RESEARCH ARTICLE

Studies on *Smilax perfoliata* and *Breynia retusa* against Experimentally Induced Cancer in Swiss Albino Mice

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ABSTRACT

The anticancer effect of methanolic extracts of *Smilax perfoliata roots* and *Breynia retusa* bark against Ehrlich ascites carcinoma (EAC) was investigated in the present study. The procured plant material was subjected to soxhlet extraction using methanol. Albino mice were divided into 7 groups of 6 mice (n = 6) in each group. Group I served as a control for about 14 days, and groups II to VII were administered EAC cells and the standard drug doxorubicin (0.3, i. p.) and test extracts at two doses 200 & 400 mg/kg daily. On last day of the experiment, mice were sacrificed for antitumor activity, tumor volume, cell viability, mean survival time and life span. Hematological and biochemical parameters were estimated. Liver sections were prepared and examined for histological changes. The antitumor effect of both the test extracts was dose-dependent. The volume of the tumor and cell viability were significantly reduced (**$p < 0.001$), whereas the mean survival time and life span were raised in a significant manner (**$p < 0.001$). The hematological parameters such as hemoglobin content, RBC, monocytes and lymphocytes were raised while WBC and neutrophils were reduced significantly (**$p < 0.001$) and the levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (SALP), and bilirubin were reduced significantly after the treatment with the test drugs. Histopathological study revealed the restoration of the structure of the liver in the post-treatment animals. It was concluded that the methanolic extract of *S. perfoliata roots* and *B. retusa* bark exhibited antitumor activity, which might be due to the presence of alkaloids, glycosides, flavonoids, phytosterols, phenolic compounds phytosterols, carbohydrates and tannins. Further investigation of bioactive compounds is needed to confirm the anticancer properties thoroughly.

Keywords: *Smilax perfoliata*, *Breynia retusa*, Ehrlich ascites cwarcinoma, Hematological profile

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INTRODUCTION

Presently, cancer is considered a blazing health concern and one of the dangerous diseases that is affecting both developed and developing countries.[1] Approximately half of all deaths are caused by malignancies of the pancreas, lung and bronchus, colorectal, and breast tissues.[2] An uncontrolled proliferation of cells and misplacement of cells inclines to cancers.[3] The genesis of cancer is linked to altered oncogenes, DNA repair genes and tumor suppressor genes.[4] Nevertheless, there are many extrinsic factors responsible for the cause of cancer such as harmful chemical substances, nicotine, radiation and spreadable organisms, whilst acquired mutations, hormones and the innate immune system stand as intrinsic factors.[5] There are various conventional treatments available such as chemotherapeutic agents, radiation, hormone replacement therapy and surgical methods, but these present severe side effects.[6] Might be because of the adverse effects and mortality rate, focussing on the research for an alternative therapy with a few side effects was motivated by the researchers. In this regard, natural products have gained importance in many of chronic diseases including cancers.[7]

In the investigation of plants used in the treatment of cancers, the use of Ehrlich ascites carcinoma cells was under research.[8] These types of cells are transplantable and were tested in mice to screen the anticancer agents. Ehrlich ascites carcinoma (EAC), an animal model of the mammary tumor, which grows aggressively with undifferentiated malignancy as they have no H-2 histocompatibility antigens, effective in all strains of mice.[9] When these cells are implanted, it induces an inflammatory reaction with the generation of ascetic fluid that progress in the murine, ascetic fluid supplies nutrition for tumor growth. Several plant-derived products have been reported against several rodents and human cancer cell lines for potent antitumor activity.[10] By using *in-vivo* and *in-vitro* methods, several natural compounds have been reported to induce cell death in Ehrlich tumor cells. Numerous studies

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have demonstrated the efficacy of these medicinal plants in both clinical and experimental cancer patients.[11]

People who suffer from cancer rely on herbal medicine which is considered as most complementary and alternative therapy. Though the evidence of clinical trials of herbal medicines is lacking for the treatment and prevention of any type of cancer, according to Chinese medicine, herbs may aid in the long life of an individual, present minute side effects and control relapse. According to the ancient medicine system, various herbal medicines exist with medicinal properties.[12] These effects are experienced when herbal medicines are used in concomitance with conventional drugs. Relapse of cancer creates stress in a patient that renders a sort of depression, illnesses associated with hormonal imbalance, altered immune system and worrisome health outcomes which might have an impact on the resistance of the host for progression of cancer. Hence, an approach has to be initiated to investigate herbal medicines to combat cancer and the bioactive principles responsible for their therapeutic effect.[13] In medicinal plants, the presence of tannins, flavonoids, coumarins and alkaloids play a critical role in oxidative stress-related diseases because oxidative stress leads to the development of inflammation which results in chronic diseases like diabetes, cardiovascular and neurological diseases.[14] Therefore, when natural products are opted for the treatment of cancers, they might control oxidative stress, and cellular proliferation and stimulate the immune system.[15] Herbal drugs are reliable for cancer treatment, as they are safe, effective and economical, particularly when the cost of the treatment is extremely high.

