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In-Vivo Anti-Neoplastic Activity of Leaves Extract of Madhuca Longifolia

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Conflicts of Interest: Nil

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ABSTRACT

The study was emphasis on anticancer activity of Hydro-Ethanolic extract of leaf of Madhuca longifolia i.e HAEML against MCF7 induced breast carcinoma. Various parameters, such as tumor size, tumor weight, mean survival time, body weight of tumor bearing mice, hematological and histological parameter ware used to evaluate the anticancer activity. Results indicate that leaf extract would significantly increase the life span of restored the altered hematological parameters. Tumor size and tumor weight ware also considerably reduced.

Keywords: Breast carcinoma, Madhuca longifolia, anticancer activity.

Introduction

The treatment of malignancy is consistently employed. Healthy cell regulate many function but malignant cells cannot be able perform normal function. So they keep on going to differentiate while healthy cells cannot. This characteristic makes malignant growth cells helpless to chemotherapeutic medications. These medications are not without their own natural issues and hence different sorts of toxicity. For instance bone marrow suppression caused by a superior anticancer agent 5-flurouracil1. It also causes damage to cardiac muscle. The different treatments have been propounded for the treatment of disease, a significant number of which used plant inferred items. The plant drug store of organic agents that may give defensive potential against malignant growth. Madhuca longifolia is a medicinal plant possess various therapeutic characterstics belonging to the family Sapotaceae.large deciduous tree that grow up to13-16 meters in height2,3. It has various properties like anti-inflammatory4,anti-malignancy5, anti-asthematic6.

Material and Methods

Collection and Authentication of the leaf

Madhuca longifolia leaves were collected from garden of Ash tang Ayurvedic College, Indore and authentication by Dr. Shakun Mishra, Head of Department, S.S.N Govt. P.G. College, Khandwaand under guidance of Dr. Manmeet Singh Saluja.

Preparation of Crude Drug for Extract

Madhuca longifolia leaves were keep and dried under shade and grounded. The grounded plant leaves were sieved through sieve no.40 and stowed in a sealed vessel for extraction7.

Preparation of extracts of Madhuca longifolia

The grounded and sieved plant leaves about 1000gm were sequentially extracted using petroleum ether, ethanol, hydroalcohalic (1:1) and distilled water in soxhlet apparatus.

Pharmcological Evaluation:

Animals

The female albino mice weighing (20-25 g) of around similar age, were procured from CSIR-CDRI, Lucknow, Uttar 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 accommodated their sustenance. Medication given orally by methods of oro gastric cannula. Creatures were exposed to fasting for least 12 hours, before any of the investigation performs, techniques for try were presented for the assessment of the institutional Animals Ethical Committee were passed by the equivalent. Giving to CPCSEA rules for care of research facility of creatures and the moral rule for examinations of exploratory agony in cognizant creatures, tests were acted in morning.

Acute toxicity study

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

Anti-cancer activity

Cell line

Cell line (MCF-7) ware obtained from the CSIR-CDRI Lucknow. They were maintained by weekly incubation of 106Cells8,9.

Experimental Design

The Female albino mice weighing (20-25 g) were divided into 5 groups consisting of 12 animals in each. 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 day 1st, normal saline (5 ml/kg) was given in group I and group II, was serve as a tumor control group and given normal saline (5 ml/kg), Methotrexate (20mg/kg)given to group III, HAEML (250mg/kg) were given to group IV, and group V were given HAEML (500mg/kg), for 14 consecutive days. On day 15th half of the mice from each group were sacrificed, 24h after last dose, for the determination of tumor weight, tumor size and hematological 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 tumor size and tumor weight

On 15th day, after 24 of dose, 6 mice from each group were dissected and tumor was collected. The tumor size is measure by Vernier calipers and worth was taken from four distinct measurements for every tumor. The tumor weight was estimated by taking the heaviness of mice before after collection of tumor8, 9.

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.8,9

$$\text{ILS (\%)} = \left[\frac{\text{Mean survival time of treated group}}{\text{Mean survival time of control group}} - 1\right] \times 100$$

$$\text{Mean survival time} = \frac{\text{1st Death + Last Death}}{2}$$

Effect of extraction on body weight

All groups (except Group I), consisting of six mice each were transplanted with 1×106MCF-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 animal8, 9.

