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Anti-Neoplastic Activities of *Polygonum hydropiper* Linn. Against Ehrlich Ascites Carcinoma in Swiss Albino Mice

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Abstract: The present study is aimed to evaluate the methanolic extract of *Polygonum hydropiper* leaves (MPHL) for antitumor activity against Ehrlich ascites carcinoma (EAC) in Swiss albino mice. Twenty four hours after intraperitoneal inoculation of tumor (EAC) cells in mice, MPHL was administered at 25, 50 and 100 mg/kg body weight for nine consecutive days. On day 10 half of the mice were sacrificed and rest were kept alive for assessment of increase in life span. The antitumor effect of MPHL was assessed by evaluating tumor volume, packed cell count, viable and non-viable tumor cell count, median survival time and increase in life span of EAC bearing mice. Hematological profiles and serum biochemical parameters were estimated. MPHL showed a significant ($p<0.05$) decrease in tumor volume, packed cell volume and viable cell count and increased the life span of EAC bearing mice. Hematological and serum biochemical profiles were restored to normal levels in MPHL treated mice as compared to EAC control mice. The present study demonstrates that *Polygonum hydropiper* leaves exhibited remarkable antitumor activity that is plausibly attributable to its augmenting endogenous antioxidant mechanisms.

Key words: Antitumor · Antioxidant · Ehrlich Ascites Carcinoma · *Polygonum hydropiper*

INTRODUCTION

Nature, immense source of pharmacologically active molecules, has been used for the treatment of various incurable diseases [1,2]. The worldwide upsurge use of the herbal preparation and medicinal plants with its isolated active compounds have provided one of the most importance sources for pharmaceutical industry for lead compound. Furthermore, over a 100 new products are in clinical development, particularly as anti-cancer agents and anti-infectives [3]. The global trend is also approaching towards natural bioactive substances due to their low toxicity and cost. The exploration of medicinal plants for their therapeutic efficacy still holds the hope for the treatment and prevention of cancer. Although, the mechanism of interaction between phytochemicals and cancer cells has been studied extensively and augmented the interest of pharmacological evaluation of various plants used in Bangladeshi traditional systems of medicine [4].

Polygonum hydropiper Linn. is an important medicinal plant belongs to the family of polygonaceae. It possesses stimulant, tonic, diuretics, anthelmintic and carminative properties [5]. Recently it has been revealed that *P. hydropiper* have remarkable antibacterial, antifungal properties [6]. The leaves of this plant have been used for cancer, colds and coughs. The infusion of an extract of this plant has been prescribed for treating rheumatism, chronic ulcers, haemorrhoids and erysipelas [7]. Presence of some insecticidal properties has also been reported [8]. Moreover, *P. hydropiper* is used for the treatment of wide range of diseases including diarrhea, dyspepsia, itching skin and excessive menstrual bleeding etc. [9]. The whole plant has been found to contain

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flavones and flavonoids glycosides such as quercetin galactosides, a sesquiterpene acid, viscosumic acid, oxymethyl anthraquinones and polygonic acid [10, 11]. From the volatile fraction, many drimane-type sesquiterpenes such as polygodial and warburganal were isolated which possesses strong insect antifeedant and antibacterial activities. [12]. Also several components of this plant have been reported to display various biological activities such as anti-inflammatory (polygonolide) and antioxidative (hyropiperoside, rhamnazin and persicarin). [13-15]. However, no studies to date have been conducted to demonstrate the antitumor activity of *Polygonum hydropiper*. The present study was carried out to evaluate the *in vivo* antitumor activity of the methanolic extract of *Polygonum hydropiper* leaves (MPHL) against Ehrlich ascites carcinoma (EAC) in mice.

MATERIALS AND METHODS

Plant Material: *Polygonum hydropiper* leaves were collected from the adjoining area of Jahangirnagar University Campus, Bangladesh during August 2011. The plant material was taxonomically identified by the National Herbarium of Bangladesh whose voucher specimen no. DACB-3124 is maintained in our laboratory for future reference.