Smilax perfoliata Lour (Family - Smilacaceae), is a climbing shrub with tuberous rhizomes, widely distributed in tropical and temperate regions that grow throughout India. It is a sturdy climber with somewhat powerful arms. The stem strengthens the gums by acting as a toothbrush. The tender shoot is used as a blood purifier and is consumed in curries. Stems and roots are used to treat bladder problems, dysentery, and cancer.[16]

Breynia retusa (Family Phyllanthaceae) is commonly known as a cup saucer plant with spreading branches, native to Southeast Asia and China. Substantially, it cures tooth pain, skin inflammation, high glucose levels, arthritis, and diarrhoea and acts as an astringent. The identification of bioactive compounds in the last investigation was carried out by gas chromatography–mass spectrometry (GC-MS methodology.[17]

Therefore, the present study investigated the anticancer properties of two herbal drugs - *S. perfoliata* and *B. retusa* - on experimental animals.

MATERIALS AND METHODS

Extract Preparation

Dr. K. Madhavachetty, a botanist, authenticated the *S. perfoliata* roots and *B. retusa* in from Sri Venkateswara University, Tirupati and a voucher specimen was (Pt 0815&Pt 0846) preserved in herbarium. Firstly, the material (5 g) was shade-dried for seven days at a temperature of 25 to 35ºC, and then powdered. Methanol extraction was carried out using soxhlet apparatus, and the content was filtered. Using a rotary evaporator, the filtrate was dried and lyophilized in powder form.

Preliminary Phytochemical Analysis

The methanolic extracts of *S. perfoliata* roots (MESP) and bark of *B. retusa* (MEBR) were analyzed for the presence of phytochemical constituents.[19]

GC-MS investigation

Based on the phytochemical analysis, the methanolic extracts of *S. perfoliata* roots and bark of *B. retusa* were investigated for the presence of different compounds by GC-MS methodology. It was analyzed and reported.[20]

Animals

Six Swiss albino mice (25–30 g) were included in each group (n = 6), with seven groups in total. To get acclimatized, animals were housed for seven days in an air-conditioning system room, 22 ± 1°C temperature, 55 ± 1% humidity, free access to water, and a pelleted diet. The protocol bearing with number as 1447/PO/Re/S/11/CPCSEA-75/A was approved by the Institutional Animal Ethics Committee (IAEC).

Acute oral toxicity studies

According to OECD guidelines 423, the rats must not be administered with a body weight of less than 2000 mg/kg. There were five groups of animals, with six (n = 6) in each group. Group I served as control, and groups II to V were administered orally with test doses as - 5, 50, 300, and 2000 mg/kg of body weight. Any potential toxic effects were observed in 24 hours following extract administration. Physiological and behavioral parameters such as body weight, urinary output, food and water consumption, respiratory rate, tremors, constipation, and ocular and dermal effects were examined for 72 hours.[21]

Anticancer Activity using *In-vivo* Method

Transplantation of cancer cells

From the National Centre for Cell Sciences (NCCS) Pune, India, Ehrlich ascites carcinoma (EAC) cells were purchased. 2×10^6 EAC cells were suspended in PBS and each mouse was transplanted by intraperitoneal route for every 10 days, on 7th of tumor growth, ascetic fluid was collected.

Grouping of animals and treatment

A total of 7 groups were divided with 6 mice each (n = 6), a group I served as a control (0.9% Nacl 5 mL/kg body weight i. p.), groups II to VII received EAC cells (2×10^6 cells/mouse, i. p.). Group II served as disease control, ensuing a 24-hour period of EAC transplantation. Groups III to VII were administered with standard drug doxorubicin 0.3 (i. p.) mg/kg, and test drugs in two doses of 200 and 400 mg/kg body weight orally for nine uninterrupted days. The animals were fasted for 18 hours after the last dosage, which was 24 hours ago. All the groups of animals except control animals were treated with the standard and test drugs at specified doses for

14 days. Animals were slaughtered by heart puncture on the fifteenth day in order to estimate the parameters related to hematology and biochemistry. The liver was dissected out for histopathological examination.

