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Pharmacological studies of anticancer, anti inflammatory activities of *Murraya koenigii* (Linn) Spreng in experimental animals

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Abstract:

The aim of the present study is to evaluate the effect of column (SU I, SU II, SU III) extracts of the plant *Murraya koenigi* against *in vitro* (short term incubation method, SU I, SU II, SU III) and in vivo (Dalton's Ascitic Lymphoma, SU II) has been evaluated in male swiss albino mice. DAL cells were injected intraperitoneally (10^6 cells) to the mice. Two days after cells injection the animals were treated with 7 mg/Kg of SU II for 15 days. 5-flurouracil (20 mg/Kg) was used as the reference. On day 16, cancer cell number compared with the same parameter in control. A significant decrease in the cancer cell number and tumour weight were noted in the tumour-induced mice after treatment with SU II. These observations are suggestive of the protective effect of extract in Dalton's Ascitic Lymphoma (DAL). In vitro studies all the column extracts showed moderate activity.

Keywords - Column extracts, Dalton's Ascitic Lymphoma cell line, Flurouracil *Murraya koenigi*

Introduction

Cancer is one of the disease which cannot be completely subdued by chemotherapy. These chemotherapeutic agents though effective against various types of tumour are not totally free from side effects *Murraya koenigi* having the main constituent of carbazole alkaloids. Furukawa *etal* [1] have studied the cytotoxic effect of pyranocarbazole and found them to be very active against leukemia and colon cancer cell lines . *Murraya koenigii* (Linn), Rutaceae family is found in common in forests often as gregarious undergrowths. The plant used as tonic, stomachic and carminative. Fresh juice of the root is taken to relieve pain associated with kidney. Leaves are used internally in dysentery and diarrhoea. The aqueous extracts of leaves, when administered parentrally to female guinea pigs, not only raised the phagocytic index but also mobilized a greater number of leucocytes to take part in phagocytosis. The green leaves are described to be eaten raw for the cure of dysentery. In Ceylon a decoction of the leaf is given internally in snake bite. In Indochina the fruit is considered astringent. *Murraya koenigi* is an aromatic more or less deciduous shrub or a small tree up to 6 m in height and 15-40 cm in diameter found throughout India up to an altitude of 1,500 m commonly in forests often as gregarious under growths. It is cultivated for its aromatic leaves. Root and bark are stimulant and are applied externally for skin eruptions and poisonous bites. Green leaves are febrifuge and are used in dysentery. The whole plant is considered to be a tonic and stomachic. The leaves are used extensively as a flavoring agent in curries and chutneys[2,3,4,5,6]. The main constituents reported are sterols, aminoacids, glycosides, proteins and flavanoids. Acytotoxic coumarin murrayatin was reported by Barik, Dey.A.K,1983a [7].Synthetic carbazole alkaloids examined 1- methoxy carbazolequinones for their potential cytotoxicity against MOLT-4 leukaemic and HOP -18 non small cell lung cancer cells [8]. Hence plants containing flavanoids are constantly being screened for antitumour activity [9].So it was decided to illustrate the ethnobotanical use of the plant and the study was planned to evaluate the SU II against DAL.

Materials and Methods

Plant materials: Whole dried stem bark of *Murraya koenigii* (Linn) were collected in the of July, Madurai district of Tamilnadu, India. Taxonomic identification made from Dr.Stephen , Botanist, American College, Madurai. The stem bark dried in the shade. Then the

shade dried stem barks were powered to get a coarse powder.

Preparation of extracts: Dried coarse powder of the stem bark (1kg) was placed into the extractor of a Soxhlet apparatus and subjected to extraction by hot percolation method. The extraction was carried out by using solvents of increasing polarity starting from petroleum ether, chloroform and acetone. The extraction was carried out with 2 liters of each solvent for a period of 72 hours. At the end of the extraction the respective solvents were concentrated by evaporation.

Preliminary phytochemical investigation: The qualitative chemical test of various extracts of *Murraya koenigii* was carried out using standard procedure [10,11,12,13]. Carbohydrate, sterols, Coumarins, Flavonoids and alkaloids are present in the extracts.