Chemicals and Reagents: Bovine serum albumin and Bleomycin from Sigma (St. Louis, MO); trichloroacetic acid (TCA) from Merck (Mumbai, India); thiobarbituric acid (TBA), nitroblue tetrazolium chloride (NBT) from Loba Chemie (Mumbai, India); 5,5'-dithio *bis*-2-nitro benzoic acid (DTNB), phenazonium methosulfate (PMS), nicotinamide adenine dinucleotide (NADH) and reduced glutathione (GSH) from SISCO (Mumbai, India). All other chemicals and reagents used were of highest analytical grade.

Preparation of Plant Extract: The plant material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve #40 and stored in an air tight container. The dried powder material (1 kg) was refluxed with MeOH for three hours. The total filtrate was concentrated to dryness, *in vacuo* at 40°C to render the MeOH extract (90 g).

Animals: Albino mice (25-30g) were used for assessing biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water *ad libitum.* The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. Animal treatment and maintenance for acute toxicity and anticancer effects were conducted in accordance with the Principle of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Animal Care and Use Guidelines of Atish Dipankar University of Science and Technology, Dhaka, Bangladesh.

Acute Toxicity Study: Acute oral toxicity assay was performed in healthy nulliparous and non pregnant adult female albino Swiss mice (25-30g) divided into different groups. The test was performed using increasing oral dose of the MPHL in water (50, 100, 200, 500, 1000 mg/kg body weight), in 20 ml/kg volume to different test groups. Normal group received water. The mice were allowed to feed *ad libitum,* kept under regular observation for 48 hrs, for any mortality or behavioral changes [16].

Transplantation of Tumor: Ehrlich ascites carcinoma (EAC) cells were obtained from Indian Institute of Chemical Biology (IICB), Calcutta, India. The EAC cells were maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation of 2×10^6 cells per mouse after every 10 days. Ascetic fluid was drawn out from EAC tumor bearing mouse at the log phase (days 7-8 of tumor bearing) of the tumor cells. Each animal received 0.1 ml of tumor cell suspension containing 2×10^6 tumor cells intraperitoneally.

Treatment Schedule: The animals were divided into five groups (n = 12) and given food and water *ad libitum*. All the animals in each group except Group-I received EAC cells (2×10^6 cells/mouse i.p.). This was taken as day '0'. Group-I served as normal saline control (5 ml/kg body weight i.p.) and Group-II served as EAC control. 24-hrs after EAC transplantation, Group-III, IV and V received MPHL at a dose of 25, 50 and 100 mg/kg b.wt., i.p. respectively and Group-VI received reference drug Bleomycin (0.3 mg/kg i.p) for nine consecutive days [17]. Twenty-four hours of last dose and 18 hrs of fasting, 6 animals of each group were sacrificed by cardiac puncture for the estimation of hematological and serum biochemical parameters and then sacrificed by cervical dislocation to measure antitumor and liver biochemical parameters and the rest were kept with food and water *ad libitum* to check percentage increase in life span of the tumor host. The antitumor activity of MPHL was measured in EAC animals with respect to the following parameters.

Determination of Tumor and Packed Cell Volume: The mice were dissected and the ascetic fluid was collected from the peritoneal cavity. The volume was measured using a graduated centrifuge tube and packed cell volume was determined by centrifuging at 1000 g for 5 min.

Viable and Nonviable Tumor Cell Count: The ascetic fluid was taken in a white blood cell (WBC) pipette and diluted 100 times. Then a drop of the diluted suspension was paled on the Neubauer counting chamber and the cells were then stained with Trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those took the stain were non-viable. These viable and non-viable cells were counted.

Cell count = (Number of cells × dilution factor)/ (Area × thickness of liquid film)

Determination of Median Survival Time and Percentage Increase in Life Span: The mortality was monitored by recording percentage increase in life span (% ILS) and median survival time (MST) as per the following formula [18].

$$\% \text{ ILS} = \left[\frac{\text{Mean survival time of the treated group}}{\text{Mean survival time of the control group}} - 1\right] \times 100$$

Mean survival time* = (Day of First death + Day of last death)/2

(*Time denoted by number of days)

Estimation of Hematological and Serum Biochemical Parameters: Collected blood was used for the estimation of hemoglobin (Hb) content, red blood cell (RBC) and white blood cell count [19]. Differential count of WBC was carried out from Leishmen stained blood smears [20].

