantial liver and kidney stress or damage resulting from bleomycin therapy. Interestingly, when animals were treated with POP (a specific protective agent), these adverse effects were markedly reversed. The levels of CPK-MB, LDH, AST, ALT, creatinine, and BUN were significantly lower in the POP-treated group compared to those treated with bleomycin alone, indicating a protective effect of POP on vital organs during chemotherapy. This suggests that POP has a potential therapeutic benefit in mitigating the organ toxicities commonly associated with bleomycin, thereby improving the overall safety profile of the chemotherapy regimen.The roles of the aforementioned biomarkers were further corroborated by detailed histopathological examination results. In the EAC solid tumor model, a significant increase in myocardial congestion and myofibrillar degeneration was observed, which was further exacerbated by bleomycin treatment. These findings align with those reported by Reddy et al. [19], who demonstrated the efficacy of POP as a cardioprotective agent in the EAC solid tumor mode [18]. Moreover, in the groups treated with EAC and bleomycin, there was a notable increase in several indicators of liver damage.

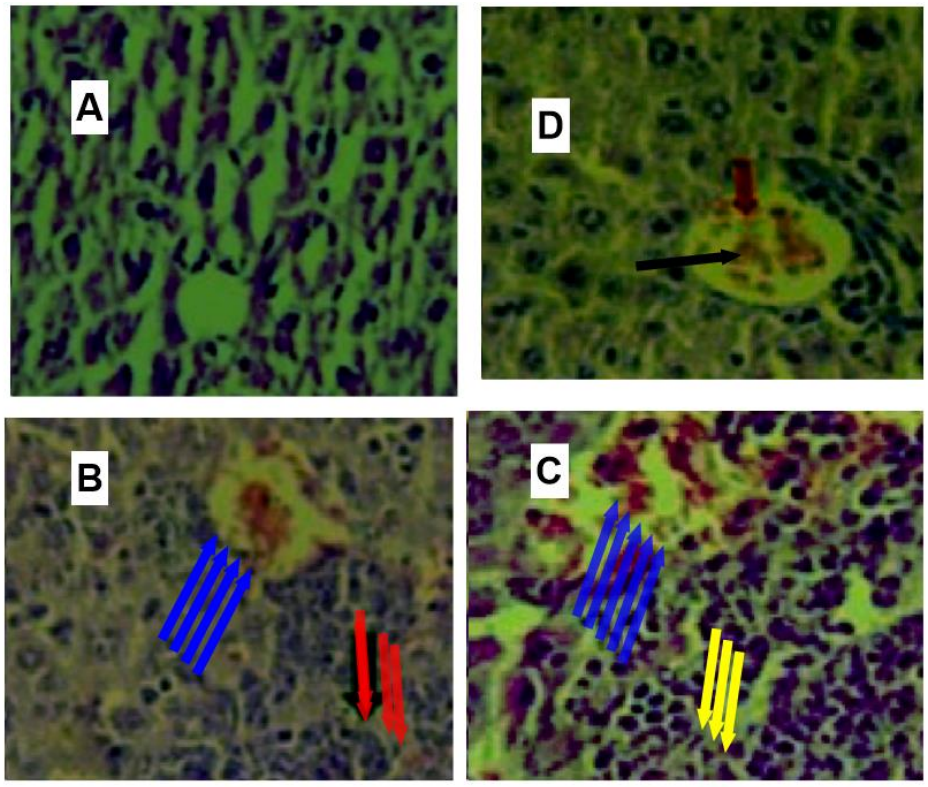

Fig. 5. The effect of POP on hepatic histology was investigated through examination of liver sections from different treatment groups stained with hematoxylin and eosin at ×40 magnification. The panels included (A) Normal, (B) EAC (Ehrlich Ascites Carcinoma), (C) Bleomycin-treated, and (D) POP (4xIC50) treated. In the EAC and Bleomycin groups (B and C), venous and sinusoidal congestion, Kupffer cell hyperplasia, as well as apoptosis and spotty necrosis were observed, indicating significant liver damage. However, in the POP-treated group (D), there was a noticeable recovery from venous congestion, suggesting a potential protective effect of POP treatment on hepatic vascular function. This indicates that POP treatment at 4xIC50 may have beneficial effects on hepatic histology, mitigating some of the pathological changes induced by EAC and Bleomycin treatments

