

Research article

***Oroxylum indicum* stem bark extract exerts antitumor potential against Ehrlich's ascites carcinoma in Swiss albino mice**Sharmila K.P.¹, Shilpa S. Shetty¹, Suchetha Kumari², Madhyastha Harishkumar³, Ashwini Prabhu⁴
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Corresponding author: **Satheesh Kumar Bhandary B.** Email: satheeshbhandary@gmail.com**ABSTRACT****Introduction and Aim:** Constant efforts are exerted to explore unique bioactive principles from natural sources that possess more effective and specific antineoplastic activities. In the present study, we aimed to evaluate the antitumor activity of stem bark extract of *Oroxylum indicum* in mice bearing Ehrlich ascites carcinoma (EAC).**Materials and Methods:** Ninety female Swiss albino mice were categorized into fifteen groups (n=6). The animals were inoculated with 1x10⁶ EAC cells. Tumor control animals received sterile water once daily for 10 consecutive days. Positive control group was injected with Cisplatin (CP) (one dose – 3.5 mg/kg body weight). The treatment groups were administered with *O. indicum* (OI) stem bark ethanol extract once daily with 50mg/kg, 200mg/kg and 400mg/kg body weight for eleven consecutive days. The blood parameters and serum hepatic enzymes activity was determined. The percentage increase in weight, the median survival time, the increase in median life span was calculated. The cytotoxic effect of CP and OI extract was determined.**Results:** There was significant reduction in the white blood cells count in OI and CP treatment group compared to increased level in EAC control group. The RBC count and Hemoglobin level which was significantly decreased (p<0.05) in the tumour mice, was enhanced in the drug treatment groups. The EAC control group showed significant increase in tumour cell count (p<0.05) whereas, treatment of EAC tumor bearing mice with OI and CP significantly increased the non-viable tumor cell count (p<0.05).**Conclusion:** OI stem bark ethanol extract reduced the toxic implications of Ehrlich ascites carcinoma, reverted the hematological and biochemical changes induced by tumour. These results call for additional research on isolating and identifying the responsible bioactive elements in order to clarify the underlying processes of the anticancer impact.**Keywords:** *Oroxylum indicum*; cisplatin; Ehrlich ascites carcinoma; hepatic enzymes; hematology.**INTRODUCTION**

The effectiveness of medicinal plants in curing practically all human illnesses has been established. Numerous recognized and unidentified phytochemicals have reportedly been extracted, isolated, and clinically investigated for their potential therapeutic benefits (1).

The bioactive chemicals that have been extracted from various plant sections have been used to create a number of contemporary medications (2). These phytomedicines are employed in traditional medical systems because they are readily available, highly specific, and effective in actions with less side effects and lower prices than synthetic pharmaceuticals. The rise of lifestyle disorders like diabetes, cardiovascular problems, and cancer has brought attention to ongoing efforts to develop potential remedies utilizing natural resources. In the world, cancer has become one of the

main causes of death, taking over 6 million lives each year (3). In the series of malignant diseases, the patients suffer from the unregulated multiplication of abnormal cells which invade the body and initiate atypical progression at other sites (4). The current treatment modality for this deadly disease (chemotherapy, radiotherapy, and surgery) possesses some drawbacks, such as high cost, unavailability in the resource-limited countries, loss of normal cells, development of secondary malignancies and serious post-treatment complications, thus increasing the demand for the introduction of novel drugs (5). The alternative approach is the screening of potential candidate plants used in traditional systems of medicine for novel pharmacologic activities. Several clinically promising phytochemicals, such as vinca alkaloids (vincristine and vinblastine), bleomycin, paclitaxin, taxol, *etc.*, are currently used for treating several neoplastic diseases in humans. Some unique bioactive compounds from natural sources possess

more effective and specific antineoplastic activities. Due to this property, continuous efforts are being exerted globally for their exploration.

Oroxylum indicum (L.) Kurz is distributed in southeast and South Asian nations. It is indigenous to India and is grown all throughout the country's forested areas, primarily in the Western Ghats and the foothills of the Himalayas. The Indian government has designated it as a medicinal plant that is fragile. The Indian people use *O. indicum* extensively to cure a variety of illnesses (6).

