



## *In-Vivo* anticancer activity of leaves extract of *Madhuca longifolia*

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### Abstract

This study was done to examine the anticancer activity of hydroalcoholic extract from the leaves of *Madhuca longifolia* ieML HA against Breast Carcinoma in mice. The activity was evaluated using tumor size, tumor weight, mean survival time, body weight of tumor bearing mice and haematological parameters. Results found that oral administration of extract would significantly increase the life span and restored the altered haematological parameters. Tumorsize and tumor weight were also considerably reduced.

**Keywords:** anticancer activity, breast carcinoma and *Madhuca longifolia*

### Introduction

Malignancy has been a consistent fight worldwide with a great deal of improvement in fixes and protection treatments. The ailment is portrayed by cells in the human body persistently increasing with the powerlessness to be controlled or halted. Therefore, forming tumors of dangerous cells with the possibility to be metastatic <sup>[1]</sup>. Treatments for malignancy include chemotherapy, radiotherapy and synthetically inferred drugs. Medicines, for example, chemotherapy can put patients under a great deal of strain and further harm their wellbeing. In this way, there is an emphasis on utilizing elective medicines and treatments against disease <sup>[2]</sup>.

For a long time, natural medications have been utilized as yet utilized in developing nations as the essential wellspring of clinical treatment. Herbs have been utilized in medication for their different therapeutic uses. Subsequently, research has been going on into exploring the therapeutic actions of herbs and their employments for illnesses including malignancy <sup>[3]</sup>. Various species of herbs are now being utilized in treatment or to inhibit the growth of malignancy. Various inventors have distinguished types of plants that have exhibited anticancer action with a great deal of spotlight on those that have been utilized in herbal treatments in growing nations <sup>[1, 4, 8]</sup>.

*Madhuca* is a well-known herbal tree belonging to family *Sapotaceae*, is an average to big sized deciduous tree widely spread in India. Its leaves possess expectorant, antioxidants, antimicrobial, and hypoglycaemic properties <sup>[9]</sup>.

### Material and Methods

#### Collection and Authentication of the leaves

The leaves of *Madhuca longifolia* was collected from Garden of Ashtang Ayurvedic College, Indore in month of July 2015 and authenticated by Dr. Shakun Mishra, Head of the Department (Botany), Shree S.N Govt. P.G. College, Khandwa. A Voucher specimen of plant has been preserved for further references.

#### Preparation of Crude Drug for Extract <sup>[10]</sup>

The authenticated plant leaves were gathered and dried under shade and then coarsely powdered. The powdered leaves were screened through sieve no. 40 and stored in an airtight container for extraction.

#### Preparation of extracts of *Madhuca longifolia* <sup>[10]</sup>

The powdered plant material about 1000gm of plant leaves were successively extracted using petroleum ether, ethanol, hydroalcoholic (1:1) and distilled water in Soxhlet apparatus. After about forty siphons of each solvent extraction step, the materials were concentrated by evaporation

### Pharmacological Evaluation

#### Animals

Female Albino mice (20-25gm) of around the same age, procured from Local shop in Nagar Nigam market area, Indore, Madhya Pradesh. Polypropylene cages were used for housing of animals, standard rodent nutrition along with water *ad libitum* and an alternate cycle of twelve hours of darkness and light were provided for their nourishment. Drug given orally by means of orogastric cannula. Animals were subjected to fasting for minimum 12hours, before any of the experiment perform; procedures for experiment were introduced for the examination of the Institutional Animals Ethical Committee and were passed by the same. Conferring to CPCSEA guidelines for care of laboratory animals and the ethical guideline for investigations of experimental pain in conscious animals, experiments were performed in morning.

#### Acute Toxic Study

This study was carried out on the basis of OECD 423 guidelines, hydroalcoholic extract of leaves were found to be nontoxic up to 5000mg/kg hence the LD50 was 5000mg/kg and 1/10 LD50 was selected as dose for the study.

## Anticancer activity

### Cells

MCF7 cells were obtained from Amala Cancer Research Centre, Thrissur. They were maintained by weekly inoculation of  $10^6$  cells [11, 12].

### Experimental Design

The animals (Female albino mice weighing 20-25 g) were divided into 5 groups, each group consisting of 12 animals. Animals were fed with basal diet and water throughout the experimental period. All the groups were injected with MCF7 cells except the group I and was considered as day zero. From first day of experiment, normal saline (5 ml/kg) was given in group I, group II was serve as a tumor control group and given normal saline (5 ml/kg), Methotrexate (20mg/kg) given to group III, MLHA (200 and 500 mg/kg) were given to group IV and group V respectively, for 14 consecutive days. On day 15<sup>th</sup> half of the mice from each group were sacrificed, 24h after last dose, for the determination of tumorsize, tumor weight, haematological parameters etc, and rest were kept with food and water *ad libitum* to check the increase in the life span of the tumor hosts.

