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EFFECTS OF METHANOLIC BARK EXTRACT OF TRADITIONALLY USED MEDICINAL PLANT *PHOEBE GOALPARENSIS* HUTCH. ON SOME OF THE HEMATOLOGICAL PARAMETERS IN DALTON'S LYMPHOMA ASCITES INDUCED SWISS ALBINO MICE

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ABSTRACT

Phoebe goalparensis Hutch. is an important medicinal plant used by the Mising tribe of Dhemaji District, Assam, to treat skin related ailments. The plant material was extracted using methanol as solvent. The methanolic bark extract of ***Phoebe goalparensis*** Hutch. (MBPG) was used for studying its effect on Dalton's lymphoma ascites (DLA) tumorigenic swiss albino mice. The activity of MBPG was studied to observe its effect on hematological parameters-RBC (Red Blood Corpuscles), White Blood Corpuscles (WBC) and haemoglobin (Hb) content in DLA induced mice. Swiss albino mice of both sexes weighing 23-27 g were taken for the study. The experimental animals were divided into groups containing 4 mice (n=4) in each group. Two different doses of MBPG – 100 mg/kgbwt and 500 mg/kgbwt were taken for the study. Other groups constituted of DLA control, 5-Fluorouracil (5-FU)- 20 mg/kgbwt and Normal Control. The study showed that MBPG could significantly increase the Hb, RBC and decrease in WBC content in DLA induced swiss albino mice deciphering the efficacy of the plant extract.

KEYWORDS: Medicinal plants, ***Phoebe goalparensis*** Hutch., Dalton's lymphoma ascites, hematological parameters.

INTRODUCTION

Natural products are indispensable sources in curing diseases as they possess many compounds having essential therapeutic properties. Herbal medicines are used as the primary source of medical treatment in most of the developing countries. From early ages, human communities are dependent on the therapeutic uses of medicinal plants which have been used in folk medicines of Asian and African populations at large. World Health organisations (WHO) has described that most of the places belonging to developing countries rely on medicinal plants based treatment used by traditional healers as sources of therapeutic purposes (Rao *et al*., 2007). Ancient folkmedicines have helped in the development of modern therapeutic drugs which have been exemplified by the beneficial effects of quinine and salicyclic acid from *Cinchona* and willow bark extracts (Hill and Rang, 2013).

The use of traditional medicinal plants has been recorded in ancient Indian medication system specifically ayurveda, siddha and unani. Likewise, Chinese traditional medicinal plants have also been profoundly followed in quest for the discovery of new drugs. Traditional medicinal systems are considered as the first-hand treatment measures in most of the rural areas and it has helped people to incorporate the use of herbal remedies against cancer (Mudur, 1995).

The search for effective therapeutic agents against cancer has led to the screening of approximately 35,000 plant species by the National Cancer Institute (NCI) and amongst them 3,000 potential species have demonstrated chemotherapeutic properties (Desai *et al*., 2008). *Vinca* alkaloids, taxanes, anthra cyclins, podophyllotoxins and its derivatives are the potential drugs against cancer. Vincristine, vinblastine, vindesine and vinorelbine are *vinca* alkaloids of the plant *Vinca rosea* whereas; paclitaxel and docetaxel are obtained from the plant *Taxus brevifolia*. Taxanes derivatives are antimitotic agents that block cancer cell growth by stopping the cell divisions (Abal *et al*., 2017). Vincristine and vinblastine are the potent anticancer drugs which are used to treat leukemia and lymphoma (Desai *et al*., 2008). The response of these drugs in malignant cells has made it popular amongst cancer combating drug discovery scenario. As cancer is one of the leading causes of deaths in the present scenario of the world, the pharmacological derivatives from plants have emerged to be alternative measures against cancer (Aung *et al*., 2017). Globally,

there is a gradual increase in the prevalence of cancer and it is believed that it will surpass other diseases to become the cause of highest numbers of death in the near future. (Murray and Lopez, 1997; Lozano *et al*., 2012; GBD, 2013; WHO, 2017).

