ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, August 2022**

In-Vitro And *In-Vivo* Antitumor Activity Study Of Dry Fruit Of *Ficus Carica Linn* In Experimental Animals

HAQUE MUDABBIRUL [1*], SHIRSAT MRUNAL KRISHNARAO[2], KAYANDE NANDU[3], AEJAZ AHMED[*4]

1) Department of Pharmacognosy, SunRise University, Bagad Rajput Dist. Alwar 301001 Rajashtan, India
2) Department of Pharmacy, SunRise University Bagad Rajput Dist. Alwar 301001 Rajashtan-India
3) Department of Pharmacognosy, Thakur Shivakumarsingh Memorial Pharmacy College, Burhanpur 450331 M.P. India
4) Department of Pharmaceutical Chemistry, Ali-Allana College of Pharmacy, Akkalkuwa 425415 Nandurbar MS India

Correspondence

Mudabbirul Haque
Research Scholar
Dept. of Pharmacognosy,
SunRise University, Bagad Rajput
dist.Alwar, Rajashtan-India
Email:-mudabbirpharma@gmail.com, aejazboraji@gmail.com
Mobile no: +919922384205

ABSTRACT:

The antitumor activity of the ethanolic extract of Dry fruit of *ficus carica* (EFC) has been evaluated against Dalton's ascitic lymphoma (DAL) in swiss albino mice at the dose of 250 and 500 mg/Kg, body weight. The experimental parameter used were tumor volume, tumor cell count, viable tumor cell count, mean survival time and increase in the life span to assess antitumor activity. The extract administered orally for 14 consecutive days to tumor bearing group of animals. The extract increases the life span of DAL treated mice and restore the hematological parameter 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 EFC showed significant antitumor activity in tested animal models.

Keywords :- Antitumor activity, Dalton's ascitic lymphoma (DAL), *Ficus Carica*,

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, August 2022

INTRODUCTION

Tumor is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body.[1] It is one of the major ailment effecting humankind and remains as one of the leading causes of mortality worldwide, for instance above 10 million new patients are diagnosed with cancer every year and over 6 million deaths are associated with it representing roughly 12 % of worldwide deaths.[2]Cancer is a health problem of global concern.[3] This uncontrolled proliferation of a normal cell which produces genetic instabilities and alterations accumulates within cells and tissues which transforms normal cell into a malignant cell. These genetic instabilities include mutations in DNA repair genes (p21, p22, p27, p51, p53) and tool box for DNA repair genes (p53, NF1, NF2, RB and biological breaks), oncogenes (MYC, RAF Bcl-2, RAS (biological accelerators) and genes involve in cell growth metabolism. Both external factors (radiation , Smoking, tobacco, pollutants in drinking water food air chemicals.[4] Worldwide, breast cancer is the second leading cause of death in women.[5] Since Cancer is associated with such high morbidity and mortality worldwide, there is an urgent need to determine ways of management of this ailment where the current treatment modalities are mainly surgery, radiation based therapy, chemotherapy, gene therapy and/or hormonal therapy.[6]The use of foods and medicinal plant to improve health is nearly as old as humanity. Among such, none may be older than the fig, which recent investigation have indicated has been cultivates for over 11,000 years.[7] Numerous *ficus* species have been used for various medicinal purposes in Siddha, Ayurvedic and traditional Chinese medicine, moreover various pharmacological studies (eg. Anticancer, anti-inflammatory and antidiabetic activities) have been supported by the ethnomedicinal uses of *ficus* species [8]Alternative and complementary medicine such as Ayurveda are playing an important role in the treatment of cancer. Eleven percent of the basic drugs considered as essential by World Health Organization (WHO), are plant derived and modified plant products.[9]Fruits and vegetables consumption have been shown by multiple epidemiology studies to reduce the risk of chronic diseases such as cancer and heart disease. [10, 11] The drying of fruit is every ancient practice for food preservation still in use nowadays. The fig is a delicious, nutritive fruit and has medicinal properties that may reduce the risk of cancer and heart disease. Fresh and dried figs are dried figs are especially rich in antioxidant polyphenols fiber, trace minerals, proteins, sugar and organics acids.[11] Eight phenols (chlorogenic acid, catechin, epicatechin, rutin, cyanidin-3-O-rutinoside, luteolin-8-C-glucoside, quercetin-3-Oglucoside, kaempferol-3-O-glucoside) were identified in fresh and dried fruits and the predominant phenolic compound was epicatechin.[11,12] Raisins are among solid fruits products having the highest concentration of total phenolic compounds and the highest level of total antioxidant activity. In raisins, the most abundant phenolic compounds are usually quercetin, kaempferol and coumaric acid. Reported the presence of 10 phenols (gallic, 3,4dihydroxybenzoic, caffeic, syringic, ferulic, salicylic and coumaric acids, catechin, quercetin and rutin) in Chinese raisins [11, 13, 14]

