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ORIGINAL ARTICLE

Protective Effect of *Madhuca longifolia* Leaves in 7, 12-Dimethylbenz(a)anthracene Induced Mammary Carcinoma in Sprague Dawley Rat model

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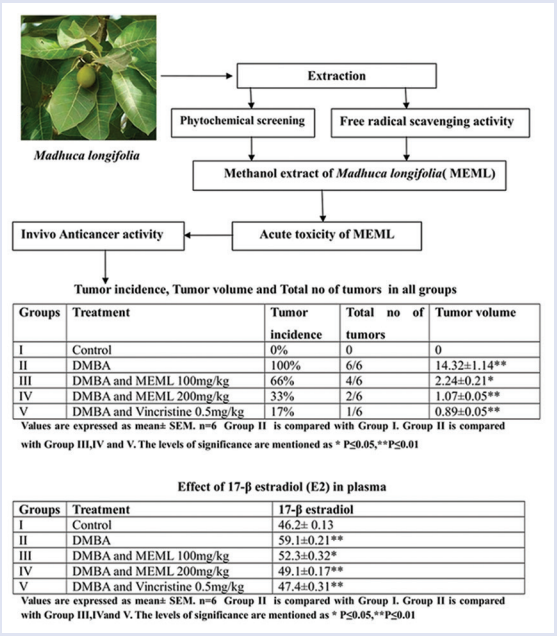
ABSTRACT

Objectives: To evaluate the pretreatment with *Madhuca longifolia* leaves on 7, 12-Dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma in Sprague Dawley rat. **Materials and Methods:** Thirty female Sprague Dawley rats were divided into five groups, and each group is having six rats. Group I rats received vehicle (1 mL of emulsion of sunflower oil and physiological saline) subcutaneously and 1 mL of 2% dimethyl sulfoxide per orally. Groups II, III, IV, and V were induced mammary carcinogenesis by giving single dose of subcutaneous injection of 25 mg of DMBA. Group III, IV, and V rats were administered with MEML 100, 200 mg/kg and Vincristine 0.5 mg/kg dissolved in 1 ml of 2% dimethylsulfoxide given 1 week before the administration of the carcinogen, respectively, and continued for 16 weeks. At the end of experiment, the animals were sacrificed and biochemical estimations were done in all groups. Mammary tissues in all groups were dissected out and used for histopathological studies. **Results:** Oral administration of 200 mg/kg of MEML to DMBA-treated rats effectively prevented the tumor incidence, total number of tumors, and tumor volume and brought back the biochemical markers to normal, which was comparable with standard group. In lower dose 100 mg/kg, the effect was very less compared to normal and standard groups. Our data showed that MEML 200 mg/kg significantly restored the breast tissue biochemically and histologically which was comparable with standard. **Conclusion:** Our results concluded that the leaves of *Madhuca longifolia* may be used in the treatment of mammary carcinoma.

Key words: Breast cancer, Dimethylbenz(a)anthracene, leaves, *Madhuca longifolia*, rat

SUMMARY

- Anticancer activity of *Madhuca longifolia* leaves in 7,12-Dimethylbenz(a) anthracene (DMBA)-induced mammary carcinoma in Sprague Dawley rat was evaluated
- MEML was selected based on the phytochemical analysis and free radical scavenging activity
- Methanol extract of this plant was found devoid of mortality of animals at the dose of 2000 mg/kg body weight
- Single subcutaneous injection of 25 mg of DMBA was given to induce mammary gland carcinogenesis
- MEML 100, 200 mg/kg (orally), and 0.5 mg/kg of Vincristine (intraperitoneally) were given 1 week before the administration of the carcinogen and continued for 16 weeks
- Vincristine was used as standard in this experiment
- The research concluded that MEML 200 mg/kg significantly prevented the breast cancer biochemically and histologically which was comparable with standard
- Chemopreventive activity may be due to flavonoids present in the methanol extract of plant leaves.



