Evaluation Of Anticancer Potential Of The Bulbs Of Urginea Indica

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***Abstract*—Objective: To evaluate anticancer activity of Methanolic Extract of Urginea Indica (MEUI) against Ehrlich Ascites Carcinoma (EAC) cells in swiss albino mice.**

Methods:Rudimentary assessment of MEUI was done to observe reduction ability of the number of EAC cells in previously inoculated Swiss albino mice. Enhancements of life span, reduction of average tumor weight have been measured to find out anti-cancer potential of MEUI.

Results: Significant weight variation was recorded at 4X doses of MEUI indicating loss of tumor weight.Tumor weight variation study of MEUI showed a 47.66 % decrease at 2x dose and 65.10% decrease in weight at 4x doses in total ascites fluid weight versus control which were statistically highly significant (p=0.003).After 30 days observation, 3 mice were alive in the group treated by MEUI at 2x dose and 5 mice were alive in the group treated with MEUI at 4x dose, which were analogous with control group in where no mice survived.This significant results ensures that MEUI has anticancer potential.

Conclusions:Our experimental results prove the anti-cancer potentiality of MEUI.

Keywords—MEUI = Methanolic Extract of Urginea Indica, EAC= Ehrlich Ascites Carcinoma, Anticancer Potential Study.

INTRODUCTION

Bangladesh has a rich and prestigious heritage of herbal medicines. More than 250 plants are being used for the treatment of various ailments. However, few of these plants have undergone chemical, pharmacological and toxicological studies [1-4].The World Health Organization estimates that 4 billion people (i.e., 80% of the World's population) use herbal medicines in some aspects of primary health care, and there is a growing tendency to “Go Natural.”[5,6].

Cancer is a major cause of mortality all over the world and represents a major public health problem. After cardiovascular disease, it is the second leading cause of death. The search for anti-cancer agents from plant sources started in earnest in the 1950s with the discovery and development of the Vinca alkaloids, vinblastine and vincristine, and the isolation of the cytotoxic podophyllotoxins [7]. It is well established that plants have always been a useful source, for the occurrence of anticancer compounds. Approximately 60% of the currently used anticancer chemotherapeutic drugs (Vinblastin, Vincristine) are derived from plant resource [7,8]. Although most of the plants used in the traditional medicine have been identified and their applications are well-documented, the anticancer efficacy of many plants is yet to be verified.

The plant *Urginea Indica* kunth under the family of Liliacea commonly known as Indian squill (British name:sea onion), is widely distributed in Chittagong, Cox’s Bazar, Bangladesh.It’s also found growing in sandy places, especially in the seacoast or in dry hills. This plant has been reported to be used as cardio-tonics, in small doses as an expectorant, digestive and in the treatment of many diseases, especially asthma, dropsy, rheumatism, leprosy and skin ailments[9].The extract of the bulbs possesses hypoglycaemic and anticancer properties[10].IAlthough anti-cancer activity of the bulbs of urginea indica was reported but no researchers have been reported yet the anti-cancer activity of *Urginea Indica* using EAC method. So our current studies documented the anticancer activity of *Urginea Indica* against Ehrlich Ascites Carcinoma in Swiss Albion mice inorder to ensure the anti-cancer activity of this plant.

MATERIALS AND METHODS

Collection of Plant

The bulbs of *Urginea Indica* was collected from Teknaf, Chittagong, Bangladesh and identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka whose accession no is DACB 42402.

Preparation of Extract

After drying and grinding, Bulb powder of UI and methanol were used in a ratio of 1:3.5, then was left for 3 days with periodically stirring. Moreover, soaked

samples were allowed to filter through white clean cloth and cotton filter successively.Obtained final filtrate was evaporated by Rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) until obtaining of constant weight and stored at 4^0C.After evaporation,a brownish viscous concentrate was found.

Evaluation of anti-cancer activity of methanolic extract of *Urginea Indica* against Ehrlich Ascites Carcinoma (EAC) cells in swiss albino mice

EAC cells are experimental tumor models used worldwide in cancer research. In 1907, Paul Ehrlich discovered this tumor in the mammary gland of a white mouse, and the tumor was named after him. The present form of EAC cells has been developed by Loewenthal and Jahn [11].

Acute toxicity study (LD_{50}) for the determination of dose

The LD_{50} value was determined by following conventional methods [12].Extract injected intraperitoneally to six groups of mice each consisting 5 mice at different doses of 500,1000,1500,2000, 3000 and 4000 mg/Kg, respectively. LD_{50} was evaluated by the recording mortality rate after 24 hours.

