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Modulatory effects of *Calotropis gigantea* latex extract on plasma hematological profiles in DMBA induced mammary carcinogenesis in Charles Foster Rats

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Abstract
Background: Mammary carcinogenesis remains a primary focus of oncology research, often accompanied by severe systemic disturbances, including altered hematological profiles. *Calotropis gigantea* (giant milkweed) is widely recognized in traditional medicine for its diverse pharmacological activities, including cytotoxic and anti-inflammatory properties. This study investigated the modulatory effects of *Calotropis gigantea* extract on plasma hematological parameter in 7,12-dimethylbenz[a]anthracene (DMBA) induced mammary carcinogenesis in female Charles Foster rats.
Method: female Charles Foster rats were divided into three distinct experimental groups: control, DMBA induced, DMBA+ *Calotropis gigantea milk* extract treated. Mammary tumor was induced via a single oral dose of DMBA (15 mg/kg body weight). Following tumor development, the treated groups received oral administration of *Calotropis gigantea* milk daily for 6 weeks. At the end of experimental period, blood samples were collected to evaluate key plasma hematological parameters, including red blood cell (RBC) count, hemoglobin (Hb) concentration, white blood cell (WBC) count, and platelet count.
Results: DMBA administration resulted in a significant drop in RBC count and Hb levels, indicating tumor-associated anemia, along with a pronounced rise in WBC count (leukocytosis), reflecting an ongoing inflammatory response to tumor progression. Conversely, treatment with *Calotropis gigantea* milk extract significantly modulated these alterations. The extract-treated groups demonstrated a remarkable restoration of RBC and HB levels toward baseline values and a controlled reduction in the elevated WBC counts, effectively suppressing DMBA –induced leukocytosis.
Conclusion: The finding suggests that *Calotropis gigantea* extract, a potent modulator of the hematopoietic system, effectively mitigate the hematotoxic effects and systemic inflammation associated with DMBA –induced mammary carcinogenesis. This protective profile underscores its potential as a supportive therapeutic agent in breast cancer management, warranting deeper molecular investigation.

Keywords: *Calotropis gigantea*, breast tumor, mammary carcinogenesis, DMBA, Charles Foster rats, hematological profile, antitumor activity

Introduction

Breast cancer among Asian population represents a staggering 40% of the global disease burden. An Asian Patient remains historically under-represented in major clinical trials. Crucially, distinct variations in tumor biology, risk presentation, and therapeutic response exist between Asian and non-Asian demographics. While the majority of standard pharmacological therapies demonstrate comparable baseline efficacy across populations, key pharmacogenetic differences alter clinical outcomes. For instance, Asian patients often experience reduced clinical benefit from tamoxifen. (Lu et al., 2021). The development of mammary gland is a highly intricate and dynamic postnatal process, regulated carefully by localized growth factors and hormones. Throughout puberty and the reproductive cycle, the tissue undergoes profound remodeling driven primarily by mammary stem cells (Ma SCs). However, dysregulation within these normal cellular pathways can lead to malignancy. Breast cancer remains one of the primary causes of cancer-related mortality in women globally. Crucial to this pathology are cancer stem cells (CSCs), which dictate tumor initiation, drug resistance, recurrence, and metastatic progression. Emerging research highlights the evolutionary conserved notch signaling pathway as a foundational regulator of both healthy mammary organogenesis and malignant breast neoplasia niche environments (Chen et al.,2021) [2]. There is a critical link between chronic stress exposure and cancer development. Specifically, stress induced neurotransmitters interact with their corresponding receptor to drive carcinogenesis (Silva et al., 2022) [11].

Breast cancer is characterized by the unregulated proliferation of cells within the mammary glands, which subsequently invade adjacent tissue. At the molecular level, the progression of oncogenesis can be monitored by evaluating specific protein biomarkers. To investigate the etiology and progression of the breast cancer, various models are widely utilized. Among these, the administration of the carcinogen DMBA is well established method for inducing mammary carcinomas. Typically exposing peripubertal mice to 4-6 weekly dose of 1mg DMBA result in mammary tumor development in 30% to 70% of subjects within 150-200 days of initial exposure, occasionally leading to lungs metastasis. Because it closely replicates the multiple step nature of human breast cancer development, DMBA induced tumorigenesis serve as a highly reliable and relevant translational model (Plante, 2020) [8].

Calotropis gigantea, a member of the Asclepiadaceae family, is well documented for its rich content of cardiac glycosides and has previously demonstrated cytotoxic

properties against several malignant cell lines. The therapeutic potential of C*alotropis gigantea* methanolic extract, focusing on its capacity to inhibit proliferation and trigger apoptotic pathways within the human breast adenocarcinoma cell line, MCF-7 (Kharat & Kharat, 2019) [6]. *Calotropis gigantea*, is widely recognized for its diverse pharmacological applications and therapeutic properties (Chitme et al., 2004) [3]. *Calotropis gigantea* long-lived shrubs, possesses a deep-rooted history in traditional folk medicine for managing diverse aliments. Anhydrosophoradiol-3-acetate (A3A) from *C. gigantea* flower to analyze its therapeutic efficacy in restriction tumor growth (Habib & Karim 2013) [5].