### Effect of extracts on tumorsize and tumor weight

On 15<sup>th</sup> day, after 24h of dose, 6 mice from each group were dissected and the tumor was collected. The tumor size is measure by Vernier callipers and value were taken from four different dimensions for each tumor. The tumor weight was measured by taking the weight of mice before and after collection of tumor [11, 12].

### Effect of extracts on Mean survival time

The mean survival time (MST) of each group, consisting of remained 6 mice after tumor study was noted. The antitumor efficacy of plants extract was compared with that of Methotrexate and control group using the following calculation [11, 12].

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

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

### Effect of extractson body weight

All groups (except Group I), consisting of six mice each were transplanted with  $1 \times 10^6$  MCF-7 cells. After 24 h, the groups were orally treated Extracts. The group I, serving as the control, received normal saline (0.9% w/v). Treatments were continued for 14 days. Body weights were recorded every 5th day till 40 days of treatment or till the death of the animal [11, 12].

### Effect of extracts hematological parameters

All the treatments were given for 14 days to each group (except group I), On the 15<sup>th</sup> day, blood was drawn by retro orbital plexus method. WBC count, RBC count, haemoglobin, protein and packed cell volume were determined [13, 14]. Cells smear was prepared in slide and stained with Lieshman stain solution [15]. Red blood cells (RBC), White blood cells (WBC) and Haemoglobin (Hb) were determined.

### Histopathological study of tumor

On 15<sup>th</sup> day, after 24h of dose, 6 mice from each group were dissected and the tumor was collected.

For fixation of tissues, ten percent formalin buffer was used. This sample was then fixed in paraffin wax. After cooling, tissues were cut into 5 $\mu$ m section. By using hematoxylin and eosin stains, the cut tissues sections were stained. Cover tissue with cover slip and by using light microscope tissues were investigated.

### Statistical analysis

All the values were expressed as mean  $\pm$  SEM (standard error of mean) for six rats. Statistical analysis was carried out by using PRISM software package. Statistical significance of differences between the control and experimental groups was assessed by One-way ANOVA followed by Newman-Keuls Multiple Comparision Test. The value of probability less than 5% ( $P < 0.05$ ) was considered statistically significant [16].

### Results and Discussion

The results of the present study show an anticancer effect of MLHA in Female albino mice. A significant enhancement of MST was observed. The effect of MLHA on the survival of tumor-bearing mice is shown in Table 1. The MST for the control group was  $20.34 \pm 1.15$  days, whereas it was  $24.45 \pm 2.56$  days,  $29.74 \pm 1.14$  days and  $38.06 \pm 2.91$  days for the groups treated with MLHA (200 and 500 mg/kg/day, p.o.) and MXT (20 mg/kg/day, i.p.) respectively. The % increase in the lifespan of tumor-bearing mice treated with MLHA and MXT was found to be 20.23% (200mg/kg), 46.23% (500mg/kg), and 87.13% (20mg/kg standard) respectively ( $P < 0.01$ ) as compared to the control group.

**Table 1:** Effect of MLHA extract treatment on the survival of tumor bearing mice

S No	Treatment	Mean Survival Time (Days)	Increase in life span (%)
1	Tumor Control	$20.34 \pm 1.15$	-
2	MXT (20mg/kg, i.p)	$38.06 \pm 2.91$	87.13
3	MLHA (500 mg/kg, p.o)	$29.74 \pm 1.14$	46.23
4	MLHA (200 mg/kg, p.o)	$24.45 \pm 2.56$	20.23

n=6 animals in each group,  $P < 0.01$  Vs control. Days of treatment = 14, Values are expressed as mean  $\pm$  SEM

There was reduction in the tumorsize and tumor weight of mice treated with MLHA ( $P < 0.001$ ) as showed in table no 3. Tumorsize of control animals was  $10.07 \pm 0.1$  mm, whereas for the extract-treated group it was  $3.53 \pm 0.23$  mm and  $3.53 \pm 0.23$  mm MLHA at 200, 500 mg/kg respectively. Tumor weight of control animals was  $2.26 \pm 0.12$  gms, whereas for the extract-treated group it was  $1.93 \pm 0.21$  and  $1.48 \pm 0.21$  g for MLHA at 200, 500 mg/kg respectively.