Therapeutic effects of medicinal plants against cancer in *in vitro* studies have been estimated using specific cell lines for the targeted cancer cells. Similarly, *in vivo* studies are also performed using animal model for elucidation of anticancer activities of certain drugs or plant extracts. There are many types of animal models used for various scientific researches and Dalton's lymphoma is a mouse tumor model used in anticancer studies. It is a murine T-cell lymphoma used in laboratories to study the potentiality of anticancer agents. Different animal models have been generated by genetic engineering, graft transplantation and viral/physical/chemical induction (Yee *et al.,* 2015). Mouse has been the traditional animal model for basic investigation on the importance and complimentary roles for cancer research (Lunardi *et al*., 2014). It has been extensively studied for haematological malignancies and diverse form of lymphoid neoplasm (Pattengle and Taylor, 1983). It is also relevant that *in vivo* preclinical test in mouse model is a source for searching anticancer agents before applying in humans (Koiri *et al*., 2017). Therefore, Dalton's lymphoma ascites tumor model was taken for the present study. This T-cell murine ascites lymphoma induced in the peritoneal cavity of mouse grows aggressively in the peritoneum and affects other organs of the body through the lymphatic system. When compared with other tumor model, dalton's lymphoma can easily develop the malignancy in albino mouse, and it has emerged as an important murine T-cell lymphoma to study and determine the potential anticancer activities of novel drugs against DLA cells at specific time intervals for proper understanding and elucidation of the lymphoma cells (Koiri *et al*., 2017).

North eastern region of India is a powerhouse of innumerable medicinal sources with its pristine biogeographic province encompassing major biomes recognized in the world (Mao and Hynniewta, 2000; Mao *et al*., 2009). It is regarded as the richest reservoir of biodiversity of India. Assam, belonging to northeastern part of India, is enriched with immense source of traditional knowledge and inhabited by large groups of tribal communities embodied with different cultures and traditions (Nath *et al*., 2009). The tribal Mising population constitutes the second largest tribal group of Assam after the Bodos (Saikia, 2015).

Phoebe goalparensis Hutch. is the medicinal plant selected for the present study on the basis of the information provided by the Mising traditional healers of Dhemaji, Assam. Traditionally this plant has been practised as a remedy for skin related ailments (supposed to be cancer by local healers) by the Mising folklore practitioners. It belongs to the family lauraceae. Locally the plant is known as Bonsum.

Dalton's lymphoma ascites proliferates aggressively in the peritoneal cavity of induced mouse. This leads the DLA bearing mice to suffer from severe anemia (the lymphoma cells create myelosuppression and anemia) (Hogland, 1982; Prince and Greenfield, 1958). The inflammation triggered by the cancer cells instigates the WBC cells for the defensive mechanism which subsequently raise its level to agitate the malignant cells (Manjula *et al*., 2010). In contrary to the rise in WBC content, there was significant decrease in the RBC content. So, this was again due to myelosuppression that has restricted the divisions of RBC content in the blood. Therefore, present study endeavours to evaluate the potential activity of the bark extract of *Phoebe goalparensis* Hutch. on the hematological parameters in Dalton's lymphoma ascites tumorigenic mice.

MATERIALS AND METHODS

Collection of plant material

Plant material for the present study was collected from Dhemaji district of Assam. Dhemaji is situated at $94^0$12'18" E and $95^0$41'32" E longitudes and $27^0$05'27" N and $27^0$57'16" N latitudes. The plant species was identified and a herbarium of the species was submitted in the Dept. of Botany, Gauhati University. Identification and authentication of the plants were done by the Botanical survey of India, shillong, NER, India.

Chemicals

Chemicals used in the present study were purchased from local vendor, N.E. Chemical Company. Conc. HCl, KCl, methanol, NaCl, RBC dilution fluid and WBC dilution fluid were obtained from crest biosystems. 5-Fluorouracil (5-FU), was obtained from HiMedia Pvt. Ltd. Other chemicals used for the experiments were obtained from Merck, Germany and GCC Biotech, India.