Serum biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT); serum glutamate pyruvate transaminase (SGPT) [21], serum alkaline phosphatase (SALP), serum bilirubin [22] and total proteins [23] were also estimated.

Statistical Analysis: All values were expressed as the mean ± SEM of three replicate experiments. The analysis was performed by using SPSS statistical package for WINDOWS (version 16.0; SPSS Inc, Chicago). All *in vivo* data are subjected to ANOVA followed by Dunnett's test and $p<0.05$ were considered to be statistically significant

RESULTS

The acute toxicity studies mainly aims at establishing the therapeutic index, i.e., the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species. MPHL was safe up to a dose of 1000 mg/kg (p.o.) body weight. Behavior of the animals was closely observed for the first 3hrs then at an interval of every 4hrs during the next 48hrs.The extract did not cause mortality in mice and rats during 48hrs observation but little behavioral changes, locomotor ataxia, diarrhea and weight loss were observed. Food and water intake had no significant difference among the group studied. MPHL at 25, 50 and 100 mg/kg body weight significantly ($p<0.05$) reduced the body weight, tumor volume, packed cell volume and viable tumor cell count whereas increased non-viable tumor cell count in a dose dependent manner as compared to EAC control group. Furthermore, the median survival time was increase to 24.81 ± 0.11 (% ILS = 33.21), 31.09 ± 0.17 (% ILS = 50.28) and 37.21 ± 0.11 (% ILS = 64.11) on administration of MPHL at a dose of 25, 50 and 100 mg/kg body weight respectively. The reference drug bleomycin showed 46.60±0.11 (% ILS = 85.81).

Table 1: Effect of MPHL on tumor volume, packed cell volume, mean survival time (MST), percentage increase life span (% ILS), viable and non-viable tumor cell count in EAC bearing mice

	EAC control ($2x10^6$ cell/mouse)	EAC+MPHL (25 mg/kg)	EAC+ MPHL (50 mg/kg)	EAC+ MPHL (100 mg/kg)	Bleomycin (0.3 mg/kg)
Tumor volume (ml)	5.31 ± 0.21	2.91 ± 0.11*	1.92 ± 0.25*	1.11 ± 0.18*	0.62 ± 0.21*
Packed cell volume (ml)	2.99 ± 0.24	1.90 ± 0.29*	1.10 ± 0.14*	0.80 ± 0.19*	-
MST (days)	20.11 ± 0.12	24.81 ± 0.11*	31.09 ± 0.17*	37.21 ± 0.11*	46.60± 0.11*
% ILS	-	33.21	50.28	64.11	85.81
Viable cell (x 10^6 cell/ml)	10.01 ± 0.22	5.01 ± 0.12*	3.01 ± 0.02*	1.01 ± 0.22*	0.52 ± 0.15*
Non-viable cell (x 10^6 cell/ml)	0.64 ± 0.24	2.42 ± 0.74*	2.22 ± 0.24*	3.98 ± 0.64*	3.32 ± 0.05*
Total cell (x 10^6 cell/ml)	10.65 ± 0.46	7.43 ± 0.86	5.23 ± 0.26	4.99 ± 0.86	3.84 ± 0.15
Viable %	93.99	67.42	57.55	20.24	13.14
Non-viable %	6.01	32.58	42.45	79.76	86.86

Each point represent the mean ± SEM. (n = 6 mice per group), *$p<0.05$ statistically significant when compared with EAC control group.