Material and Methods

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, August 2022

Plant materials and extraction [15]

The dried fruits of *ficus carica*, were collected from Nimar region of Madhya Pradesh, India and identified and authenticated at Govt. PG College, Dept. of Pharmacognosy, Mandleshwar. Small pieces of fruit (750gm) were subjected to extract with soxhlet apparatus using ethanol within 72 hours at 75-80°C. 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.

Tumour cell lines

Daltonsasciticlymphoma (DAL) and mouse lung fibroblast (L-929) cells were obtained through the courtesy of the cancer Research Centre, Adyar, Chennai and National Institute of Virology, Pune, India respectively. DAL cells were maintained by weekly intraperitoneal (*i.p*) inoculation of 1 X 10^6 cells/mouse [16]

***In-vitro* Cytotoxicity**

Long term cytotoxicity of the EFC to L-929 was determined by seeding 1 X 10^5cells (L-929) in a culture bottle containing 10 ml minimum essential medium (MEM) supplemented with 10% heat inactivated goat serum and 100mg streptomycin. After 24hr incubation at 37°C, the cells were exposed to different (1-100µg) concentration of the extract or camptothecin (a reference drug). Inhibition of the cell proliferation was assessed after 6 days by trypsin sing and counting the cells with hemocytometer [17]

Antitumor activity in mice

After acclimatization mature male swiss albino mice divided into four groups (n=10) and given food and water ad libitum. The total group mentioned in (**Table 1**) except group were injected with DAL cells (1 X 10^6cells/mouse. i.p). This was taken as day zero. Group I served as normal saline control. On day first, the EFC at a dose of 250 and 500 mg/Kg body weight (Gr- III & IV) were administered orally and continued for 14 consecutive days. The dose of EFC was selected based on previous study on hepatoprotective activity [18] On day 15, five mice of each group were sacrificed 24 hrs after the last dose and the rest 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.[16,19]

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.

Determination of tumor cell count

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, August 2022

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 Neubauer counting chamber and the number of cells in 64 small squares was counted.

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.

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 [20]

ILS (%) = [(Mean survival of treated group / Mean survival of control group) - 1] x 100

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 concentration was estimated by lowry's method and packed cell volume (PCV) was determined by the method described by Docie *et al*. [21, 22]

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

Results

Results of the preliminary phytochemical analysis carried out on the crude ethanol extract indicated the presence of alkaloids, glycosides, lignin flavonoids and saponins. In long term chemosensitive cytotoxic assay, 75 µg/ml of the extract produced 50% death of L-929 cells whereas 2 µg/ml camptothecin produced the same result. The effect of EFC on survival of tumor bearing mice showed MST (table 1) for the tumor control group (DAL treated) to be 23.60±1.3 days, while it was 32.42±1.03 days (46.40%) and 37.58 ±1.07 days (72.90%) for the group treated with EFC at the dose of 250 & 500 mg/Kg respectively. The average number of tumor volume (table 1) in DAL treated animals was found to be 3.81±0.11. EFC treatment at both dose level significantly (P<0.05) reduced tumor volume which was found to be 2.20±0.11 and 0.83±0.04 respectively. Viable cell counts of the tumor bearing mice was significantly decreased while non-viable cell count were increase in EFC treated group in dose dependent fashion when compared with DAL treat group. Moreover, hematological parameters of (table 2) tumor bearing mice on day 15 were found to be significantly alters from normal group. The total WBC count, protein and PCV were found to be increased with a reduction of the hemoglobin and RBC. In a differential count of WBC, the percent of neutrophils increased while the lymphocyte count decreased. At the same time interval EFC treatment could change those altered parameters to near normal.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, August 2022