Dose of administration

For the EAC study, the extracts and standard Cyclophosphamide were administered at a dose of 1-2g/kg and 10 mg/kg, respectively.

Experimental Animal

Adult Swiss albino male mice (20-35 g) were used for the experiment. The animal was collected from Jahangirnagar University and fed with standard food with water ad libitum.

Tumor Cell line and their maintenance

The EAC cell line inoculated mice was supplied by animal house of the Department of Pharmacy, Jahangirnagar University, Bangladesh. Full grown tumor cell-line was collected from the mice peritoneum, washed thrice with 0.9% saline and suspended in PBS. About $1x10^6$ cells were injected into a new healthy mouse [13].

Induction of experimental tumor

The EAC cells were collected from the tumor bearing mice aseptically. The cells were diluted in a manner of $1x10^6$ EAC cell in a 0.3 ml phosphate buffer saline (PBS), were injected intraperitoneally to obtain ascites tumor in mice. The EAC re-inoculated mice of six groups containing 12 animals in each group were treated with water, standard and extract samples from 6 hours after the inoculation of EAC for 10 days at 24 hours interval.

Table-1: Protocol for treatment

S.I	Group	No. of Animals	Drug	Treatment
I	Control	12	EAC mice+water	10 ml/Kg B.W
II	Standard	12	EAC mice+cyclophosphamide	1X
V	MEUI	12	EAC mice+ extract	2X
VI	MEUI	12	EAC mice+ extract	4X

1X=Equal to body weight, 2X=Double of body weight, 4X=Four times of body weight

Determination of tumor growth response

The anti-tumor potential of *Urginia Indica* was evaluated by following analysis:

Body weight analysis

All mice were weighed on the day of tumor inoculation and monitored daily [14,15].

Estimation of tumor weight

Tumor weight of EAC tumor bearing mice was measured on the day of sacrifice by comparing the body weight of sacrificed animals with its dry weight. To dry out the ascitic fluid, small cotton balls was inserted into the peritoneal cavity.

Tumor cell count

The ascites fluid withdrawn from the peritoneal cavity of the mice was taken in WBC pipette and diluted 100 times with normal saline and 10 times with PBS. A drop of a diluted cell suspension was placed on the neubauers chamber and the number of cells in the 64 square was counted [15].

Statistical analysis of data

All results are expressed as mean±SEM and one way ANOVA method is used in case of data containing two groups as well as control group.

RESULTS

Effect of MEUI on the body weight variation of EAC treated mice

From the weight variation results, it was found that at the 9^{th} day of the study, the body weight variations between MEUI at 4x dose and a control group were highly significant (p=0. 01). This indicates that this plant extracts have potency to treat cancer.