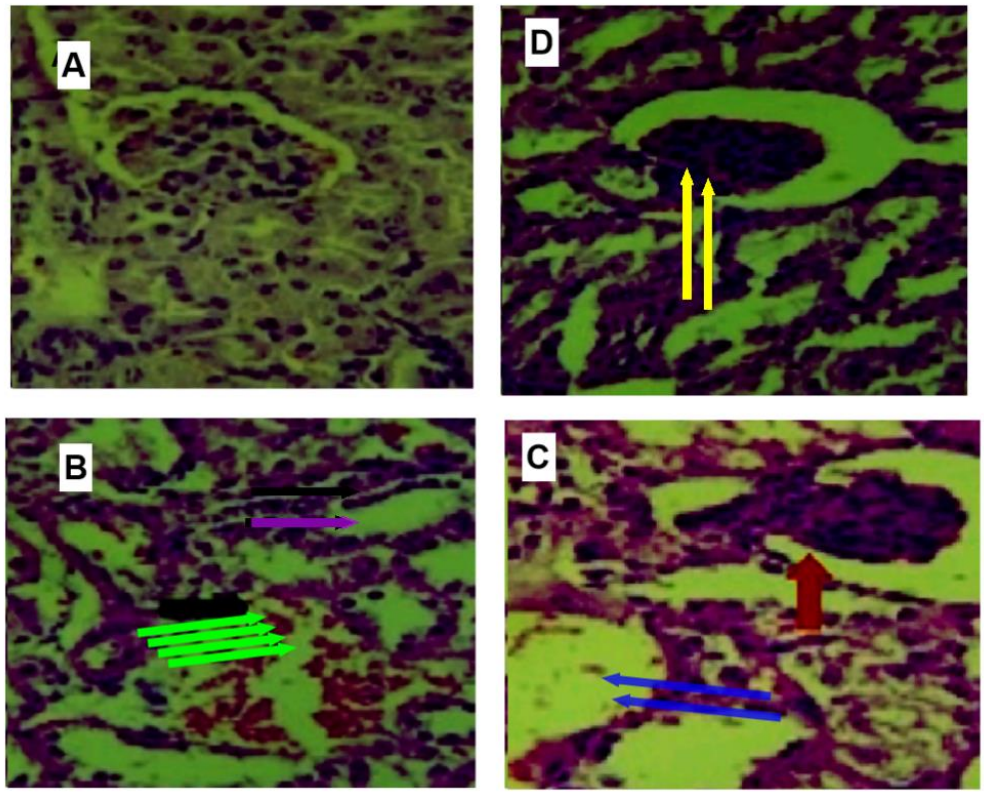

Fig. 6. The investigation into the effect of POP on kidney histology involved a comprehensive analysis of kidney sections from different treatment groups, each stained with hematoxylin and eosin and examined under a microscope at ×40 magnification. The experimental panels encompassed (A) the Normal group, (B) the EAC (Ehrlich Ascites Carcinoma) group, (C) the Bleomycin-treated group, and (D) the POP (4xIC50) treated group. Upon examination, distinct histopathological alterations were observed in the EAC and Bleomycin groups (B and C). These alterations included tubular congestion, represented by an increased presence of cellular material within the renal tubules (green), and glomerular congestion, indicated by an accumulation of blood cells within the glomerular capillaries (pink). Furthermore, signs of inflammation, characterized by infiltrating immune cells and tissue edema, were noted, contributing to the compromised renal architecture. Additionally, the presence of glomerular atrophy (red) and tubular cell swelling (blue) underscored the severity of renal damage in these groups. Conversely, in the POP-treated group (D), a distinct reversal of glomerular atrophy was observed, suggesting a potential protective effect of POP treatment on glomerular structure. This observation indicates that POP treatment at 4xIC50 may exert beneficial effects on kidney histology, possibly mitigating some of the pathological changes induced by EAC and Bleomycin treatments. Overall, these findings provide valuable insights into the potential therapeutic role of POP in preserving renal function and mitigating renal injury under pathological conditions.

Specifically, we observed significant necrosis, apoptosis, inflammation, hepatocellular dysplasia, venous and sinusoidal congestion, and Kupffer cell hyperplasia in hepatic tissues [19]. These histological changes indicate severe liver injury induced by bleomycin. However, animals treated with POP showed significant cellular regeneration in the liver, suggesting that POP mitigates the extent of tissue damage.