The wide traditional usage of *O. indicum* and the lack of scientific study on this medicinal plant were the main factors that encouraged us to study the antitumor activity of stem bark extract of *O. indicum* in mice bearing Ehrlich ascites carcinoma. Further, the antineoplastic effects in this study was particularly carried out in the advanced stage of tumor growth with Ehrlich ascites carcinoma, since cancer is mostly detected in late stages.

MATERIALS AND METHODS

The entire experiments were carried out at the Central Research Laboratory, K.S Hegde Medical Academy after obtaining ethical clearance from the Institutional Animal Ethics Committee, K.S Hegde Medical Academy (Ref: KSHEMA/IAEC/17/2017).

Plant material and extraction

Oroxylum indicum (OI) stem bark was collected from Mangaluru region. The fresh stem bark was cut into small pieces, thoroughly cleaned with distilled water to eliminate any remaining plant matter before being allowed to air dry for five days at 27°C–30°C. The dried stem bark was ground into a fine powder using a mixer grinder and then subjected to Soxhlet extraction using 99% ethanol for 72 hours. The stem bark extract was dried using a rotary flash evaporator (Rotavap superfit PBU-6) for 15 minutes at 60°C before being placed in an airtight container and kept in the fridge until needed. A taxonomist from Alva's College in Moodubidri, Dakshina Kannada, recognized and verified the stem bark.

Experimental work

Female Swiss albino mice (n=90), ranging in age from four to six weeks and weighing 25 ± 5 g, were chosen from the animal house facility. The experimental chamber, which had a temperature of $23 \pm 2^\circ\text{C}$, controlled humidity levels, and a cycle of light and dark lasting 12:12 hours, was acclimated by the animals. The mice were kept in sterile polypropylene cages with bedding made of sterile rice husk, with a maximum of six mice per cage. The mice were given free access to water and autoclaved normal mice diet pellets. The animal experiments were carried out according to the guidelines of the Institutional Animal

Ethics Committee (IAEC) after obtaining the ethical clearance (Ref: KSHEMA/IAEC/17/2017).

Ehrlich Ascites Carcinoma (EAC) cells were initially procured from the Radiobiology laboratory, KMC, Manipal, Karnataka, India. They were maintained by weekly intraperitoneal (*i.p.*) inoculation of 10^6 cells/mouse.

Acute toxicity studies

The acute toxicity studies were conducted to determine the safe dose of *O. indicum* (OI) stem bark extract according to OECD guidelines (7). Six female Swiss albino mice were allowed to fast by removing food and water for 18 hours. The oral drug administration (OI - 2000 mg/kg body weight) was followed immediately by the administration of food and drink to the animals. To prepare the doses, distilled water was used, and the dose volume was limited to 1 mL per 100 g of body weight. Following administration of the test drugs, the animals were continually monitored for the first 4 hours, 24 hours, and 48 hours to observe for any changes in overall behavior or physiological activity and mortality (8).

Analysis of hematological parameters

Female Swiss albino mice weighing 20 ± 5 g and aged 4–6 weeks were used as the test subjects. Each mouse received 1×10^6 EAC cells in 0.1 ml of physiological saline. They were divided into the subsequent groups (n = 6).

- Group I - Tumor control: The animals of this group were orally administered sterile water once per day for a total of 11 days.
- Group II – Positive control: The animals in this group received a single injection of 3.5 mg/kg body weight of cisplatin.
- Groups III, IV and V were orally administered with *O. indicum* stem bark extract at doses of 50 mg/kg, 200 mg/kg, and 400 mg/kg body weight respectively once daily for 11 consecutive days, with the first administration beginning 24 hours after tumour inoculation.

All the test animals were routinely checked for changes in body weight, toxicological symptoms, and mortality. After tumour inoculation, the weights of the animals were measured every third day across all groups. Blood was drawn from each mouse by cardiac puncture with the anticoagulant heparin. The total white blood cell count (WBC), differential leukocyte counts, total red blood cell count, and hemoglobin content was measured using veterinary blood counter. (ERMA INC. model PCE-210VET, Japan).

Determination of hepatic enzymes activity and biochemical markers

Serum was separated, and using a semi-automatic analyzer (Star 21 Plus Rapid Diagnostics) and a

commercial kit in accordance with the manufacturer's instructions, the activity of SGOT, SGPT, and ALP was assessed. A semi-automatic analyzer was used to determine the amounts of total protein, uric acid, and triglycerides in the separated serum using a commercial kit and following the manufacturer's instructions.

