



ANTITUMOR ACTIVITY OF METHANOLIC EXTRACT OF *CYNODON DACTYLON* LEAVES AGAINST EHRlich ASCITES INDUCED CARCINOMA IN MICE

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ABSTRACT

The aim of the present study is to evaluate the effect of methanolic extract of leaves of *Cynodon dactylon* against Ehrlich ascitic Lymphoma (ELA) in Swiss albino mice. The tumor was induced in mice by intraperitoneal injection of EAC (1X10⁶cells/mouse). Methanolic extract of *cynodon dactylon* was administered to the experimental animals at a dose of 80µg/kg/day after 24 h of tumor inoculation. The antitumor effect of extract was evaluated by assessing *in vitro* cytotoxicity, hematological parameters and liver enzymes. The methanolic extract brought back the altered levels of the hematological parameters and liver enzymes. Thus the present study revealed that methanolic extract of *Cynodon dactylon* possessed significant antitumor activity and hepatoprotective effect.

Keywords: *Cynodon dactylon*, ELA, liver enzymes, Cytotoxicity, Hematological parameters

1. INTRODUCTION

Cancer is a disorder developed due to some molecular changes within the cell. It becomes the second major cause of death in the human after cardiovascular disease [1]. It is a hyperproliferative disorder that involves transformation, dysregulation of apoptosis, proliferation, invasion, angiogenesis and metastasis. Cancer is a group of more than 100 different diseases, characterized by uncontrolled growth, local tissue, invasion and distant metastases [2]. Chemotherapy, radiotherapy and surgery are only three major existing modes of treatment in modern medicine. Chemotherapy is still a major challenge to the cancer patients because such highly potent drug can be toxic and less than 1% of injected drug molecules can reach their target cells, whereas the rest may damage healthy cells and tissue [3]. Drug discovery from the medicinal plants has played an important role in the treatment of cancer and indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last half-century have been made towards combating cancer [4].

The *cynodon dactylon* (Family; Poaceae), commonly known as 'doob' (Hindi), 'arogum pillo' (Tamil), is called creeper in India. It is an important medicinal plant which is used for treatment of various ailments in Ayurvedic system of medicine. *Cynodon dactylon* extracts possess antiulcer activity [5] hepatoprotective activity [6] cardioprotective effect [7] and also protein fractions of *cynodon dactylon* possessed immunomodulatory activity and antioxidant activity in Swiss

albino mice [8, 9] has been reported. We have already reported the antioxidant activity of Phenolic fractions of *Terminalia catappa* in ELA propagated Swiss albino mice [10]. The objective of the present study is to evaluate the antitumour activity of methanolic extract of *cynodon dactylon* in ELA induced Swiss albino mice.

2. MATERIAL AND METHODS

2.1. Plant Material

Fresh leaves of *Cynodon dactylon* were collected in area, free of pesticides and other contaminants from the area surrounding of Coimbatore Dt, Tamilnadu. The collected leaves were washed thoroughly and blotted dry with filter paper and used for the preparation of the extract.

2.2. Preparation of Methanol Extract

The extraction was undertaken with 20 g of powdered plant material and 200ml. of light petroleum ether (B.P. 40°C – 60°C) in a Soxhlet apparatus for 18 hours to remove the chlorophyll and lipid de waxing. The treated material was dried and extracted with methanol using Soxhlet apparatus for 4 hours. The extract was concentrated in vacuo using a rotary evaporator.

2.3. Tumour Cell lines

Ehrlich ascites carcinoma (EAC) cells were obtained under the courtesy of Amala Cancer Research Center, Thrissur, India. They were maintained by weekly intra-peritoneal inoculation of 10^6 cells/per mouse.

2.4. Animals

In bred Swiss albino mice weighing on an average 20-25 g procured from Small Animal Breeding Station, Medical College, Perundurai, and Tamilnadu were used to evaluate the antitumorigenic effect in ELA tumor induced Swiss albino mice. These animals were maintained for two weeks under environmentally controlled conditions with free access to standard food (Lipton, India) and water *ad libitum*, prior to the experiments. All animal experiments were carried out according to the guidelines prescribed by Animal Welfare Board and with the approval of Animal Ethic Committee (Register no: 623/02/b/CPCSEA). The mice were acclimatized for two weeks and cells were propagated by intraperitoneal transplantation of 1×10^6 cells in 100 μ l of PBS. After 10-15 days, the cells were drawn from the intraperitoneal cavity and used for the *in vitro* studies.

2.5. Determination of in vitro cytotoxic activity using EAC cell lines

In vitro cytotoxic studies were carried out to find out the 50 per cent effective concentration (EC_{50}) of methanolic extract of *Cynodon dactylon* by trypan blue exclusion method [11]. The number of (stained) dead cells randomly in every 200 cells was counted [12]. The results were recorded as percent protection against tumor growth calculated as the difference between the number of dead cells in treated and untreated animals expressed as a percentage of the number of dead cells in untreated (control) animals.

2.6. Antitumor activity

Male Swiss albino mice were divided into three groups (n = 6). All the groups were injected with EAC cells (1×10^6 cells/mouse) intraperitoneally except Group I. This was taken as day Zero.

Group I - Normal control.

Group II - Disease Control, EAC cell line (1×10^6 cells /mouse).