Tumor and packed cell volume estimation

After the sacrifice of mice, ascetic fluid was collected by centrifugation at 1000 × g at 4°C for 5 minutes for estimation of tumor and packed cell volume.[22]

Cell viability estimation

Ascetic fluid was collected and diluted 100 times with PBS. trypan blue (0.4% in normal saline) was used for staining, and a drop of the diluted suspension was used for counting in Neubauer counting chamber. Cells that absorbed the dye were non-viable, while other cells were considered viable.[23]

Viability was calculated using the formula below:

$$\text{Cell count} = (\text{cell number} \times \text{dilution factor})/(\text{area} \times \text{thickness of liquid film})$$

$$\text{Cell count} = \frac{\text{Cell number X Dilution factor}}{\text{Area X thickness of liquid film}}$$

The %ILS and the median survival time (MST), were calculated using the below formula [29]:

$$\text{ILS\%} = \frac{\text{Avg of survival time in treated group}}{\text{Avg of survival time in treated group}} \text{ X } 100$$

$$\text{Where, Mean survival time} = (\text{first mortality day} + \text{last mortality day})/2$$

Hematological parameters and biochemical parameters

The amount of hemoglobin (Hb), red blood cells (RBC), and white blood cells (WBC) in the collected blood were measured. Leishman-stained blood smears were used to perform differential counts of white blood cells. After allowing the blood to coagulate, the serum was extracted and centrifuged for 15 minutes at 2500 rpm to measure the levels of bilirubin, total proteins, ALP and SGPT.[24,25]

Histopathological study

The treated and control animals were sacrificed and dissected fast. The liver was removed, then washed in normal saline properly, processed, and fixed in paraffin. A thickness of 4 µm liver sections were prepared and stained with hematoxylin and eosin, then examined under a light microscope and taken photographs using a digital microscope.

Statistical Analysis

All the values were represented as Mean ± SEM. One-way ANOVA followed by post hoc Dunnett's test was applied using Graph Pad Prism 5. A *p-value* < 0.05 was considered statistically significant.

RESULTS

The presence of various phytoconstituents, such as flavonoids, carbohydrates, cardiac glycosides, phytosterols, tannins, phenols, amino acids, alkaloids, steroids, proteins and terpenoids was noticed in the ethanolic extracts of *S. perfoliata* roots and *B. retusa* bark.

Acute Oral Toxicity Studies

After 72 hours of dosing the animals used in the main study were observed for any toxic signs or death. Animals were not killed during these days, and physiological and behavioral parameters were normal. Both the extracts showed no toxicity symptoms at the dose of 2,000 mg/kg body weight for further *in-vivo* studies, two test doses for each extract - 200 and 400 mg/kg body weight were selected as therapeutic dose which were 1/10th and 1/5th of this dose. The effect of extracts MESP and MEBR on the body weight at different doses was analysed on days 0, 7 and 14. Not much difference in the weight on 0 days was noticed as compared to 14th day. All the weights were displayed in Table 1.

In the control animals, there was no evidence of tumor and cell volume viability count as indicated by zero. It was not relevant to study the median survival time, so it was not recorded. In the disease-control animals, there was a significant raise in ($^{**}p$ <0.001) tumor volume (5.1 mL), and substantial viable cell count (9.4 million), and this group served as a reference for untreated illness progression. The median survival time was 19.3 days with no increase in lifespan. Doxorubicin-treated animals (0.3 mg/kg, i. p.) presented a significant reduction ($^{**}p$ <0.001) in the tumor volume and viability count. There was an increased median survival time to 41 days with an ILS of 95.8%. MESP at 200 mg/kg showed moderate inhibition of tumor volume (3.5 mL), and reduced the viable count though not significant. The median survival time increased significantly to 24.3 days, which is comparable

Table 1: Determination of body weight of animals on treatment with the test extracts

Treatment& Dose	*MESP*			*MEBR*		
	Day 0 (g)	*Day 7 (g)*	*Day 14 (g)*	*Day 0 (g)*	*Day 7 (g)*	*Day 14 (g)*
Control	34.7 ± 0.23	37.6 ± 0.21	39.6 ± 0.24	33.6 ± 0.18	36.5 ± 0.45	38.4 ± 0.66
5 mg/kg	32.7 ± 0.24	35.6 ± 0.18	38.6 ± 0.36	31.7 ± 0.24^{ns}	34.6 ± 0.25^{ns}	37.4 ± 0.34^{ns}
50 mg/kg	36.6 ± 0.28	38.6 ± 0.17	40.6 ± 0.35	35.5 ± 0.19^{ns}	37.4 ± 0.18^{ns}	39.4 ± 0.38^{ns}
300 mg/kg	32.7 ± 0.15	34.7 ± 0.35	36.6 ± 0.39	31.7 ± 0.24^{ns}	33.6 ± 0.11^{ns}	35.5 ± 0.32^{ns}
2000 mg/kg	36.6 ± 0.16	38.6 ± 0.24	41.6 ± 0.18	35.5 ± 0.37^{ns}	37.4 ± 0.27^{ns}	40.3 ± 0.25^{ns}

Mean ± SEM; ^{ns}p >0.05 considered as non-significant; compared with the control