Determination of mean survival time (MST) and average body weights of tumor bearing mice

Six mice per group were used in a separate experiment to determine how cisplatin and OI extract affected the length of survival time. The percentage increase in body weight was calculated using the formula - percent increase in weight = [(animal weight on corresponding day/animal weight on day 0) - 1] 100. (9). The formula: [first death + final death in the group]/2 was used to compute the median survival time (MST), as previously stated by Uma Devi et al. The formula (MST of treated mice - MST of control) x100/MST of control was used to calculate the increase in median life span (percent IMLS) as previously described by Uma Devi et al. (10).

Effect on the tumor cell viability in vitro

Six mice per group were used in a separate experiment to investigate the cytotoxic effects of the test substances as detailed earlier by Mazumdar et al., 1997. (11). On the 11th day of tumour development, 0.1 mL of ascitic fluid was aseptically aspirated. Trypan blue and cell suspension were combined thoroughly in equal parts. Hemocytometer was charged with the diluted suspension. Under a microscope, the viable cells (unstained) in the WBC chamber were counted, and the average number of cells in the four chambers was determined as follows:

Total number of cells = mean number of cells × dilution factor × 10⁴.

Statistical analysis

The obtained results were analyzed by ANOVA test and expressed as Mean ± SEM. Tukey's multiple comparison test was used for intergroup comparison by Prism 7.0 software. A 'p' value < 0.05 was considered statistically significant. For all *in vivo* experiments, six animals were used per group.

RESULTS

Effect of OI Ex on body weight changes in EAC bearing mice

After the tumour started to develop on day 5, a continuous rise in body weight was seen up to end of the study (11th day). The maximum gain of body weight was observed in the EAC control group. In case of OI 200 mg treated group, a significant decrease in body weight was observed (p < 0.001) on day 9 and in CP treatment group a significant decrease

in body weight was observed (p < 0.001) on day 6 and day 9 compared to EAC mice (Table 1).

The WBC count in the EAC tumor bearing mice was found to be elevated compared to the control mice which was significantly different (p<0.05). OI-200 mg, OI - 400mg/kg body weight and CP 3.5 mg significantly reduced (p<0.05) the WBC count compared to EAC group. RBC count and hemoglobin level which was significantly decreased (p<0.05) in the tumor mice was enhanced in the drug treatment groups. The significantly decreased level of platelet counts in the tumor bearing mice did not show any improvement in the drug treatment groups (Fig.1).

Effect of (OI Ex) on hepatic enzyme activity in EAC bearing mice

We observed elevated activity of SGPT, SGOT, ALP in EAC control group. However, the SGPT activity in the control and Cisplatin treatment groups decreased significantly (p < 0.05) compared to tumor control. Whereas the OI extract treated groups did not exhibit significant changes in SGPT activity. The tumor control group exhibited increased SGOT activity whereas the control, CP and OI Ex treated animals showed significant decrease in the SGOT activity (p<0.05). No significant changes were observed in alkaline phosphatase activity in the Control, CP, OIEx treated groups compared to tumor control (p>0.05) (Fig. 2). Total protein level also did not exhibit any significant changes between the tumor control and treatment groups. There was a significant increase in the uric acid level of the tumor control group whereas the control and treatment groups showed significant decrease in the uric acid level (p<0.05). The serum triglyceride level decreased in the tumor control group whereas the control and treatment groups showed increased level of triglycerides (p<0.05) which was significantly different (Fig. 3).

Table 1: Effect of OI Ex on body weight changes in EAC bearing mice

Group	Percent increase in weight as compared to 0 th day		
	Day 3	Day 6	Day 9
EAC control	6.42±0.45	19.94±4.71	27.54±5.06
OI-50	4.48±1.74	14.20±2.28	23.42±3.65 ^a
OI-200	4.60±0.40	7.65±1.73	10.85±1.0 ^a
OI-400	3.66±2.32	11.18±4.44	11.86±3.42
CP-3.5	2.81±1.07	3.99±1.59 ^a	4.51±1.47 ^a

In the present study, significant increase in body weight, ascitic tumor volume, tumor weight and tumor cell count were observed in EAC control (p<0.05). Treatment of EAC tumor bearing mice with OI extract and cisplatin significantly decreased the body weight, viable tumor cell count and increased the non- viable tumor cell count (p<0.05).

