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In Vivo Anticancer Activity of *Porteresia coarctata* Methanolic Extract on Ehrlich Ascites Carcinoma (EAC) Cells

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Abstract: *Porteresia coarctata* (family Poaceae) grass plant with profound medicinal properties is native of India. Cancer is an evil spirit that produces terrifying death-related symptoms. Mangroves are rich in potentially bioactive compounds, they are extremely important ecologically. as well as bioactive components that have a wide range of applications in cancer eradication. The present work aimed to evaluate methanolic extract of *Porteresia coarctata* (MEPC) whole plants for its anti-cancer activity using *in vivo* experimental animal model Ehrlich ascites carcinoma (EAC) cells. *Porteresia coarctata* whole plant crude extract was prepared. Flavonoids, alkaloids, carbohydrates, tannins, and other phytochemicals were removed from the extract using screening. On EAC cells, *in vivo* cytotoxic research was conducted. On EAC cells, the anti-cancer activity was examined *in vivo* at two different MEPC concentrations: 250 mg/kg and 500 mg/kg. By monitoring the drug-induced changes in biochemical and haematological markers, the cytotoxicity of the samples was evaluated *in vivo* in mice bearing normal, standard, and EAC conditions. Current study found that MEPC exhibits exceptional anticancer properties. As a result, the plant could serve as a source of potential of targets for cancer therapy efforts.

Keywords: *Porteresia coarctata*, Cancer, Cytotoxicity, Ehrlich ascites carcinoma, Phytochemicals

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Introduction

Cancer is spreading throughout the world and is an evil spirit that gives frightening signs of death. It was estimated that 14,61,427 cases of cancer (crude rate: 100.4 per 100,000) (Sathishkumar *et al.*, 2022) would occur in India in 2022. In India, cancer affects one in nine people at some point in

their lives. Lung and breast cancers were the most frequent cancers in men and women, respectively. According to estimates, the number of instances of cancer would rise by 12.8% in 2025 compared to 2020. Cancer is a deadly disease that spreads over the world and is considered an evil force.

According to a recent assessment, there would be approximately 10.9 million new cases of incidence, 6.7 million deaths, and over 24.6 million cancer patients worldwide by 2022 (Siegel *et al.*, 2022). In the United States in 2022, there was expected to be 1,918,030 new cases of cancer and 609,360 cancer deaths. This is an increase of 8.8% in cases and 0.41% in deaths above 2019 figures.

As per GLOBOCAN 2020, approximately expected 19.3 million additional cancer diagnoses and about 10 million died from the disease occurred worldwide. With 11.4% of cases, lung cancer is still the most frequent type of cancer followed by colorectal (10%), prostatic (7.3%), and gastric (5.6%). Eastern Asia reported the highest number of cases—6.0 million, or 31.1% of the total—and the highest number of deaths-- 3.6 million, or 36.3%—according to the GLOBOCAN data with region-specific information. North America reported 2.6 million cases (13.3%) and 7% of cancer-related deaths, compared to 1.95 million cases (10%) and 1.3 million fatalities (12.6%) in South and Central Asia. Of the 4.4 million cases reported in Europe, 1.9 million (20%) resulted in fatalities (Sung *et al.*, 2021).

Due to declined favourable outcomes of conventional therapies like high rates of morbidity and death from cancer treatment, including radiation, chemotherapy, immunological modulation, and surgery, highlight the urgent need for innovative methods of managing cancer (Tannock, 1998). In view of this raising prevalence there is now a growing issue and burden for the creation of novel anticancer medications in the treatment of cancer, as well as for the relief of cancer-related illnesses.

Because mangroves are rich in potentially bioactive compounds, they are extremely important ecologically. These possess bioactive components that have different facets of applications in cancer eradication. Majority of the medications used and licenced for treatment for cancer are of natural origin, paving the route for the creation of novel chemical compounds from natural sources over time. These plants include

anticancer chemicals that are effective against breast, stomach, lung, colon, prostate, and leukaemia cancers (Umamaheshwari *et al.*, 2009).