Table 2: Effect of MESP and MEBR on EAC-bearing mice on tumor-related parameters

Treatment & dose (mg/kg)	*Tumor volume (mL)*	*Packed cell volume (mL)*	*Viable cell count (cells×10^6 /mL)*	*Non-viable cell count (cells×10^6 /mL)*	*Median survival time (days)*	*%Increase of lifespan (ILS%)*
Control	0	0	0	0	-	-
Disease/Positive control	5.1 ± 0.29	3.1 ± 0.27	9.4 ± 0.25	0.5 ± 0.21	19.3 ± 0.27	0
Doxorubicin (0.3, i.p.)	0.4 ± 0.23**	0	0.5 ± 0.25**	1.4 ± 0.22**	41 ± 0.89**	95.8
MESP (200, p.o.)	3.5 ± 0.38 ^{ns}	1.4 ± 0.2**	4.3 ± 0.25 ^{ns}	0.7 ± 0.17**	24.3 ± 1.21**	26.3
MESP (400, p.o.)	2.1 ± 0.23 ^{ns}	1.1 ± 0.1**	2.5 ± 0.33 ^{ns}	0.8 ± 0.2**	27.2 ± 1.17**	41
MEBR (200, p.o.)	3 ± 0.54^{ns}	1.2 ± 0.15**	3.6 ± 0.46 ^{ns}	1 ± 0.21**	30.7 ± 1.03**	59.2
MEBR (400, p.o.)	1.1 ± 0.38**	0.5 ± 0.2**	1.2 ± 0.15**	1 ± 0.24**	33 ± 1.26**	71.3

Mean ± SEM; $p < 0.001$**, $^{ns}p > 0.05$ considered as significant and non-significant; comparable to control

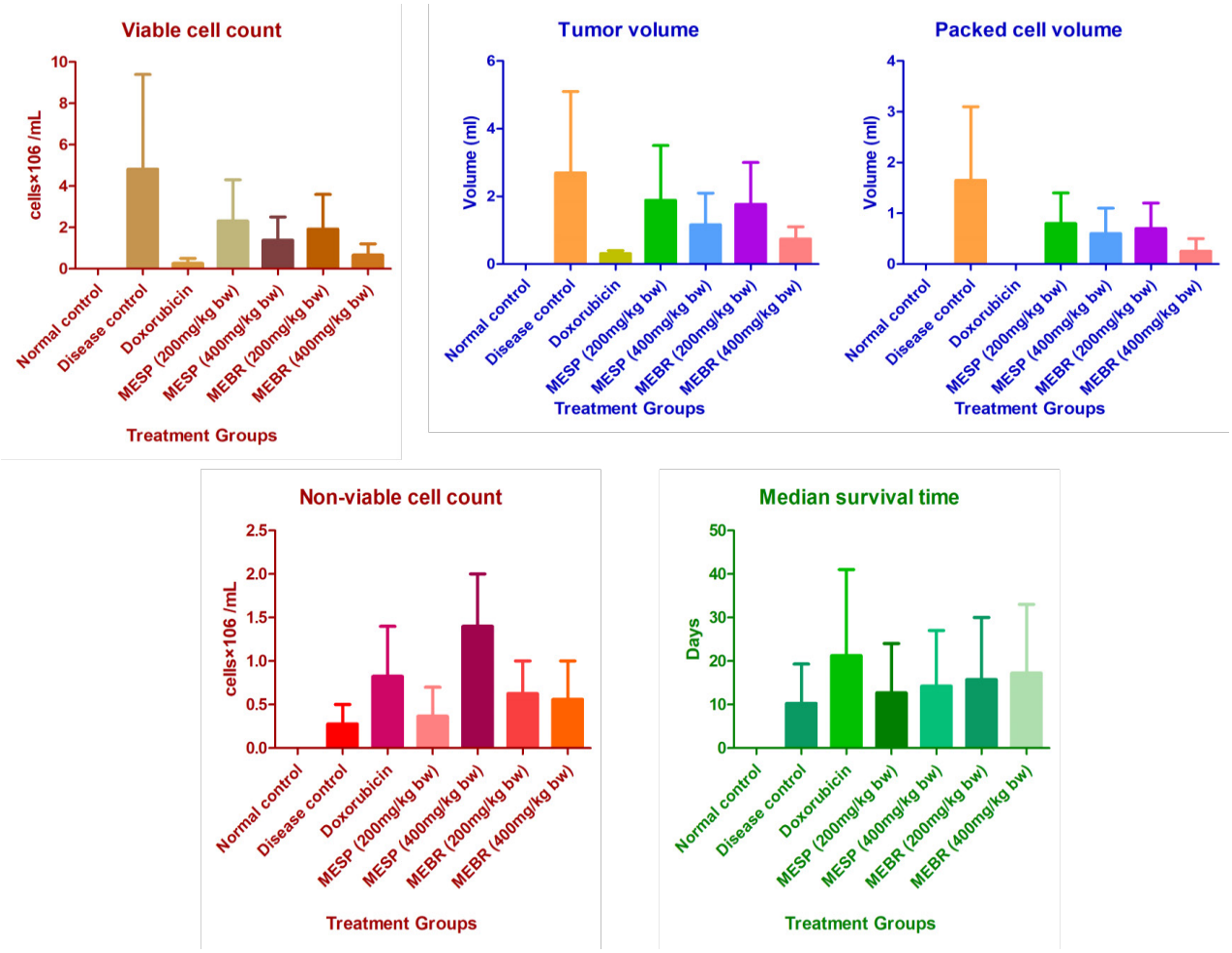

Figure 1: Effect of MESP and MEBR on EAC-bearing mice on tumor related parameters