Group III - EAC cell line (1×10^6 cells/mice) treated with 80 μ g of extract in 100 μ l of Dimethyl sulphoxide (DMSO) /kg.bw.

All this treatments were given 24 h after the tumor inoculation, once daily for 14 days after the last dose and 24 h fasting, six mice from each group were sacrifice. The blood was collected from the animals by retro-orbital puncher under slight anesthesia (diethyl ether) conditions; and the

hematological parameter such as hemoglobin (Hb) content, red blood cell (RBC) and white blood cell count [13] and the remaining blood was centrifuged and serum was used for the estimation of liver enzymes like ALT, AST [14] and ALP [15].

3. STATISTICAL METHODS

All values are expressed as mean \pm SD

4. RESULTS

4.1. Cytotoxicity of methanolic extract of *Cynodon dactylon* towards ECA cell lines

The methanolic leaf extract of *Cynodon dactylon* was found to be cytotoxic towards Ehrlich ascites carcinoma cells only at higher concentration (Table 1). At concentration of 80 μ g and 90 μ g it produced 100% cell death. The methanolic leaf extract produced a concentration dependent cytotoxic effect to EAC cells.

Table 1: Effect of methanolic extract of *Cynodon dactylon* on cytotoxicity of Ehrlich ascites carcinoma

S.no	Concentration /ml	% of cytotoxicity EAC cells
1	Control	8
2	10	18
3	25	20
4.	50	24
5	60	55
6	70	75
8	80	100
9	90	100

4.2. Effect of on methanolic extract of *Cynodon dactylon* on Hematological parameter

As shown in Table 2 the total WBC was found to be increased with a reduction in RBC and Hb in tumor control animals. At the same time treatment with extract at the dose of 80 μ g/kg could change those altered parameter to near normal. The present investigation was carried out to evaluate the antitumor activity of methanolic extract in EAC tumor bearing mice.

4.3. Effect of methanolic extract of *Cynodon dactylon* on liver enzymes

As shown in Table 3, the AST, ALT and ALP activities were increased in ELA treated mice when compared to control mice. Co-administration of methanoloic extract of *Cynodon* to

ELA tumor induced mice showed significant decreases the activities of AST, ALT and ALP.

Table 2. Effect of methanolic extract of *Cynodon dactylon* on the hematological parameters in Swiss albino mice

Groups	Heamoglobin (%)	RBC 1×10^6 cells/mm ³	WBC 1×10^3 cells/mm ³
Group I Normal control	12.4±0.289	5.35±0.361	10.91±0.43
Group II Disease control	8.63±0.216	3.28±0.194	20.11±0.729
Group III ELA+Cynodon extract	9.88±0.147	4.30±0.228	17.05±0.578

The values are the mean ± SD of 6 animals

Table 3. Activities of serum AST, ALT and ALP in control and experimental Swiss albino mice

Groups	AST(U/ L) ^a	ALT(U/ L) ^a	ALP (U / L) ^b
Group I Normal control	48.76 ±0.585	92.83 ±3.12	125.83 ±3.48
Group II Disease control	84.55 ± 1.42	98.58±3.07	195.66 ±3.44
Group III ELA+Cynodon extract	53.51±0.818	94.5± 2.23	135.0±4.30

The values are the mean ± SD of six animals

^a micromole of pyruvate formed / minute

^b micromole of phenol formed / minute

5. DISCUSSION

Plants have served as a good source of antitumor agents. A large number of plants possessing anticancer properties have been documented. In cancer chemotherapy the major problems are of myelosuppression and anemia. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC and Hb% [16, 17] and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions [18].

Enzymes in serum have been studied for many years as possible early indicators of neoplasia and as aids in following the progression and regression of disease [19]. Hepatotoxicity may occur due to cytotoxic agent itself, or due to its toxic metabolites. The levels of serum AST, ALT and ALP activities were significantly increased in ELA tumor induced mice when

compared to controls. The activities of AST, ALT and ALP were decreased on treatment with the extract of *cynodon dactylon*. The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals [20]. The increase in the activities of these enzymes in serum might be due to the leakage of these cytosolic enzymes into the in circulatory system resulting from hepatocellular damage during ELA propagation. This is indicative of the onset of hepatocellular damage due to liver dysfunction and disturbance of the biosynthesis of these enzymes, with alteration in the permeability of liver membrane. Treatment with the extract significantly reversed the alterations in the status of these markers to normal levels, possibly by maintaining the hepatocellular membrane integrity. This is an indicator of possible hepatoprotective property offered by methanolic extract of *Cynodon dactylon*.

6. CONCLUSION

It was reported that the presence of tumor in the human body or in the experimental animal is known to affect many function of the liver. The significantly elevated level of AST, ALT, ALP in serum of tumor inoculated animal indicated liver damage and loss of functional integrity of cell membrane. The significant reversal of these changes towards the normal by methanolic extract treatments in the present study, the biochemical examination of ELA inoculated animal showed marked changes indicating the toxic effect of the tumor. The normalization of these effects observed in the serum treated with methanolic extract of cynodon supported the potent antitumor and hepatoprotective effect of the extracts. Preliminary photochemical study of methanolic extract of cynodon indicated the presence flavanoids, phenolic compounds, Tannins' and phytosterols. The observed antitumor activity may be due to the presence of the any of these compounds.

7. REFERENCES

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