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Anti-tumor potential of cucurbitacin triterpenoids of *Momordica dioica* Roxb. fruit by EAC induced ascites tumor model

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ABSTRACT

Momordica dioica Roxb. (*Cucurbitaceae*) is commonly known as spiny gourd and traditionally used as astringent, febrifuge, antiseptic, anthelmintic, spermicidal and also used in bleeding piles, urinary infection and as a sedative. Studies indicate that it possesses antioxidant, hepatoprotective, antibacterial, anti-inflammatory, anti-lipid peroxidative, hypoglycaemic and analgesic properties. In this study, the anticancer efficacy of *Cucurbitacins* obtained from *Momordica dioica Roxb. (MDR)* has been evaluated. Based on previous *in-vitro* studies performed, *in-vivo* studies were carried out on mice model. Ehrlich ascites carcinoma (EAC) cells were inoculated into swiss albino mice intraperitoneally to form a liquid tumor and then treated with oral administration of 50, 100, 200mg/kg. Evaluation parameters involved the mean survival time (MST), body weight, hematological parameters, Percentage increase in life span were measured in normal control, EAC control and *Cucurbitacin* treated groups (n = 6). Treatment with *Cucurbitacins* enriched fraction has shown anti-tumor effects against liquid tumor as indicated by a significant ($P < 0.05$) reduction in body weight. Interestingly, the enriched bio fraction restored the altered hematological parameters of tumor-bearing animals and significantly increased their life span. These data indicate the cytotoxic potential effects of MDR on tumor cells opening new opportunities for further studies on the anti-cancer effects of this agent.

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INTRODUCTION

Cancer therapy has faced problems for several years due to the adverse effects associated with various chemotherapeutic agents, mainly due to poor tumor specificity (Kenis and Reutelingsperger, 2009). Though there has been significant progress in medical research, there is still an increasing demand of safe and specific anticancer agents in clinical practice. Several existing therapies, such as radiotherapy, chemotherapy, as well as patient non-compliance, have been related to tumor resistance mechanisms. This has propelled research in the direction of identifying newer moieties in cancer management, which would prove as reliable agents with lesser side effects (Kumar *et al.*, 2014).

Momordica dioica Roxb. (MDR) is a dioecious and cucurbitaceous plant which is native to Asia and extensively been studied for its preventive and curative properties. The pharmacological properties which were tested using the extracts were antioxidant activity, analgesic activity, nephropro-

tective, neuroprotective, anti-allergic, anti-ulcer, anti-microbial, anti-diabetic, antimalarial, anti-inflammatory, hepatoprotective, antifertility and anti-epidemic activity. The two main models to study the anti-tumor potential are EAC induced liquid tumor model and DLA induced solid tumor model.

The Ehrlich ascites carcinoma (EAC) is a spontaneous murine mammary adenocarcinoma adenocarcinoma (Jaganathan *et al.*, 2010), which in its ascites form is carried in outbred mice in passages done intraperitoneally. The main reason for its proliferative activity is the absence of H-2 histocompatibility antigens (Chen and Watkins, 1970), which allows it to multiply rapidly inside host mice (Patt and Straube, 1956).

In the present work, the cucurbitacin fraction of the fruits of MDR was used to explore and evaluate the effect on EAC induced tumors in mice models.

MATERIALS AND METHODS

Animals

This study used Swiss Albino mice, which were obtained from Biogen laboratory facility, Bangalore and which weighed around 25-25g. This study was approved by the Institutional Animal Ethics committee of JSS College of Pharmacy, Mysuru (Approval no: 204/2016).

Plant material

The plant material used for this study received suitable authentication, followed by deposition into the Dept. of Pharmacognosy of JSS College of Pharmacy, Mysuru. The initial process of extraction involved the washing of the fruit regions and shade drying the parts. The dried fruits were suitably stored in air-tight containers.

METHODS

Preparation and standardization of Cucurbitacin rich bio-fraction

After 20 days of air drying, the dried fruits were utilized for preparing the cucurbitacin triterpenoids rich bio fraction of *Momordica dioica* Roxb.(CTRB). Moisture content was reduced to less than 4%.