to diseased control animals. Similarly, at a higher dose (400 mg/kg), there was a reduced packed cell volume (PCV) of 1.1 mL which was significant ($^{**}p < 0.001$) as compared to diseased control. The median survival time was also increased to 27.2 days with an increase in %ILS of 41. MEBR at the dose of 200 mg/kg showed a moderate inhibition of tumor volume (3 mL), and reduced the viable count though not significant. The median survival time increased significantly to 30.7 days compared to the diseased control. Similarly, at higher dose (400 mg/kg), there was a reduced packed cell volume of 0.5 mL which was significant ($^{**}p < 0.001$) as compared to diseased control. The median survival time was also increased to 33 days with an increase in %ILS from 59 to 71.3% (Tables 1 and 2, Figure 1).

The control animals showed normal baseline values in Hb content, RBCs, WBCs, monocytes, lymphocytes, and neutrophils. A significant decrease ($^{**}p < 0.001$) in Hb content, RBC content, an abrupt increase in WBCs, and with a notable

Table 3: Effect of MESP and MEBR on hematological parameters

Treatment & Dose (mg/kg)	*Hb (g/dl)*	*RBC (million/mm^3)*	*WBC (/mm^3)*	*Monocytes (/mm^3)*	*Lymphocytes/µL*	*Neutrophils/µL*
Control	14.4 ± 1.23	5.2 ± 0.27	4700 ± 0.25	2 ± 0.28	76.8 ± 3.61	22.7 ± 1.07
Disease/Positive control	6.8 ± 0.38	3 ± 0.17	9700 ± 0.51	1.5 ± 0.2	34.4 ± 1.96	97 ± 5.53
Doxorubicin (0.3, i. p.)	12.7 ± 0.86**	4 ± 0.46**	5300 ± 0.13**	1.8 ± 0.25**	71.9 ± 3.89**	32 ± 3.77**
MESP (200, p. o.)	7.7 ± 0.56 ^{ns}	3.1 ± 0.18**	8200 ± 0.44 ^{ns}	1.6 ± 0.21**	39.5 ± 2.26**	86.4 ± 1.02^{ns}
MESP (400, p. o.)	8.6 ± 0.36**	3.2 ± 0.2**	7300 ± 0.35 ^{ns}	1.6 ± 0.22**	42.7 ± 2.44**	80.5 ± 4.05^{ns}
MEBR (200, p. o.)	8.3 ± 0.43**	3.1 ± 0.08**	7100 ± 0.21 ^{ns}	1.6 ± 0.22**	42.1 ± 1.62**	78.9 ± 3.97^{ns}
MEBR (400, p. o.)	9.9 ± 0.42**	3.6 ± 0.09**	6000 ± 0.18**	1.7 ± 0.24**	58.8 ± 3.37**	42.2 ± 1.32**

Mean ± SEM; $p < 0.001$**, $^{ns}p > 0.05$ considered as significant and non-significant; compared with the control

Table 4: Effect of MESP and MEBR on biochemical variables

Treatment & Dose (mg/kg)	*SGOT(IU/L)*	*SGPT (IU/L)*	*SALP (IU/L)*	*Total protein (mg/dL)*	*Bilirubin (mg/dL)*
Normal control	33.1 ± 1.57	28.6 ± 0.81	78.4 ± 1.31	9.1 ± 0.48	1.2 ± 0.1
Disease control	80.6 ± 3.79**	76.4 ± 1.4**	119.6 ± 2.25**	4.6 ± 0.25**	3.7 ± 0.21**
Doxorubicin (0.3, i. p.)	37 ± 1.83**	32.7 ± 0.93**	79.4 ± 1.75**	7.7 ± 0.41**	1.6 ± 0.13**
MESP (200, p. o.)	80.8 ± 4.30	71.4 ± 2.08	112.2 ± 2.0	4.7 ± 0.21	3.3 ± 0.06
MESP (400, p. o.)	74.3 ± 2.09**	62.9 ± 1.83**	109.2 ± 2.87**	5.6 ± 0.13	3 ± 0.14**
MEBR (200, p. o.)	54.5 ± 1.95**	64.3 ± 1.88**	96.1 ± 2.53**	6.5 ± 0.15**	3 ± 0.17
MEBR (400, p. o.)	50.1 ± 1.79**	49.9 ± 2.49**	88.4 ± 2.33**	7.4 ± 0.15**	2.5 ± 0.15

