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Article *in* International Journal of Research in Ayurveda and Pharmacy · June 2013

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TOXICOLOGICAL STUDIES AND CYTOTOXIC ACTIVITY OF ETHANOL AND ETHYL ACETATE EXTRACTS OF *TECOMARIA CAPENSIS* LEAVES

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Received on: 17/01/13 Revised on: 10/02/13 Accepted on: 20/03/13

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DOI: 10.7897/2277-4343.04324

Published by Moksha Publishing House. Website www.mokshaph.com

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ABSTRACT

In the present study ethyl acetate and ethanol extracts of leaves of *Tecomaria capensis* was screened for cytotoxic activity. The cytotoxic activity was performed by two models. One was short term cytotoxicity and another was long term cytotoxicity. In short term cytotoxicity assay Dalton's lymphoma ascites (DLA) and Ehrlich ascites carcinoma (EAC) cell lines were used and for long term L929 cell lines (Lungs fibroblast) were used. In both methods ethyl acetate and ethanol extracts showed protective action against the cell lines. Comparing both extracts, ethanol extract has shown better cytotoxic activity than the ethyl acetate extract and in comparison of standard both extracts have moderate cytotoxic activity.

Keywords: *Tecomaria capensis,*T oxicological studies, Cytotoxicity.

INTRODUCTION

Tecomaria capensis (family:Bignoniaceae) also known as Cape-honeysuckle is a fast growing, scrambling shrub which may grow up to 2-3m high and spread more than 2.5m. *Tecomaria capensis* is an evergreen plant in warm climate areas but loses its leaves in colder areas. It has pinnately compound leaves that have oval leaflets with blunt teeth. Flowering time for this shrub is very erratic and often it flowers round the year. Flowers are orange in color. Plant is used as a traditional medicine to relieve pain and sleeplessness. Dried powdered bark infusions are taken for sleeplessness[1] and reported to induce sleep[2]. It is included in the list of African plants evaluated for in vitro antiplasmodial activity[3].

MATERIALS AND METHODS

Plant materials and Preparation of Extracts

The leaves of *Tecomaria capensis* were collected from Guntur, Andhra Pradesh. It was authenticated by professor Dr. S.M. Khasim, Department of Botony and Microbiology, Acharya Nagarjuna University, Nagarjuna nagar, Guntur, India. The leaf part of *Tecomaria capensis* was dried at room temperature and grounded into powder and passed through 60# sieve. The powder (500gm) was extracted successively in soxhlet by ethanol and ethyl acetate. The sediments were filtered and the filtrate was dried at 40°C in an oven to get dried product. The different fractions obtained were used for further study.

Acute oral toxicity study and selection of doses

Acute Toxicity Study

Healthy Wistar albino rats of both sexes weighing between 120-150 g maintained under standard laboratory conditions were used for the acute toxicity test according to the Organization for Economic Cooperation and Development (OECD) guidelines 423 (OECD guideline, 2002). A total of ten animals of equal numbers of male and female rats were used and each received a single oral-dose of 2000 mg kg-1 body weight of ethyl acetate and ethanol extracts of *Tecomaria capensis*. Animals were kept overnight fasting prior to drug administration by oral gavage. After administration of drug sample, food was withheld for further 3-4 hour. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 hour (with special attention during the first 4 h) and daily thereafter for a period of 7 days. Daily observations on the changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes were noted (OECD, 2002)[4]. Experimental protocol was approved by the institutional animal ethics committee IAEC PROTOCOL.NO: 9/IAEC/VPC/pharma/RES/2011-2012.

Sub-Acute Toxicity Study

Wister albino rats of either sex weighing 150-200gms were assigned to each group (6 per group). Group 1 received distilled water for 28 days and group 2 received the test drug ethanol extract at the dose of 200 mg/kg P.O. once daily for 28 days. Group 3 received the test drug ethyl acetate extract at the dose of 200 mg/kg body weight once daily for 28 days. Body weight, food intake, and water intake were monitored. The animals were sacrificed on the day 29, blood samples were collected for haemotogical parameters like haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), erythrocyte sedimentation rate (ESR), differential leucocytes count and biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate

pyruvate transaminase (SGPT), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and serum creatinine[5].