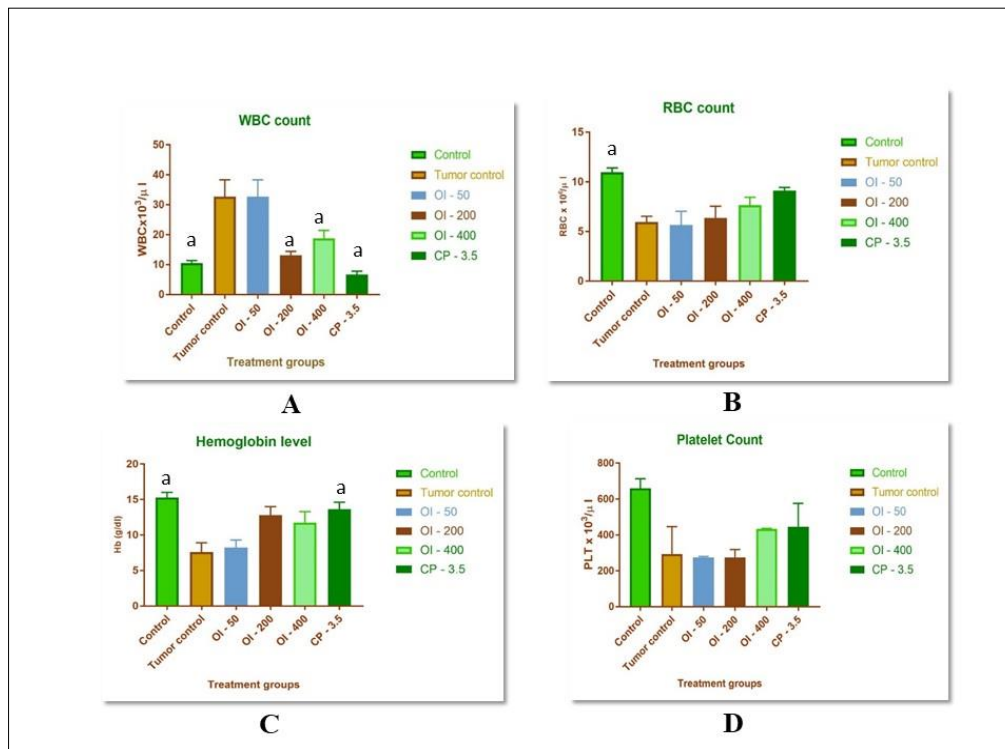


Fig. 1. Effect of OI Ex on Hematological parameters such as WBC count (1A), RBC count (1B), Hb level (1C) and Platelet count (1D) in EAC bearing mice. Values are expressed as Mean ± SEM, (n=6 per group), a = p<0.05 as compared to EAC tumor control group.