Mean ± SEM; $p < 0.001$**, $^{ns}p > 0.05$ considered as significant and non-significant; compared with the control

reduction in lymphocytes and neutrophils was observed in disease control animals. With the treatment of standard doxorubicin, there was an improvement in the Hb content and RBC content near normal values. The WBC count in standard drug-treated animals was found to be closer to normal, with a significant reduction ($^{**}p < 0.001$) in neutrophils and a distinct rise in lymphocytes. The treatment of MESP at low and high doses (200 & 400 mg/kg) showed a slight improvement in the Hb content RBC count as compared to disease control. The number of monocytes and lymphocytes was significantly more ($^{**}p < 0.001$) with both MESP doses than the disease control. The treatment of MEBR at low and high doses (200 & 400 mg/kg) showed an improved RBC count, while the Hb content was raised significantly ($^{**}p < 0.001$) compared to disease control. The number of monocytes and lymphocytes were significantly more ($^{**}p < 0.001$) with both doses of MEBR as compared with the disease control. At a high dose (400 mg/kg), MEBR showed a significant reduction ($^{**}p < 0.001$) in neutrophils, with an improvement in the monocytes and lymphocytes (Table 3).

In disease induced animals, there was a significant elevation ($^{**}p < 0.001$) in serum SGOT of 80.6 IU/L, SGPT of 76.4 IU/L, SALP of 119.6 IU/L and bilirubin of 3.7 mg/dL. However, there was a significant decline in protein content ($^{**}p < 0.001$). There was a significant reduction in the levels of biochemical indices ($^{**}p < 0.001$) in the standard treatment group. When the test drug MESP was given at 400 mg/kg, there was a significant decrease in biochemical variables, and the effect was dose-dependent because there was no significant effect at the dose of 200 mg/kg. The treatment of MEBR at the dose of 200 and 400 mg/kg decreased ($^{**}p < 0.001$) the levels of biochemical parameters. A low level of bilirubin suggests improved liver function or reduced hemolysis (Table 4 & Figure 2).

DISCUSSION

In the histopathological study of liver tissue, positive control liver showed an enlargement of hepatic sinusoids and necrotic tissue. Standard drug treatment showed a remarkable necrotic tissue, which was improved with the treatment. Treatment with the MESP (200 & 400 mg/kg) revealed that there was a reduced inflammation of liver cells with the treatment (Figure 3). Globally, cancer is one of the deadliest diseases with a high death rate, yet the available treatments for it are not up to the mark. Cancer treatment includes a long history of use of plants, currently more than 70% of the anticancer agents are of natural origin.[26] More than 50% of the patients rely on complementary and alternative therapy, especially on herbal medicines. Consequently, researchers and pharmaceutical companies are always looking for natural compounds that are safer to use in the treatment of cancer. Patients' survival times will be extended as cancer treatment approaches advance; however, more and more palliative and curative cancer care options are required. In the present study, albino mice were used for evaluation of the roots of *S. perfoliata* and bark *of B. retusa* at both the doses of 200 & 400 mg/kg for cancer. The test extracts increased the % of life span and survival time significantly as compared to the disease control. A reliable criterion to determine the potential of any anticancer drug was a prolonged lifespan and decline in WBCs. The EAC-bearing mice exhibited a robust increase in ascetic tumor volume was