Effect of extracts hematological parameters

All the treatments were given for 14 days to each group (except group I), on the 15th day, blood was drawn by retro orbital plexus method. WBC count, RBC count, heamoglobin, protein and packed cell volume were determined. Cells smear was prepared in slide and stained with Lieshman stain solution. Red blood cells (RBC), White blood cells (WBC) and Heamoglobin (Hb) were determined.

Histopathological study of tumor

On 15th 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 5µm 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 □ SEM (standard error of mean) for six mice. Statistical implication of contrasts between the control and investigational sets was evaluated by One-way ANOVA. The value of probability values ($P < 0.05$) as compared to the control group.10

Results and Discussion

The results of the research work reveal an anticancer effect of HAEML in mice. A significant enhancement of MST was observed shown in Table 1. The MST for the control group was 20.34±1.15 days, whereas it was29.74±1.14, 24.45±2.56, 38.06±2.91 days for the groups treated with HAEML (500 and 200 mg/kg/day, p.o.) and MXT (20 mg/kg/day, i. p.) respectively. The percentage increase in the life span of tumor bearing mice treated with HAEML and MXT was found to be 20.23% (200 mg/kg), 46.23% (500 mg/kg), and 87.13% (20 mg/kg standard) respectfully ($P<0.01$) as compared to the control group.

Table No 1. Effect of HAEML Ext Treatment on the Survival of Tumor bearing mice

S No	Treatment	Mean Survival Time (Days)	Increase in life span (%)
1	Tumor Control	20.34±1.15	-
2	MXT (20mg/kg, i.p)	38.06±2.91	87.13%
3	HAEML (500 mg/kg, p.o)	29.74±1.14	46.23%
4	HAEML (250 mg/kg, p.o)	24.45±2.56	20.23%

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

There was reduction in the tumor size and tumor weight of mice treated with HAEML ($P<0.01$) as shown in Table 2. The tumor size control group was 10.07±0.1mm, whereas the extract treated group, it was 3.53±0.23 mm and 6.97±0.14 mm, HAEML at (500mg/kg and 200 mg/kg)respectively. Tumor weight of control group was 2.26±0.12gms, whereas for the extract treated group, it was 1.48±0.21gm and 1.93±0.21gm for HAEML at (500mg/kg and 200 mg/kg) respectively.

Table No. 2: Effect of HAEML on tumor size and tumor weight

S No	Treatment	Tumor Size (mm)	Tumor weight (gm)
1	Tumor Control	10.07±0.1	2.26±0.12
2	MXT (20mg/kg, i.p)	2.21±0.09	0.88±0.10
3	HAEML (250 mg/kg, p.o)	6.97±0.14	1.93±0.21
4	HAEML (500 mg/kg, p.o)	3.53±0.23	1.48±0.21

*P<0.01 Vs tumor control, n=6 animals in each group, No. of days = 14, Values are expressed as mean ± SEM.

There was significant decrease in the tumor weight gain by the HAEML treated mice when compare with control groups shown in Table 3. The analysis of hematological parameters shows minimum toxic effect in mice treated with HAEML. After 14 days of transplantation, HAEML extract were able to restore the change in the hematological parameters consequent to tumor inoculation. The total WBC count, protein and PCV were found to increase with a reduction in the hemoglobin 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, HAEML at (500mg/kg/day and 200 mg/kg/day, p. o.) treatment could change these altered parameters to near normal shown in Table 4.

Table No.3: Effect of HAEML Standard Treatment on Body Weight of Tumor bearing mice

n= 6 in each group. Values were expressed as mean± SEM, **P<0.01Vs normal, *P<0.01 Vs Tumor

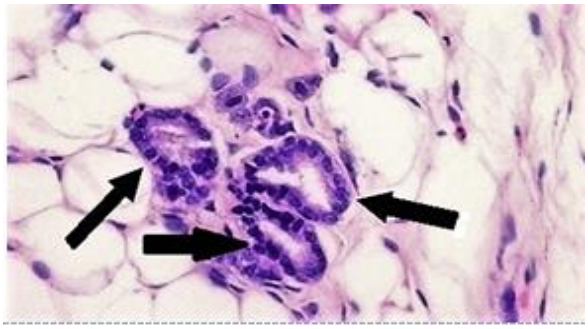
Parameter	Normal	Tumor control	MXT (20 mg/kg)	HAEML 250mg/kg	HAEML 500mg/kg
Hb(g/dl)	14.18±0.15	8.1±0.09*	13.97±0.08	9.48±0.07	12.8±0.09
RBC (million/mm^3)	4.71±0.01	2.5±0.06	4.22±0.01	3.12±0.03	3.54±0.06
WBC(million/mm^3)	7.51±0.02	24.81±0.04	8.29±0.03	11.22±0.04	9.24±0.03
Protein %	8.22±0.03	13.77±0.38	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, *P<0.001 vs normal, $P<0.001 vs tumor