The study analyzed the hematological and biochemical data of 56 breast cancer Patients. The result indicated that advanced-stage patients (stage 3 &4) had significant higher random blood sugar (RBS), alkaline phosphatase (ALP), and urea levels compared to early stage patients. Additionally, advanced progression was strongly associated with decrease hemoglobin and anemia (Shreya et al., 2023) [10]. Alkaline phosphatase is a widely distributed member associated glycoprotein responsible for catalyzing the hydrolysis of phosphate monoesters under alkaline conditions (Sharma et al., 2014) [9]. Analyzing hematological profile serve as a dependable paraclinical approach for diseases assessment; these blood-based parameters offer valuable predictive insights regarding diseases severity, Patients mortality, and therapeutic monitoring. The effectively differentiated breast cancer patients from healthy controls (Divsalar et al., 2021) [4].

Materials and Methods

Ethical approval

The experimental protocols involving animals were officially reviewed and sanctioned by the Institutional Animal Ethical Committee (IAEC) located at the Mahavir Cancer Sansthan in Patna, Bihar, India (Approval No. 2023/ID-01/11/23). All animal procedures were carried out in strict compliance with the guideline set forth by government of India's committee for animal experimentation control and supervision. Every effort was made to reduce animal suffering throughout the study.

Chemicals and Reagents

The carcinogenesis 7,12-dimethylbenz[a]anthracene (DMBA), used as the oncogenic agent for breast tumors inductions, was procured from sigma- Aldrich, USA (product No. D3254-1G; CAS NO.57-97-6; Lot No. PXLNG2901) via an accredited located distributor in Patna, Bihar. All additional solvent and chemical reagents utilized during the investigation were of analytical grade, possessing a purity level of approximately 99%.

Experimental animal

To minimize epigenetic variation and eliminate confounding environmental stressors, the experimental animals were housed in a strictly regulated, barrier-sustained vivarium facility. The microenvironmental parameters were continuously monitored and standardized according to laboratory animal welfare guidelines.

Upon procurement from the certified breeder, the rats were subjected to a mandatory 7-day acclimatization period under standard laboratory conditions to stabilize physiological baseline prior to the initiation of any experimental procedures.

Animals were housed in a bio-clean, autoclavable polypropylene cages, 2-3 rats per cage. Group housing was prioritized to prevent isolation induced stress, which can heavily skew behavioral and metabolic data.

Cages were lined with steam- sterilized, dust free corn cob or rice husk bedding, which was completely renewed twice weekly. All handling, cage changes, and sanitation routine was performed at a fixed time during the light cycle by the same personnel to maintain a low-stress environment.

Nutritional variables were strictly controlled to eliminate dietary interference with metabolic parameters or biochemical assay. The animals were provided ad libitum access to a standardized, pathogen free rodent pellet diet. The diet was structurally formulated to provide optimal macro-and micronutrients profiles, typically consisting of fat, protein and fibers. Clean, acidified or reverse-osmosis purified drinking water was made available ad libitum via automated watering valves or sterilized poly carbonated bottles equipped with sipper tube.

Induction of breast tumor

The 7,12-dimethylbenz[a]anthracene (DMBA) induced mammary carcinogenesis model is a highly reliable, widely utilized chemical induction system in experimental oncology. It closely mimics human breast cancer, particularly Hormone dependent luminal subtypes, and is characterized by the high tumor incidence and a highly predictable latency period. For experimental purpose, animals were orally treated with DMBA (manufactured by Sigma Aldrich, USA, product number D3254-1G, (CAS Number: 57-97-6), lot# PXLNG2901, P code: 10093303440) dissolved in olive oil at a dose of 15mg/ml. All the other solvents and chemicals used were of analytical grade 99%. Beginning in the fourth week following DMBA oral administration, treated rats were palpated weekly to detect the growth of tumors. First tumor emerged at week 18. By week 20th, all the DMBA-treated rats had tumors. DMBA treated rats were divided into two groups, n=6 each.

Tumor kinetics and growth profile: Tumor kinetics is highly predictable, multi-stage timeline.

Tumor volume

Tumour burden was determined by calculating the total number of tumour present per animal. Tumour volume was measured weekly using a digital Vernier calliper by recording tumour length and width. The tumour volume was calculated using the following formula:

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

Where, L=length (longest dimension)

W=width /breadth (shortest dimension) (Faustino et al., 2013)

Collection of *Calotropis gigantea* Latex

Fresh latex of *Calotropis gigantea* was collected from fully grown plants located in and around Phulwari Sharif, Patna, and Bihar.