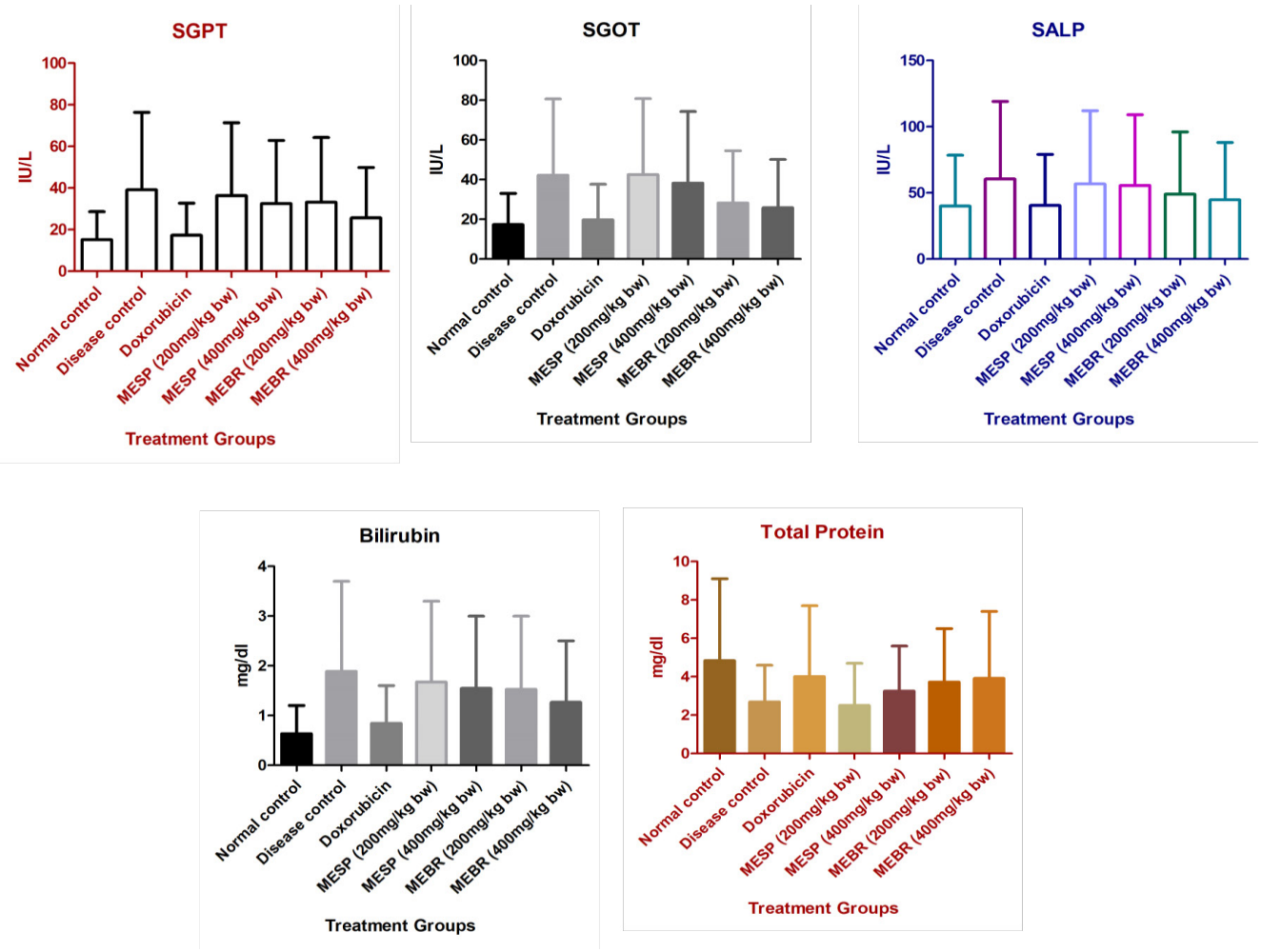

Figure 2: Effect of MESP and MEBR on biochemical variables

noted, as ascetic fluid supplies nutrition to the tumor cells. When the mice were treated with the test drugs MESP and MEBR at lower and higher doses (200 & 400 mg/kg), there was a reduction in tumor and cell volume with the viability, thereby improving EAC-bearing mice's life span. There is a direct connection between the test extracts and tumor cells since an anticancer drug lyses the cells by directly entering the peritoneal cavity and then entering the tumor cells.[27] Ascites carcinoma has been known to frequently exhibit anemia and myelosuppression. In myelopathic or hemolytic disorders, anemia in ascites carcinoma is mostly caused by iron shortage, which can result in and ultimately lower red blood cell counts.[28] The administration of the extracts MESP and MEBR at lower and higher doses increased of hemoglobin and RBC, with a reduced WBC count significantly, so corroborating its protective function against hematopoietic cells without causing myelotoxicity, which was a common adverse reaction to cancer chemotherapy.

The evaluation of the phytochemical constituents revealed the presence of glycosides, flavonoids, phenolic compounds, and tannins in both test extracts. Flavonoids are effective in inflammation and act as antioxidants that conquer free radicals.[29,30] These generated free radicals contribute to carcinogenesis and cause damage to cells by assaulting molecules like proteins, nucleic acids and lipids. According to the studies, flavonoids were reported to possess anticancer effects: The arrest of the cell cycle, apoptosis and autophagy activation, suppression of the growth and dissemination of cancer cells, and acts as scavengers in oxidative stress.[31] Estimation of serum enzymes play a role as diagnostic markers in the diagnosis of diseases. As they are present in other body tissues, they stand as potential biomarkers for identifying several diseases. Abnormal liver enzymes might even lead to mortality and this could be in a non-liver disease such as cancer. Substantial toxins and chronic inflammation cause liver damage at low-grade and also cancers.[32] Whenever an

