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Anti-Carcinogenic Activity of Ethanolic Seed Extract of *Zizipus Jujuba* in DMBA Induced Charles Foster Rat

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Abstract

The anti-carcinogenic potential of ethanolic extract derived from *Ziziphus jujuba* seeds against breast cancer induced by 7,12-Dimethylbenz[a]anthracene (DMBA) in Charles Foster rats. Mammary tumoriogenesis was initiated via oral gavage of DMBA, solubilised in olive oil, at a dose of 15 mg per kilogram of body weight through oral administration. Once tumors started to appear, the rats were given the ethanolic seed extract of *Ziziphus jujuba* at a dose of 300 mg/kg body weight every day for five weeks. The development of tumors and the response to treatment were regularly checked by measuring the size of the tumors using Vernier calipers.

The results showed that the group of rats treated with the extract demonstrated a significant reducing in tumor within the extract-treated cohort relative to the untreated control animals. This reduction suggests that the extract has a protective effect against DMBA-induced mammary tumor development against cancer caused by chemical exposure. These outcomes suggest the ethanolic extract of *Ziziphus jujuba* seeds possesses substantial anti-tumor properties, highlighting its potential viability as a therapeutic agent of natural origin for managing mammary carcinoma.

Keywords: DMBA, Breast tumor, Mammary Carcinoma, Ziziphus jujuba, Charles Foster Rats, Tumor Volume, Anti-carcinogenic Activity

INTRODUCTION:

Breast cancer is one of the most common types of cancer affecting women worldwide and continues to be a serious public health issue. Over the years, a lot of research has been done to understand its causes, how to prevent it, and how to treat it effectively (Singh et al., 2000). According to the global data from the World Health Organization and the 2020 GLOBOCAN statistical report, malignancies of the breast represent the most frequently diagnosed oncological condition worldwide, positioning it is a leading determinant of cancer-related mortality. This accounts for an estimated annual incidence of 2.3 million emerging cases each year (Sung et al., 2021). Beyond mortality figures, the disease imposes a massive global epidemiological strain when quantified via disability-adjusted life years (DALYs), underscores its profound socio-economic and public health ramifications (WHO, 2022).

projections indicate an upward trajectory in the absolute volume of breast cancer diagnoses, particularly within developing and low- to middle-income countries (LMICs). This shifting epidemiological trend in primarily driven by accelerated urbanization and the widespread adoption of westernized behavioural patterns. This induced delay childbearing, shortened breastfeeding durations, increasingly sedentary lifestyle, dietary transitions, and an earlier onset of menarche. Conversely, heightened public awareness, systematic screening programs, and sophisticated diagnostic innovations have also elevated the clinical detection and documentation rates of these cases (Porter, 2008).

Among established epidemiological risk factors, female biological sex remains paramount, as mammary tumor development is inherently modulated by endocrine signalling. Ovarian steroids, specifically estrogen and progesterone, serve as critical regulators of mammary epithelial cell proliferation, and homeostatic disruptions in these hormonal pathways can induce neoplastic, transformation. Furthermore, an inherited familial predisposition drastically elevates individual vulnerability, with approximately about 13–19% of diagnosed patients presenting at least one affected first-degree relative with the disease (Appleby et al., 2013).

Breast cancer is not a solitary condition; rather it is an umbrella term for a diverse group of malignancies, each distinguished by its own unique genetic profile and molecular features, how aggressive they are, their appearance under a microscope, and their outcome in patients. The World Health Organization has identified several histological subtypes, each with different progression patterns and responses to treatment (Tavassoli et al., 2003). The condition occurs when abnormal breast epithelial cells grow out of control, which can spread to surrounding tissue and more distant area of the body in later stages. However, some abnormal cell changes may not become malignant and may appear as benign growths such as cysts, atypical hyperplasia, or intraductal papillomas (Sinha, 2018).

