



Phytochemicals Screening and Evaluation of Anticancer, Antioxidant Activity of Dry Fruit Extract of *Ficus carica* Linn in Experimental Animals

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Abstract

The antioxidant and anticancer activity dry fruit extract of *ficus carica linn* evaluated against free radical scavenging activity (RSA) [reactions with 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and anticancer activity against Dalton's ascitic lymphoma cell line and assessment of the influence of extracts on the enzyme xanthine oxidase (XO), and Fe³⁺ reducing ability. Antitumor activity was studied on swiss albino mice at various dose such as 250, 500 and 1000 mg/kg, body weight. The experimental parameter used were tumor volume, tumor cell count, viable tumor cell count, mean survival time and increase in life span to assess antitumor activity. The extract was administered orally for 14 consecutive days to tumor bearing group of animals. It increases the life span of DAL treated mice and restore the hematological parameters as compared with the DAL bearing mice in dose dependent manner. The study revealed that the extract of *ficus carica* (EFC) showed significant antitumor activity in tested animal models.

Keywords

Ficus carica linn, Antioxidant, Anticancer, Xanthine oxidase, RSA, DPPH.

INTRODUCTION:

Cancer remains one of the leading causes of morbidity and mortality globally. Amongst the non-communicable disease, cancer is the second leading cause of death, after cardiovascular disease.¹ Remedies to treat such chronic state are available in nature in the form of herbal medicine or drug which is minimal adverse effect when compared to available synthetic drugs². Throughout the history of medicine, many effective drugs were derived from natural extracts of plants or animals. For example, the anti-malarial drug-quinine is extracted from the bark of the cinchona tree. This fact might suggest to us that more primary anticancer drugs could well be

found in nature. In the East since ancient times, especially in China and Korea, people have been using plant rhizomes, leaves or bark and other natural materials soaked in alcohol or wine as drugs to treat illness³. A plant-based diet also protects against chronic oxidative stress-related diseases⁴. Managements of cancer are a global problem and successful treatment is very much essential preventing or at least delaying the onset of long-term complication of the disorder. Remedies to treat such chronic state are available in nature in the form of herbal medicine or drug which is minimal adverse effect when compared to available synthetic drugs². Such herbal drugs as therapeutic agent is a boon

when compared to the severe adverse effect of the allopathic medical practice for cancer, though the quest for a complete and permanent cure for the diseases is being pursued relentlessly by eluding physician and researcher.

The benefits from achieving our goal will be to reduce the cancer mortality rate and improve the quality of life of those who develop cancer in the future.

The aim of research thesis is to identify and evaluate new, more effective, natural active principal.

The aim of projects is to identify new cancer drugs. We plan to do this by examining recently discovered biological targets that are predicted to offer the opportunity to develop drugs that are more potent and less toxic than the existing therapies. For instance, the reference with some targets is predicted to kill cancer cell without affecting normal ones and therefore the resulting drug is expected to have few side effects.

MATERIAL AND METHODS:

Plant materials and extraction⁵

The dried fruits of *Ficus carica*, were collected from Nimar region of Madhya Pradesh, India in the month November 20017 and identified and authenticated at Govt. PG College, Dept. of Pharmacognosy, Mandleshwar. A voucher specimen has been kept in Govt. PG College, Dept. of Pharmacognosy. The sun dry and coarsely powdered of fruit (600gm) extracted with Soxhlet apparatus using ethanol within 72 hours. The extracts were found brown and semisolid in nature.

Animals

The experimental protocol was approved by IACUC of Nimar Institute of Pharmacy, Dhamnod and Mature male Swiss albino mice weighing 20-25g were housed in standard isolation cages (45×35×25 cm) under environmentally controlled conditions with 12-h light/12-h dark cycle. They were allowed free access to water, standard laboratory chow (Patanjali Pvt. Ltd Haridwar) given food and water *ad libitum*. After sufficient period of acclimatization, they were used to evaluate anticancer activity.

Tumor Cell Line

Dalton's ascitic lymphoma (DAL) cells were obtained through the courtesy of the Cancer Research Centre, Indore, India. DAL cells were maintained by weekly intraperitoneal (i.p.) inoculation of 1×10^6 cells/mouse.