Tumor incidence, Tumor volume and Total no of tumors in all groups

Groups	Treatment	Tumor incidence	Total no of tumors	Tumor volume
I	Control	0%	0	0
II	DMBA	100%	6/6	14.32±1.14**
III	DMBA and MEML 100mg/kg	66%	4/6	2.24±0.21*
IV	DMBA and MEML 200mg/kg	33%	2/6	1.07±0.05**
V	DMBA and Vincristine 0.5mg/kg	17%	1/6	0.89±0.05**

Values are expressed as mean± SEM. n=6 Group II is compared with Group I. Group II is compared with Group III,IV and V. The levels of significance are mentioned as * P≤0.05,**P≤0.01

Effect of 17-β estradiol (E2) in plasma

Groups	Treatment	17-β estradiol
I	Control	46.2± 0.13
II	DMBA	59.1±0.21**
III	DMBA and MEML 100mg/kg	52.3±0.32*
IV	DMBA and MEML 200mg/kg	49.1±0.17**
V	DMBA and Vincristine 0.5mg/kg	47.4±0.31**

Values are expressed as mean± SEM. n=6 Group II is compared with Group I. Group II is compared with Group III,IVand V. The levels of significance are mentioned as * P≤0.05,**P≤0.01

Abbreviations used: DMBA: 7, 12-Dimethylbenz(a)anthracene; MEML: Methanol extract of *Madhuca longifolia*; SOD: Superoxide dismutase; CAT: Catalase; TBARS: Thiobarbituric acid reactive substances; GSH: Glutathione; GPx: Glutathione peroxidase; CPCSEA: Committee for the purpose control and supervision in experimental animals.

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INTRODUCTION

Mammary carcinoma is the second most common carcinoma in women across the world, nearly 2 million new cases were diagnosed in 2018,

accounting for almost one in four cancer cases among women.[1] Breast cancer develops in the lobules are called as lobular carcinoma and develops in cells lining of milk ducts are called as ductal carcinoma. Several studies have reported that 7, 12-Dimethylbenz(a)anthracene (DMBA) is used to induce mammary carcinomas in rats. In the mammary gland, DMBA produces epoxides and active metabolites with a capacity for damaging the DNA molecule leading to carcinogenesis. With the higher cellular proliferative index of Types 1 and 2 lobules, there is higher metabolic activity and more epoxide formation.[2,3]

Physicians and patients are in need of maximum therapeutic value with no or less side effects to improve the quality of the life of breast cancer patients. Several medicinal herbs constitute such a group. In recent years, many scientists have examined the effect of herbals used traditionally by herbalist and indigenous healers to treat different types of cancer. Several hundred plants have been studied for the treatment of different types of cancer. There have been only a handful of plants fairly well researched. After the extensive search of medicinal plant for the treatment of mammary carcinoma, the plant selected for the study is *Madhuca longifolia*, which comes under *Sapotaceae* family. It is a large-sized tropical deciduous tree, grown up to 16–17 m height and distributed in India, Sri Lanka, and Nepal. Different parts of this plant were reported to contain sapogenins, steroids, saponins, flavonoids, tannins, β-amyrin, betulinic acid, ethyl cinnamate, ursolic acid, stigmasterol, β-carotene, xanthophylls, quercetin, dihyroquercetin, β-sitosterol, sesquiterpene alcohol, triterpenoids, α-terpineol, Mi-saponin A and B, 3-β-monocaprylic ester of erythrodiol, myricetin, erythrodiol, and glycosides.[4,5] Leaves are used for chronic bronchitis, cancer, and Cushing's disease.[6,7]

Based on the literature review, there was no established work in the protective activity of *Madhuca longifolia* leaves in mammary carcinoma. Hence, the study was planned to evaluate the chemopreventive activity of *Madhuca longifolia* leaves in DMBA-induced mammary carcinoma in rat.

MATERIALS AND METHODS

Drugs and chemicals

DMBA and Vincristine were purchased from Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru, India. All other chemicals used in the study were purchased from local sources and were of analytical grade.

Collection, authentication, and extraction of plant

The fresh leaves of *Madhuca longifolia* were collected from Sankarankovil, Tamil Nadu, India. The plant was authenticated by Botanical Survey of India, Coimbatore, and the preserved specimen of an identified plant has been kept in Pharmacognosy Department of R.V.S. College of Health Sciences, Coimbatore, India.

Extraction

After authentication, the fresh leaves of *Madhuca longifolia* were properly dried in shade for 2–3 weeks. It was reduced to fine particles in a blender, sieved, and used for the further experimental studies. About 2 kg of shade dried plant leaves of *Madhuca longifolia* was extracted in Soxhlet successively extracted with n-hexane, chloroform, ethyl acetate, and methanol. Each extract was evaporated using rotary vacuum evaporator. The extracts collected from each solvent was weighed, and the percentage yield was calculated. The consistency and color of the plant leaf extracts were noted.