Preparation of plant extracts

The methanolic bark extract of *Phoebe goalparensis* Hutch. was prepared by cold extraction method as described by Abdul *et al*., 2008 and Khalili *et al*., 2012 with slight modification. The extract was filtered with Whatman no. 41 filter paper bearing pore size 20-25 µm. Filtrates were concentrated in a rotary vacuum evaporator (BUCHI) under reduced pressured at 40^0 C and finally the yield obtained was transferred to glass vials for longer storage period at 4^0 C in a refrigerator.

Animals

Swiss albino mice of both sexes weighing 23-27 g were considered for the study. The animals were regularly fed with proper diet containing maize bran, rice bran, salt, vitamins, proteins and water *ad libitum*. They were maintained at 27± 2^0C temperature conditions. The experiment was allowed to perform with due permission granted by the IAEC (Institutional Animal Ethics Committee) of Gauhati University bearing the no. IAEC/

PER/ 2012-13/ 158, according to the rules laid and supervised by "Committee for the purpose of control and supervision of experiments on animals" (CPCSEA).

Experiments on animals

Induction of Dalton's lymphoma ascites cells

Dalton's lymphoma ascites cells were maintained *in vivo* in swiss albino mice. Parent cell line of Dalton's lymphoma ascites (DLA) was obtained as a gift from Tumor and Cell Biology laboratory, Dept. of Zoology, NEHU. Ascites cells were washed with Phosphate buffered saline and inoculated into the peritoneum of healthy mice for tumor maintenance. Counting of cells was done in the RBC chamber of Neubauer's haemocytometer slide (Kuttan, 1985). The ascites tumor cell numbers were adjusted to 1×10^6 cells/mouse and induced intraperitoneally into the experimental animals. After 24 h of tumor inoculation the experimental mice were treated with different doses of the test samples.

Administration of test samples

The test samples of different concentrations of the extract were suspended in phosphate buffered saline (PBS) and administered orally. Oral administration was done by feeding the experimental animals with a gastrostomy feeding tube of size 5F G.

Experimental groups

The experimental animals for the study were divided into five different groups. Both male and female swiss albino mice were considered for the experiments and inoculated with the ascites cells for tumor development. The day of tumor induction in the mouse was designated as day '0'. Mice were grouped for 10 days and 18 days experiments.

Table 1: Animal groups for *in vivo* experiment against different treated doses.

Groups' Name (n=4)	Types
I	Normal mice + PBS (Normal control)
II	DLA induced mouse + PBS (DLA control)
III	100 mg/kgbwt of MBPG
IV	500 mg/kgbwt of MBPG
V	Standard Drug 5-FU 20 mg/kgbwt

Preparation of Doses

Phosphate Buffered Saline (PBS) was used as a vehicle for oral administration of different concentrations of MBPG in the study. The dried extract for preparing different doses were weighed with respect to the body weight of each single mouse in a group and prepared accordingly by following the OECD guideline.

Haematological parameters

The activity of Methanolic bark extract of ***Phoebe goalparensis*** (MBPG) was studied to observe its effect on haematological parameters - red blood corpuscles (RBC), white blood corpuscles (WBC) and haemoglobin content in DLA induced mice. The parameters were estimated as per the method followed by Shrivastava and Das, 1987; Mohan, 2005 and Venkateshwarlu *et al*., 2012. Neubauer's haemocytometer chambers were used for counting RBC and WBC cells. Haemokwik kit was used for estimating the values of haemoglobin.

Statistical analysis

Statistical analysis for all the data obtained in the study were analysed by using Microsoft office Excel 2007 and graphpad prism software. The results were expressed as Mean ± Standard error of mean (X ± SEM). The means in negative control and positive control groups were compared with the treated groups and were analysed for significance differences using independent student's *t*-test. Values of $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.001$ were considered statistically significant for all the tests in the study.