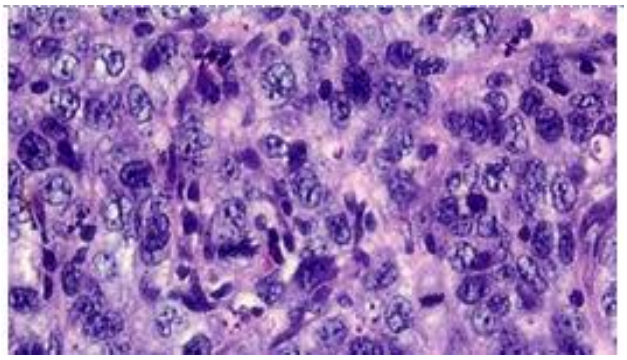
Histopathological study of tumor

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.

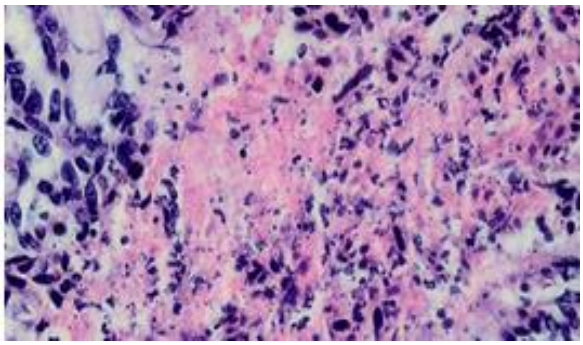
Figure.1: Shows effect of extracts and Methotrexate on tumor cells.



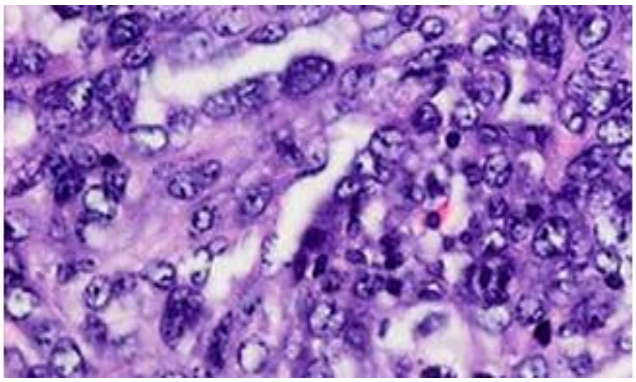
Normal Breast (Arrow indicate normal duct)



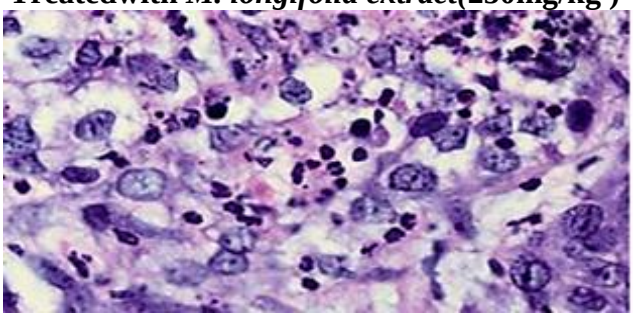
MCF7 induced breast cancer



Treated MXT



Treatedwith *M. longifolia extract(*250mg/kg)



Treated with *M. longifolia extract(*500mg/kg

Conclusion

Anticancer activity of Madhucalongifolia in an in-vivo of MLC7 cells induced breast adenocarcinoma has been evaluated. Treatment with Madhucalongifolia extract delayed tumor formation, reduced tumor size and tumor weight and increase mean survival time of tumor bearing mice. It also helpful in restoring haematological parameters nearby their normal value. Thus by this study, it is clear that Madhucalongifolia possess anticancer potential and an option for management of breast cancer.

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