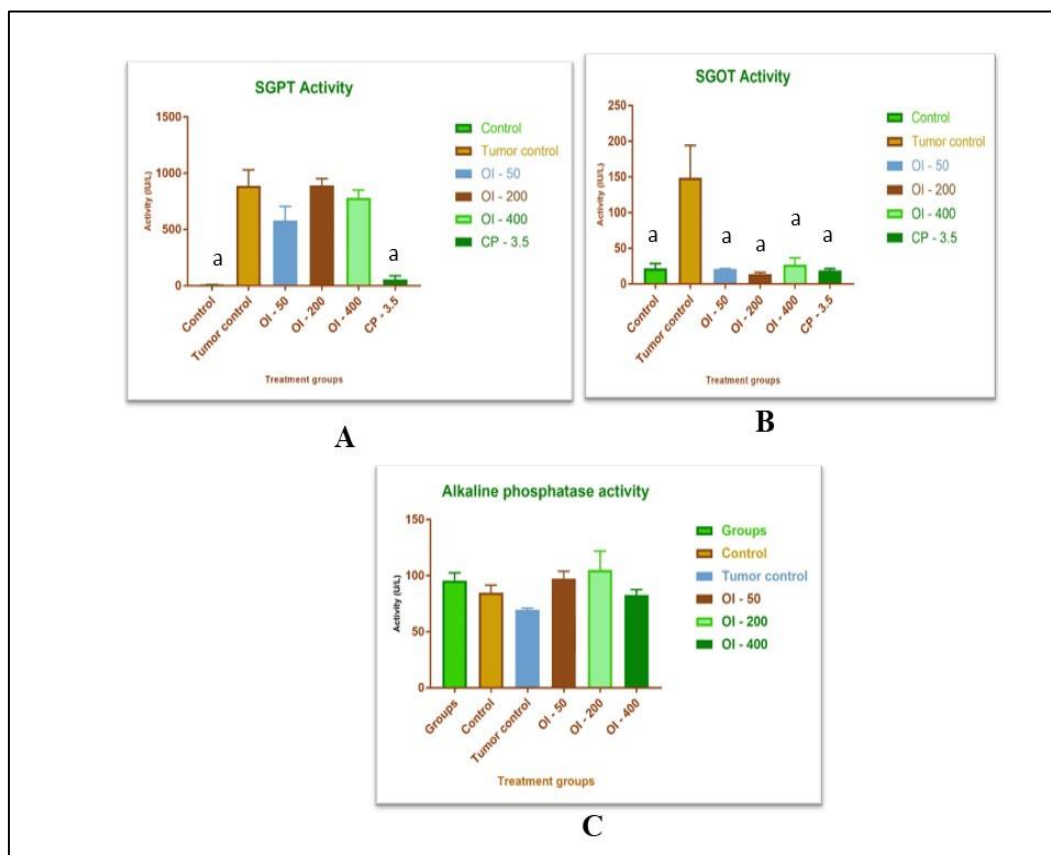


Fig.2: Effect of OI Ex on hepatic enzyme activity such as SGPT (2A), SGOT (2B) and alkaline phosphatase (2C) in mice with EAC. Values are expressed as Mean ± SEM, (n=6/ group), a= p<0.05 as compared to EAC tumor control group.