RESULT

Effect of MBPG on Haemoglobin (Hb) content

The Hb content of the animals was recorded to find the comparative increase in haemoglobin level in different groups of DLA bearing swiss albino mice with the treatment of MBPG as shown in Table.2. The DLA control animals were found to suffer from low Hb content leading to anemia. It was found that different doses of MBPG- 100 mg/kgbwt and 500 mg/kgbwt subsequently elevated Hb content in the DLA induced mice. 500 mg/kgbwt dose daily administered for 10 days and 18 days, significantly ameliorate Hb constituents in the blood of ascites induced mice. In 10 days DLA control (PBS treated) mice, the result of the Hb level, showed only 6.83 ± 0.46 g/dL which directs the condition to be anemic. Restoration of proper Hb constituent was seen in MBPG treated groups with a rise up to 11.5 ± 0.55 g/dL and 11.6 ± 0.67g/dL. This significant ($p<0.001$, $p<0.0001$) elevation was found in the animals administered with 500 mg/kgbwt MBPG concentration. The rise of haemoglobin content was highly significant ($p<0.0001$) in the standard group treated with 5-FU (20 mg/kgbwt).

Table 2: Hb content in the MBPG treated DLA induced mice.

Groups	10 days Hb content (g/dL)	18 days Hb content (g/dL)
DLA Control	6.83 ± 0.46	5.5 ± 0.23
MBPG 100 mg/kgbwt treated group	^{ns}7.1 ± 0.19	^{ns}6.86 ± 0.46
MBPG 500 mg/kgbwt Treated group	***11.5 ± 0.55**	****11.6 ± 0.67**
5-FU (20 mg/kgbwt)	****12.9 ± 0.53**	****13.3 ± 0.22**
Normal control	14.2 ± 0.21	14.03 ± 0.18

The data are expressed as mean ± SEM where n=4 (triplicate measurements) *p<0.001, **p<0.0001 and ns= non significant, compared to DLA control.

Effect of MBPG on RBC content in DLA induced mice

The analysis for RBC content showed that control DLA bearing animals showed low level of RBC in the blood with values 8.54 ± 0.75 $\times10^6$ cells/mm^3 and 6.8 ± 0.39$\times10^6$ cells/mm^3 for 10 days and 18 days studies. Different doses of MBPG were administered orally for 10 days and 18 days. The lowest MBPG dose (100 mg/kgbwt) significantly (p<0.001) resulted in the elevation of RBC content after 18 days treatment, whereas the 10 days treated groups showed non significant result. The higher dose (500 mg/kgbwt) treated animals significantly (p<0.001; p<0.0001) exhibited profound increase in the RBC content up to 13.83 ± 0.53$\times10^6$ cells/mm^3 and 14.93 ± 0.25 $\times10^6$ cells/mm^3 in 10 days and 18 days dose administration. In the standard drug 5-FU treated groups the results were highly significant (p<0.0001) both for 10 days and 18 days treatment.

Table.3: RBC content in 10 days and 18 days MBPG treated DLA induced groups.

Groups	RBC content (10 days) $\times10^6$ cells/mm^3	RBC content (18 days) $\times10^6$ cells/mm^3
DLA Control	8.54±0.75	6.8±0.39
MBPG 100 mg/kgbwt treated group	^{ns}8.9±1.61	*10.9±0.21
MBPG 500 mg/kgbwt treated group	****13.83±0.53**	*****14.93±0.25**
5-FU (20 mg/kgbwt) treated	***15.67±0.58	***15.7±0.33
Normal control	16.67±0.42	16.66±0.23

The values are expressed as mean ± SEM (n=4) (Triplicate measurement). *P<0.01, **p<0.001, ***p<0.0001 and ns= non significant when compared to DLA control.

Effect of MBPG on WBC content in DLA induced mice

Study on the WBC content showed that the control group mice expressed excessive rise in WBC content in 10 days and 18 days studies (Table.4). MBPG administered for 10 days significantly decreased the WBC content bringing it down to normal level in the DLA bearing mice. The high dose (500 mg/kgbwt) was highly significant (p<0.001) when compared against the DLA control group and the level of WBC was 11.87 ± 0.53 $\times10^3$cells/mm^3. Animal groups exposed to two different doses of MBPG for 18 days recorded comprehensive reduction of the WBC content to normal. 500 mg/kgbwt dose of MBPG showed significant (p<0.001) decrease in the WBC content up to 11.11 ± 0.65 $\times10^3$cells/mm^3. Also, the standard 5-FU (20 mg/kgbwt) significantly reduced the WBC content in 10 days and 18 days with values 10.14 ± 0.45 $\times10^3$cells/mm^{3}and 10.00 ± 0.47$\times10^3$cells/mm^3 respectively.