Endogenous genetic variants also exert a profound influence on the onset and progression of the malignancy. Specifically, pathogenic germline mutation within the BRCA1 and BRCA2 tumor suppressor genes exhibit a robust correlation with hereditary breast cancer syndrome. These genetic anomalies drastically elevate to nearly 85%, and frequently precipitate an earlier clinical onset compared to sporadic, non-inherited cases (Hu et al., 2022).

Medicinal plants have gained growing scientific attention as potential sources of anticancer compounds due to their bioactive chemicals and relatively low toxicity. Ziziphus jujuba, commonly known as jujube, has been used for both nutritional and medicinal purposes. It contains various active components, such as vitamin C, flavonoids, phenolic compounds, triterpenic acids, and polysaccharides. These components are known for their antioxidant, anti-inflammatory, calming, wound-healing, and anticancer properties (Preeti et al., 2014).

7,12-Dimethylbenz[a]anthracene (DMBA) is a potent polycyclic aromatic hydrocarbon used widely in experimental research to induce mammary tumors in laboratory animals. Its effectiveness in causing mammary adenocarcinoma has made it a common model for studying breast cancer (Kwon et al., 2018). DMBA-induced cancer has been associated with the disruption of neuroendocrine regulation, including changes in the hypothalamic–pituitary–gonadal and hypothalamic–pituitary–adrenal systems, as well as disturbances in melatonin production before the tumors appear (Kerdelhué et al., 2016). Additionally, DMBA can penetrate the blood–brain barrier, where it upregulates cytochrome P450 expression within the cerebral vascular endothelium, suggesting its biological impact extends well past the mammary tissue alone (Granberg et al., 2003). Promoted by these observations, this investigation was designed to assess the chemopreventive potential of an ethanolic seed extract derived from *Ziziphus jujuba* against DMBA-induced breast tumor in Charles Foster rats.

MATERIALS AND METHODS

Ethical Approval

All animal testing protocols received formal clearance from the Institutional Animal Ethics Committee (IAEC) hosted at the Mahavir Cancer Sansthan (Patna, Bihar, India; Approved No; 2023/ ID-01/11/23). The research was executed strictly adhering to the regulatory frameworks established by the government of India's committee for the purpose of control and supervision of experiments on animals. Throughout the investigation, meticulous care was taken to minimize animal distress and uphold rigorous ethical standards.

Chemicals and Reagents

The oncogenic agent 7,12-Dimethylbenz[a]anthracene (DMBA), utilized for the induction of breast tumors, was sourced from Sigma-Aldrich, USA (Product No. D3254-1G; CAS No. 57-97-6; Lot No. PXLNG2901) through an authorized scientific supplier in Patna, Bihar. All solvents and reagents used in the experiments were of analytical grade and had a purity of about 99%.

Preparation of Ethanolic Seed Extract of Ziziphus jujuba

Fresh, fully mature fruits of Ziziphus jujuba belonging to the family Rhamnaceae were collected from local orchards near Patna, Bihar, during the natural fruiting season. The collected fruits were thoroughly washed with distilled water to

remove surface contaminants and adhering debris. After cleaning, the seeds were manually separated from the fruit pulp and shade-dried at room temperature (25–28°C) for 10–12 days to preserve heat-sensitive bioactive compounds.

The dried seeds were mechanically ground into a coarse powder and stored in airtight containers under dry conditions until further extraction. Approximately 200 g of seed powder was subjected to Soxhlet extraction using 95% ethanol as the extraction solvent. Continuous extraction was carried out for 24 hours, and the process was considered complete when the solvent in the siphon chamber became clear, indicating efficient extraction of soluble bioactive constituents.

The obtained extract was filtered through Whatman No. 1 filter paper to remove particulate matter and solid residues. The filtrate was subsequently concentrated under reduced pressure using a rotary evaporator at a controlled temperature of 40–45°C, resulting in the formation of a semi-solid crude extract. The concentrated extract was further dried in a desiccator to remove residual solvent. The dried ethanolic seed extract was accurately weighed, stored in dark-colored airtight containers, and preserved for further experimental use.

