ABSTRACT

Objective: The purpose of the study was to evaluate the antitumor and antioxidant status of ethanolic extract of *Cyperus kyllingia* Endl. on Ehrlich ascites carcinoma (EAC)-treated mice.

Methods: The determination of *in vivo* antitumor activity was performed using EAC cells inoculated mice groups (n=12). The groups were treated for 9 consecutive days with ethanolic extract of *C. kyllingia* (EECK) at the doses of 20 and 40 mg/kg/b.w., respectively. After 24 h of the last dose, half of the mice were sacrificed and the rest were kept alive for assessment of increase in life span. The antitumor potential of EECK was assessed by evaluating tumor volume, viable and non-viable tumor cell count, tumor weight, hematological parameters, and biochemical estimations. Furthermore, antioxidant parameters were assayed by estimating liver tissue enzymes.

Results: EECK showed direct cytotoxicity on EAC cell line in a dose-dependent manner. EECK exhibited significant (p<0.05) decrease in the tumor volume, viable cell count, tumor weight, and elevated the life span of EAC tumor-bearing mice. The hematological profile, biochemical estimations, and tissue antioxidant assay were reverted to normal level in EECK-treated mice.

Conclusion: Experimental results revealed that EECK possesses potent antitumor and antioxidant properties. Further, research is going on to find out the active principle(s) of EECK for better understanding of mechanism of its antitumor and antioxidant activity.

Keywords: *Cyperus kyllingia* Endl, Ehrlich ascites carcinoma, Antioxidant, Antitumor, Superoxide dismutase, glutathione.

INTRODUCTION

Cancer is one of the most life-threatening diseases in which a group of abnormal cells grows uncontrollably by disregarding the normal rules of cell division. Cancer is characterized by uncontrolled growth, invasion (intrusion on and destruction of adjacent tissues), and metastasis (spread to other locations in the body through lymph or blood). It is one of the leading causes of the death worldwide. It is estimated that 12.5% of the population dies due to cancer [1]. Nowadays, chemotherapy is the most effective treatment for cancer, but existing chemotherapeutic agents adversely affect our host cell also, especially bone marrow, epithelial tissues, gonads, and the reticulo-endothelial system [2]. Natural products have been the mainstay in cancer chemotherapy for the past 30 years. Over 60% of the clinically used anticancer drugs are of natural origin and most of them are derived from plants [3]. Phytochemicals have minimal toxicity on host cell compared to chemotherapeutic agent. Therefore, researches are still going on medicinal plants in search of new effective anti-carcinogenic agent which can reverse progression of cancer.

*Cyperus kyllingia* Endl. (Cyperaceae) is a perennial herb with creeping underground stem commonly found in tropical regions of the world. According to ethnomedical investigation, this plant was found to have numerous biological activities such as anti diarrheal, diuretic, stomastic, anthelmintic, and expectorant activities, and it is also used in fever, hepatopathy, splenopathy, diabetes, and tumors [4]. Previously, phytochemical analysis was performed and terpenes such as α-cyperone, β-selinene, and α-humulene were found in underground part of this plant [5]. The present study was carried out to evaluate the anticancer effect of root extract of *C. kyllingia* Endl. against Ehrlich's ascites carcinoma (EAC) in Swiss albino mice. There is no scientific report available on biological effect of root extract from *C. kyllingia* Endl. Therefore, this effort has been made to investigate the antiproliferative effect of *C. kyllingia* Endl.
under reduced pressure in a rotary vacuum evaporator (Buchi R-210). The concentrated extract was stored in vacuum desiccators.

Animals
Male Swiss albino mice, an average body weight 20–22 g, were taken for this experiment. The mice were obtained from the animal house, B. N. Ghosh and Co, Kolkata, India, and were grouped and housed in polycrylic cages (38 cm×23 cm×10 cm) with not more than six animals per cage. Animals were maintained under standard laboratory conditions (temperature 25 ± 2 °C and dark/light cycle 14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The mice were acclimatized to laboratory conditions for 7 days before commencement of the experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee.

Transplantation of tumor cell
EAC cells were obtained from Chittaranjan National Cancer Institute, Kolkata, India. The EAC cells were maintained in vivo in Swiss albino mice by intraperitoneal transplantation of 2×10⁶ cells per mouse after every 10 days. Ascetic fluid was drawn out from EAC cell-bearing mouse at the log phase (days 7–8 of tumor bearing) of the tumor cells. Each animal injected intraperitoneally with 0.1 ml of tumor cell suspension containing 2×10⁶ tumor cell [6].

Determination of acute toxicity
As per OECD guideline 425, the acute oral toxicity of EECK in Swiss albino mice was performed as per previously described method Gangopadhyay et al., 2017 [7,8].