Table 2: Effect of MPHL on hetatological parameters in EAC bearing mice

	Hb content (g%)	RBC (cellsx10^6/mm^3)	WBC (cellsx10^6/mm^3)	Differential count		
				Monocyte (%)	Lymphocyte (%)	Neutrophil (%)
Normal saline (5 ml/kg)	12.02 ± 0.86	5.32 ± 0.16	4.13 ± 0.12	2.11 ± 0.06	76.10 ± 0.07	21.01 ± 0.19
EAC control (2x10^6 cell/mouse)	4.21 ± 0.36$^{\#}$	2.01 ± 0.16$^{\#}$	6.98 ± 0.32$^{\#}$	1.22 ± 0.16$^{\#}$	30.10 ± 0.67$^{\#}$	69.01 ± 0.29$^{\#}$
EAC+MPHL (25 mg/kg)	5.82 ± 0.11*	2.42 ± 0.16*	6.03 ± 0.19*	1.45 ± 0.19*	44.80 ± 0.23*	41.21 ± 0.10*
EAC+MPHL (50 mg/kg)	7.72 ± 0.18*	3.02 ± 0.12*	5.93 ± 0.59*	1.54 ± 0.72*	54.10 ± 0.13*	31.62 ± 0.47*
EAC+MPHL (100 mg/kg)	9.82 ± 0.13*	4.02 ± 0.12*	5.43 ± 0.19*	1.78 ± 0.12*	68.21 ± 0.19*	23.72 ± 0.17*
Bleomycin (0.3 mg/kg)	10.02 ± 0.63*	4.52 ± 0.22*	4.71 ± 0.19*	1.81 ± 0.22*	78.93 ± 0.26*	21.02 ± 0.11*

Each point represent the mean ± SEM. (n = 6 mice per group), $^{\#}p<0.05$ statistically significant when compared with normal saline group.$^{*}p<0.05$ statistically significant when compared with EAC control group.

Table 3: Effect of MPHL on serum biochemical parameters in EAC bearing mice

	SGOT(IU/L)	SGPT(IU/L)	SALT(IU/L)	Total protein (mg/dL)	Bilirubin(mg/dL)
Normal saline (5 ml/kg)	38.12 ± 1.41	28.52 ± 4.32	77.01 ± 2.24	9.27 ± 0.24	0.96 ± 0.19
EAC control(2x10^6 cell/mouse)	74.02 ± 1.11$^{\#}$	66.52 ± 5.32$^{\#}$	122.10 ± 3.24$^{\#}$	5.48 ± 0.14$^{\#}$	3.95 ± 0.12$^{\#}$
EAC+ MPHL (25 mg/kg)	55.52 ± 3.51*	48.12 ± 5.12*	108.31 ± 1.24*	6.78 ± 0.19*	2.35 ± 0.75*
EAC+ MPHL (50 mg/kg)	42.52 ± 5.11*	38.32 ± 2.32*	93.19 ± 5.24*	6.18 ± 0.34*	1.95 ± 0.18*
EAC+ MPHL (100 mg/kg)	34.12 ± 1.01*	36.39 ± 1.12*	83.10 ± 1.27*	7.98 ± 1.34*	1.25 ± 1.18*
Bleomycin (0.3 mg/kg)	31.13 ± 1.91*	33.01 ± 1.31*	73.90 ± 1.92*	8.38 ± 1.14*	0.98 ± 1.18*

Each point represent the mean ± SEM. (n = 6 mice per group), $^{\#}p<0.05$ statistically significant when compared with normal saline group.$^{*}p<0.05$ statistically significant when compared with EAC control group.

Hematological parameters (Table 2) of tumor bearing mice were found to be significantly altered compared to the normal group. The total WBC count was found to be increased ($p<0.05$) with a reduction of Hb content and RBC count in EAC control animals when compared with the normal saline group. Treatment with MPHL at 25, 50 and 100 mg/kg body weight significantly ($p<0.05$) increased Hb content and RBC count towards the normal levels. In the differential count, lymphocytes and monocytes were found to be decreased and the neutrophils were increased in the EAC control group when compared with the normal group. MPHL treatment at different doses significantly ($p<0.05$) changes these altered parameters approximately to the normal values.