Preparation of column chromatography: After screening the various extracts obtained from 1 kg of coarse bark powder, the petroleum ether extract and chloroform extract were found to be promising. The petroleum ether (8gm) was a dark green residue. He chloroform extract was dark brown residue (20gm) were pooled and chromatographed over silica gel (100-200mesh). Then the compounds separated starting with solvents like n-hexane, petroleum ether, benzene, chloroform, acetone, ethyl acetate and methanol and the compounds isolated as (Su -I, Su-II, Su-III)

Animals used: Albino wistar rats 120-150 gms and Swiss albino mice 25-30 gms of either sex were obtained from the standard animal house, Madurai. The animals were maintained in a well ventilated room with 12:12 hour light / dark cycle in polypropylene cages [14]. The animals were fed with standard pellet feed and water was given *ad libitum.*

Screening of Anti-Inflammatory activity: Anti-inflammatory Activity of *Murraya koenigii* was studied in adult albino rats of either sex weighing between 120-150gms by Carrageenan Induced hind paw edema method [15,16].Animal were divided into four groups containing 6 animals per each. Group I (control) was given a 1% Sodium carboxy methyl Cellulose solution. Group II of animals received 10mg/kg of Diclofenac sodium which was considered as standard. Group III and Group IV were treated with 250 mg/kg and 500 mg/kg of methanol extract respectively dissolved in Sodium carboxy methyl Cellulose solution. Doses were given orally with the help of an oral catheter. 0.1 ml of 1% solution of carragenan was administered to the rats into the plantar surface of the right hind limb to induce paw edema. Paw volume was measured plethysmographically after 1h, 2h and 3h, 4h of carragenan injection and paw swelling in groups of drug treated were compared with control. Percentage inhibition of oedema was calculated by using the following formula

$$\%\text{inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

where

Vt- means increase in paw volume in rats treated with test compounds

Vc-means increase in paw volume in control group of rats.

Determination of anti tumour activity [17,18]

***In vitro* Studies:** *In vitro* cytotoxic studies was carried out by short term incubation method. The extracts SU I, SU II and SU III were used. The Daltons Lymphoma Ascites Cell line were used.1 ml of tumour cells was diluted with 5 ml of ice cold normal saline and 1 ml of the drug were incubated for 3 hours at 37° C. The dead cells were determined by using trypan blue. The living and dead cells were conted using haemocytometer.

***In vivo* Studies:** The animals acclimatized to our laboratory conditions were divided into four groups viz G1,G2,G3 and G4 each with 3 mice and used for the study [19].The DAL cells were obtained through the courtesy of Amala Cancer Institute, Thrissur, Kerala and injected

intraperitoneally (10^6 cells per mice) to all the mice of the three groups except G1.On the second day the animals of G4 were treated with SU II 7 mg/kg of column extract of *Murraya koenigii*. Intraperitoneally while the mice of G3 were treated with 5-FluroUracil (20mg/kg) and the treatment was continued for 10 days.G2 was not allocated any treatment after inoculation with DAC cells. The mice were observed for next 10 days for the development of ascitic tumour. On day 16, the cancer cell count was estimated.

Results

In vivo studies the cancer cells of the cancer control group was found to be 2.4 lakhs/ cu mm. For the standard (5-Flurouracil) the cancer cell count was fond to be 70,000 cells/cu mm. After treatment of SU II , the cancer cell count for test was found to be 1,50,000 cells/cu mm. *In vitro* studies all the column extracts showed moderate activity. The percent inhibition of inflammation at the third hour was in the rank order of SU II (60%)> SU III(58.42%) > SUI (57.36%) when compared with the standard drug Diclofenac sodium (60.5%). All the three extracts possess significant anti-inflammatory activity [Fig 1]

Table 1: Effect of extract of *Murraya koenigii* on DAL induced mice *(In vivo)*

Treatment	Cancer cell number ($\times 10^6$)
G1 (normal Control)	-
G2 (cancer)	2.4 lakhs cells/cu mm
G3 (5-FU Treated)	70,000 cells/cu mm
G4 (Test control)	1,50,000 cells/cu mm

G1 – normal control
G2 - control (DAL induced, non-treated)
G3 -20mg/kg of 5- fluorouracil treated group
G4 -7 mg/kg of SU II treated group.

Table 2: Effect of extract of *Murraya koenigii* on DAL induced mice *(In vitro)*

Drug	Dead Cells
Control	25%
SU- I	30%
SU-II	55%
SU-III	40%

Su -I, Su-II, Su-III are isolated compounds

Discussions

Intraperitoneal inoculation of DAL cells in the mice produced an enormous increase in the cancer cell count which indicated that there is progression of cancer in the animals. The decrease in the cancer cell number observed in the ether extract of *Murraya koenigii* the treated mice of G4 indicates that the test drug is having significant inhibitory effect on the tumour cell proliferation. The increase in tumour weight of G2 may be due to accumulation of peritoneal fluid as an abnormal enlargement of peritoneal cavity was observed in tumour-induced mice. Treatment with extract of *Murraya koenigii* reduced the tumour weight and hence increased the life span. These observations on the effect of extract of *Murraya koenigii* on parameters studied to evaluate the antitumour activity enabled to conclude that it has significant antitumour activity. However further investigations are essential for the isolation of the principle of extract of *Murraya koenigii* and it's mechanism of action.