Phytochemical analysis and free radical scavenging activity

The n-hexane, chloroform, ethyl acetate, and methanol extracts of the leaf powder of *Madhuca longifolia* were subjected to qualitative chemical analysis based on the method of Takeda *et al.*[8] Diphenylpicrylhydrazyl, superoxide and nitric oxide free radical scavenging activity were determined by the method of Sanchez-Moreno *et al.*[9]

Experimental animals and acute toxicity

Adult female Sprague Dawley rats weighing 100–150 g were purchased from R.V.S. College of Health Sciences, Coimbatore, India. Animals were maintained in well-ventilated housing conditions and fed with commercial rodent diet. They were provided with water *ad libitum* during the experiment. The Institutional Animals Ethics Committee (Register number 1012/c/06/CPCSEA) permitted the study. Acute toxicity study was done according to Organisation for Economic Co-operation and Development guidelines 423.[10,11]

Experimental design

A total of thirty female Sprague Dawley rats were divided into five groups and each group consisting of six rats.

Group I (vehicle-treated control group) – administered with excipient (single dose of 1 mL of emulsion of sunflower oil and physiological saline) subcutaneously and 1 mL of 2% dimethyl sulfoxide per orally for 16 weeks.

Group II (negative control group) – administered with single subcutaneous injection of 25 mg of DMBA in 1 mL of emulsion of sunflower oil and physiological saline.

Group III (test group lower dose) – administered per orally with 100 mg/kg of MEML, dissolved in 2% dimethyl sulfoxide, started 7 days before the exposure of the DMBA.

Group IV (test group higher dose) – administered per orally with 200 mg/kg of MEML, dissolved in 2% dimethyl sulfoxide, started 7 days before the exposure of the DMBA.

Group V (positive control group) – administered intraperitoneally once per week with 0.5 mg/kg of standard drug Vincristine, started 7 days before the exposure of the DMBA.

Groups III, IV, and V induced mammary carcinogenesis by giving single subcutaneous injection of 25 mg of DMBA in 1 mL of emulsion of sunflower oil and physiological saline and continued for 16 weeks. The experiment was terminated at 16th week to determine the protective activity of *Madhuca longifolia* during DMBA-induced mammary carcinogenesis. The rats from all groups were sacrificed at the end of experiment by cervical dislocation method for biochemical analysis and histopathology.

Biochemical analysis and histopathology

Blood samples were collected from all groups and used for biochemical estimations. Half portion of tumor tissues from rats were fixed in formaldehyde for histopathological study and the remaining portion were used for biochemical analysis. Thiobarbituric acid reactive substances (TBARS) in plasma sample were determined using the method of Yagi[12] Tissue lipid peroxidation was estimated using the method of Ohkawa *et al.*[13] The activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were determined using the method of Kakkar *et al.*,[14] Sinha,[15] and Rotruck *et al.*,[16] respectively. Glutathione (GSH) in mammary tissues and plasma was estimated using method of Beutler and Kelly[17] Chemiluminescent immunoassay was used for the estimation of serum 17 β-estradiol (E2) using the method of Buscarlet *et al.*[18]

Statistical analysis

The results were given as mean ± standard error of the mean of six animals from all groups. The statistical analyses were done using

one-way analysis of variance. $P < 0.05$ was considered as statistically significant.

RESULTS

Phytochemical analysis and *in vitro* free radical scavenging activity

Methanolic extract showed high extractive yield of 7.7% w/w when compared to other extracts of *Madhuca longifolia* [Table 1]. In phytochemical screening, most of the active constituents responsible for the biological activity are present in methanol extract compared to other extracts. Methanol extract showed the presence of alkaloids, terpenoids, carbohydrates, flavonoids, phenols, saponins, proteins, and glycosides [Table 2]. Methanol extract showed the dose-dependent free radical scavenging activity in all *in vitro* assay models compared to other extracts [Table 3].