The Poaceae family has a wide range of different actions in the medication cure of many maladies like cardiovascular disease, Parkinsonism, and auto immune diseases. The Poaceae family is a key hub of nutritional qualities, with a core of chemical components that have various functions such as antibacterial, anti-inflammatory, antifungal, antioxidant, and many more (Abdelkader *et al.*, 2016). It has been reported that mangroves contain secondary metabolites with anti-cancer properties. Despite having access to abundant chemically enriched resources, the mangrove flora remain mainly unexplored in terms of anticancer substances. Therefore, in order to support the mangrove flora-based anticancer research, the research has to be done on mangrove plants (Das *et al.*, 2015). It paves the door for substantial research into the development of novel chemical entities in order to discover newer medications.

Porteresia coarctata is a tetraploid wild rice (Syn = *Oryza coarctata*), extensively found along the coasts of India and some other Asian countries. (Sengupta and Majumder, 2010). *Porteresia coarctata* (Roxb.) *Tateoka* of Poaceae is a perennial grass that roots from lower nodes and has culms that are erect. The majority of the leaves are coriaceous, ligulate, and have eciliate membranes. The leaf blade has ribbed surface, spinulose, cartilaginous edges, and an attenuated apex. The panicle-like, lanceolate inflorescence is constricted. The fruit is oblong, isodiametric, biconvex, and has a linear hilum. It has an attached pericarp and Caryopsis. It is noteworthy because it is a relative of oryza, or rice, which is found in coastal areas' mangrove areas and intertidal zones. It can withstand both water logging and salinity. Previous studies of *Porteresia coarctata* extracts indicated its efficacy as an effective insecticidal and antifeeding property against *Spodoptera litura* (F.), a nuisance insect (Ulrichs *et al.*, 2008).

The present work aimed to evaluate methanolic extract of *Porteresia coarctata* (MEPC) whole plants for its anti-cancer activity using *in vivo* experimental animal model Ehrlich ascites carcinoma (EAC) cells.

Materials and Methods

Collection of plants:

The medicinal plants *Porteresia coarctata* (Roxb.) Tateoka of the Poaceae family was collected in October, 2019 from several locations in the Koringa Mangrove woods of Kakinada, Andhra Pradesh, India. Dr. V.S. Gopal Rao Naidu, Principal Scientist, Central Tobacco Research Institute (CTRI), Rajahmundry, India, verified and authenticated the plant. After removal of adherent particles of *Porteresia coarctata* (Roxb.) Tateoka (Poaceae) has been carefully cleaned, shade dried for a week, and powdered. For additional examination, the powdered samples were kept in airtight receptacles.

Extraction:

25 g of dried powdered sample of *Porteresia coarctata* was extracted using a Soxhlet process and 200 ml of methanol over a ten-hour period in order to get the extract. For this purpose, conventional Soxhlet extraction is still regarded as one of the most popular methods, and it is crucial to the entire analytical procedure (Castro and Jiménez-Carmona, 2000; Abdel-Aal *et al.*, 2015). Following the condensing of the plant source extract using a rotary evaporator, the samples were reconstituted in their corresponding solvent to create a stock solution of 100 mg/ml of *Porteresia coarctata*, which was then kept refrigerated. The anticancer activity and qualitative phytochemical analysis of the extracts were subsequently studied.

Phytochemical Screening:

For the identification of phytoconstituents, a first examination of the methanolic extracts' phytochemical composition was performed. Secondary metabolite identification included alkaloids (tested by Mayer and Dragendorff),

flavonoids (tested by Shinoda), terpenes (tested by Salkowski), tannins (tested by Ferric chloride), saponins (tested by Frothing), and cardiac glycosides (tested by Keller-Killani), phenols (Dos, 2009; Yu *et al.*, 2021).

Both ash and extract values were obtained using the standard methods with in Indian Pharmacopeia and the World Health Organization recommendations. Aluminum chloride calorimetric assay for total flavonoid concentration and Folin-Ciocalteu technique for total phenolic content were quantified (Ainsworth and Gillespie, 2007).