Dose Preparation and Administration

The ethanolic seed extract of *Ziziphus jujuba* was prepared as a dosing solution and administered orally at a dose of 300 mg/kg body weight. The selected dose was based on previous pharmacological studies reporting the biological activity and safety of plant extracts in experimental animal models.

Experimental Animals

Healthy adult female Charles Foster rats, weighing between 150 and 200 grams, were procured from the animal house facility at Mahavir Cancer Sansthan, Phulwari Sharif, Patna, Bihar, India.The subjects were housed in a regulated laboratory facility maintained at a stable temperature of 22 ± 2°C, a relative humidity between 50–60%, and a 12-hour light/dark cycle to maintain a stable physiological setting. The rats had constant assess to conventional laboratory chow and purified clean drinking water throughout the study.

Before starting the experimental procedures, the animal underwent a one-week habituation period to adapt to laboratory environment and minimize stress. All aspects of animal care, maintenance, and handling were conducted in strict conformance with the regulatory frameworks established by the committee for the purpose of control and supervision of experiments an animal, Government of India. The study was approved by the Institutional Animal Ethics Committee (IAEC) prior to the commencement of any procedures.

Experimental Design

A total cohort of 18 healthy female Charles Foster rats was utilized for this investigation. The rats were randomly divided into three distinct groups, each comprising six animals.

Group I (Normal Control):

Serving as a baseline control, this group received no therapeutic interventions or exposure to carcinogenic agents for the entire duration of the study.

Group II (DMBA Control):

To induced mammary carcinoma, the rats in this category were administrated a solitary single oral dose of 7,12-Dimethylbenz[a]anthracene (DMBA), which was prepared in olive oil at a dosage of 15 mg/kg body weight.

Group III (DMBA + *Ziziphus jujuba* Treated Group):

Tumor development was initiated in these rats using the identical DMBA protocol applied to Group II. Once the presence of tumors was verified, the animals received an ethanolic extract of *Ziziphus jujuba* seeds. This extract was administered orally at a daily dose of 300 mg/kg body weight once daily for five weeks.

Tumor development was first confirmed in DMBA-administered animals before initiating treatment with *Ziziphus jujuba* seed extract. Following confirmation of tumor formation, tumor volume was monitored at 7-day intervals during the 35-day treatment period using Vernier caliper measurements. This group setup allowed research to evaluate the chemotherapeutic potential of *Ziziphus jujuba* seed extract against DMBA-induced mammary carcinogenesis by tracking and comparing tumor advancement between the treated and untreated cohorts.

Tumor Induction

Mammary tumors were triggered in the experiment's subjects via oral gavage of DMBA, dissolved in olive oil at a concentration of 15 mg/kg body weight. This carcinogen was delivered as a single dose to stimulate mammary tumor growth. DMBA administration, the rat were systematically monitored for physical indicators of tumor presence. The first palpable tumor in the mammary region was observed approximately six weeks after the carcinogen was introduced. The number of tumor-bearing animals increased gradually as the study progressed, which aligns with previous findings in the literature (Anderson et al., 1999). This model was used to evaluate the potential anti-carcinogenic effects of the ethanolic extract of *Ziziphus jujuba* seeds on chemically induced mammary tumors in female Charles Foster rats.

RESULT

Measurements of mammary tumor volume

The effect of *Ziziphus jujuba* seed extract on mammary tumor size within DMBA-induced rats models was monitored across a five-week duration. Representative gross morphological images of mammary tumors from DMBA-treated and DMBA + *Ziziphus jujuba* seed extract-treated Charles Foster rats are shown in Figure 1. The progression of tumors was assessed on a weekly basis via using Vernier calipers measurements, with final volume determined by following equation.:

$$\text{Tumor Volume} = \frac{L \times W^2}{2}$$

Where, L=length (longest dimension)

W= width /breadth (shortest dimension)