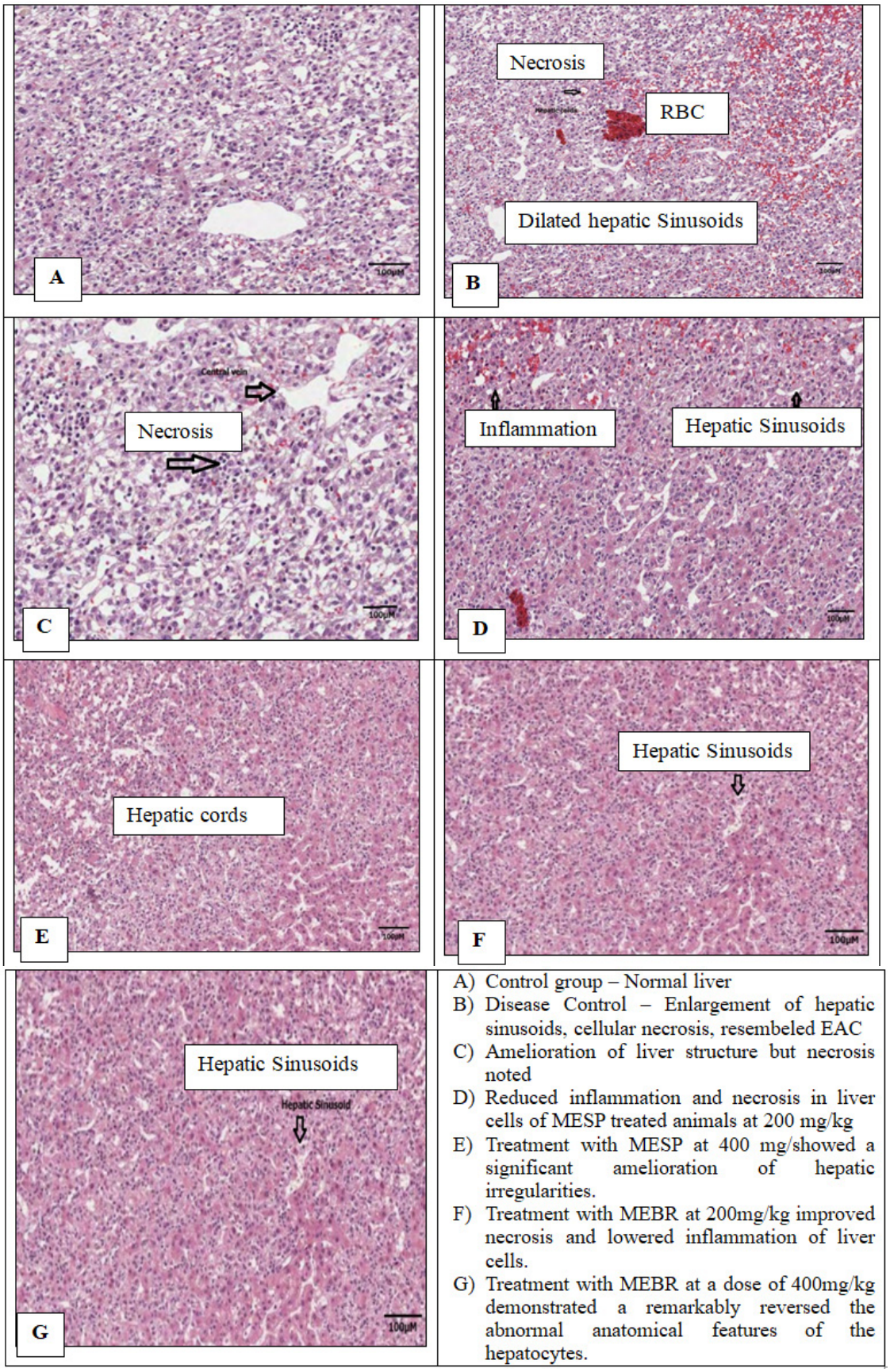

Figure 3: Effect of MESP and MEBR on anatomical features of HE-stained rat liver

imbalance between pro and anti-inflammatory responses exists, this becomes a possible catalyst for the development of cancer.[33] Studies revealed that cancer cells cause changes in serum enzyme activity due to liver damage and disturbances in metabolism. Similar changes were noticed in SGPT, SGOT, SALP, total protein and bilirubin levels in disease-control animals. With the treatment of test extracts MESP and MEBR at lower and higher doses reduced the levels of SGPT, SGOT, SALP and bilirubin significantly, thus showing promising protection against cancer cell lines.

In regard to the histopathological study of liver sections, the diseased control liver was evident with the enlargement of hepatic sinusoids and necrotic tissue. With the standard drug treatment, necrosis was prominent, although there was an improvement in the structure of the liver. Treatment with the test drug MESP (200 & 400 mg/kg) revealed that there was an improvement in inflammation and necrosis of liver cells also with MEBR treatment (200 & 400 mg/kg), there was a reduction in inflammation, necrosis and there was a remarkable reversal of the abnormal anatomical features in the hepatocytes. GC/MS analysis of the test extracts in their previous study identified the existence of active compounds. Hence, further evaluation of these bioactive constituents will yield a thorough pharmacodynamic action of the anticancer effect.

CONCLUSION

Researchers are still working to discover a viable cancer treatment, which is a deadly illness that affects people all over the world. This study proved the effectiveness of *S. perfoliata* roots and *B. retusa* bark as anticancer agents, evident from the presence of phytochemical constituents in the extract. As compared to the EAC cells, both the extracts reduced the tumor volume and viability, whilst the median survival time and %increase in life span was significantly raised. However, further investigations are needed to analyze the activity of isolated compounds.

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