Discussion

In the above results the *in-vitro* cytotoxic activity was more pronounced in long term exposure of fibroblasts (L-929) to the EFC. Although the cytotoxicity of camptothecin to L-929 cell was more than that of the extract it should be noted that camptothecin is a pure compound where the extract is a crude one containing numerous compounds the reliable criterion for judging the value of any anticancer drug is the prolongation of lifespan of the animal and disappearance of WBC from blood. [23, 24] the above result demonstrated the antitumor effect of EFC against DAL is 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 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 10^6 intraperitoneal cells, 50 % of which are macrophage. EFC treatment was found to enhance non-viable cell count in peritoneal exudates and decrease the viable cell count. It might be due to the absorption of EFC by viable cells which leads to lysis of cell through to the activation of macrophages or some cytokine production in peritoneal cavity usually, in cancer chemotherapy, the major problems that are being encountered are of *myelosuppression* and anemia [25, 26] but the results have clearly shown that EFC has not only brought back hemoglobin content to normal but also the RBC count to normal. Analysis of the other hematological parameters showed minimum toxic effect in the mice which were treated with EFC. After 14 days of transplantation, EFC-treated groups were able to reverse the changes in the hematological parameter consequent to tumor hematological parameters consequent to tumor inoculation. All these data point to the possibility of developing an ethanolic extract of dry fruit of *ficus carica* as a novel potential agent in the area of cancer chemotherapy and management. The phytochemicals study indicates the presence of flavonoids, alkaloids and terpenoids in EFC. Flavonoids have been shown to possess antimutagenic and antimalignant effects [27, 28] Furthermore, flavonoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis [29, 30] According to the previous reports, Dry fruit of *ficus carica* possess antioxidant ability [31] Thus antitumor effect produced by the EFC may be due to its flavonoids as well as its antioxidant potential. The ethanolic extract of *ficus carica* restore the mean survival time our present study suggests that EFC possess potent anticancer activity and increase life span. Further studies to characterize the active principle and elucidate the mechanism of action of EFC are in progress using different cell lines.

Table 1: Effect of ethanolic extract of *ficus Carica* on survival time, life span, tumor volume, Viable and non-viable cell count in DAL bearing mice

Sr. No	Treatment group	Survival time (Days)	Increase of life span	Tumor volume (ml)	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 Cell)	23.67± 1.4	-	3.84±0.11	10.31±0.14	3.66±0.21

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, August 2022

3	DAL (1 x 10^6 cell) + EFC (250 mg/kg *p.o*)	32.51±1.12*	46.46	2.23±0.11 *	3.69±0.04*	1.64±0.09*
4	DAL (1 x 10^6cell) + EFC (500mg/kg *p.o*)	37.49±1.13*	72.98	0.87±0.04 *	2.21±0.08*	1.78±0.19*

Statistical significance *(p value)* calculated by one-way ANOVA followed by Dunnett's test.

*$p < 0.01$ calculated by comparing treated groups with DAL control group.

Table 2. Effect of ethanolic extract of ficus Carica on hematological parameter in DAL bearing mice

Sr. No	Treatment group	Hb (g %)	RBC (Million/mm^3)	WBC (10^3 cells/mm^3)	Protein (g %)	PCV (mm)	Different count %		
							Lymphocytes	Neutrophils	Monocytes
1	Normal saline (5ml/kg)	14.13 ± 0.2	6.59±0.2	7.39±0.2	8.59±0.2	17.61±0.6	70.14±1.31	29.62±1.1	2.42±0.4
2	DAL control (1 x 10^6 cell)	7.65± 0.6^{a**}	3.79±0.1^{a**}	15.28± 1.3^{a**}	14.79± 1.4^{a**}	27.31±0.4^{a**}	30.22±0.4^{a**}	68.59± 1.6^{a**}	3.62±0.5^{a**}
4	DAL (1 x 10^6 cell) + EFC (250 mg/kg p.o)	10.47±0.6^{b**}	5.98±0.5^{b**}	11.29 ±0.7^{b*}	11.81± 0.1^{b**}	21.32±0.4^{b**}	55.58±1.1^{b**}	42.41± 1.3^{b**}	$2.81±0.4^{ns}$
5	DAL (1 x 10^6)+ EVN (500mg/kg p.o)	12.24±0.4^{b**}	5.78±0.3^{b**}	8.59±0.7^{b**}	9.39±0.1^{b**}	18.12±0.1^{b**}	67.49±2.1^{b**}	30.23± 2.2^{b**}	$2.81±0.3^{ns}$