**Table 2:** Effect of MLHA on Tumor size and Tumor weight

S No.	Treatment	Tumor Size (mm)	Tumor weight (gm)
1	Tumor Control	$10.07 \pm 0.1$	$2.26 \pm 0.12$
2	MXT (20mg/kg, i.p)	$2.21 \pm 0.09$	$0.88 \pm 0.10$
3	MLHA (500 mg/kg, p.o)	$6.97 \pm 0.14$	$1.93 \pm 0.21$
4	MLHA (200 mg/kg, p.o)	$3.53 \pm 0.23$	$1.48 \pm 0.21$

$P < 0.001$  Vs tumor control, n=6 animals in each group, no of days = 14, Values are expressed as mean  $\pm$  SEM.

There was a significant decrease in the weight gain by the MLHA treated mice when compare with tumor control as showed in table no 3. The analysis of the haematological

parameters showed minimum toxic effect in mice treated with MLHA. After 14 days of transplantation, MLHA extracts were able to reverse the changes in the haematological parameter's consequent to tumor inoculation. The total WBC count, proteins and PCV were found to increase with a reduction in the haemoglobin

content of RBC. The differential count of WBC showed that the percentage of neutrophils increased ( $P<0.001$ ) while that of lymphocytes decreased ( $P<0.001$ ). At the same time interval, MLHA (200, 500 mg/kg/day, p.o.) treatment could change these altered parameters to near normal showed in table no. 4.

**Table 3:** Effect of MLHA and standard treatment on body weight of tumor bearing mice

Treatment/dose	5 <sup>th</sup> Day	10 <sup>th</sup> Day	15 <sup>th</sup> Day	20 <sup>th</sup> Day	25 <sup>th</sup> Day	30 <sup>th</sup> Day	35 <sup>th</sup> Day	38 <sup>th</sup> Day
Normal	22.97±1.17	23.25±1.16	23.65±1.13	23.97±1.17	24.84±1.31	25.64±1.33	25.92±1.34	26.54±1.39
Tumor Control	23.43±0.78	25.02±0.77	27.02±0.68	31.04±1.30	-	-	-	-
MXT (20mg/k, i.p)	23.93±1.04	24.63±1.04	25.33±1.04	26.04±1.01	27.52±1.02	28.98±0.86	30.30±0.88	31.10±0.66
MLHA (500 mg/kg, p.o)	23.49±1.08	24.52±0.80	25.65±0.60	26.70±0.66	28.38±0.37	-	-	-
MLHA (200 mg/kg, p.o)	23.42±1.13	24.92±1.08	26.51±0.76	28.01±0.76	-	-	-	-

n= 6 in each group. Values were expressed as mean± SEM,

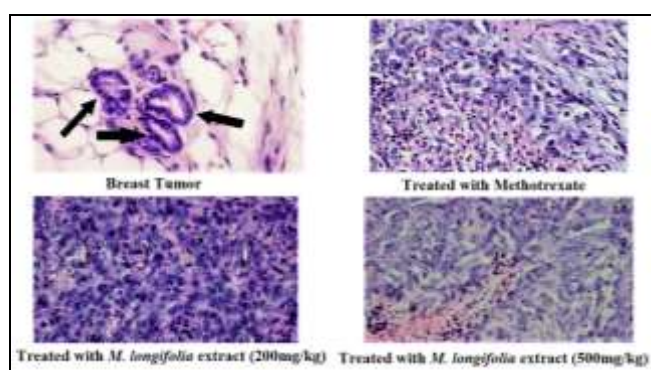
**Table 4:** Effect on Haematological Parameters of tumor bearing mice

Parameter	Normal	Tumor control	MXT (20 mg/kg)	MLHA 200mg/kg	MLHA 500mg/kg
Hb(g/dl)	14.18±0.15	8.1±0.09	13.97±0.08	9.48±0.07	12.8±0.09
RBC (million/mm <sup>3</sup> )	4.71±0.01	2.5±0.06	4.22±0.01	3.12±0.03	3.54±0.06
WBC (million/mm <sup>3</sup> )	7.51±0.02	24.81±0.04	8.29±0.03	11.22±0.04	9.24±0.03
Protein %	8.22±0.03	13.77±0.38	8.72±0.03	11.86±0.03	9.18±0.06
PCV (mm)	16.62±0.09	30.63±0.25	20.58±0.19	27.92±0.12	22.97±12
Neutrophils %	30.02±0.26	66.45±0.19	31.62±0.27	48.55±0.27	48.47±0.32
Lymphocytes %	67.6±0.28	31.2±0.09	65.33±0.21	47.43±0.35	56.53±0.27
Monocytes %	1.16±0.01	2.19±0.01	1.25±0.02	1.91±0.04	1.52±0.03

N= 6 in each group, Values are expressed as Mean ± SEM,

### Histopathological study of tumor

Histopathology of tumor was done by using haematoxylin and eosin staining. Development of cancer in mice cause the mammary duct disrupt, leads to excessive development of cells which lines the duct in breast that is generally known as hyperplasia in epithelial duct. In groups treated with extracts and standard drug, mammary tissues become enriched and disruption in cancer cells observed. Figure 1 shows effect of extracts and Methotrexate on tumor cells.