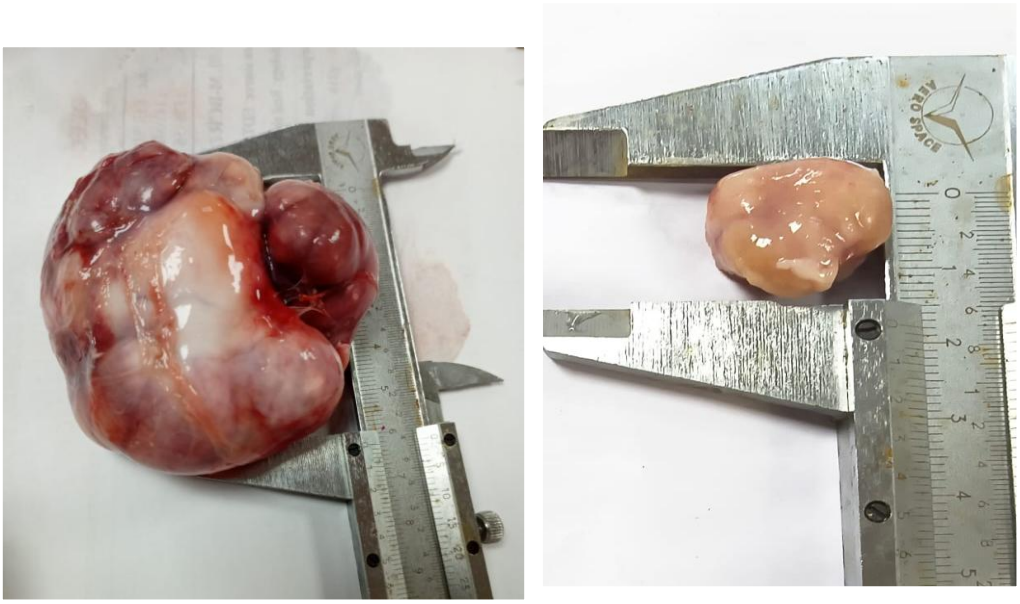

Figure 1: Gross morphological appearance of mammary tumors in experimental groups.

(A). Mammary tumor from DMBA-treated groups.

(B). Mammary tumor from DMBA + *Ziziphus jujuba* seed extract-treated group showing comparatively reduced tumor size.

The changes in tumor volume among different experimental groups are presented in Table 1. DMBA-treated rats showed a progressive increase in tumor volume throughout the observation period, whereas treatment with *Ziziphus jujuba* seed extract resulted in comparatively reduced tumor volume.

Table 1 : Effect of Ziziphus jujuba seed extract treatment on tumor volume during the 35-day post-treatment observation period

Volume of tumor (mm^3)	7 days	14 days	21 days	28 days	35 days
Control	0	0	0	0	0
DMBA treated	2	2.75	3.5	4.25	5.0

DMBA+*Ziziphus jujuba* treated	0.35	0.224	0.288	0.40	0.3645

STATISTICAL ANALYSIS

Data were expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was performed for comparison of tumor volume between experimental groups at each observation time point. A p-value < 0.05 was considered statistically significant.

Table 2: Effect of Ziziphus jujuba Seed Extract on Mammary Tumor Volume During the 35-Day Observation Period

Experimental Group	Day 7	Day 14	Day 21	Day 28	Day 35
Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
DMBA treated	2.01 ± 0.06	2.76 ± 0.08	3.52 ± 0.10	4.26 ± 0.12	5.03 ± 0.05
DMBA + *Ziziphus jujuba* treated	0.35 ± 0.02	0.22 ± 0.01	0.29 ± 0.02	0.40 ± 0.02	0.36 ± 0.01

Values are expressed as mean ± SEM (n = 6 animals/group).