Antitumor activity in mice

After acclimatization, mature male Swiss albino mice divided into five groups (n=10) and given food and water *ad libitum*. All the groups (Table 1) except group I were injected with DAL Cells (1×10^6

cells/mouse. i.p.). This was taken as day 0. Group I served as normal saline control (5 ml/kg, p.o.) and Group II served as DAL control. On day 1, the EFC at a dose of 250, 500 and 100 mg/kg body weight (Gr-III, IV & V) were administered orally and continued for 14 consecutive days^{6, 7}. The dose of EFC was selected based on previous study on hepatoprotective activity.¹⁰ On day 15, five mice of each group were sacrificed 24 h after the last dose and the rest were kept with food and water *ad libitum* to check the increase in the life span of the tumor hosts. The effect of ethanol extract on tumor growth and host's survival time were examined by studying the parameters like tumor volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, mean survival time and increase in life span.^{6, 7, 8}

Determination of tumor volume

The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and packed cell volume determined by centrifuging at 1000 g for 5 min.^{8, 9}

Determination of tumor cell count

The ascitic fluid was taken in a RBC pipette and diluted 1000 times. Then a drop of the diluted cell suspension was placed on the Neuburger counting chamber and the number of cells in 64 small squares was counted.¹⁰

Estimation of viable tumor cell count

The cells were then stained with Trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were nonviable. These viable and non-viable cells were counted.^{9, 10, 11}

Cell count = (No. of cells x Dilution) / (Area x Thickness of liquid film).

Percentage increase life span

Recording the mortality monitored the effect of the EFC on tumor growth and percentage increase in life span (ILS %) were calculated.^{12, 13}

ILS (%) = [(Mean survival of treated group/ Mean survival of control group)-1] x100

Mean survival time = [1st Death + Last Death] / 2

Hematological studies

The effect of EFC on peripheral blood was investigated. RBC, WBC counts and estimation of hemoglobin were done by standard procedures from freely flowing tail vein blood. Serum protein conc. was estimated by Lowry's method and packed cell volume (PCV) was determined by the method described by Docie et al.^{14, 15}

Antioxidant activity

For measuring radical scavenging activity^{16,17} (RSA) against the stable radical N, Ndiphenyl-N'-

picrylhydrazyl, 0.1 and 0.2 ml of plant extract was added to 2.9 ml of DPPH 10⁻⁴ M solution in ethanol and the absorbance (A) was measured at 517 nm after 30 min incubation at 30°C (Brand-Williams et al., 1995). RSA was calculated in percent by the following formulae:

$$RSA = (A \text{ Contr.} - A \text{ Sample} / A \text{ Contr.} - A \text{ Blank}) \times 100.$$

Inhibition of xanthine oxidase was expressed as decreasing of uric acid generation (Noro et al., 1983).

The mixture of 2.6 ml of 0.225 M xanthine solution in 0.65 M PBS (pH=7.4) with 0.1 ml and 0.2 ml of plant extract (30 mg ml⁻¹) in ethanol (control ñ 0.1 ml ethanol) was incubated 5 min at 37°C. Afterwards 0.2 ml of XO (0.15 U ml⁻¹) in 0.65 M PBS (pH=7.4) was added and absorbance (A) at 290 nm was measured after 5 min. The inhibition was calculated in percent by the formulae: ^{17, 18, 19}

$$IE = 100 \times (A \text{ Sample} - A \text{ Control}) / A \text{ Sample}.$$

Statistical analysis

The experimental results were expressed as the mean ± S.E.M. Data were assessed by the method of one-way ANOVA test. P value of <0.05 was considered as statistically significant.

RESULTS:

The significant antioxidant and anticancer activity of dry fruit of *Ficus carica* Linn. Extract. The Phytochemical findings revealed the presence of, glycosides, flavonoids, and alkaloids.^{5, 6} The EFC treated group shown that decrease in mortality rate as compared to non-treated group. There is no significant increase in activity above the optimum concentration of extract. The viable cell count in controlled (group II) was significantly decreased while non-viable cell count was significantly

increased in EFC treated group. Hematological parameter was altered in group II as compare to normal group on 15th day. WBC, protein and PCV parameter were decrease and RBC and hemoglobin parameter was increase in EFC treated group. EFC extracts reflects the activity towards week free radicals and ability to decrease ferric ions into ferrous along with inhibition of XO in both concentrations.

DISCUSSION:

The Phytochemical finding indicated the presence of alkaloids, flavonoids, and terpenoids in EFC. Flavonoids have been shown to possess antimutagenic and effects. The above results demonstrated the antitumor effect of EFC against DAL in Swiss albino mice. A significant (P<0.05) enhancement of MST and non-viable cell count in peritoneal exudates (P<0.05) was observed due to EFC treatment. To evaluate whether EFC treatment indirectly inhibited tumor cell growth, the effect of EFC treatment was examined on the viable & non-viable cell counts against tumor bearing mice. Normally, each mouse contains about 5 x 10⁶ intraperitoneal cells, 50% of which are macrophage. EFC treatment was found to enhance nonviable cell counts in peritoneal exudates and decrease the viable cell count. EFC extracts increase the level of hemoglobin and RBC to normal level while decrease the WBC, protein, and PCV as compared to control group although there is anemia which usually occurred in cancer chemotherapy. The presence of flavonoids and antioxidant activity of *Ficus carica* linn shows the possible potent therapy for cancer treatment.