In vivo Anti-cancer activity studies:

According to the 2001 OECD recommendations, acute toxicity assessments were conducted (Jonsson *et al.*, 2013). In accordance with usual practice, normal Swiss albino mice were given extracts in various dosages up to 2000 mg/kg b wt after being denied food for 18 h in order to determine the highest tolerable dose of the extracts. The animals were continuously watched for any signs of toxicity for 4 h, then for 24 h, and lastly for 72 h, when the number of survivors was recorded. The therapeutic doses for additional research were chosen based on the results (1/10th to 1/20th of the highest tolerated dose).

Ehrlich Ascites Carcinoma (EAC) model and animal groups:

A suspension into the peritoneum of healthy albino mice was injected intraperitoneally with EAC cells (2 x 10⁶ cells/ml). ranging between 25 and 30 g to cultivate Ehrlich's Ascites Carcinoma cells. On day 15, the cells were aseptically removed from the mice's peritoneal cavity, cleaned in normal saline, and centrifuged for 15 min at 1,500 rpm. The procedure was carried out three times after the pellet was re-suspended in regular saline. Lastly, the cell count was corrected to (2 x 10⁶ cells/ml) after the cells were suspended in a predetermined volume of normal saline. A sample with more than 90% viability was selected for the transplanting process. A 0.5 ml intraperitoneal dose of a tumor cell suspension

comprising 2×10^6 cells was given to each animal (Sunil *et al.*, 2013).

Sixty female albino mice were chosen and obtained to investigate the *in vivo* anticancer activity of the methanolic extract of *Porteresia coarctata* (Roxb.) Tateoka (MEPC). 10^6 EAC (Ehrlich's Ascites Carcinoma) cells were injected intraperitoneally (i.p.) into each of them in a volume of 0.2 ml. These mice were divided into five groups at random, each with 12 animals, the same number of mice in the control and standard groups (Islam *et al.*, 2018). Mice were given cisplatin intraperitoneally (i.p.) every other day for 48 h following tumour inoculation, using the following protocol:

Group I (Normal): For 13 days, 0.2 ml of physiological saline (0.9 g/dl) was given intraperitoneally (i.p.) into 12 normal mice every other day.

Group II (EAC Control): For 13 days, 0.2 ml of physiological saline injections were given to 12 EAC-bearing mice every other day.

Group III (Cisplatin): For 13 days, 12 EAC-bearing mice received cisplatin (0.25 mg/kg) every other day.

Group IV (Treated group 1): For 13 days, a methanolic extract of *Porteresia coarctata* (Roxb.) Tateoka (MEPC-250 mg/kg) was given to 12 EAC-bearing mice every other day.

Group V (Treated group 2): 12 EAC-bearing mice were treated with with *Porteresia coarctata* (Roxb.) Tateoka (MEPC-500 mg/kg) every other day for 13 days.

Every three days, the animals with ascitic tumors were weighed. The experiment lasted 25 days in total. On the 14th day, six mice from each group were sacrificed in order to perform hematological and biochemical tests as well as assess the anti-tumor potency of *Porteresia coarctata* (Roxb.) Tateoka methanolic extracts.

II - V Group mice (n = 12/group) that remained were maintained to calculate their median survival time (MST) and percentage

increase in life span (% ILS).

Gathering of ascitic liquid:

Ascites tumor volume, packed cell volume (PCV), Ehrlich ascites tumor cell viability, and total count were immediately determined using the ascetic fluid from the treatment and control groups. The dye-exclusion technique and hemocytometer were used for these analyses, respectively.

MST and ILS percentage calculations:

Every day, the mortality of each group was recorded in order to track their MST. The animals' natural deaths marked the experiment's end, and the following equation was used to compute MST:

MST is equal to (days of death one and last)/2:

Equation $(ILS\% = (T - C)/C \times 100)$ was used to determine the percentage of ILS, where T stands for the MST of the treated animals and C for the MST of the control group.

On day 15, hematological analyses involved the collection of blood samples from twelve animals in each group into microfuge tubes containing EDTA. Additionally, biochemical investigations were performed with serum.