Short Term *in-vitro* Cytotoxicity assay by Trypan Blue Dye Exclusion Technique

Cells were aspirated from the peritoneal cavity of tumour bearing mice. The cells were washed three times using phosphate buffer saline (PBS). The viability of the cells was checked using trypan blue. The cell suspension was added to tubes containing various concentrations of the test compounds and the volume was made up to 1ml using phosphate buffer saline (PBS). These assay mixtures were incubated for 3hours at 37^0C and then 1ml of trypan blue was added after incubation and the number of dead cells was counted using a haemocytometer[6].

Table 1: Effect of 28 days oral administration of the extracts (Ethanol and Ethyl acetate) on organ weights in rats

Group	Treatment	Organ weight (g/100g body weight)		
		Liver	Kidney	Heart
1.	Control	2.78±2.1	0.70±1.2	0.33±0.5
2.	Ethyl acetate	2.98±0.7	0.69±1.0	0.34±0.7
3.	Ethanol	3.0±0.6	0.68±0.8	0.36±1.1

Mean ± S.E. of 6 animals (one-way ANOVA)

Table 2: Effects of 28 Days Administration of the extracts (Ethanol and Ethyl acetate) on Haematological Parameters in Rats

Group	Treatment	Hb (%)	RBC (mm^3)	WBC (mm^3)	Differential count Percentage			
					N	L	E	B
1.	Control	11.5±0.2	7.38±2.3	9.9±12.4	68.2±2.1	28.2±2.1	1.2±0.4	--
2.	Ethyl acetate	12.6±0.9	7.53±5.1	10.0±8.8	69.3±5.6	28.2±2.1	1.0±0.8	--
3.	Ethanol	11.5±0.2	7.48±2.6	10.1±9.0	68.2±2.1	28.8±2.4	1.1±0.6	--

Mean ± S.E. of 6 animals (one-way ANOVA)

HB-Haemoglobin, RBC-Red Blood Cells, WBC-White Blood Cells, N-Neutrophils, L- Lymphocytes, E-Eosinophils, B-Basophils

Table 3: Effects of 28 Days Administration of the extracts (Ethanol and Ethyl acetate) on Hepatic Function in Rats

Group	Treatment	Liver Glycogen (mg %)	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)
1.	Control	134.8±0.6	72.0±1.39	53.67±1.91	33.0±1.53
2.	Ethyl acetate	138.2±3.4	70.2±1.19	52.73±0.81	32.4±1.87
3.	Ethanol	136.2±5.3	71.4±1.29	53.67±1.91	32.9±3.4

Mean ± S.E. of 6 animals (one-way ANOVA)

SGPT-Serum Glutamate Pyruvate Transaminase**, SGOT-** serum glutamate oxaloacetate transaminase**, ALP-** Alkaline Phosphatase**.**

Table 4: Effects of 28 Days Administration of the extracts (Ethanol and Ethyl acetate) on Renal Function in Rats

Group	Treatment	Blood Urea (mg %)	Serum Creatinine (mg %)
1.	Control	16.2±0.5	0.98±1.2
2.	Ethyl acetate	17.1±0.5	1.04±0.8
3.	Ethanol	16.8±1.0	1.0±1.1

Mean ± S.E. of 6 animals (one-way ANOVA)

Table 5: Effect of ethyl acetate and ethanol extracts of *Tecomaria capensis* leaves against DLA and EAC cell lines by Trypan blue dye exclusion method

Sample	Concentration µg/ml	Percentage cytotoxicity of DLA cell lines	Percentage cytotoxicity of EAC cell lines
Ethyl acetate	10	18.13 ± 2.87	24.13 ± 2.87
	20	24.58 ± 3.18	39.62 ± 1.41
	50	37.15 ± 3.51	59.81 ± 4.23
	100	54.95 ± 2.98	71.73 ± 4.21
	200	62.58 ± 3.60	83.95 ± 8.57
Ethanol	10	23.13 ± 2.97	27.52 ± 2.41
	20	32.45 ± 3.51	42.14 ± 7.52
	50	51.38 ± 4.90	64.85 ± 1.43
	100	73.62 ± 3.60	79.42 ± 3.52
	200	94.37 ± 3.00	96.38 ± 4.72
Curcumin	10	42.17 ± 2.12	47.21 ± 3.45
	20	55.49 ± 6.12	64.38 ± 1.87
	50	70.62 ± 7.14	85.71 ± 1..43
	100	80.75 ± 7.15	91.42 ± 2.21
	200	100 ± 1.14	100 ± 4.62