Healthy and disease-free plants were selected for latex collection. A small incision was gently made on the stem or leaf, and the milky latex released from the plant was collected carefully in clean glass containers.
Due to the irritant nature of latex, proper precautions were taken during handling. The collected latex was transferred immediately into airtight containers and stored at low temperature until further experimental use.

Preparation of Fresh Latex Samples for Experimental Use

Freshly collected latex of *Calotropis gigantea* was diluted using sterile distilled water. A two-fold dilution was performed to obtain a 50% concentration of the original latex sample. The diluted latex was mixed thoroughly to ensure uniform distribution of its components.
The prepared latex dilution was filtered through filter paper to remove any unwanted impurities and to obtain a clear solution. The filtrate was collected and used for further experimental analysis (Aliyu et al., 2015) [1].
The prepared latex dilution was stored under cold conditions to maintain its stability and biological activity. After filtration, the solution was transferred into clean, sterile, properly labelled airtight containers and stored at −20°C until further use. Repeated freezing and thawing cycles were avoided to prevent possible degradation of active components.
Fresh *Calotropis gigantea* latex was administered orally at a dose of 1mL per animal once daily for six consecutive weeks.

Result

Effect of *Calotropis gigantea* on Haematological Parameters

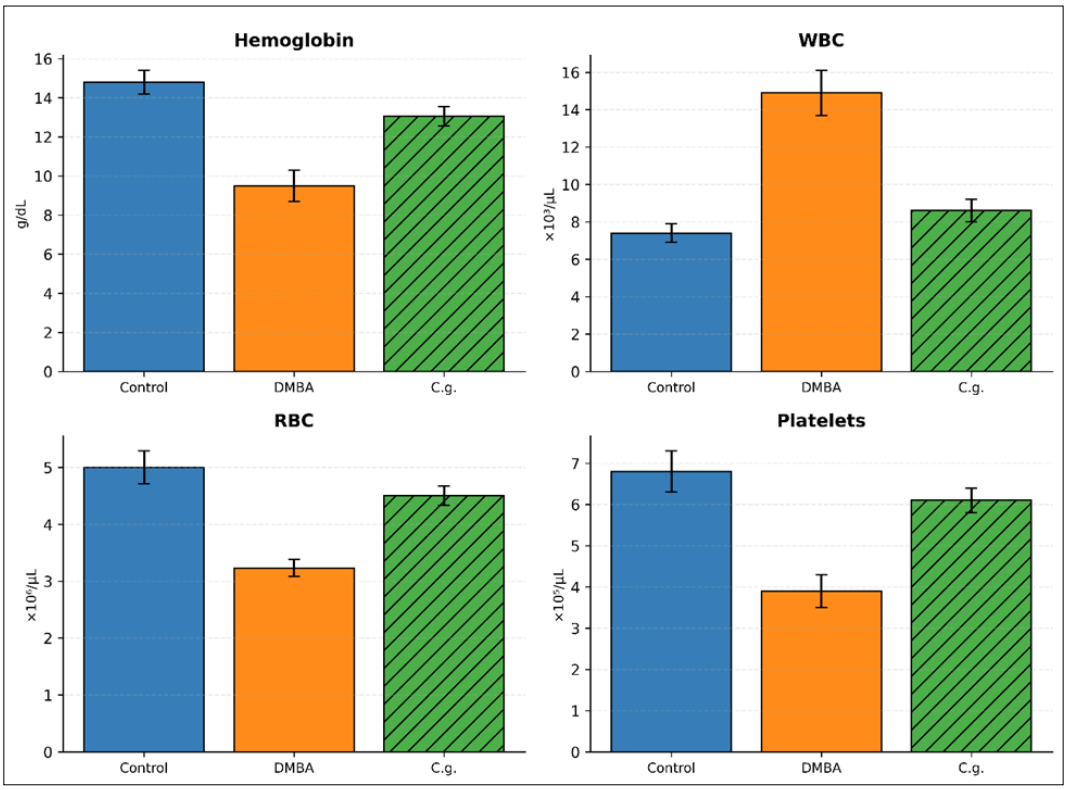

Fig 1: Effect of *Calotropis gigantea* latex on haematological parameters (haemoglobin, white blood cells, Red blood cell and platelets) in DMBA-induced mammary carcinogenesis in Charles Foster rats. Data are presented as mean ± SD. RBC