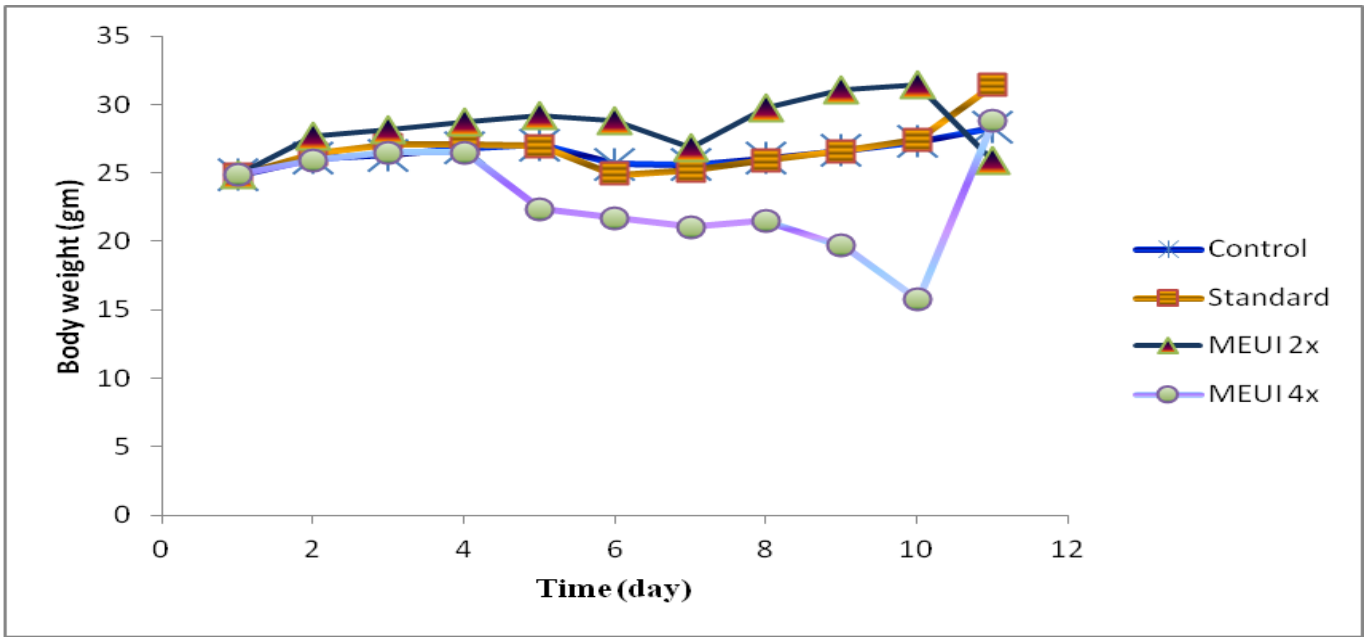

Figure-1: Body Weight variation.

Effect of MEUI on tumor weight variation of EAC treated mice

MEUI caused 47.66 % decrease at 2x dose and 65.10 % reduce in weight at 4x dose in total ascites fluid weight versus control which were statistically highly significant (p=0.003).

Table-2. Effect of MEUI on tumor weight variation of EAC treated mice

Group	Tumor fluid weight(gm)
Control (Mean±SEM)	2.3500±.47234 (n=12)
STD (Mean±SEM)	3.0000±.52501(n=12)
MEUI 2x (Mean±SEM)	1.2333±.15538**(n=12)
MEUI 4x (Mean±SEM)	0.8167±.21945***(n=12)

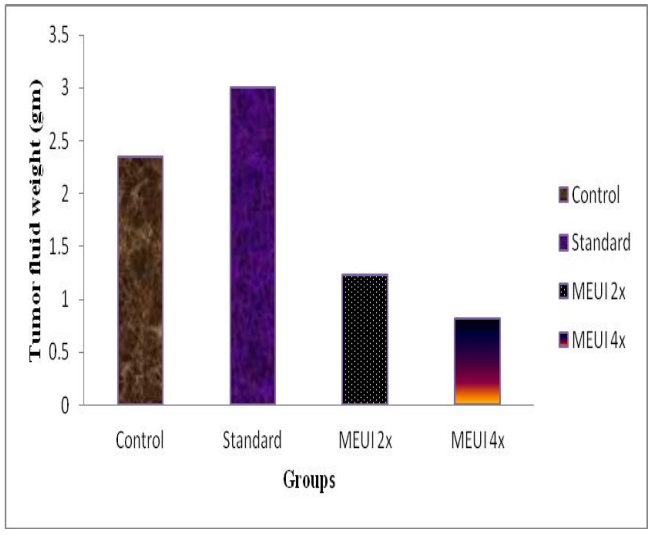

Figure-2 : Tumor Fluid Variation.

Effect of MEUI on cancer cell count

In this study of cancer cell count in the hemocytometer, subside of cancer cells was 71.3% and 82.7% at 2x and 4x doses, respectively in the MEUI treated mice while in case control group, it was 41.23%. The decrease in cancer cell count was statistically highly significant (p<0.001).

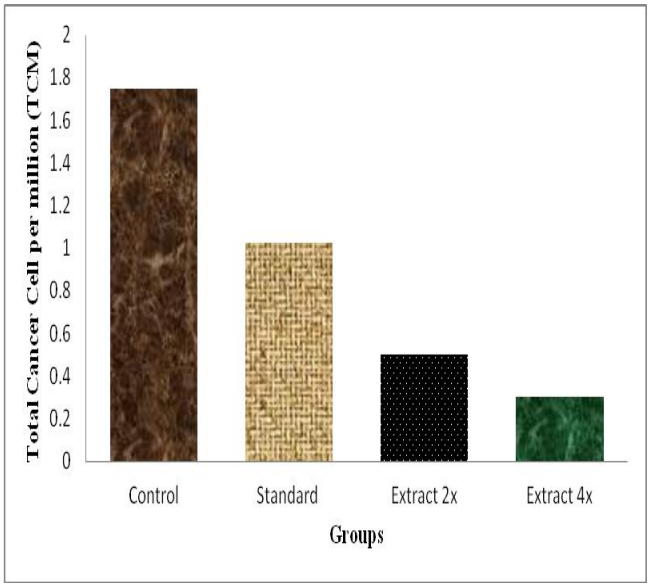

Figure-3: Total Cancer Count (Per million).