Table 3 demonstrated that the biochemical parameters like SGOT, SGPT, SALP and bilirubin in the EAC control group were significantly ($p<0.05$) increased as compared to the normal group. Treatment with MPHL at doses of 25, 50 and 100 mg/kg significantly decreased ($p<0.05$) the SGOT, SGPT, SALP and bilirubin to approximately normal levels in a dose dependent manner. The total protein was found significantly decreased in the EAC control group when compared with the normal group ($p<0.05$). Administration of MPHL at different doses in EAC bearing mice significantly ($p<0.05$) induced the total protein as compared with the EAC control group.

DISCUSSION

In EAC tumor bearing mice, a regular rapid increase in ascetic tumor volume was observed. A rapid increase in ascetic fluid, the direct nutritional source for tumor cells, with tumor growth would be a means to meet the nutritional requirement of tumor cells [24]. Treatment with MPHL reduced the intraperitonial tumor burden, thereby reducing the tumor volume, tumor weight, viable tumor cell count and increased the life span of the tumor bearing mice. Prolongation of life span of animals is the steadfast criteria for judging the potency of any anticancer drug [25]. It can therefore be inferred that MPHL increased the life span of EAC bearing mice may be due to decrease the nutritional fluid volume and delay the cell division [26]. Anemia and myelosuppression have been frequently observed in ascites carcinoma [27] mainly due to iron deficiency, either by haemolytic or myelopathic conditions which finally lead to reduced RBC number [28]. Treatment with MPHL brought back the hemoglobin content, RBC and WBC count more or less to normal levels, thus supporting its haematopoietic protecting activity without inducing myelotoxicity, the most common side effects of cancer chemotherapy.

Preliminary phytochemical study indicated the presence of steroids, tannins, phenolic and flavonoid compounds and glycosides in crude extract of

Polygonum hydropiper. A number of scientific reports indicate certain steroids and phenolic compounds such as tannins, caumarins and flavonoids have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis [29]. Furthermore, flavonoids such as quercetin, kaemferol and their glycosides have been shown to possess antimutagenic and antimalignant effect [30]. They also have been shown to enhance *in vitro* human peripheral blood lymphocyte and T-cell proliferation, which suggests a possible stimulation of the immune system function [31]. The anticancer activities of MPHL are probably due to the presence of phenolic compounds, as well as flavonoids and their synergistic effects.

Plant derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells [32] and antitumor activity in experimental animals [33]. The cytotoxic and antitumor activity of plant derived product is either through induction of apoptosis or inhibition of neovascularization [34]. In the present study it was noted that MPHL significantly reduced tumor growth and viability of tumor cells and normalized the hematological and serum biochemical profiles, raising life span as compared with those of EAC control mice.

CONCLUSION

It can be concluded that the methanolic extract of *Polygonum hydropiper* leaves demonstrated remarkable antitumor activity against Ehrlich ascites carcinoma in mice Now our next aim is to explore the isolation and characterization of lead compound liable for aforementioned activity from this plant.