Active constituents such as flavonoids and alkaloids are responsible for most of the biological activity which includes cancer. Many studies have been reported that flavonoids can exert chemopreventive effects in estrogen-dependent or independent breast cancer. Flavonoids are present in methanol extract of test plant. Hence, methanol extract was selected based on phytochemical analysis and *in vitro* free radical scavenging effect. In acute toxicity study, the MEML was found devoid of mortality of animals at the dose of 2000 mg/kg body weight. Hence, 1/20 th (lower dose 100 mg/kg, per orally) and 1/10th (higher dose 200 mg/kg, per orally) of the doses were selected for the screening of anticancer activity.

Vincristine, a commonly used anticancer agent in the treatment of breast cancer, was used as standard in this experiment.

Table 1: The percentage yield of successive extracts of the leaves of *Madhuca longifolia*

Name of the extract	Color of the extract	Physical nature	Percentage yield (w/w)
n-Hexane	Green/sticky mass	Waxy semisolid	1.9
Chloroform	Green/sticky mass	Semisolid	2.1
Ethyl acetate	Brownish green solid	Solid	3.3
Methanol	Brownish green solid	Solid	7.7

Table 2: Phytochemical screening of *Madhuca longifolia*

Test	n-Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloids	–	–	–	+
Carbohydrate	–	–	+	+
Glycosides	–	–	+	+
Phytosterol	–	–	–	–
Fixed oils and fats	–	–	–	–
Tannins	–	–	–	–
Phenols	–	–	–	+
Proteins	–	–	+	+
Gums and mucilages	–	–	–	–
Flavonoids	–	–	–	+
Terpenoids	–	–	+	+
Steroids	+	+	–	–
Saponins	–	–	+	+

+ve: Positive result; –ve: Negative result

Table 3: Free radical scavenging activity of various extracts of *Madhuca longifolia*

Name of the extract	Concentration (Mcg/ml)	Scavenging activity (%)		
		DPPH	Superoxide radicals	Nitric oxide radicals
n-Hexane	50	13.14±0.12	25.18±0.15	9.14±0.21
n-Hexane	100	28.11±0.31	33.01±0.23	14.12±0.13
Chloroform	50	31.14±0.14	19.12±0.24	19.23±0.26
Chloroform	100	38.32±0.28	28.21±0.14	21.12±0.09
Ethyl acetate	50	26.04±0.22	28.12±0.14	16.12±0.15
Ethyl acetate	100	41.31±0.22	33.13±0.18	27.12±0.08
Methanol	50	64.43±0.24	51.18±0.27	48.12±0.19
Methanol	100	85.69±0.21	71.33±0.21	55.09±0.12

DPPH: 2,2-diphenyl-1-picrylhydrazyl

Anticancer activity

Table 4 showed the incidence of mammary tumors volume and total no of tumors in DMBA, MEML 100 mg/kg, and MEML 200 mg/kg treated rats. One hundred percent tumor incidence was observed in Group II (treated with only DMBA). Oral administration of MEML 100 mg/kg and 200 mg/kg to DMBA-treated rats decreased the tumor incidence (66% and 33%, respectively) and tumors in this group (40% and 20%). The size of the tumor and tumor volume were very small in 200 mg/kg-treated rats and standard group rats [Table 4].

The level of plasma 17 E2 was significantly increased in DMBA-treated rats as compared to control rats. Oral administration of methanol extract of 200 mg/kg to DMBA-treated rats as well as control rats significantly ($P < 0.01$) reduced which was comparable with standard group [Table 5].

In plasma, TBARS was increased significantly whereas GSH and activities of CAT, SOD, and GPx were decreased in DMBA-treated rats as compared to control rats in our study. Oral administration of 200 mg/kg of methanol extract to DMBA-treated rats significantly ($P < 0.01$) restored the status to near normal which was comparable with vincristine-treated group rats [Table 6].

Levels of TBARS, GPx, and GSH were increased, and the CAT and SOD activities were decreased in mammary gland tumor tissue groups as compared to normal tissues of control rats. Oral administration of 200 mg/kg of MEML to DMBA-treated rats significantly restored the status to near to standard group [Table 7].