Similarly, kidney tissues in the EAC- and bleomycin-exposed groups exhibited signs of significant renal damage. There was evidence of tubular and glomerular congestion, glomerular atrophy, tubular cell swelling, and inflammation. POP treatment, however, markedly ameliorated these pathological changes, indicating its protective effect on kidney tissues. Furthermore, the cytotoxic effects of the treatments were confirmed by detailed histopathological analysis

of the tumor tissues. Bleomycin treatment resulted in significant devastation of the tumor mass, characterized by reduced tumor growth, decreased mitotic activity, increased necrosis, and the presence of apoptotic nuclei. These findings indicate high levels of cytotoxicity. In contrast, treatment with POP led to a pronounced reduction in these adverse effects, highlighting its potential in reducing tumor burden while protecting normal tissues. These comprehensive histopathological findings underscore the protective and therapeutic potential of POP in mitigating organ toxicity and enhancing overall treatment outcomes in chemotherapy. The ability of POP to promote cellular regeneration and protect against tissue damage makes it a promising adjunctive treatment in cancer therapy, particularly in minimizing the side effects associated with conventional chemotherapeutic agents like bleomycin.

5. CONCLUSION

This study demonstrated the in vivo anticancer activity of Pleurotus ostreatus polysaccharide (POP) by effectively targeting EAC cells while simultaneously reducing toxicity to vital organs such as the heart, liver, and kidneys. Our results showed that treatment with POP led to a significant reduction in tumor size and mass, indicating its strong anticancer efficacy. Additionally, POP treatment was associated with a notable decrease in biomarkers indicative of organ toxicity, suggesting that POP can mitigate the adverse side effects commonly associated with conventional chemotherapeutic agents like bleomycin. Further analysis revealed that POP has the ability to neutralize free radicals generated by EAC cells. This antioxidant property helps in maintaining cellular integrity and preventing oxidative damage, which is often a consequence of aggressive cancer treatments. The inherent anticancer properties of POP, coupled with its capacity to scavenge free radicals, contribute to the prolongation of survival time in EAC-bearing mice. Animals treated with POP not only showed reduced tumor growth but also exhibited improved overall health and longevity compared to those receiving bleomycin alone. Histopathological examinations supported these findings by showing significant reductions in myocardial, hepatic, and renal damage in the POP-treated groups. In the heart, there was less congestion and myofibrillar degeneration; in the liver, there was reduced necrosis, apoptosis, and inflammation; and in the kidneys, there was less tubular and glomerular congestion, and reduced signs of glomerular atrophy and tubular cell swelling. These protective effects highlight POP's potential as a multi-organ protective agent. Overall, Pleurotus ostreatus (POP) exhibits promising nutraceutical properties with cardioprotective, hepatoprotective, and nephroprotective effects. These findings suggest that POP could be a valuable adjunct in cancer therapy, particularly in reducing the side effects associated with bleomycin-based chemotherapy. However, to fully establish POP as a health supplement for cancer patients, further confirmatory studies at the clinical level are needed. These studies should aim to validate the safety, efficacy, and protective benefits of POP in a larger and more diverse patient population.

ETHICAL APPROVAL

University granted permission for the collection and maintenance of mice exclusively for research purposes. This authorization was provided under strict adherence to ethical guidelines and protocols to ensure the humane treatment of the animals. All research activities involving mice complied with institutional policies and relevant ethical standards, which were designed to minimize distress and ensure the welfare of the animals. The use of mice in research was closely monitored and regulated by the university’s ethics committee to ensure that all procedures were conducted responsibly and ethically. All animal procedures strictly adhered to the guidelines set forth by the Institutional Animal Ethics Committee at Primeasia University in Dhaka, Bangladesh.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and

major patterns in GLOBOCAN 2012. International Journal of cancer. 2015;136 (5):E359-86.