Table 4: WBC content in 10 days and 18 days MBPG treated animal groups.

Groups	WBC content (10 days)$\times10^3$ cells/mm^3	WBC content (18 days) $\times10^3$ cells/mm^3
DLA Control	14.83 ± 0.34	15.86 ± 0.18
MPG 100 mg/kgbwt treated group	^{ns}13.70 ± 0.28	^{ns}14.31 ± 0.43
MBPG 500 mg/kgbwt treated group	***11.87 ± 0.53**	***11.11 ± 0.65**
5-FU (20 mg/kgbwt) treated group	**10.14 ± 0.45	**10.00 ± 0.47
Normal	10.16 ± 0.28	9.19 ± 0.29

The data are expressed as mean ± SEM (n=4). All the analyses are of three replicate measurement. *p<0.001, **p<0.0001 and ns= non significant compared to DLA control.

DISCUSSION

Present study showed that the methanolic bark extract of ***Phoebe goalparensis*** can restore the normalcy of hematological parameter in the DLA induced mice during the 10 days and 18 days study. In DLA induced mice depletion of haemoglobin content is one of the major detrimental affects owing to development of malignant ascites cell in the animal body. Study on

haemoglobin (Hb) parameter in DLA induced mice scheduled for 10 days and 18 days unveiled the probable antitumor activity by MBPG. The ascites cell proliferation in the DLA bearing mice, is caused due to the lymphoma cells which create myelosuppression and anemia (Hogland, 1982; Prince and Greenfield, 1958). In the study it was found that the dose of 500 mg/kgbwt MBPG, elevated the Hb content in the blood up to 11.5 ± 0.55 g/dL and 11.6 ± 0.67 g/dL in 10 days and 18 days treated animals respectively. This stipulated that micronutrients Fe and Cu present in the extract restored Hb content in DLA carrying mice. Anemia in mice has been caused by the reduction of Hb or erythocytes which is due to deficiency of iron or other physiological conditions such as haemolytic or myelopathic formation in the blood (Fenninger and Mider, 1954). Thavamani *et al*. in 2014 when working with the methanolic extract of the plant *Cissampelos pareira* against DLA induced mice found similar result which restored the Hb content of the blood in DLA induced mice. Also work on *Nathopodytes nimmoniana* against DLA bearing mouse revealed similar findings that has restored the Hb content (Dharmalingam *et al*., 2014). The presence of secondary metabolites or the mineral constituents in MBPG has simultaneously helped in fighting against the ascites cells for homeostasis in the haematological parameters.

The infiltration of ascites cells in the animals sought invasion of foreign body that leads to the stimulation of certain immunological responses, increasing WBC level in the site of inflammation. There was significant rise in the WBC content which has been exaggerated by the reactions to minimize the lymphoma ascites cells in the peritoneal cavity. The inflammation triggered by the cancer cells instigates the WBC cells for the defensive mechanism which subsequently raise its level to agitate the malignant cells (Manjula *et al*., 2010). In contrary to the rise in WBC content, there was significant decrease in the RBC content. So, this was again due to myelosuppression that has restricted the divisions of RBC content in the blood. As we have already discussed that bark material of ***P. goalparensis*** possessed Fe, Cu along with Mg and these have helped to produce and develop normal RBC values in the DLA induced groups. Whereas Mg acts against inflammatory cells by producing significant role in immune defence system and has helped to diminish the elevated WBC in blood to normal (Shenkin, 1995).

CONCLUSION

The findings from the study highlight the efficacy of the methanolic bark extract of *Phoebe goalparensis* Hutch. (MBPG) in restoring the normalcy of hematological parameters in Dalton's lymphoma ascites induced mice. This study gives an insight into the constituents of the plant material that might have helped in gaining the normal level of Hb and RBC and diminishing the elevated WBC. Through these findings it can be said that the traditionally used medicinal plant ***Phoebe goalparensis*** Hutch. could be considered for further interesting studies against Dalton's lymphoma ascites induced mice.

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