Histopathology of mammary tissues in control and experimental groups

In histopathological studies, normal ductal epithelium was observed in control group [Figure 1]. Abnormal cellular proliferation and ductal hyperplasia were observed in DMBA-treated groups [Figure 2]. Rats treated with higher dose of methanol extract of 200 mg/kg of rats

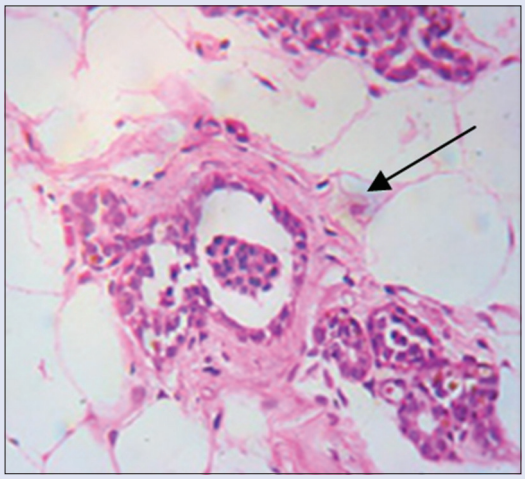

Figure 1: Control groups showing normal ductal epithelium

Table 4: Tumor incidence, tumor volume, and total number of tumors in all groups

Groups	Treatment	Tumor incidence (%)	Total number of tumors	Tumor volume
I	Control	0	0	0
II	DMBA	100	6/6	14.32±1.14**
III	DMBA and MEML 100 mg/kg	66	4/6	2.24±0.21*
IV	DMBA and MEML 200 mg/kg	33	2/6	1.07±0.05**
V	DMBA and vincristine 0.5 mg/kg	17	1/6	0.89±0.05**

Values are expressed as mean±SEM. *n*=6 Group II is compared with Group I. Group II is compared with Group III, IV, and V. The levels of significance are mentioned as *$P \leq 0.05$; **$P \leq 0.01$. DMBA: 7, 12-Dimethylbenz(a)anthracene; MEML: Methanol extract of *Madhuca longifolia*; SEM: Standard error of mean

Table 5: Effect of plasma 17-β estradiol in control and experimental groups

Groups	Treatment	17-E2
I	Control	46.2±0.13
II	DMBA	59.1±0.21**
III	DMBA and MEML 100 mg/kg	52.3±0.32*
IV	DMBA and MEML 200 mg/kg	49.1±0.17**
V	DMBA and vincristine 0.5 mg/kg	47.4±0.31**

Values are expressed as mean±SEM. *n*=6 Group II is compared with Group I. Group II is compared with Group III, IVand V. The levels of significance are mentioned as *$P \leq 0.05$; **$P \leq 0.01$. E2: β estradiol; DMBA: 7, 12-Dimethylbenz(a) anthracene; MEML: Methanol extract of *Madhuca longifolia*; SEM: Standard error of mean

showed improved ductal architecture [Figure 3]. In 100 mg/kg, the effect was very less and there was no improvement in the ductal hyperplasia. However, 200 mg/kg effectively inhibited the ductal hyperplasia.

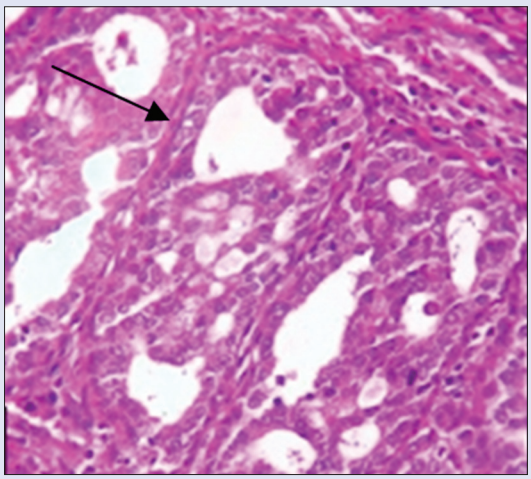

Figure 2: Abnormal ductal hyperplasia in dimethylbenz(a) anthracene-treated groups

DISCUSSION

Breast development at puberty is stimulated by 17 E2, which is the most biologically active ovarian steroid hormone in breast tissue and involved in pathogenesis of breast cancer.[19,20] Several studies reported that decreased serum estrogen concentration produces lower incidence of DMBA-induced mammary carcinoma in rats. In our experiment, the level of plasma 17 E2 was significantly increased in DMBA-treated rats, decreased in Vincristine MEML treated rats which indicates protective activity against breast cancer.