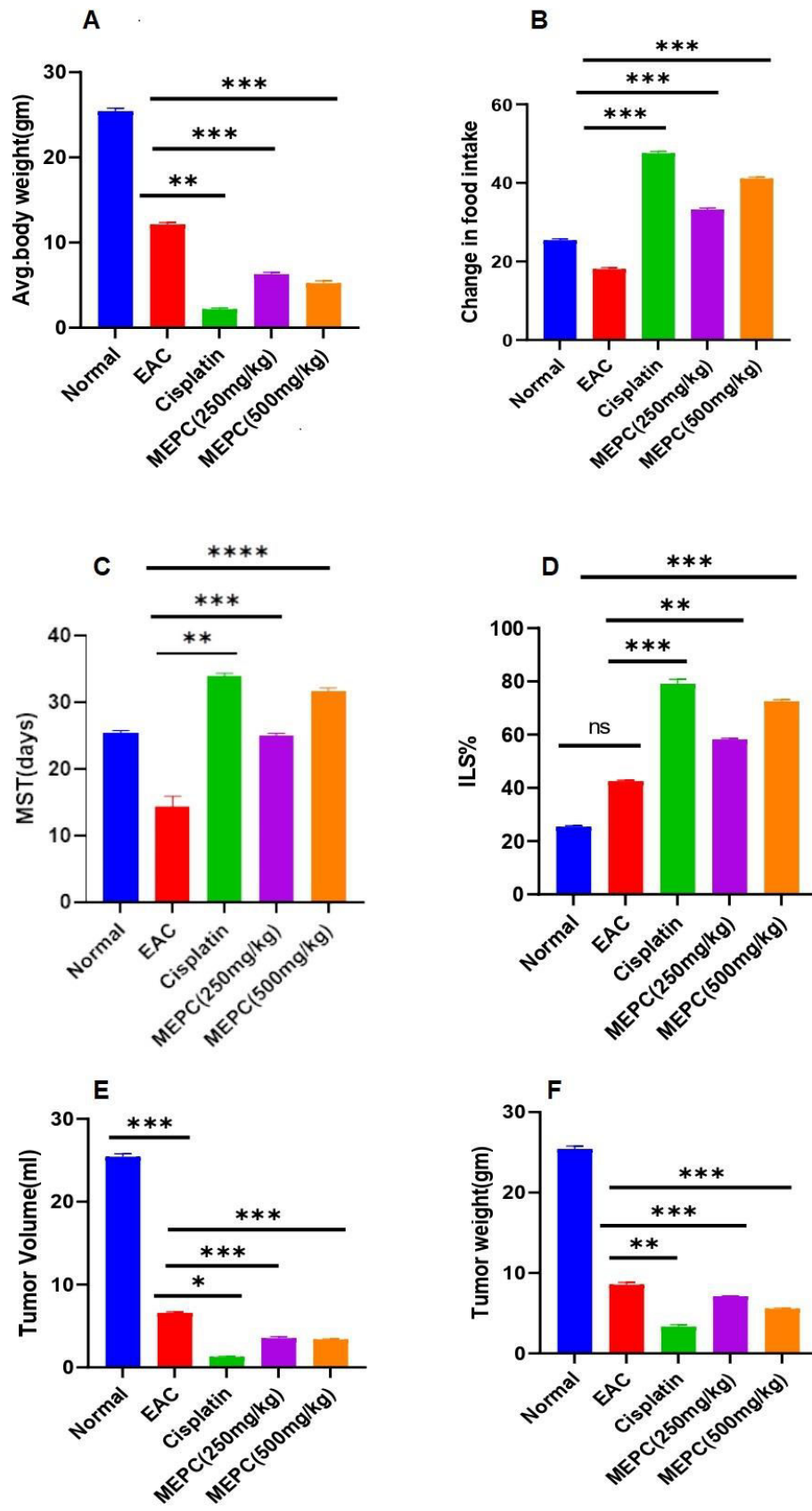
Results and Discussion

Effect of MEPC on Tumor Growth and Survival:

The administration of MEPC at the lowest allowable dose of 250 mg/kg and the maximum dose of 500 mg/kg to EAC-bearing mice resulted in a substantial ($P < 0.05$) decrease in the tumor volume, tumor weight, PCV, and mouse body weight as compared to the non-treated animals (Fig. 1). These findings suggest that MEPC at 250 mg and 500 mg has notable anti-tumour effects. As opposed to control tumour-bearing mice, however, MST was higher by 25.8 days and 31.8 days, while ILS was higher by 59.5% and 73.3%, respectively.