Survival time of the mice

The host survival time of the mice was recorded.After 30 days observation, 3 mice were alive in the group treated by MEUI at 2x dose and 5 mice were alive in the group treated with MEUI at 4x dose, which were comparable with control group in where no mice survived. So,it is clear that methanolic extract of *urginea indica* possesses anticancer activity.

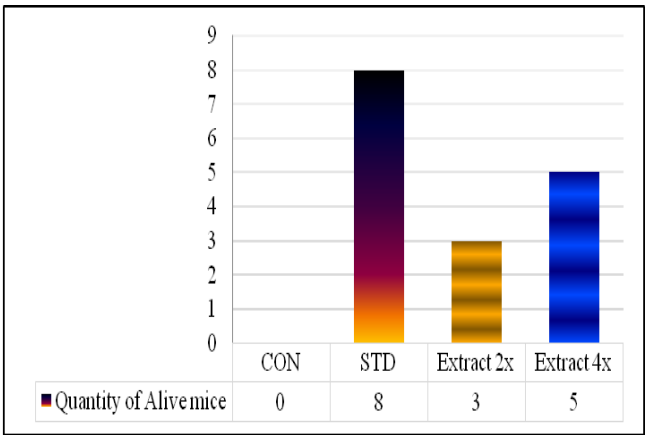

Figure-4: Quantity of Alive mice after 30 days observation.

DISCUSSION

Uncontrolled growth of cell is called cancer. Natural products render one of the most significant sources for the development of novel chemotherapeutic in the areas of infectious diseases and cancer. Cragg and colleagues showed that, for the period 1989 to 1995, over 60% of the approved drugs developed for the treatment of cancerous and infectious diseases were of natural origin, including biological, natural and natural derived products [16,17].

Ehrlich ascites carcinoma (EAC) is a widely used animal model for the evaluation of anticancer activity of plant extracts. Ehrlich ascitic tumor implantation induces a local inflammatory reaction, with increasing vascular permeability, which results in an intense edema formation, cellular migration, and a progressive ascitic fluid formation and accumulation [18]. The ascitic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells [19]. Anticancer effect is quantified by attenuation of EAC-induced weight gain, decreasing in ascites volume and in viable cell count [19].

Experimental results explicated above proved that the methanolic extract of Urginea Indica at its different doses can slow down the growth of tumor satisfactorily, reduce tumor weight markedly and increase life span considerably.

Most reliable criteria for ensuring the anticancer activity of plant extracts is prolongation of life span and decrease [20]. In this study, MEUI showed a significant prolongation of life span of mice bearing cancer cell and that was 3 mice were alive at 2x dose and 5 mice were alive at a 4x dose while 8 mice were alive in the group treated with standard and no mice were alive in the control group.

Intraperitoneal fluid volume reduction by MEUI (47.66% at 2x dose and 65.10% at 4x dose) indicated a toxic effect on these cells that resulted in cell death. It assured that the drug was absorbed directly by the EAC cells in the peritoneal cavity and this herbal drug induced the lysis of the EAC cell by direct cytotoxic mechanism [21].

Reduction of cancer cell count also assured that MEUI has anticancer potential to treat cancer. In this study of cancer cell count by hemocytometer, cancer cell was reduced 71.3% and 82.7% at 2x dose and 4x dose of MEUI, respectively while standard group reduced 41.23%.

CONCLUSION

Present research finding was to evaluate anticancer activity of MEUI, experimented on different animal model at different doses. Results suggest that there is potent anticancer resource in MEUI but to ensure specific compound responsible for such activity, further study is needed.

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AUTHOR CONTRIBUTIONS

SH and KZ were involved in conception and design of the experiments. AFI and NH contributed to perform the experiments. SH and KZ analyzed data. SR contributed to drafting the article. SH and SR contributed to revising it critically for important intellectual content. KZ made the final approval of the version to be published.

CONFLICTS OF INTEREST

All the authors declare no competing interests.

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