Lipid peroxidation was assayed by determining the production of TBARS. Free radicals induce lipid peroxidation of unsaturated fatty acids in patients with breast cancer.[21,22] Numerous studies have shown increased levels of different markers of lipid peroxidation in plasma, serum, urine, and cancer tissue of women suffering from breast cancer and showed that raised oxidative stress played an important role in breast cancer development and progression. Since breast cancer is largely associated with lipid peroxidation, it may be hypothesized that the disease progression or response to treatment may highly relay on patient's individual ability to scavenge either lipid peroxidation products or reactive species that lead to lipid peroxidation. Natural antioxidant defense mechanism consists of both enzymatic and nonenzymatic systems. In that, important antioxidant enzymes include SOD, GPx, and CAT. SOD catalyze dismutation of superoxide anion into hydrogen peroxide, whereas CAT and GPx reduce hydrogen peroxide, thus preventing production of highly toxic hydroxyl radical.[21] One study reported that increased plasma lipid peroxidation in cancer was accompanied by the increased activity of GPx 1 and GPX 1 polymorphism; this may be an important factor modifying oxidative stress response in breast cancer individuals.[23]

In our study, TBARS was increased significantly whereas GSH and activities of CAT, SOD, and GPx were decreased in DMBA-treated rats as compared to control rats in plasma. Oral administration of 200 mg/kg of MEML to DMBA-treated rats significantly restored the status to near normal.

Enhanced lipid peroxidation in breast cancer tissue was reported in many studies. Similarly, antioxidants GPx in tumor tissues was significantly increased, and CAT activity was significantly decreased in breast cancer patients.[24,25] Many studies proved that upregulation of GSH in breast cancer tissue is the potential biomarker for the diagnosis and treatment of breast cancer. Lower SOD activity and higher GSH level have been reported in breast cancer tissue compared to unaffected healthy tissue of mammary gland.[26,27]

Our present research reported that the levels of TBARS, GPx, and GSH were increased, and the CAT and SOD activities were decreased in mammary gland tumor tissue groups as compared to normal tissues of control rats. Oral administration of 200 mg/kg of MEML to DMBA-treated rats significantly restored the status to near to normal.

Leaves of *Madhuca longifolia* have been evaluated in various preclinical studies for its anticancer activity. The DMBA-induced breast cancer in rats is one of the standard preclinical animal models for studying chemopreventive drug development against breast cancer. In present study, we evaluated the preventive effect of MEML extract against DMBA-induced breast cancer, and MEML was given 1 week before the DMBA administration and continued till the end of experimental period.[28-30] Many studies reported that pretreatment of the extract (before administration of carcinogen) has more potent tumor suppressive activity than posttreatment of the extract (after administration of Carcinogen). Many phytochemicals have strong antioxidant activity and suppress either the initiation or promotion step of carcinogenesis. Therefore, antioxidant activity of the plant extract might be expected to suppress initiation of carcinogenesis. Before tumor induction, Pretreatment of MEML extract suppressed carcinogenesis and growth of DMBA induced breast cancer in a dose dependent manner. In our study, we found that MEML pretreatment

Table 6: Effect of plasma thiobarbituric acid reactive substances and antioxidants in control and experimental groups

Groups	Treatment	TBARS (nmoles/mL plasma)	SOD (U/mL)	CAT (U/mL)	GPx (U/L)	GSH (mg/dL)
I	Control	1.55±0.12	2.84±0.22	1.43±0.12	157±0.08	31±0.12
II	DMBA	3.18±0.14**	1.80±0.15**	1.09±0.11**	108±0.12**	18±0.11**
III	DMBA and MEML 100 mg/kg	2.89±0.09*	2.27±0.20*	1.29±0.09*	134±0.19*	23±0.10*
IV	DMBA and MEML 200 mg/kg	1.84±0.10**	2.56±0.22**	1.38±0.11**	142±0.13**	28±0.12**
V	DMBA and vincristine 0.5 mg/kg	1.21±0.05**	2.81±0.15**	1.41±0.08**	155±0.15**	30±0.07**

Values are expressed as mean±SEM. *n*=6 Group II is compared with Group I. Group II is compared with Group III, IV, and V. The levels of significance are mentioned as *$P \leq 0.05$; **$P \leq 0.01$. TBARS: Thiobarbituric acid reactive substances; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; GSH: Glutathione; DMBA: 7, 12-Dimethylbenz(a)anthracene; MEML: Methanol extract of *Madhuca longifolia*; SEM: Standard error of mean