DLA- Dalton's lymphoma ascites cell lines, **EAC**-. Ehrlich ascites carcinoma cell lines

Table 6: Effect of ethyl acetate and ethanol extracts of *Tecomaria capensis* leaves against L929 cell lines by MTT assay

Sample	Concentraction µg/ml	Absorption at 570nm	Percentage (%) of cytotoxicity
Control		0.318 ± 0.10	
Ethyl acetate	10	0.254 ± 0.092	20.12
	20	0.231 ± 0.017	27.35
	50	0.217 ± 0.032	31.76
	100	0.209 ± 0.054	34.27
	200	0.197 ± 0.068	38.05
Ethanol	10	0.182 ± 0.026	42.76
	20	0.093 ± 0.067	70.75
	50	0.047 ± 0.054	87.41
	100	0.012 ± 0.047	96.22
	200	0.000 ± 0.000	100
Curcumin	10	0.017 ± 0.041	94.65
	20	0.009 ± 0.017	97.16
	50	0.003 ± 0.023	99.05
	100	0.000 ± 0.000	100
	200	0.000 ± 0.000	100

Long term *in vitro* cytotoxicity by MTT assay

Cells were seeded in 96-well flat-bottom plates and allowed to adhere for 24hours at 37^0C with 5% CO_2 atmosphere. Different drug concentration was added and incubated further for 48 hours. Before 4hours of the completion of incubation, 20µl of MTT (5mg/ml) was added. Percentage of dead cells was determined using an ELISA plate reader set to record absorbance at 570nm[7].

RESULTS

Acute Toxicity Study

In the acute toxicity study of both extracts (Ethanol and Ethyl acetate) treatment, no related mortalities recorded in animals treated with a single dose of 2000mg/kg body weight. Therefore, the approximate lethal dose (LD_{50}) of both extracts in the experimental rats was higher than 2000 mg/kg. There were no clinical signs in the skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) among rats administered 2000 mg kg-1 body weight of both extracts (Ethanol and Ethyl acetate). According to organization for economic cooperation and development (OECD) guidelines for acute oral toxicity, an LD_{50} dose of 2000mg/kg and above is categorized as unclassified and hence the drug is found to be safe.

Sub-Acute Toxicity Study

In the sub-acute toxicity study, both the extracts (Ethanol and Ethyl acetate) treated groups did not show any significant changes in body weight at weekly intervals compared to the control group. The weights of the liver, kidney, and heart (Table 1) were unaltered in the experimental groups compared with the control group. The haematological and biochemical parameters (hepatic and renal function test) (Table 2 and 3) did not show any significant changes in the both extracts (Ethanol and Ethyl acetate) treated groups when compared to the control group. The histopathological section of various organs such as the liver, heart, kidney, pancreas and uterus revealed normal architecture on comparison with the control group. In the sub-acute toxicity study, both the extracts (Ethanol and Ethyl acetate) treated groups did not show any significant changes in body weight, indicating that it did not have any adverse effects on body weight, which is used to assess the response to therapy of drugs and to indicate the adverse effects of a drug. The organ (liver, kidney and heart) weights in the test drug treated group remained normal, indicating that both the extracts (Ethanol and Ethyl acetate) were not toxic in these vital organs. Furthermore, the histopathology results indicated that it was not toxic in the liver, kidney, heart, adrenals, pancreas and uterus since they all exhibited normal architecture. There were no significant changes in any liver function parameters, such as SGOT, SGPT, ALP and liver glycogen, compared to the control group. Increase in these parameters would have indicated hepatocyte damage. The normal levels of blood urea and serum creatinine (Table 4) indicate that the test drug did not interfere with renal function and that renal integrity was preserved. Also, there were no significant changes in various haematological parameters such as Hb, RBC, WBC, ESR and differential count compared to the control group, which indicates that both the extracts (Ethanol and Ethyl acetate) may not be toxic and does not affect circulating red cells, hematoieses, or leucopoiesis.