Cucurbitacin triterpenoids rich bio fraction of Momordica dioica Roxb.(CTRB)

The extraction process was performed in 5 separate batches with the removal of the solvent by means of rotatory evaporator and concentration of the extract with dichloromethane and toluene to obtained enriched cucurbitacin fractions(CTRB).

EAC inoculated Ascites tumor model

Induction of Ascites tumor (EAC)

(Manjula *et al.*, 2010) EAC cells were removed from the peritoneal region of mice bearing it and around 0.25ml of the suspension was administered intraperitoneally containing 2.5×10^6 EAC cells. Dosing was started after 24 hrs of inoculation followed by 15 days dosing regimen (Kumar *et al.*, 2014),(Table 1).

RESULTS AND DISCUSSION

(Table 2) depicts the effect of CTRB on changes in body weight in EAC inoculated mice; it was found that maximum weight gain was there in the control group animals when compared to that of normal group animals as the day progresses. CTRB has not shown an increase in the body weight as that of control and the change in the body weight is the same as that of the standard group (Sharma *et al.*, 2015).

Hematological parameters

A significant decrease in total RBC counts was observed in EAC induced control mice when compared to normal mice. CTBR dose-dependently and cisplatin significantly reversed the decrease in the total RBC count. A significant increase in WBC count was observed in EAC induced control mice when compared to normal mice Whereas the CTRB at 100, 200 and 400 mg/kg showed a significant decrease in WBC counts when compared to control. The same was observed in the hemoglobin level. CTRB showed a dose-dependent significant increase in hemoglobin level, which was very much similar to standard (Table 3), (Sharma *et al.*, 2015).

Percentage increase in a life span

Significant decrease in mean survival time (MST) was observed in control, where has there was an increase in MST in cisplatin and CTRB treated mice. The treatment with CTRB at 400 mg/kg showed a significant increase in life span and it is very much similar to cisplatin (Figure 1).

Cancer therapy is complex in terms of its detection and diagnosis and effective treatment may be carried out only when detected at the early stages. (Martin-Cordero *et al.*, 2012) In cases where the cells of primary tumors have undergone metastasis and spread to other parts of the body, the primary form of treatment is chemotherapy, which involves the delivery of drugs directly into the system to target cancer cells but is associated with numerous adverse effects. A major challenge in modern medicine is promoting selectivity and decreasing the number of adverse effects (Denny,

Table 1: G rouping and treatment schedule of experimental animals in EAC model

Group	Treatment	Evaluation
Normal	0.5%Sodium CMC (Vehicle) p.o. for 15 days.	Change in body weight was noted on 3rd, 6th, 9th, 12th, and 15th day7.
Control + EAC cells	Vehicle p.o. for 15 days.	Haematological parameters7. Mean Survival studies7
Standard + EAC cells	Cisplatin in vehicle (4mg/kg) i.p. for 2 alternative days.	
CTRB D1 + EAC cells	50mg/kg in vehicle p.o for 15 days	
CTRB D2 + EAC cells	100mg/kg in vehicle p.o for 15 days	
CTRB D3 + EAC cells	200mg/kg in vehicle p.o for 15 days	

CTRB- Cucurbitacin rich biofraction of MDR

Table 2: Effect of CTRB on body weight change in EAC inoculated Mice

	%increase in body weight				
Group	Day 3	Day 6	Day 9	Day 12	Day 15
Normal	28.85±2.36	29.83±2.25	29.95±1.74	31.23±1.59	31.58±1.61
Control	39.16±1.95a	39.71±1.98a	40.40±1.88a	43.0±1.06a	49.10±1.47a
Standard	35.0±1.63	33.11±1.36	33.96±1.65	34.18±1.28	31.60±1.14
CTRB (100mg/kg)	35.50±2.90a,c	35.78±2.9a,c	37.41±3.19a,c	41.03±3.55a,c	45.38±3.57a,c
CTRB (200mg/kg)	33.38±0.62b	33.29±0.72b	34.24±0.68b	33.37±0.19b	32.16±0.51b
CTRB (400mg/kg)	29.51±0.45b	29.63±0.72b	31.02±0.65b	29.76±1.18b	28.98±0.37b