Table 7: Effect of mammary tissue thiobarbituric acid reactive substances and antioxidants in control and experimental groups

Groups	Treatment	TBARS (nmols/100 g of tissue)	SOD (U/mg of protein)	CAT (U/mg of protein)	GPx (U/mg of protein)	GSH (mg/100 g of tissue)
I	Control	0.55±0.12	12.84±0.22	52.17±0.12	15.6±0.08	11.3±0.12
II	DMBA	1.18±0.14**	9.80±0.15**	31.09±0.11**	28.12±0.12**	18±0.11**
III	DMBA and MEML 100 mg/kg	0.89±0.09*	10.27±0.20*	41.29±0.09*	22.21±0.19*	15±0.10
IV	DMBA and MEML 200 mg/kg	0.69±0.10**	11.56±0.22*	49.38±0.11*	17.2±0.13**	12.8±0.12**
V	DMBA and vincristine 0.5 mg/kg	0.62±0.05**	12.81±0.15**	51.41±0.08**	16.5±0.15**	11.80±0.07**

Values are expressed as mean±SEM. *n*=6 Group II is compared with Group I. Group II is compared with Group III, IV and V The levels of significance are mentioned as *$P \leq 0.05$; **$P \leq 0.01$. TBARS: Thiobarbituric acid reactive substances; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; GSH: Glutathione; DMBA: 7, 12-Dimethylbenz(a)anthracene; MEML: Methanol extract of *Madhuca longifolia*; SEM: Standard error of mean

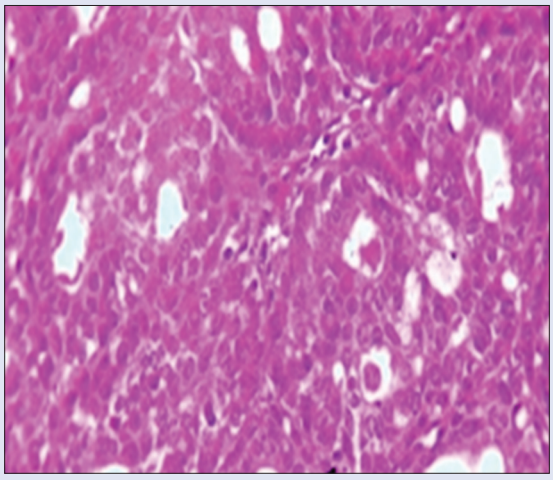

Figure 3: Two hundred milligram/kg of methanol extract of *Madhuca longifolia* extract treated rats showing improved ductal architecture

prevented the carcinoma against DMBA-induced mammary cancer. Flavonoids such as quercetin, Kaempferol, myricetin, isorhamnetin, and genistein can potentially contribute to breast cancer prevention and treatment by antioxidant activity and apoptotic activity. Quercetin is found to be an effective chemotherapeutic agent in the treatment of breast cancer, and it is present in the leaves of *Madhuca longifolia*; this may be the reason for anticancer activity. The bioactive compounds responsible for the prevention of mammary carcinoma have not been well determined in this study. Hence, further research to be carried out to find the biologically active compounds present in the leaves of this plant for antibreast cancer activity.