2. Turan I, Demir S, Misir S, Kilinc K, Mentese A, Aliyazicioglu Y, Deger O. Cytotoxic effect of Turkish propolis on liver, colon, breast, cervix and prostate cancer cell lines. Tropical Journal of Pharmaceutical Research. 2015;14(5): 777-82.
3. Pang CL, Zhang X, Wang Z, Ou J, Lu Y, Chen P, Zhao C, Wang X, Zhang H, Roussakow SV. Local modulated electro-hyperthermia in combination with traditional Chinese medicine vs. intraperitoneal chemoinfusion for the treatment of peritoneal carcinomatosis with malignant ascites: A phase II randomized trial. Molecular and clinical oncology. 2017; 6(5):723-32.
4. Liang Z, Guo YT, Yi YJ, Wang RC, Hu QL, Xiong XY. Ganoderma lucidum polysaccharides target a Fas/caspase dependent pathway to induce apoptosis in human colon cancer cells. Asian Pacific Journal of Cancer Prevention. 2014;15 (9):3981-6.
5. Gan QX, Wang J, Hu J, Lou GH, Xiong HJ, Peng CY, Huang QW. Modulation of apoptosis by plant polysaccharides for exerting anti-cancer effects: A review. Frontiers in Pharmacology. 2020; 11:792.
6. Friedman M. Mushroom polysaccharides: chemistry and antiobesity, antidiabetes, anticancer, and antibiotic properties in cells, rodents, and humans. Foods. 2016;5(4):80.
7. Kalmıs E, Azbar N, Yıldız H, Kalyoncu F. Feasibility of using olive mill effluent (OME) as a wetting agent during the cultivation of oyster mushroom, Pleurotus ostreatus, on wheat straw. Bioresource Technology. 2008;99(1):164-9.
8. Tong H, Xia F, Feng K, Sun G, Gao X, Sun L, Jiang R, Tian D, Sun X. Structural characterization and *in vitro* antitumor activity of a novel polysaccharide isolated from the fruiting bodies of Pleurotus ostreatus. Bioresource Technology. 2009;100(4): 1682-6.
9. Jayakumar T, Thomas PA, Geraldine P. Protective effect of an extract of the oyster mushroom, Pleurotus ostreatus, on antioxidants of major organs of aged rats. Experimental gerontology. 2007;42(3):183-91.
10. Adebayo EA, Oloke JK. Oyster mushroom (Pleurotus species); a natural functional food.
11. Pinheiro F, Faria RR, De Camargo JL, Spinardi-Barbisan AL, Da Eira AF, Barbisan LF. Chemoprevention of preneoplastic liver foci development by dietary mushroom Agaricus blazei Murrill in the rat. Food and Chemical Toxicology. 2003;41(11):1543-50.
12. Lavi I, Friesem D, Geresh S, Hadar Y, Schwartz B. An aqueous polysaccharide extract from the edible mushroom Pleurotus ostreatus induces anti-proliferative and pro-apoptotic effects on HT-29 colon cancer cells. Cancer letters. 2006;244(1):61-70.
13. A. Barnes D, Barlow R, Singh Nigam P, Owusu-Apenten R. Antioxidant, Anticancer and Antibacterial Activity of Withania somnifera Aqueous Root Extract. J. Adv. Biol. Biotechnol. 2015;5(1): 1-6.
    Available:https://journaljabb.com/index.php/JABB/article/view/264
    [Accessed on:2024 May 28]
14. Vinitha V, Meignanalakshmi S, Tirumurugaan KG, Sarathchandra G, Sundaram SM. Poly-unsaturated Fatty Acid, Biodiesel Property and Anticancer Activity Analysis of Monoraphidium griffithii. Curr. J. Appl. Sci. Technol. 2022;41(41): 44-55.
    Available:https://journalcjast.com/index.php/CJAST/article/view/3996
    Accessed on:2024 May 28];
15. Wang X, Yuan S, Wang J, Lin P, Liu G, Lu Y, Zhang J, Wang W, Wei Y. Anticancer activity of litchi fruit pericarp extract against human breast cancer *in vitro* and in vivo. Toxicology and applied pharmacology. 2006 Sep 1;215(2):168-78.
16. Uddin Pk MM, Islam MS, Pervin R, Dutta S, Talukder RI, Rahman M. Optimization of extraction of antioxidant polysaccharide from Pleurotus ostreatus (Jacq.) P. Kumm and its cytotoxic activity against murine lymphoid cancer cell line. PLoS One. 2019;14 (1):e0209371.
17. Mitry MA, Edwards JG. Doxorubicin induced heart failure: Phenotype and molecular mechanisms. IJC heart & vasculature. 2016;10:17-24.

18. dos Santos DS, dos Santos Goldenberg RC. Doxorubicin-induced cardiotoxicity: from mechanisms to development of efficient therapy. Cardiotoxicity. 2018:3-24.

19. Shivakumar P, Rani MU, Reddy AG, Anjaneyulu Y. A study on the toxic effects of doxorubicin on the histology of certain organs. Toxicology international. 2012;19 (3):241.

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