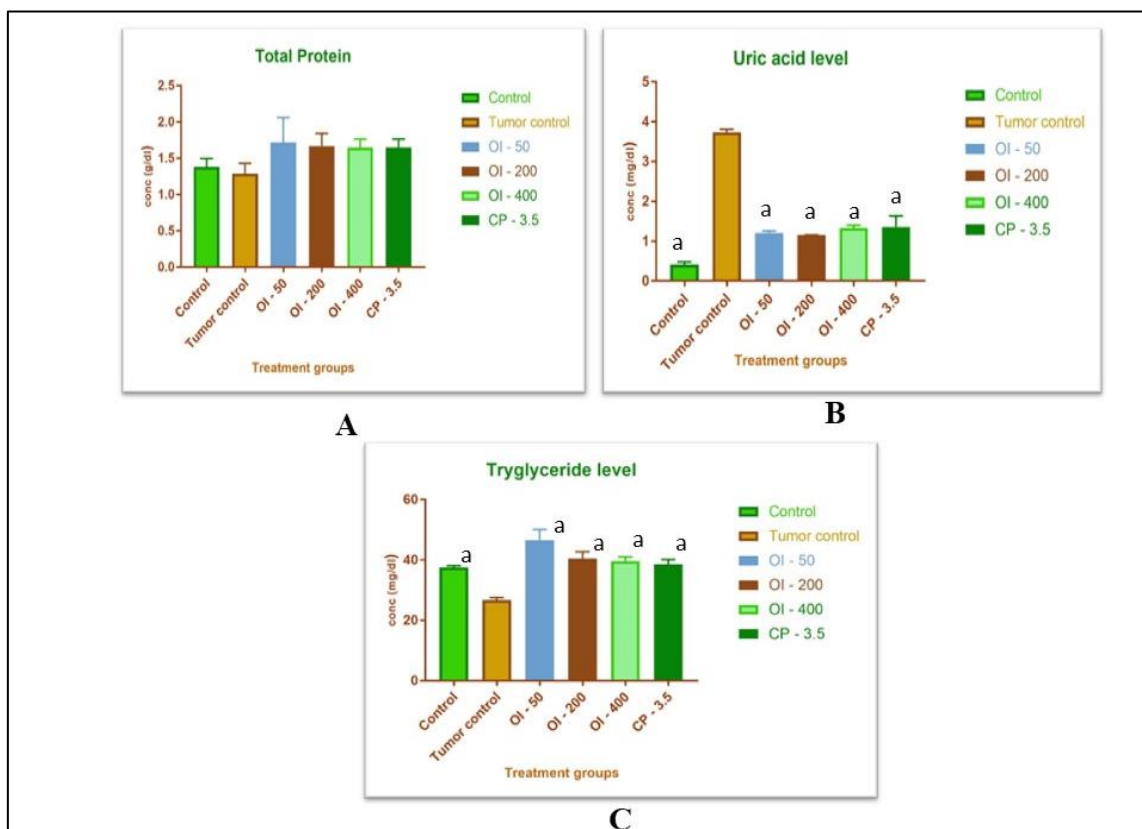


Fig. 3: Effect of OI Ex on Total Protein (3A), Uric acid level (3B) and Triglyceride level (3C) in EAC bearing mice. Values are expressed as Mean \pm SEM, (n=6 per group), a = p<0.05 as compared to EAC tumor control group

Table 2: Effect of OI extract on survival time in EAC bearing mice

Groups	Median Survival time (MST) in days	Increase in median life span (%IMLS)
EAC control	15	—
OI 50	15	0
OI 200	20	33.33
OI 400	17.5	16.66
CP-3.5	27.5	83.33

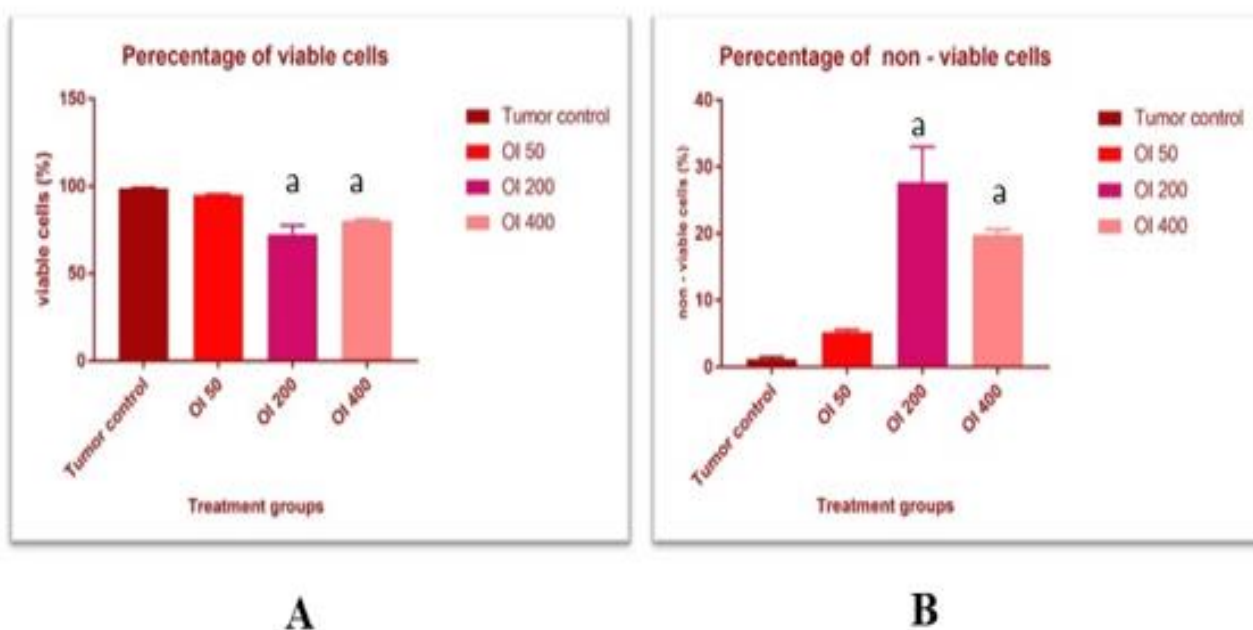


Fig.4. Effect of OI Ex on cell viability in EAC bearing mice (4A & 4B) by trypan blue dye exclusion assay. Values are expressed as percentage, (n=6 per group), a = p<0.05 as compared to EAC tumor control group

DISCUSSION

Ascitic fluid is an essential nutritional requirement for the growth of EAC cells and a rapid increase in ascitic fluid would be a means to meet the nutritional requirement of tumor cells (12).