REFERENCES

1. Najiah, M., M. Nadirah, Z. Arief, S. Zahrol and L.W. Tee, A.D. Ranzi, A.S. Amar, A.R. Laith, M. Mariam, S. Suzana and R.J. Aida, 2011. Antibacterial activity of Malaysian edible herbs extracts on fish pathogenic bacteria. Research Journal of Medicinal Plants, 5: 772-778.
2. Nithya, V. and A. Baskar, 2011. A preclinical study on wound healing activity of *Lausonia ulba* Linn. Research Journal of Phytochemistry, 5: 123-129.
3. Hafidh, R.R., F. Abas, A.S. Abdulamir, F. Jahanshiri, F.A. Bakar and Z. Sekawi, 2009. A review: Cancer research of natural products in Asia. International Journal of Cancer Research, 5(2): 69-82.
4. Kumar, R.S., B. Jayakar and B. Rajkapoor, 2007. Antitumor activities of *Indigofera trita* on Ehrlich ascites carcinoma induced mice. International Journal of Cancer Research, 3(4): 180-185.
5. Sharma, R., 2003. Medicinal plants of India-An encyclopedia. Daya publishing house. Delhi-110035, pp: 46-47.
6. Hasan, M.F. and M.M. Rahman, 2011. Screening of antibacterial, antifungul and cytotoxic activities of *Polygonum hydropiper* L. stem extracts. International Journal of Biosciences, 6(1): 47-53.
7. Rahman, E., S.A. Goni, M.T. Rahman and M. Ahmed, 2002. Antinociceptive activity of *Polygonum hydropiper*. Fitoterapia, 73: 704-706.
8. Kundu, B.R., R. Ara, M.M. Begum and Z.I. Sarkar, 2007. Effect of Bishkatali, *Polygonum hydropiper* L. plant extracts against the red flour beetle, *Tribolium castaneum* Herbst. University Journal of Zoology Rajshahi University, 26: 93-97.
9. Chevallier, A., 1996. The Encyclopedia of Medicinal Plants. Dorling Kindersley. London, pp: 185.
10. Furukawa, T., A. Eshima, M. Kouya, S. Takio, H. Takano and K. One, 2002. Coordinate expression of genes involved in catchin biosynthesis in *Polygonum hydropiper* cells. Plant Cell Reports, 21: 385-389.
11. Yanga, X., B.C. Wanga, X. Zhangb, S.P. Yangc, W. Lia, Q.T. Gurinder and K. Singha, 2011. Simultaneous determination of nine flavonoids in *Polygonum hydropiper* L. samples using nanomagnetic powder three-phase hollow fibre-based liquid-phase microextraction combined with ultrahigh performance liquid chromatography-mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis, 54: 311-316.
12. Miyazawa, M. and N. Tamura, 2007. Inhibitory Compound of Tyrosinase Activity from the Sprout of *Polygonum hydropiper* L. (Benitade). Biology and Pharmaecutical Bulletin, 30(3): 595-597.
13. Peng, Z.F., D. Strack, A. Baumert, R. Subramaniam, N.K. Goh, T.F. Chia, S.N. Tan and L.S. Chia, 2003. Antioxidant flavonoids from leaves of *Polygonum hydropiper* L. Phytochemistry, 62: 219-228.
14. Kiem, P.V., N.X. Nhiem, N.X. Cuong, T.Q. Hoa, H.T. Huong, L.M. Huong, C.V. Minh and Y.H. Kim, 2008. New phenylpropanoid esters of sucrose from *Polygonum hydropiper* and their antioxidant activity. Archives of Pharmacal Research, 31: 1477-1482.

