Anticancer Activity of Extracts of Leaf of *Ophiorrhiza mungos* L. on Dalton's Ascitic Lymphoma in Mice

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Abstract

The present investigation was aimed at evaluating the anticancerpotential of the alcohol and aqueous extracts of leaf of Ophiorrhiza mungos L. on DAL in mice. Animals were inoculated with DAL cells (106 cells/mouse, i.p.) The extracts wereadministered at doses 400 and 800 mg/kg p.o. All the groups except the normal control received DAL cells. The parameters estimated were: Cancer cell count, Packed Cell Volume (PCV), Tumor mass, Increase in Life Span (ILS), Hematological parameters - RBC count, WBC count. HPTLC profiles for Camptothecinin the extracts were also obtained. Following, inoculation with DAL cells, there was profound proliferation of tumor cells in peritoneal cavity of animals which was significantly normalized by the extracts. Significant ($p<0.001$) changes were observed in tumor and haematological parameters.

Key Words: Ophiorrhiza Mungos, Dalton's Ascitic Lymphoma, Camptothecin

1. INTRODUCTION

Cancer is a scourge afflicting mankind from time immemorial. In spite of spectacular advances made by medical science during present century, treatment of cancer remains an enigma [1]. Many naturally occurring substances were tested for anticancer activity on experimental animals resulting in present availability of some 30 effective anticancer drugs [2]. Camptothecin is a pyrrolo quinoline alkaloid, used in the treatment of cancer. It was originally identified in extracts of Camptotheca acuminata Descne [3]. Subsequently Camptothecin was also isolated from Ophiorrhiza mungos Linn [4].

O. mungos belongs to the family Rubiaceae. Decoction of roots, leaves and bark are given as stomachic. Leaves are used for dressing ulcers, as anthelmintic, to counteract poisonous effects of scorpion sting, rat and snake poisoning. Leaves and stems of O. mungos contain hydrocyanic acid. Leaves contain Camptothecin, 10-methoxycamptothecin and β-sitosterol. Roots contain starch and a light brown resin [5-7].

The objective of the present study was to evaluate the anticancer property of the alcohol and aqueous extracts of leaf of O. mungos. Such studies have not been carried out on the leaves of O. mungos and hence the present investigation [8].

2. METHODOLOGY

2.1 Plant material

Leaves of *O. mungos*were collected from the forests of Thenmalai, Kerala during November 2007, taxonomically identified and authenticated by Dr. S. N. Yoganarasimhan, Taxonomist using local floras [9,10]. A voucher herbarium specimen, Christin Rachael 017, was prepared and has been deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy; a sample of the crude drug has been deposited in the crude drug museum of the institution. The air dried powdered drug material was subjected to soxhlet extraction with different solvents in the order of increasing polarity. The extracts were subjected to preliminary phytochemical screening [11]. HPTLC method was developed for fingerprinting of Camptothecin in alcohol and aqueous extracts of *O. mungos* [12].

2.2 Preparation of extracts for anticancer activity studies

Total alcohol extract was prepared by soxhlation method using 95% v/v ethanol and total aqueous extract was prepared by maceration with chloroform water.

2.3 Animals

Swiss albino mice of either sex weighing 20-25 g were used for the acute toxicity and anticancer studies.

2.4 Acute toxicity study

Acute toxicity studies were carried out following the methods described by Ghosh [13].

2.5 Anti - cancer study [14-16]

The animals were divided in to 7 groups containing 8 animals each. Group 1: Normal control group treated with distilled water; Group 2: Positive control group inoculated with DAL cells; Group 3: Standard group inoculated with DAL cells and treated with 5-Fluorouracil (20 mg/kg p.o); Group 4: Test group inoculated with DAL cells and treated with alcoholextract of drug (400 mg/kg p.o); Group 5: Test group inoculated with DAL cells and treated with alcohol extract of drug (800 mg/kg p.o); Group 6: Test group inoculated with DAL cells and treated with aqueous extract of drug (400 mg/kg p.o); Group 7: Test group inoculated with DAL cells and treated with aqueous extract of drug (800 mg/kg p.o.).

DAL cells were injected intraperitoneally (106 cells/mouse, i.p.) to 6 groups of animals (only normal group did not receive the DAL cells). On the second

day onwards respective treatment was started for each group. The treatments were continued for the next 14 days, with 24 h intervals. On 15th day, blood was withdrawn by puncturing retro orbital plexus for determination of RBC and WBC counts. Then 3 animals from each group were kept for observing the life span and remaining animals (n = 5) from each group were sacrificed and cancer parameters were determined. Sample was collected by aspiration from the peritoneal cavity of the animals. All animals were weighed once daily from the day of inoculation, to the 15th day. The parameters estimated were: Cancer cell count, Packed Cell Volume (PCV), Tumormass, Increase in Life Span (ILS), Hematological parameters - RBC count, WBC count.

2.6 Statistical analysis

The values of all groups were compared with positive control and data were expressed as mean values ± S.E.M and tested with one way ANOVA followed by Tukey- Kramer multiple comparisons test for anticancer activity.

3. RESULT AND DISCUSSION

Preliminary phytochemical screening revealed the presence of alkaloids; carbohydrates and glycosides; phenolic compounds and tannins; flavonoids in both alcohol and aqueous extracts; phytosterols in the alcohol extract and gums and mucilage in the aqueous extract. The alcohol and aqueous extracts revealed spots at Rf 0.53and 0.54 which was corresponding to that of standard Camptothecin Rf 0.53 when scanned at 254 and spots at Rf 0.51 and 0.50 at 366 nm (Fig. 1).Both the standard Camptothecin andCamptothecin in *O. mungos*leaf extracts exhibited bluefluorescence under 254 nm, bright blue fluorescenceunder 366 nm and no fluorescence under 425 nm [17].