Values are Mean ± SEM,n=6, Statistical analysis- One way ANOVA
a – (P<0.05) comparedwith the normal group
b – (P<0.05) comparedwith the negative control group
c- (P<0.05) comparedwith the standard treated group

Table 3: Effect of CTRB on hematological parameters in EAC inoculated mice

Groups	RBC count (106cells/mm)	WBC count (cells/mm)	Hb Content (gm %)
Normal	11.39±0.43	6283.3±202.34	14.51±0.24
Control	05.76±0.31a	23400.0±286.95a	7.77±0.24a,b
Standard	10.26±0.64b	7616.6±650.85b	12.81±0.24a,b
CTRB (100mg/kg)	08.01±0.26a,b,c	14333.3±334.33a,b,c	9.66±0.49a,b,c
CTRB (200mg/kg)	08.59±0.42a,b	9050.0±248.66b	12.28±0.36a,b
CTRB (400mg/kg)	10.06±0.26b	6983.3±564.16b	14.38±0.35b,c

Values are Mean ± SEM, n=6,

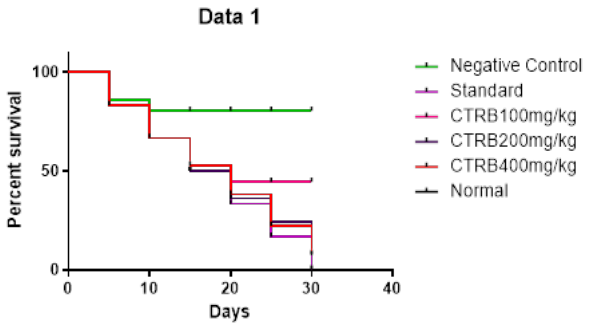

Figure 1: Effect of CTRB on MST in EAC bearing mice by Log-rank (Mantel-Cox) Test

1995).

Natural plant sources have been used in the treatments as therapeutic agents (Carvalho *et al.*, 2012) or as the basis of synthesizing new moieties. (Newman and Cragg, 2012) In search of sources for novel anti-cancer agents, several extracts have been studied extensively for their cytotoxic potential in both *in-vitro* and *in-vivo* models and correlated (Cragg and Newman, 2009).

This fruit has already been studied for its anti-diabetic and hyperlipidemic properties, which showed promising results that of Momordica charantia and the major component identified from these species was found to be Momordicoside (Enslin and Rehm, 2009).

In the present study, the cucurbitacin triterpenoid bio fraction (CTRB) of *Momordica dioica* Roxb. fruits were taken to evaluate the anti-cancer activity in transplantable tumor models like EAC liquid tumor. The CTRB concentrations of 100, 200 and 400mg/kg were tested on EAC induced liquid tumor model in mice. The maximum gain in body weight was observed in control and maximum growth reduction was seen in cisplatin and CTRB treated group. Due to its anti-oxidant and cytotoxic properties, it reduced inflammation in the peritoneal cavity and further infiltration of proliferative cells decreased, hence reducing the body weight. The maximum bodyweight reduction was seen at the dose of 400mg/kg.

Hematological parameters in EAC induced mice showed an increase in WBC cells and decreased RBC and Hb. Due to the presence of inflammatory mediators, there was more proliferation of WBC cells in the body.

Due to myelosuppression or Haemolysis, RBC synthesis in the body was decreased along with the presence of immature RBC cells in the blood. This, in turn, leads to low Hb content. All 100,200, and 400mg/kg doses of CTRB showed dose-dependent activity in modifying hematological parameters compared with the cisplatin group.

After 15^{th} day of treatment, the life span assessment was noted and the cisplatin-treated group showed a 100% increase in life span when compared to the control and CTRB 400mg/kg showed a prominent increase in %ILS in mice when compared with 100 and 200mg/kg.

CONCLUSIONS

The results of this study are in line with the *in-vitro* result of previously carried out experiments out at our laboratory on CTRB. CTRB has shown dose-dependent anti-tumor potentials on EAC induced liquid tumors and which was similar to cisplatin. The study is in the initial step in the identification of a novel and selective herbal antitumor agent devoid of many of the adverse effects of anticancer chemotherapy. However, further study is required to establish the molecular level antitumor potential of CTRB in higher animal models.

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