Mice with tumours gained more weight because of an increase in ascitic fluid volume. In the current study, the EAC control group showed an increase in body weight, ascitic tumour volume, tumour weight, and tumour cell count. OI extract and cisplatin treatment dramatically reduced body weight, the number of viable tumour cells, and raised the number of non-viable tumour cells in mice carrying EAC tumours. These findings point to either a direct cytotoxic effect of OI extract and CP on tumour cells or a local indirect effect that may include macrophage activation and suppression of vascular permeability (13).

Anemia and myelosuppression caused by hemolytic or myelopathic diseases have frequently been seen in ascites carcinoma due to iron shortage, which ultimately results in a decrease in the amount of RBC. The Hb content, RBC count, and WBC count all returned to normal following treatment with OI extract. These findings from our study indicate the haematoprotective properties of OI extract (14).

Serum enzymes are crucial neoplastic diagnostic indicators and for understanding the state of the disease. According to a number of studies, tumour cells harm the liver and disrupt the metabolism of hepatic cells, which alters the activity of serum enzymes. Similar findings were found in our investigation, which revealed higher SGPT, SGOT, and ALP levels in the EAC control group. It is possible to interpret the elevated levels of these biochemical indicators because of EAC-induced hepatocellular damage. Treatment with cisplatin and OI extract at all three doses returned the increased SGOT activity to normal range, showing protection against hepatotoxicity caused by tumour cell generated toxicity. (15).

Total protein levels significantly decreased in tumor-bearing animals, which may be explained by enhanced neoplastic cell mitosis, high blood flow and capillary permeability, which allow plasma proteins to escape into the peritoneal cavity, as well as hepatic cell necrosis (16). The nuclear degenerative alterations could be the cause of reduced total protein level that were seen in both the current investigation and previous studies (17). Similarly, Habib *et al.* (18) found that mice carrying EAC had significantly lower levels of total protein and albumin.

The constant standards for evaluating the effectiveness of any anticancer medication have been life expectancy extension and a drop in WBC (19). The life span of the mice with EAC was clearly extended when OI extract was given to the animals by limiting

the activity of the EAC cells. Animals with cancer may live longer if nutritive fluid volume is reduced and tumour growth is stopped. A similar effect was shown in EAC-bearing mice treated with OI extract (20). The plant extract was successful in lowering the ascites fluid volume, increasing the percentage of life span, and decreasing the number of viable cells, all of which support its anticancer capabilities. Trypan blue dye exclusion assay for *in vitro* cytotoxicity studies revealed that *O. indicum* extract is toxic to the EAC (21).

Patients with malignancy frequently experience disturbances in their glucose metabolism, and hypoglycemia is a hallmark of all cancers (22). Uncontrolled gene expression raises the concentration of proteins, and as cells divide more quickly, the level of uric acid rises in EAC-bearing animals. Since liver is the immediate target organ affected by fast growing EAC cells, it causes alteration in the lipid profile (23). In our study, we found that the OI extract treated animals restored the biochemical alterations caused by EAC cells. This demonstrates the anticancer efficacy of OI extract on EAC cells.

The bioactive substances Scutellarein, Baicalein, Oroxylin A-7-O-beta-D-glucuronide, Hispidulin, Oroxylin B, Chrysin derivative, and Oroxylin A, which were found in the extract by LC-MS, may be the cause of the anticancer potency of OI stem bark extract (24).

CONCLUSION

The current discovery that *Oroxylum indicum's* ethanolic stem bark extract has tumour growth inhibitory activity is highly encouraging and verifies the plant's traditional use as a medicine, which may be explained by the combined effects of the phytochemicals it contains. The potency of the active substances (Scutellarein, Baicalein, Oroxylin A-7-O-beta-D-glucuronide, Hispidulin, Oroxylin B, Chrysin derivative, and Oroxylin A) may be increased by certain minor compounds, which may have an additive or synergistic effect, lessen the toxic effects of the treatment, reverse the changes in haematological parameters caused by tumours, and provide significant benefit. These findings call for additional research on isolating and identifying the bioactive component(s) that are responsible for the anticancer action in order to clarify the underlying mechanism(s).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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