**Fig 1**

### Conclusion

Antitumor activity of *Madhuca longifolia* in an *in-vivo* model of MCF-7 cells induced breast adenocarcinoma has been evaluated. Treatment with *M. longifolia* extract delayed tumor formation, reduce tumor size and tumor weight and increase mean survival time of tumor bearing mice. It also helpful in restoring haematological parameters nearby their normal values. Thus, by this study, it is clear that *Madhuca longifolia* possess anticancer potential and an option for management of breast cancer.

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### References

- Ochwang'I DO, Kimwele CN, Oduma JA, Gathumbi PK, Mbaria JM, Kiama SG, *et al.* Medicinal plants used in treatment and management of cancer in Kakamega County Kenya. *Journal of Ethnopharmacology*. 2014; 151:1040-1055.
- Cancer Research UK [Accessed 23 January 2015]; What is cancer, 2014. Available at: <http://www.cancerresearchuk.org/about-cancer/what-is-cancer>.
- Sivaraj R, Rahman PKSM, Rajiv P, Vanathi P, Venkatesh R. Biosynthesis and characterization of *Acalypha indica* mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2014; 129:255-258.
- Freiburghaus F, Kaminsky R, Nkunya MHH, Brun R. Evaluation of African plants for their *in vitro* trypanocidal activity. *Journal of Ethnopharmacology*, 1996; 55:1-11.
- Costa-Lotufo LV, Khan MTH, Ather A, Wilke DV, Jimenez PC, Pessoa C, *et al.* Studies of the anticancer potential of plants used in Bangladeshi folk medicine. *Journal of Ethnopharmacology*, 2005; 99:21-30.
- Cai YZ, Sun M, Xing J, Luo Q, Corke H. Structure-radical scavenging activity relationships of phenolic

- compounds from traditional Chinese medicinal plants. *Life Sciences*, 2006; 78:2872-2888.
7. Fouche G, Cragg GM, Pillay P, Kolesnikova N, Maharaj VJ, Senabe J, *et al.* *In vitro* anticancer screening of South African plants. *Journal of Ethnopharmacology*, 2008; 119:455-461.
  8. Kamatou GPP, Van Zyl RL, Davids H, Van Heerden FR, Lourens ACU, Viljoen AM, *et al.* Antimalarial and anticancer activities of selected South African *Salvia* species and isolated compounds from *S. radula*. *South African Journal of Botany*, 2008; 74:238-243.
  9. Akshatha KN, Murthy SM, Lakshmidēvi N. Ethnomedical uses of *Madhuca longifolia* - A review. *International Journal of Life Science and Pharma Research*. 2013; 3(1):44-53.
  10. Trease GE, Evans WC. *Text book of Pharmacognosy*. 13th (eds). Alden Press, Oxford, London, 2003, 512-513.
  11. Kuttan G, Vasudevan DM, Kuttan R. Effect of a preparation from *Viscum album* on tumor development in vitro and in mice. *J Ethnopharmacol*, 1990; 29:35-41.
  12. Mazumder UK, Gupta M, Maiti S, Mukherjee M. Antitumor activity of *Gyrophila spinosa* Ehrlich ascites carcinoma and sarcoma-180 induced mice. *Indian. J Exp. Biol*, 1997; 35:473-77.
  13. D'Amour FF, Blood FR, Belden DA. *The Manual for laboratory work in mammalian physiology* (3<sup>rd</sup> ed.), The University of Chicago Press, Chicago, 1965, 4-6.
  14. Lowry OH, Rosenbrough NT, Farr AL. Protein measurement with Folin – Phenol reagent. *J Biol. Chem*, 1951; 173:265-75.
  15. Docie JV. *Practical haematology* (2nd ed. J&A Churchill Ltd), London, 1958, 38-48.
  16. Amin A, Lotfy M, Shafiullah M, Adeghate E. The Protective Effect of *Tribulus terrestris* in Diabetes. *Annals of the New York Academy of Sciences*, 2006; 1084:391-401.