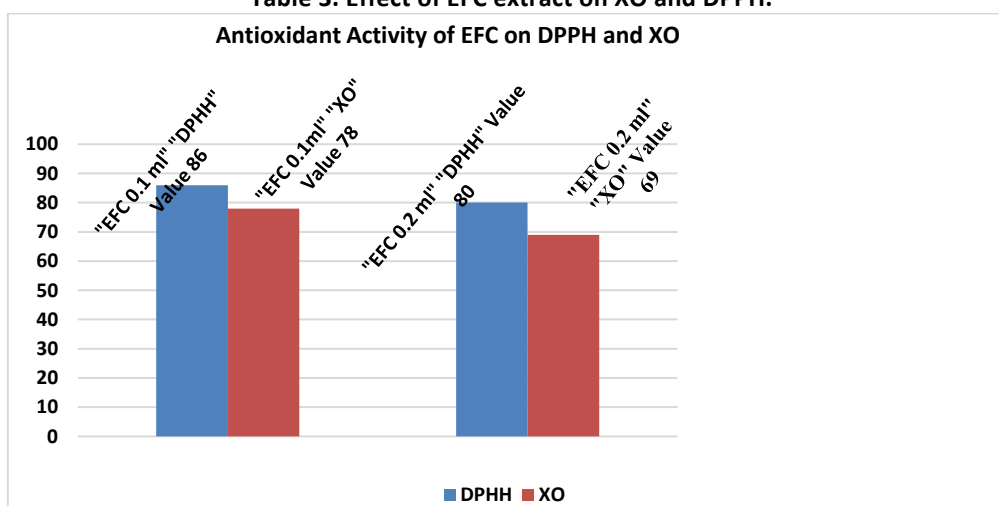
Table 1. Effect of Ethanolic extracts of dry fruit extract of *ficus carica* on survival time, life span, tumour volume, viable and non-viable cell count in DAL bearing mice.

Sr. no	Treatment group	Survival time	Increase of life span	Tumor volume	Viable cell count x 10 ⁶ cells/ml	Non-Viable cell Count x 10 ⁶ cells/ml
1	Normal Saline (5ml/kg P.O)	-	-	-	-	-
2	Dal control (1 x 10 ⁶ cells)	24.10±0.20	-	3.60±0.80	10.26±0.20	3.49±0.20
3	DAL control (1 x 10 ⁶ cells) + EFC (250mg/kg p.o)	31.56±1.02	45.60±1.56	2.80±0.01	7.60±1.05	2.70±1.20
4	DAL control (1 x 10 ⁶ cells) + EFC (500mg/kg p.o)	40.10±1.02	72.10±0.50	2.00±2.05	4.60±1.02	1.90.105
5	DAL control (1 x 10 ⁶ cells) + EFC (1000mg/kg p.o)	42.10±0.80	73.60±0.90	1.50±0.20	2.3±0.10	2.51±0.20

Table 2. Effect of EFC extracts on hematological parameter in DAL bearing mice.

Sr. no	Treatment	Hb(g%)	RBC (10 ⁶ /mm ³)	WBC (10 ³ cells/mm ³)	Proteins (g%)	PCV (mm)	Differential count%		
							Lymphocytes	Neutrophils	Monocytes
1	Normal Saline (5 ml/kg P.O)	13.97±0.2	6.50±0.2	7.7±0.2	9.50±0.2	21.0±0.2	69.90±1.20	30.10±1.20	2.20±0.2
2	DAL control (1 x 10 ⁶ cells)	6.78±0.2	3.80±0.5	14.60±0.5	16.70±0.2	32.30±1.50	40.20±1.06	68.70±1.6	4.50±0.2
3	DAL control (1 x 10 ⁶ cells) + EFC (250 mg/kg p.o)	11.30±0.5	5.87±0.2	10.70±1.02	13.60±1.06	23.60±1.00	46.60±0.2	42.0±0.6	3.3±0.6
4	DAL control (1 x 10 ⁶ cells) + EFC (500 mg/kg p.o)	13.60±0.20	6.21±0.2	9.30±0.2	11.30±0.9	21.81±1.06	59.90±1.6	38.10±1.80	2.3±0.2
5	DAL control (1 x 10 ⁶ cells) + EFC (1000 mg/kg p.o)	13.80±0.90	6.60±0.5	8.34±1.02	10.78±0.2	17.54±0.2	55.32±1.8	38.10±0.2	2.2±0.5

Statistical significance calculated by one-way ANOVA followed by Dunnett's test.

Table 3. Effect of EFC extract on XO and DPPH.


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CONCLUSION:

The present research work concludes that *Ficus Carica* is important medicinal plant with varied pharmacological spectrum. The phytochemicals screening revealed chemicals constituents that form the foundation of their pharmacological activity. The ethanolic extraction of *Ficus carica* has good

antitumor and antioxidant activity. The DPPH assay indicate that dry fruit are a significant source of natural antioxidant, which might be helpful in preventing the various diseases associated with oxidative stresses. The cytotoxicity exerted against cancer cell lines suggests bioactive principles in the fruit. This shows that the fruit could be useful as anticancer and antioxidant activity.

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