CONCLUSION

Our research concluded that the leaves of *Maduca longifolia* have potential anticarcinogenic activity against breast cancer and may act as a potent chemopreventive agent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A, *et al.* Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Kerdelhué B, Forest C, Coumoul X. Dimethyl-benz(a)anthracene: A mammary carcinogen and a neuroendocrine disruptor. Biochim Open 2016;3:49-55.
3. Wang Z, Zhang X. Chemopreventive activity of honokiol against 7, 12-dimethylbenz[a] anthracene-induced mammary cancer in female sprague dawley rats. Front Pharmacol 2017;8:320.
4. Yadav P, Singh D, Mallik A, Nayak S. *Madhuca longifolia* (Sapotaceae): A Review of its traditional uses, Phytochemistry and Pharmacology.Int J Biomed Res 2012;3;291-305.
5. Verma N, Jha KK, Kumar U, Deepak K, Singh NK, Singh AK, *et al.* Biological properties, phytochemistry and traditional uses of Mahua (*Madhuca longifolia*): A review. Int J Adv Res Innov 2014;2;630-8.
6. Bhaumik A, Upender Kumar M, Khan KA, Srinivas CH. The bioactive compounds obtained from the fruit-seeds of *Madhuca longifolia* (L) act as potential anticancer agents. Sch J App Med Sci 2014:2;1235-8.
7. Shaban A, Verma SK, Nautiyal R, Singh SK, Purohit R, Chimata ML, *et al. In vitro* cytotoxicity of *Madhuca indica* against different human cancer cell lines. Int J Pharm Sci Res 2012;3;1385-7.
8. Takeda Y, Matsumoto T, Terao H, Shingu T, Futatsuishi Y, Nohara T, *et al.* Orthosiphol D and E, minor diterpenes from *Orthosiphon stamineus*. Phytochemistry 1993;33:411.
9. Sanchez-Moreno C, Larrauri JA, Saura-Calixto F. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food Res Int 1999;32;407-12.
10. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol 1983;54:275-87.
11. Ecobichon DJ. The Basis of Toxicology Testing. 2nd ed. New York: CRC Press; 1997. p. 43-60.
12. Yagi K. Lipid peroxides and human diseases. Chem Phys Lipids 1987;45:337-51.

13. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
14. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984;21:130-2.
15. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972;47:389-94.
16. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG, *et al.* Selenium: Biochemical role as a component of glutathione peroxidase. Science 1973;179:588-90.
17. Beutler E, Kelly BM. The effect of sodium nitrite on red cell GSH. Experientia 1963;19:96-7.
18. Buscarlet L, Volland H, Dupret-Carruel J, Jolivet M, Grassi J, Créminon C, *et al.* Use of free radical chemistry in an immunometric assay for 17 beta-estradiol. Clin Chem 2001;47:102-9.
19. Russo J, Russo IH. The role of estrogen in the initiation of breast cancer. J Steroid Biochem Mol Biol 2006;102:89-96.
20. Jerry DJ, Shull JD, Hadsell DL, Rijnkels M, Dunphy KA, Schneider SS, *et al.* Genetic variation in sensitivity to estrogens and breast cancer risk. Mamm Genome 2018;29:24-37.
21. Kedzierska M, Olas B, Wachowicz B, Jeziorski A, Piekarski J. The lipid peroxidation in breast cancer patients. Gen Physiol Biophys 2010;29:208-10.
22. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem 2015;97:55-74.
23. Jablonska E, Gromadzinska J, Peplonska B, Fendler W, Reszka E, Krol MB, *et al.* Lipid peroxidation and glutathione peroxidase activity relationship in breast cancer depends on functional polymorphism of GPX1. BMC Cancer 2015;15:657.
24. Tas F, Hansel H, Belce A, Ilvan S, Argon A, Camlica H, *et al.* Oxidative stress in breast cancer. Med Oncol 2005;22:11-5.
25. Wang C, Yu J, Wang H, Zhang J, Wu N. Lipid peroxidation and altered anti-oxidant status in breast adenocarcinoma patients. Drug Res (Stuttg) 2014;64:690-2.
26. Aryal B, Rao VA. Specific protein carbonylation in human breast cancer tissue compared to adjacent healthy epithelial tissue. PLoS One 2018;13:e0194164.
27. Gamcsik MP, Kasibhatla MS, Teeter SD, Colvin OM. Glutathione levels in human tumors. Biomarkers 2012;17:671-91.
28. Sahin K, Tuzcu M, Sahin N, Akdemir F, Ozercan I, Bayraktar S, *et al.* Inhibitory effects of combination of lycopene and genistein on 7,12- dimethyl benz(a)anthracene-induced breast cancer in rats. Nutr Cancer 2011;63:1279-86.
29. Bishayee A, Mandal A, Thoppil RJ, Darvesh AS, Bhatia D. Chemopreventive effect of a novel oleanane triterpenoid in a chemically induced rodent model of breast cancer. Int J Cancer 2013;133:1054-63.
30. Kolanjiappan K, Manoharan S. Chemopreventive efficacy and anti-lipid peroxidative potential of *Jasminum grandiflorum* linn. On 7, 12-dimethylbenz(a)anthracene-induced rat mammary carcinogenesis. Fundam Clin Pharmacol 2005;19:687-93.