Effect of MEPC on Serum Parameters:

The results suggest that the tumor's progression was accompanied by shifts in the hematological parameters as compared to normal, which was



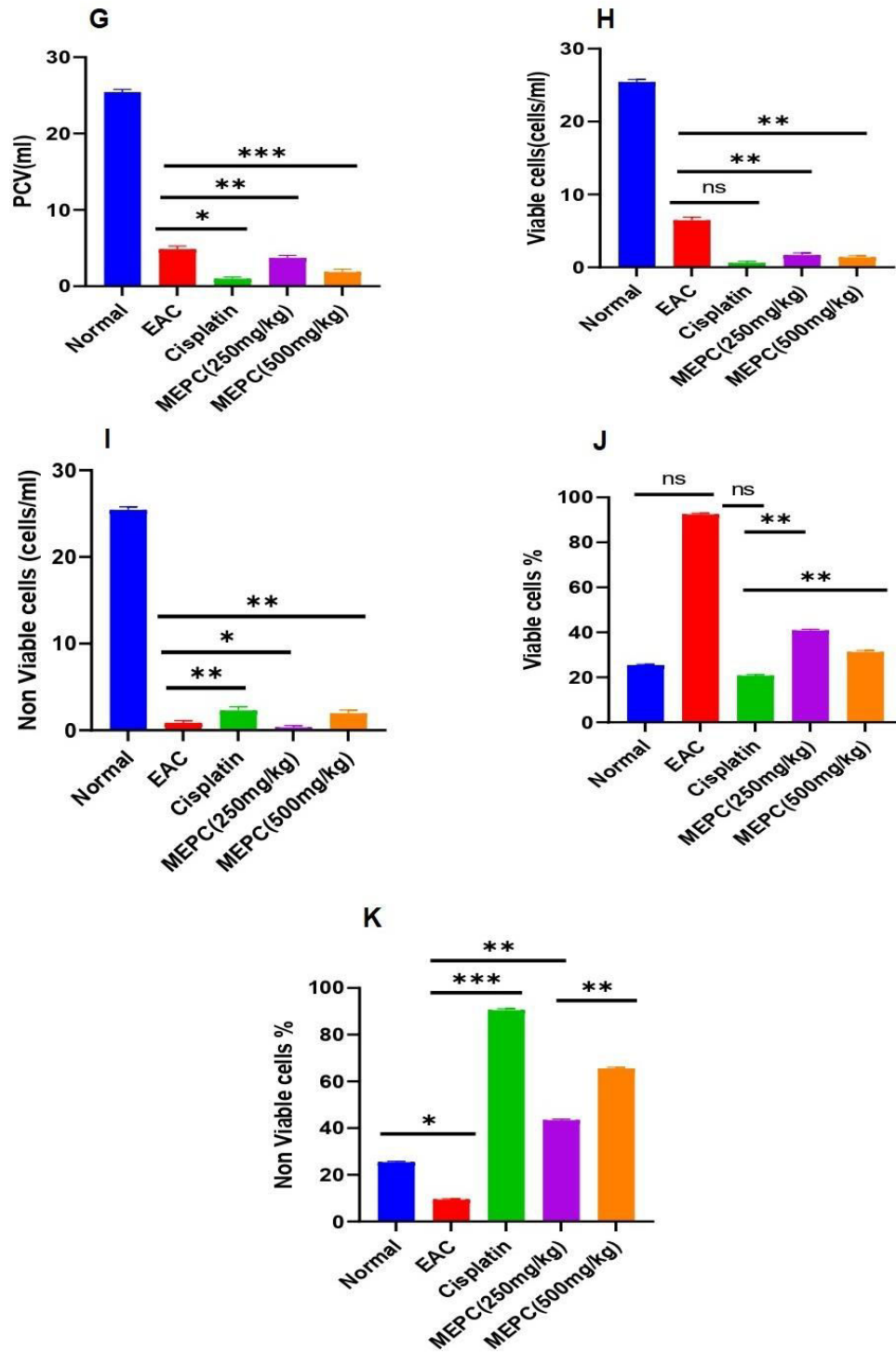


Fig. 1: Effect of MEPC in treatment groups. (A) Avg. body weight, (B) Change in food intake, (C) MST, (D) ILS%, (E) Tumor volume, (F) Tumor weight, (G) PCV, (H) Viable cells, (I) Nonviable cells, (J) Viable Cells %, (K) Nonviable cells%. All results are expressed as mean $\pm$ SEM and one way ANOVA with Dunnett's multiple comparison tests (significance at $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$). The results are compared with those of the EAC group.