Treatment schedule
A total of 60 Swiss albino mice were divided into five groups (n=12) and given food and water ad libitum. All the animals in each group, except Group-I (normal control), received EAC cells (2×10⁶ cells/mouse i.p.). This was taken as day “0”. Group-I served as normal saline (5 ml/kg i.p.) and Group-II served as EAC control. 24 h after EAC transplantation, Group-III and IV received ethanolic extract of C. kyllingia (EECK) root at a dose of 20 and 40 mg/kg i.p. for 9 consecutive days, respectively. Group-V received reference drug 5-FU (20 mg/kg i.p.) for 9 consecutive days. This was taken as day “0”. Group-I served as normal saline (5 ml/kg i.p.) and Group-II served as EAC control. 24 h after EAC transplantation, Group-III and IV received ethanol extract of C. kyllingia (EECK) root at a dose of 20 and 40 mg/kg i.p. for 9 consecutive days, respectively. Group-V received reference drug 5-FU (20 mg/kg i.p.) for 9 consecutive days [9]. 24 h of last dose, six animals of each group were sacrificed by cervical dislocation to measure antitumor, hematological, and biochemical parameters (livers) and rest of the animals were kept with food and water ad libitum to check percentage increase life span of the tumor host. The antitumor activity of EECK was measured in EAC animals with respect to the following parameters.

Determination of biochemical parameters
Serum biochemical parameter such as total proteins, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), and serum bilirubin was done using commercially available kits manufactured by the Span Diagnostics Ltd., Surat, India. Determination of tissue antioxidant parameters
Blood was collected from mice by cardiac puncture and was kept for 15 min for clotting and then centrifuged at 5000 rpm for 10 min. The supernatants (serum) were collected and total protein, bilirubin, ALT, AST, and ALP were determined using the diagnostic reagent kit (Span Diagnostics Ltd., Surat, India), using the autoanalyzer instrument (SELECTRA PRO XS, Merck Limited, Germany).

Statistical analysis
Statistical analysis was performed using GraphPad Prism (version 5.0, GraphPad Software Inc, San Diego, CA) software. The experimental results were expressed as mean ± standard error of mean. Statistical significance was analyzed by one-way ANOVA followed by Dunnett’s post hoc test of significance. p<0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Determination of acute toxicity
The LD₅₀ of the C. kyllingia ethanolic root extract was found to be 25.3 ± 0.14 mg/kg body weight for i.p. According to OECD guidelines, it is considered as a LD₅₀ cutoff value. Dose selected for pharmacological studies by fixed dose methods is mentioned below. Ethanolic extracts of C. kyllingia roots used for anticancer study were 20 and 40 mg/kg body weight which were much lower than the LD₅₀.

Determination of direct tumor-related parameters
Antitumor activity of EECK against EAC tumor-bearing mice was assessed by the parameters such as tumor volume, packed cell volume, cell count (viable and non-viable), mean survival time, and percentage increase of life span. The results are shown in Table 1. Tumor volume and packed cell volume were significantly decreased in EECK-treated group in compare with normal control group. Viable cell count was assessed by the parameters such as tumor volume, packed cell volume, and tumor weight which were much lower than the LD₅₀.

Hematological parameters
RBC level was decreased in EAC control group and WBC level was increased in EAC control group as compare with normal group. It was seen that RBC and Hb content was increased and WBC count was significantly reduced in EECK-treated group as compared with EAC control group. Furthermore, mean survival time and percentage increase of life span were increased on EECK-treated group in a dose-dependent manner. From these results, it can be concluded that EECK extract has antitumor activity on EAC-induced mice in a dose-dependent manner.

Biochemical parameters
Biochemical parameters such as TP, SGOT, SGPT, and SALP levels were discussed in Table 3. SGOT, SGPT, and SALP levels are lower in normal group compare to EAC control group and TP level is higher in normal group than EAC control group. It was estimated that SGOT
Table 1: Determination of direct tumor-related parameters

| Groups                  | Tumor Volume (ml) | Packed cell volume (ml) | Cell count (×10⁶/ml) | MST (day) | ILS% |
|-------------------------|-------------------|-------------------------|----------------------|-----------|------|
| EAC control             | 2.40±0.05         | 2.02±0.03               | 7.70±0.22            | 0.66±0.06 | 19   |
| EAC+20 mg/kg            | 1.78±0.04*        | 1.62±0.02*              | 6.40±0.22*           | 3.94±0.09*| 23   |
| EAC+40 mg/kg            | 0.70±0.04*        | 0.28±0.03*              | 5.33±0.23*           | 4.4±0.23* | 26   |
| EAC+5-FU (20 mg/kg)     | 2.71±0.15*        | 5.33±0.23*              | 2.71±0.15*           | 5.33±0.23*| 38   |
| EAC+20 mg/kg 5-FU       | 71.67±6*          |                         |                      |           |      |
| EAC+40 mg/kg 5-FU       | 5.33±0.23*        |                         |                      |           |      |
| EAC+20 mg/kg 5-FU       | 4.47±0.23*        |                         |                      |           |      |
| EAC+40 mg/kg 5-FU       | 5.48±0.10         |                         |                      |           |      |
| EAC+20 mg/kg 5-FU       | 73.33±6.09        |                         |                      |           |      |