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

*$P<0.05$, **$P<0.01$, $^{ns}P<0.05$; a *vs. Normal group,* b *vs.* DAL Control. n=5.

CONCLUSION

Based on the results of *in-vivo* and *in-vitro* antitumor activity studies demonstrate that *ficuscarica* fruit extract promising effect on tumour cell and shown arrest the proliferassion. It might be due to the absorption of EFC by viable cells which leads to lysis of cell through to the activation of macrophages or some cytokine production in peritoneal cavity usually, in cancer chemotherapy, the major problems that are being encountered are of *myelosuppression* and anemia but the results have clearly shown that EFC has not only brought back hemoglobin content to normal but also the RBC count to normal. The phytochemicals study indicates the presence of flavonoids, alkaloids and terpenoids in EFC. Flavonoids have been shown to possess antimutagenic and antimalignant effects.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, August 2022

Acknowledgement

The authors are thankful for encouragement and support provided by M/S. Nadiya Firdose, M/S. Sabiha Khatoon

REFERENCE

1) Kohler BA, Sherman RL, Howlader N, Jemal A, Ryerson AB, Henry KA. (2015) Annual report to the nation on the status of cancer, 1975–2011, featuring incidence of breast cancer subtypes by race ethnicity, poverty, and state. J Natl Cancer Inst, 107
2) Rady I, Siddiqui IA, Rady M, Mukhtar H. Melittin.(2017) A major peptide component of bee venom, and its conjugates in cancer therapy. Cancer Lett, 402:16–31.
3) Graidist P, Martla M, SukpondmaY. (2015) Cytotoxic activity of *Piper cubeba* extract in breast cancer cell lines. Nutrients. 7:2707-2718.
4) Iqbal Javed, Abbasi A Banzeer, MahmoodTariq,KanwalSobia, Ali Barkat, Shah A Sayed, Khalil Talha Ali (2017) Review article Plant-derived anticancer agents: A green anticancer approach. Asian Pac.J Trop Biomed, 7(12):1129-1150.
5) Farshori NN, Al-Sheddi ES, Al-qail MM, Musarra J, AlKhedhairy AA, Siddiqui MA. (2013) Anticancer activity of *Petroselinumsativum* seed extracts on MCF-7 human breast cancer cells. Asian Pacific Journal of Cancer Prevention, 14:5719-5723.
6) Rady Islam, Mohamed Hadir, RadyMohamad,Imtiaz A. Siddiqui, MukhtarHasan. (2018) Review article, Cancer preventive and therapeutic effects of EGCG, the major polyphenol in green tea. Egyptian J. of basic and applied science 5, 1-23.
7) KislevM.E,HartmannAnat, Bar-Yosef. (2006) early domesticated fig in the Jordan Valley. Science 312, 1372–1374.
8) Lansky P. Ephraim, Paavilainen M. Helena, Pawlus D. Alison, Newman A. Robert. (2008) *ficus spp.*(fig) Ethnobotany and potential as anticancer and anti-inflammatory agents. J. of Ethnopharmacology 119, 195-213.
9) RaiVaishali, PaiR.Vinitha, KedilayaPratapchandra (2016) A preliminary evaluation of two traditional medicinal plant from *Lamiaceae-Pogostemonheyneanus* and *plectranthus amboinicus*.J.of applied pharmaceutical science vol.6 (08), 073-078.
10) Vinson A Joe, Zubik, L., Bose P., Samman N., &Proch, J. (2005)Dried fruits: excellent in vitro and in vivo antioxidants. Journal of the American College of Nutrition, 24:1, 44-50.
11) OuchemoukhSalim, Hachoud Said, BoudrahamHamou, MokraniAbderrahmane, louailecheHayette.(2012) Antioxidant activities of some dried fruits consumed in algeria.LWT-Food science and technology 49, 329-332.
12) Slatnar, A., Klancar U., Stampar F. and Veberic R. (2011) Effect of drying of figs (*ficuscarica L.*) on the contents of sugars, organic acids and phenolic compounds. Journal of Agricultural and Food Chemistry59, 11696-11702.
13) Williamson G., Carughi A. (2010) Polyphenol content and health benefits of raisins. J. Nutrition Research, 30, 511-519.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, August 2022