Carrageenan induced paw edema was taken as a proto type of exudative phase of acute inflammation. Inflammatory stimuli microbes, chemicals and necrosed cells activate the different mediator leaves through a common trigger mechanism. The development of carrageenan induced edema is believed to be biphasic. The early phase is attributed to the release of histamine and serotonin [20,21,22] and the delayed phase is sustained by the leucotrienes and prostoglandins [23]. Flavonoids and tannins are reported to inhibit PG Student synthesis [24]. Most of the NSAIDS have well balanced anti inflammatory and ulcrogenic activities,

Table 3: Anti inflammatory activity of *Murraya Koenigii*

Sl. No.	Drug	Paw value in ml ± SEM and percentage of inhibition				
		0 Min	30 MIN	1 Hour	2 Hours	3 Hours
1	Control	0.543 ± 0.02	0.624 ± 0.05	0.672 ± 0.008	0.702 ± 0.001	0.76 ± 0.01
2	Standard (Diclofenac Sodium)	0.498 ± 0.01	0.513 ± 0.02	0.403 ± 0.06	0.358 ± 0.04	0.302 ± 0.01
3	Su – I	0.489 ± 0.04	0.496 ± 0.001 20.5%	0.424 ± 0.087* 36.9%	0.372 ± 0.0025** 47%	0.324 ± 0.05** 57.36%
4	Su –II	0.403 ± 0.06	0.520 ± 0.05 16.6%	0.476 ± 0.001* 29.16%	0.367 ± 0.02** 47.7%	0.304 ± 0.01** 60%
5	Su – III	0.520 ± 0.06	0.522 ± 0.04 16.34%	0.515 ± 0.04* 23.1%	0.380 ± 0.04** 45.86%	0.316 ± 0.05** 58.42%

* - P < 0.01 significant
** - P < 0.001 highly significant

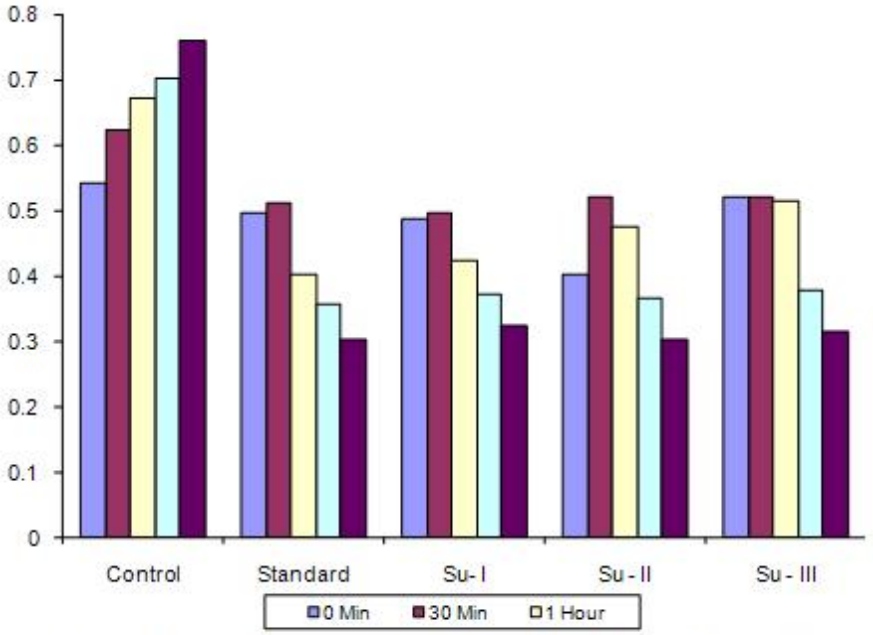

Fig 1: Anti inflammatory activity of *Murraya Koenigii*

which are considered to be due to PG synthetase inhibitor activity. The plant extract possess a marked anti-inflammatory activity and hence may pose itself as very good anti-inflammatory drug. Still further investigation with respect to pharmacological and phytochemical profile of the drug needs to be carried out. Three distinct phases are observed during inflammation which are the histamine and serotonin released in the first phase, Kinin and Prostoglandin are released in the second and third phases respectively [25]. Carrageenan induced hind paw edema in the standard experimental model of acute inflammation. Carrageenan in the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. The extract *Murraya koenigii* produced significant inhibition of Carrageenan induced paw edema. The inhibition was however less than that of the standard drug.

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