15. Takasaki, M., S. Kuroki, M. Kozuka and T. Konoshima, 2001. New phenylpropanoid esters of sucrose from *Polygonum lapathifolium*. Journal of Natural Products, 64: 1305-1308.
16. Alam, M.B., M.S. Rahman, M. Hasan, M.M. Khan, K. Nahar and S. Sultana, 2012. Antinociceptive and antioxidant activities of the *Dillenia indica* bark. International Journal of Pharmacology, 8: 243-251.
17. Osman, M.A., M.M. Rashid, M.A. Aziz, M.R. Habib and M.R. Karim, 2011. Inhibition of Ehrlich ascites carcinoma by *Manilkara zapota* L. stem bark in swiss albino mice. Asian Pacific Journal of Tropical Biomedicine, 1: 448-451.
18. Islam, M.S., M.B. Alam, R. Zahan and G.C. Sarkar, N.S. Chowdhury, M.M. Khan, M. Hasan, M.S. Rahman, M.A. Mosaddik, M. Jesmin and M.E. Haque, 2011. *In vitro* antioxidant and anti-neoplastic activities of *Ocimum sanctum* leaves in Ehrlich ascites carcinoma bearing mice. International Journal of Cancer Research, 7: 209-221.
19. Zahan, R., M.B. Alam, M.S. Islam, G.C. Sarker, N.S. Chowdhury, S.B. Hosain, M.A. Mosaddik, M. Jesmin and M.E. Haque, 2011. Anticancer Activity of *Alangium salvifolium* flower in Ehrlich Ascites Carcinoma Bearing Mice. International Journal of Cancer Research, 7(3): 254-262.
20. Khanam, J.A., M.F. Islam, M. Jesmin and M.M. Ali, Antineoplastic activity of acetone semicarbazone (ASC) against Ehrlich ascites carcinoma (EAC) bearing mice. Journal of National Science Foundation SriLanka, 38: 225-231.
21. Sen, S.K., M. Gupta, U.K. Mazumder, P.K. Haldar, P. Saha and A. Bala, 2011. Antitumor activity of citrus maxima (Burm.) Merr. Leaves in Ehrlich's ascites carcinoma cell treated mice. ISRN Pharmacology, pp: 138737.
22. Malloy, H.T. and K.A. Evelyn, 1937. The determination of bilirubin with the photometric colorimeter. Journal of Biological Chemistry, 119(2): 481-490.
23. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.I. Randall, 1951. Protein measurement with the folin-phenol reagent. Journal of Biological Chemistry, 193(1): 265-272.
24. Radha, R., S. Kavimani and V. Ravichandran, 2008. Antitumor activity of methanolic extract of *Plumeria alba* L. leaves against Dalton lymphoma ascites in mice. International Journal of Health Research, 1: 79-85.
25. Sodde, V., N. Dashora, K.S. Prabhu and R. Lobo, 2011. Evaluation of anticancer activity of *Macrosolen parasiticus* (L.) Danser on Ehrlich's ascites carcinoma treated mice. International Journal of Cancer Research, 7: 135-143.
26. Jesmin, M., M.M. Ali and J.A. Khanam, 2010. Antitumor activities of some Schiff bases derived from benzoin, salicylaldehyde, aminophenol and 2,4-di nitrophenyl hydrazine. Thai Journal of Pharmaceutical Sciences, 34: 20-31.
27. Morshed, M.H., M.F. Islam, M.A. Yousuf, G.M.G. Hossain, M.R. Habib and J.A. Khanam, 2010. Cytotoxic nature of three triazole derivatives. Journal of Engineering Sciences, 1: 121-125.
28. Gupta, M., U.K. Mazumder, P.K. Halder, C.C. Kandar, L. Monikandan and G.P. Senthil, 2007. Anticancer activity of *Indigofera aspalathoides* and *Wedelia calendulaceae* in Swiss albino mice. Iranian Journal of Pharmaceutical Research, 6(2): 141-145.
29. Kumar, R.S., B. Rajkapoor, P. Perumal, T. Dhanasekaran, M.A. Jose and C. Jothimanivannan, 2011. Antitumor activity of *Prosopis glandulosa* Torr. on Ehrlich ascites carcinoma (EAC) tumor bearing mice. Iranian Journal of Pharmaceutical Research, 10: 505-510.
30. Jones, P.J. and S.S. AbuMweis, 2009. Phytosterols as functional food ingredients: linkages to cardiovascular disease and cancer. Current Opinion of Clinical and Nutritional Metabolism Care, 12(2): 147-151.
31. Viral, D., P. Shivanand and N.P. Jivani, 2011. Anticancer evaluation of *Adiantum venustum* Don. Journal of Young Pharmacists, 3: 48-54.
32. Rajkumar, V., G. Guha and R.A. Kumar, 2011. Antioxidant and anti-neoplastic activities of *Picrorhiza kurroa* extracts. Food and Chemical Toxicology, 49: 363-369.
33. Kim, H.J., H.K. Choi, J.Y. Moon, Y.S. Kim, A. Mosaddik and S.K. Cho, 2011. Comparative antioxidant and antiproliferative activities of red and white pitayas and their correlation with flavonoid and polyphenol content. Journal of Food Sciences, 76: C38-C45.
34. Azab, M.E., H. Hishe, Y. Moustafa and E.S.E. Awady, 2011. Anti-angiogenic effect of resveratrol or curcumin in Ehrlich ascites carcinoma bearing mice. European Journal of Pharmacology, 652: 7-14.