Table 3: One-way ANOVA Analysis of Tumor Volume Among Experimental Groups

ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	150.010	2	75.005	11958.90	<0.001
Within Groups	0.094	15	0.006		
Total	150.104	17			

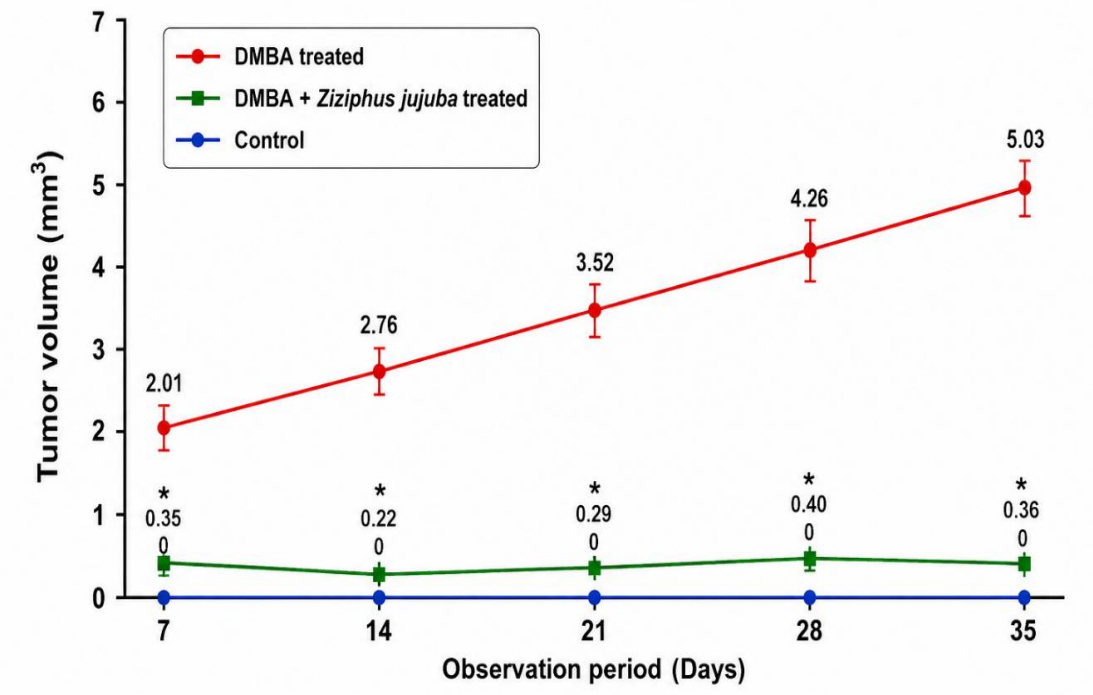

Figure 2: Effect of Ziziphus jujuba Seed Extract on DMBA-Induced Mammary Tumor Volume

Overall, these findings indicate that administration of *Ziziphus jujuba* seed extract significantly attenuated DMBA-induced mammary tumor progression, as evidenced by the reduction in tumor volume compared with the DMBA-treated group.

DISCUSSION

DMBA administration resulted in a gradual increase in mammary tumor volume throughout the experimental period, confirming its tumor-inducing potential in the rat model. In contrast, rats treated with *Ziziphus jujuba* seed extract showed a noticeable reduction in tumor volume compared with the DMBA-treated group. The observed protective effect may be related to the presence of various bioactive compounds in *Ziziphus jujuba*, particularly those with antioxidant and anti-inflammatory properties. These compounds may help in reducing oxidative stress and limiting processes involved in tumor development and progression.

CONCLUSION

The present study suggests that *Ziziphus jujuba* seed extract has potential chemoprotective effects against DMBA-induced mammary tumor development in rats. The reduction in tumor volume observed after extract treatment indicates its possible role in controlling tumor progression. However, further studies involving histopathological evaluation and molecular investigations are required to better understand the mechanisms responsible for these protective effects and to establish its therapeutic potential.

The present study has certain limitations, including the absence of histopathological evaluation and phytochemical characterization of the ethanolic seed extract. Identification and quantification of active bioactive compounds, along with molecular investigations, are required to understand the underlying mechanisms responsible for the observed anti-carcinogenic effects.

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