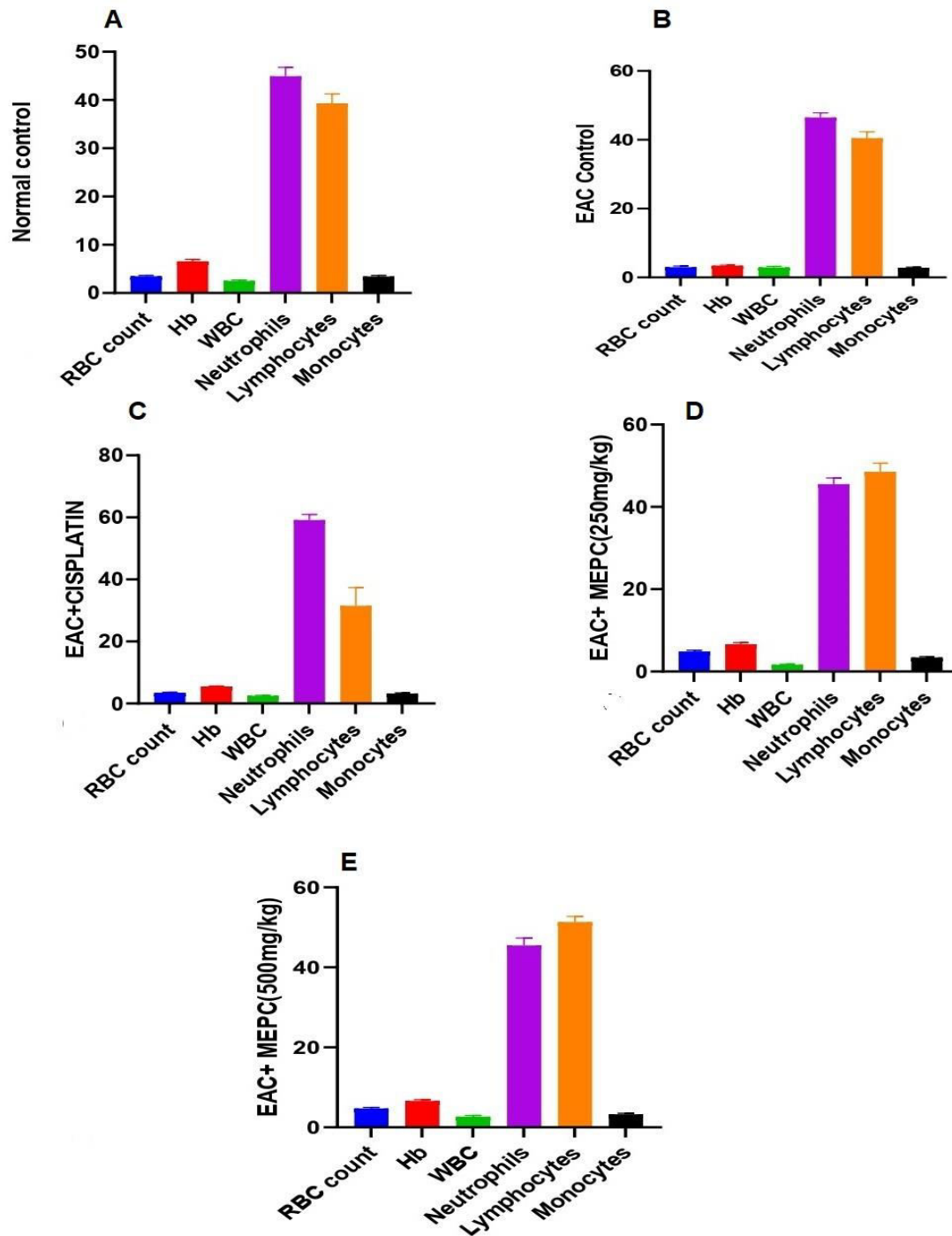


Fig. 2: Effect of MEPC on serum parameters. (A) Normal control, (B) EAC Control, (C) EAC + CISPLATIN, (D) EAC + MEPC (250 mg/kg), (E) EAC + MEPC (500 mg/kg). All results are expressed as mean $\pm$ SEM and one-way ANOVA with Dunnett's multiple comparison tests (significance at $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$). The results are compared with those of the EAC group.

shown in control EAC-bearing mice by a gradual increase in WBC count, erythrocyte count, and hemoglobin content (Fig. 2). Almost all of the RBC count was recovered to normal after receiving 500

mg/kg of MEPC treatment. The groups which received treatment with MEPC (250 mg/kg) had haemoglobin levels within the normal range. The WBC level may decrease with MEPC at a 500