**Table 2: Hematological parameters**

| Parameters | Normal | EAC control | 20 mg/kg EECK | 40 mg/kg EECK | 20 mg/kg 5-FU |
|------------|--------|-------------|---------------|---------------|--------------|
| RBC (cells 10⁶/mm³) | 5.48±0.10 | 2.45±0.17** | 3.15±0.14     | 4.15±0.17**   | 5.35±0.25**  |
| WBC (cell×10³/mm³) | 4.43±0.23 | 7.5±0.28**  | 6.4±0.23      | 5.33±0.24     | 5.0±0.10**   |
| Hb (g/dl)    | 11.79±0.23 | 4.47±0.29** | 5.44±0.28**   | 6.73±0.27**   | 8.4±0.29**   |

Effect of different concentration of the extracts on hematological parameters. Values are represented as mean±SEM. *p<0.05 when EAC control compared to normal, **p<0.05, EAC control compared with treated groups. EECK: Ethanolic extract of Cyperus kyllingia, EAC: Ehrlich ascites carcinoma, SEM: Standard error of the mean.

**Table 3: Biochemical parameters**

| Parameters | Normal | EAC control | 20 mg/kg EECK | 40 mg/kg EECK | 20 mg/kg 5-FU |
|------------|--------|-------------|---------------|---------------|--------------|
| TP (g/dl)  | 8.6±0.31 | 4.25±0.24  | 5.0±0.26      | 6.70±0.37     | 8.3±0.28     |
| SGOT (IU/L)| 77.3±14.5  | 164.3±5.60* | 156.7±4.11*   | 98.3±4.11*    | 85.6±2.96*   |
| SGPT (IU/L)| 30.00±4.93 | 95±8.66*    | 86.6±8.01*    | 71.6±6*       | 45±7.63*     |
| SALP (KA/U)| 73.3±6.09  | 155±12.58*  | 131.7±6.09    | 110±5.77*     | 81.6±17.40* |

Effects of different concentrations of the extract on serum parameters. Values are represented as mean±SEM. *p<0.05 when EAC control compared to normal, **p<0.05 when EAC control compared with treated groups. SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, SALP: Serum alkaline phosphatase, EAC: Ehrlich ascites carcinoma, SEM: Standard error of the mean.

**DISCUSSION**

The present investigation was carried out to evaluate the antiproliferative property of ethanolic extract of indigenous plant of West Bengal region, i.e., C. kyllingia Endl. in EAC tumor-bearing mice. The EECK-treated animals at the doses 20 and 40 mg/kg significantly inhibited the tumor volume, packed cell volume, viable tumor cell count, and back the hematological parameters toward normal levels. In EAC tumor-bearing mice, rapid increase in ascites tumor volume was observed. Nutritional source for tumor cells is ascites fluid, and a rapid increase in ascites fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells [3]. Treatment with EECK decreased the tumor volume, viable tumor cell count, and increased the life span of the tumor-bearing mice. The main criteria for any anticancer drug are the prolongation of the life span of animal [10]. In the present study, EECK increases the life span of EAC-bearing mice.

Usually, in cancer chemotherapy, the major problems that are being encountered are hematological problems. To restore the hematological parameter toward normal level by treatment with EECK clearly indicates that the extract having protective action on hemopoietic system also.

The lack of balance between the reactive oxygen metabolites and the antioxidant defense systems leads to "oxidative stress" which deregulates various cellular functions causing pathological conditions. Reactive oxygen species formed in cancer tissues result in lipid peroxidation and subsequent increase in MDA and other TBARS levels. MDA, the end product of lipid peroxidation, a biomarker of oxidative stress, was reported to be higher in cancer tissues than in the non-diseased organ [12]. The present study showed that there is an increased level of TBARS in the EAC-bearing liver tissues but after treatment with EECK inhibited hepatic lipid peroxidation as revealed by reduction of MDA levels toward normal levels (Fig. 1). This indicated the reduction in free radical generation by EECK in tumour-bearing mice.
CONFLICTS OF INTEREST STATEMENT
We declare that we have no conflicts of interest.

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