Short term *in vitro* cytotoxicity assay

Short term cytotoxicity was evaluated by trypan blue dye exclusion method. Viable cells which remained unstained by trypan blue were counted in a haemocytometer. Cytotoxicity of ethyl acetate and ethanolic extracts of *Tecomaria capensis* leaves to DLA and EAC cells culture was showed in Table 5.

The percentage cytotoxicity of the DLA cells at different concentrations ranging from 10µg/ml to 200µg/ml showed a dose dependent inhibition of the growth of DLA cells. The percentage cytotoxicity of the EAC cells at different concentrations ranging from 10µg/ml to

200µg/ml showed a dose dependent inhibition of the growth of EAC cells.

Curcumin was used as the reference drug for the DLA and EAC cell lines and it produced 100% cytotoxicity at 200µg/ml. In DLA cell line ethyl acetate extract showed 62.58% and ethanol extract showed 94.37%. Whereas in EAC cell line ethyl acetate extract showed 83.95% and ethanol extract showed 96.38%. Compared to standard, ethyl acetate and ethanolic extracts of leaves of *Tecomaria capensis*leaves was found to have cytotoxic effect but slightly weaker when compared to standard drug.

Ethanol extract showed better action comparing to ethyl acetate extract whereas the ethyl acetate extract showed moderate action. Results were comparable to that of the standard. This result emphasized cytotoxic nature of ethyl acetate and ethanolic extracts of *Tecomaria capensis* leaves against DLA and EAC cells.

Long term *in vitro* cytotoxicity assay

Long term cytotoxic effect of ethyl acetate and ethanolic extracts of *Tecomaria capensis* leaves on L929 cells was investigated by MTT assay. Cells were treated at concentrations ranging from 10-200µg/ml for 48hours and then the percentage of cell viability was analysed. Results are showed in Table 6.

Ethyl acetate and ethanolic extracts of *Tecomaria capensis*leaves were significantly inhibited the proliferation of L929 cells in a dose dependent manner. Extracts showed a concentration dependent cytotoxicity to cultured L929 cells. Reference drug used was curcumin as it showed 100% protection, whereas ethyl acetate showed 38% and ethanol extract showed 100% at 200µg/ml concentration. The ethanol extract showed better action like curcumin but minimal difference in concentration. Comparing ethyl acetate and ethanolic extracts, Ethanol extract showed better cytotoxic action.

DISCUSSION

Cancer is the leading cause of mortality worldwide, and the failure of conventional chemotherapy to effect a major reduction in mortality indicates that new approaches are critically needed[8]. Plants have played an important role as a source of effective cancer agents and it is significant that 60% of currently used anti-cancer agents were derived from natural sources, including plants, marine organisms and microorganisms. The control of cell proliferation is crucial in maintaining cellular homeostasis and loss of this mechanism is a principle hallmark of cancer cells. Thus, the inhibition of tumour cell growth without side effects is recognized as an important target for cancer therapy[9].

The results of the Trypan blue dye exclusion assay indicated that ethyl acetate and ethanolic extracts of *Tecomaria capensis* leaves could inhibit the growth of DLA and EAC cells in a dose dependent manner. The current investigation was also designed to explore the ethyl acetate and ethanolic extracts of *Tecomaria capensis* leaves for their oncolytic properties using standard MTT assay. The MTT reduction as a cell viability measurement is now widely chosen as the most advantageous end point[10]. The results of the MTT assay indicated that ethyl acetate and ethanolic extracts of *Tecomaria capensis* leaves significantly inhibited the proliferation of L929 cells in a dose-dependent manner.

CONCLUSION

In this study we conclude that no toxicological sign was shown by ethyl acetate and ethanol extracts of *Tecomaria capensis* in acute and sub-acute toxicological studies. In cytotoxic activity both extracts have better activity. Comparing the extracts ethanol extract showed a better activity than the ethanol extracts and they are comparable to that of standard.

ACKNOWELEDGMENT

Authors are thankful to Vignan Pharmacy College principal Dr. P. Srinivasa Babu for providing the lab facility to perform this work.

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Cite this article as:

E. Tamil Jothi, G. Vimala Devi, Ch. Vamsi Anil Krishna and V. Suba. Toxicological studies and cytotoxic activity of ethanol and ethyl acetate extracts of Tecomaria capensis leaves. Int. J. Res. Ayurveda Pharm. 2013;4(3):426-429

Source of support: Nil, Conflict of interest: None Declared

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