14) Meng, J., Fang Y., Zhang A., ChenS.,Xu T., RenZ., Han G.,Liu J., Li H., Zhang Z., Wang H., (2011) Phenolic content and antioxidant capacity of chinese raisins produced in Xinjiang Province.J. Food Research International 44 (2011) 2830-2836.
15) Ram Rastogi and B N Malhotra, Indian Medicinal Plants, Volume (4), Publication and information distribution, CSIR, New Delhi, PP 354-379.
16) Gothoskar S.V, Ranadive K.J. Anticancer screening of SAN-AB. (1971) an extract of marking nut, *Semicarpusanacardium*, Indian Journal of Experimental Biology, 9, 372-375.
17) Babu T.D, Kuttan G, Padikkala J. (1995) Cytotoxic and antitumor property of certain taxa of *Umbelliferae* with special reference to *Centella asiatica (L.)* Urban. Journal of Ethnopharmacology. 48, 53–57.
18) Tandon V.R, Khajuria V, Kapoor B, Kour D, Gupta S. (2008) Hepatoprotective activity of *Vitexnegundo* leaf extract against anti-tubercular drugs induced hepatotoxicity. Fitoterapia, 79, 533– 538.
19) Mazumder U.K, Gupta M, Maiti S, Mukherjee M. (1997) Antitumor activity of Gygrophilaspinosa on Ehrlich ascites carcinoma and sarcoma-180 induced mice. Indian Journal of Experimental Biology.35, 473-477.
20) Sur P and Ganguly D.K. (1994) Tea plant root extract (TRE) as an antineoplastic agent. Planta Med, 60, 106-109.
21) Dacie JV, Lewis SM. Practical Hematology. 2nd ed. J and A Churchill, London. 1958. p. 38-48.
22) D'Armour F.E, Blood F.R and Belden D.A. (1965) The Manual for Laboratory Work in Mammalian Physiology. 3rd ed. The University of Chicago Press, Illinois. p.4-6.
23) Clarkson B.D, Burchenal J.H. (1965) Preliminary screening of antineoplastic drugs. Progress in Clinical Cancer 1, 625-629.
24) Obiling C and Guerin M. (1954) The Role of Viruses in the Production of Cancer. Advances in Cancer Research II. Academic Press, New York. 406-411.
25) Price V.E, Greenfield R.E. Anemia in cancer. In: Grenstein, J.P., Haddow, A. (Eds.), Advance in Cancer Research, vol. 5. Academic Press, New York. p. 199-200.
26) Hogland H.C. (1982) Hematological complications of cancer chemotherapy. Sem Oncology, 9, 95– 102.
27) Brown J.P. (1980) A review of the genetic effect of naturally occurring flavonoids, anthraquinones and related compounds. Mutation Research.75, 243-247.
28) Hirano T, Oka K, Akiba M. (1989) Antiproliferative effect of synthetic and naturally occurring flavonoids on tumor cells of human breast carcinoma cell lines, ZR-75-1. Research in Community Pathology and Pharmacology, 64, 69-78.
29) Weber G, Shen F, Prajda N, Yeh Y.A, Yang H. (1996) Increased signal transduction activity and down regulation in human cancer cells. Anticancer Research. 16, 3271-3282.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** UGC CARE Listed (Group -I) Journal Volume 11, Iss 6, August 2022

30) Fotsis T, Pepper M.S, Aktas E, Breit S, Rasku S, Adlercreutz H. (1997) Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro angiogenesis. Cancer Research. 57, 2916- 2921

31) Tiwari O.P, Tripathi Y.B. (2007) Antioxidant properties of different fractions of *Vitexnegundo Linn.* Food Chemistry.100, 1170–1176.