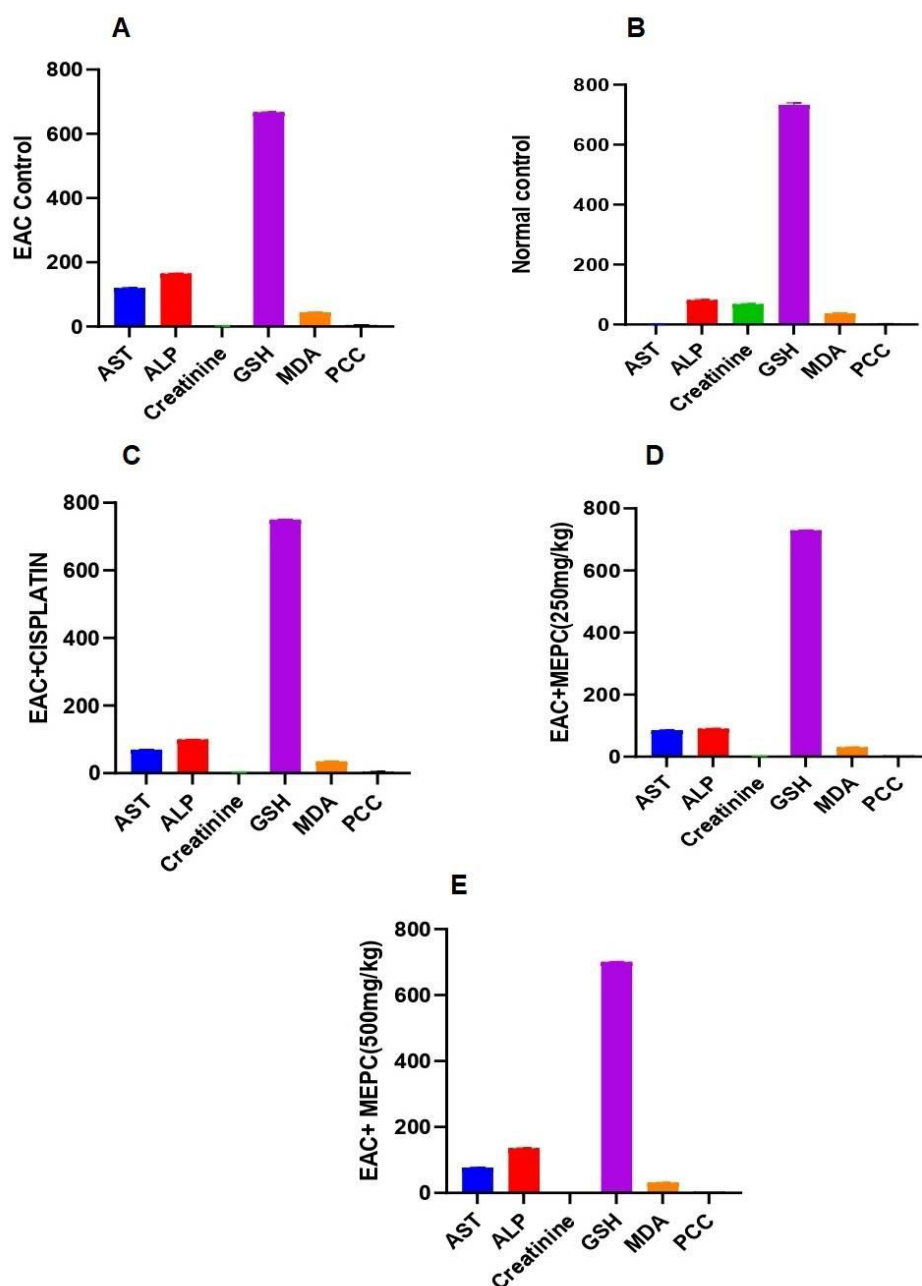


Fig. 3: Effect of MEPC on biochemical parameters. (A) Normal control, (B) EAC Control, (C) EAC + CISPLATIN, (D) EAC + MEPC (250 mg/kg), (E) EAC + MEPC (500 mg/kg). All results are expressed as mean $\pm$ SEM and one-way ANOVA with Dunnett's multiple comparison tests (significance at $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$). The results are compared with those of the EAC group.

mg/kg dosage, although not as much as with conventional cisplatin.

Effect of MEPC on Biochemical Parameters:

It is commonly known that individuals with chronic liver disorders have markedly elevated

levels of alkaline phosphatases or ALPs. Liver disorders are associated with increased transaminases, with serum glutamic pyruvic transaminase (SGPT) much more significant than serum glutamic oxaloacetic transaminase (SGOT) (Fig. 3). The levels of prothrombin complex

concentrates (PCC), malondialdehyde (MDA), and glutathione (GSH) at the lowest and maximum quantities of the plant extract did not differ substantially from those of the regular group.

Cancer has been more common throughout time, and most occurrences are linked to a variety of lifestyle factors, including smoking, eating little to no fruits and vegetables, consuming excessive amounts of alcohol, not exercising, staying outside in the sun, and exposure to toxins in the environment. While there is a moderate global increase in the incidence of cancer, even early detection and diagnosis can improve survival rates and lower fatality rates. New medications or chemical substances mainly targeting cancerous cells must now be developed.

In the present study, an experimental model of Ehrlich ascites carcinoma (EAC) cells was used to examine the anticancer efficacy of *Porteresia coarctata* methanol extracts. Comparing the methanolic extract of *Porteresia coarctata*, a member of the Poaceae family of mangroves, to the control and standard groups, the results indicate a substantial anti-cancer activity against Ehrlich ascites carcinoma (EAC) cells in an animal model. Cisplatin, the conventional medication, significantly raised MST in mice with EAC.