The alcohol and aqueousextracts of *O. mungos*leaves were safe up to the dose of 2000 mg/kg body weight in swiss albino mice.The alcohol and aqueous extracts at doses of 400 and 800 mg/kg body weight showed significant reduction in the cancer cell number ($p < 0.001$) and tumor weight ($p < 0.001$). Following, inoculation with DAL cells, there was profound proliferation of tumor cells in peritoneal cavity of animals, which were indicated by high packed cell volume. Administration of the extracts significantly reduced PCV ($p < 0.001$). An increase in life span was also noted in the animals ($p < 0.001$), which were treated by the alcohol and aqueous extracts (Table 1). It was found that the tumor bearing mice showed reduction in RBC count and an increase in WBC count compared to normal control animals. Following treatment with alcohol and aqueous extracts, RBC count was significantly elevated, whereas WBC count was reduced significantly both at ($p < 0.001$), when analyzed by Tukey-Kramer multiple comparisons test (Table 2).

Usually, in cancer chemotherapy the major problems encountered are myelosuppression and anaemia. Anaemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions [18]. Treatment with both extracts significantly brought back the reduced RBC count closer to normal value ($p < 0.001$) The WBC count was also significantly reduced ($p < 0.001$), indicating protective action on the hemopoietic system.

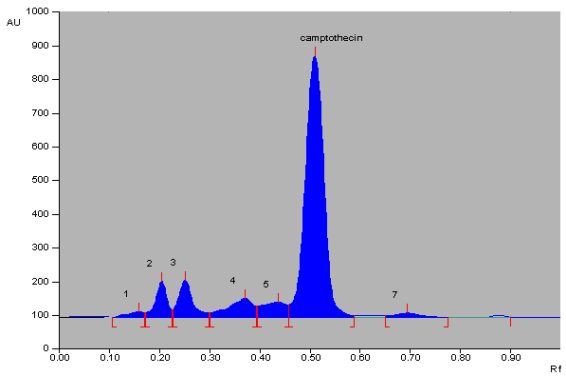

Fig. 1 HPTLC fingerprint of Camptothecin in alcohol and aqueous extracts of *O. mungos* at 366 nm

Camptothecin, a pyrrolo quinoline alkaloid present in *O. mungos* is an anticancer agent [19]. It acts by inhibiting the enzyme, topoisomerase 1, which is involved in DNA replication [20]. Camptothecin also blocks a specific step in the processing of ribosomal precursor RNA, allowing the conversion of 45S RNA to 32S RNA, but inhibiting the conversion of 32S RNA to 28S RNA [21].

4. CONCLUSION

The study substantiated the anticancer activity of leaves of *O. mungos* and Camptothecin, a chemotherapeutically important phytoconstituent solely responsible for anticancer property of the drug.

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Table 1. Effect of alcohol and aqueous extracts of leaves *of O. mungos* on DAL induced mice

Sl. No.	Groups	Dose mg/kg	Cancercell no. ($\times 10^6$/ mm^3)	PCV (%)	Tumor mass (g)	ILS (%)
01	Normal control	--	--	--	--	--
02	Positive control (DAL cells)	--	1.832 ± 0.058	61.80 ± 1.281	8.644 ± 0.097	--
03	Standard (5-Flurouracil)	20	0.591 ± 0.015***	27.80 ± 1.068***	2.874 ± 0.144***	89.470 ± 3.037***
04	Alcohol extract	400	0.958 ± 0.022***	44.60 ± 1.030***	6.614 ± 0.054***	35.083 ± 1.757***
05	Alcohol extract	800	0.725 ± 0.012***	36.00 ± 1.871***	3.778 ± 0.104***	64.906 ± 4.642***
06	Aqueous extract	400	0.923 ± 0.011***	48.00 ± 1.00***	7.182 ± 0.065***	29.820 ± 6.325**
07	Aqueous extract	800	0.762 ± 0.014***	37.60 ± 1.720***	4.432 ± 0.084***	57.890 ± 3.037***

Values are expressed as Mean ±SEM; Tukey-Kramer multiple comparisons test: *** $p < 0.001$ or ** $p < 0.01$, in comparison with the positive control.

Table 2. Effect of alcohol and aqueous extracts of leaves of *O. mungos* on hematological parameters

Sl. No.	Groups	Dose	RBC ($\times 10^6$/ mm^3)	WBC ($\times 10^3$/ mm^3)
01	Normal control	--	5.832 ±0.073	7.037 ±0.059
02	Positive control (DAL cells)	--	3.265 ±0.102	13.212 ±0.127
03	Standard (5-Flurouracil)	20	5.156 ±0.071 ***	8.137 ±0.123 ***
04	Alcohol extract	400	4.011 ±0.057 ***	11.412 ±0.200 ***
05	Alcohol extract	800	4.596 ±0.142 ***	9.550 ±0.217 ***
06	Aqueous extract	400	3.982 ±0.129 ***	11.781 ±0.366 ***
07	Aqueous extract	800	4.493 ±0.068 ***	9.837 ±0.192 ***

Values are expressed as Mean ±SEM; Tukey-Kramer multiple comparisons test: *** $p < 0.001$ or ** $p < 0.01$, in comparison with the positive control.