It was noticed that MEPC, at both doses, extended the research animals' survival period. Nonetheless, the most notable improvement was noted at the highest MEPC dose (500 mg/kg). Control mice showed a progressive increase in body weight, up to a maximum rise of 13.18%. The mice treated with MEPC (500 mg/kg) showed a weight gain of just 7.12%, indicating that the treatment effectively delayed the malignancy growth.

The data provide the specific information gathered for each haematological and biochemical parameter. It is commonly known that individuals with chronic liver disorders have markedly elevated levels of alkaline phosphatases or ALPs. Liver disorders are associated with increased transaminases, with serum glutamic

pyruvic transaminase (SGPT) much more significant than serum glutamic oxaloacetic transaminase (SGOT). When toxic hepatitis is present, very high levels are typically found. Compared to the usual cisplatin group, the SGPT and SGOT readings in the MEPC-treated groups stayed within the normal range, indicating the drug's efficacy with minimal adverse consequences. An essential component of evaluating chronic liver disorders is plasma protein content. Since the liver is the repository for synthesised proteins, including albumin and globulins, changes in the protein range are frequently observed in severe liver disorders.

Conclusion

The current study showed that *Porteresia coarctata*'s methanolic extract has remarkable anticancer effects. Therefore, the plant may provide a source of possible targets for studies aimed at cancer therapy. Further investigation on this plant is desperately needed to identify and extract its potent anticancer ingredients. Furthermore, additional research is required to define the intermediary implicated in the mechanism of growth inhibition activity.

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