DMBA-induced mammary carcinogenesis markedly altered the hematological profile of Charles Foster rats (Figure 1). The hemoglobin concentration decreased from 14.8 ± 0.6 g/dL in the control group to 9.5 ± 0.8 g/dL in the DMBA-treated group, indicating the development of tumor-associated anemia. Treatment with *Calotropis gigantea* latex restored hemoglobin levels to 13.06 ± 0.5 g/dL. Similarly, the white blood cell count increased from 7.4 ± 0.5 in the control group to 14.9 ± 1.2 following DMBA administration, whereas treatment reduced the count to 8.6 ± 0.6, approaching the normal value. Lymphocyte percentage also declined after DMBA treatment (51.3 ± 4.1) compared with the control (72.1 ± 3.4), while *Calotropis gigantea* treatment restored the value to 68.5 ± 2.9. Platelet count was reduced in the DMBA-treated group (3.9 ± 0.4) compared with the control (6.8 ± 0.5), whereas treatment improved the platelet count to 6.1 ± 0.3, suggesting a protective effect against DMBA-induced hematological alterations.

Table 1: Modulatory effects of *Calotropis gigantea* milk on key hematological parameter

Parameter	Control	DMBA treated	DMBA + C. gigantea treated
Hemoglobin (g/dL)	14.8 ± 0.6	9.5 ± 0.8	13.06 ± 0.5
White blood cells (×10³/µL)	7.4 ± 0.5	14.9 ± 1.2	8.6 ± 0.6
Red blood cells (×10⁶/µL)	5.0 ± 0.29	3.23 ± 0.15	4.50 ± 0.17
Platelets (×10⁵/µL)	6.8 ± 0.5	3.9 ± 0.4	6.1 ± 0.3

Effect of *Calotropis gigantea* on Plasma Biochemical Parameters

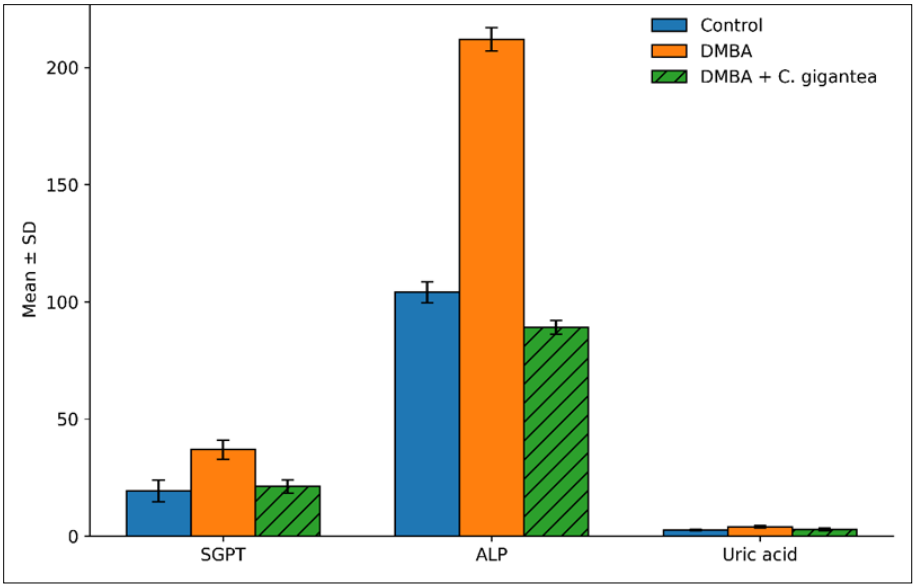

Fig 2: Effect of *Calotropis gigantea* latex on plasma biochemical parameters (SGPT, ALP, and uric acid) in DMBA-induced mammary carcinogenesis in Charles Foster rats. Data are presented as mean ± SD.

DMBA administration significantly affected plasma biochemical parameters in experimental animals (Figure 2). The SGPT level increased from 19.30 ± 4.70 U/L in the control group to 36.95 ± 4.05 U/L following DMBA treatment, indicating hepatic dysfunction. Administration of *Calotropis gigantea* latex reduced the SGPT level to 21.20 ± 2.89 U/L, approaching the control value. Likewise, ALP activity increased markedly from 104.05 ± 4.50 U/L in the control group to 212.00 ± 5.00 U/L in the DMBA-treated group, whereas treatment reduced ALP activity to 89.17 ± 2.89 U/L. Uric acid concentration also increased from 2.62 ± 0.38 mg/dL in the control group to 4.00 ± 0.58 mg/dL after DMBA administration. Treatment with *Calotropis gigantea* decreased the uric acid level to 2.83 ± 0.58 mg/dL, indicating improvement in the biochemical alterations induced by DMBA.

Table 2: Modulatory effects of *Calotropis gigantea* on key biochemical parameter

Parameter	Control	DMBA treated	Calotropis gigantea treated
SGPT	19.30±4.70	36.95±4.05	21.20 ± 2.89
ALP	104.05±4.50	212.00±5.00	89.17±2.89
Uric acid	2.62±0.38	4.00±0.58	2.83±0.58

Conclusion

The present study concludes that DMBA- induced mammary carcinogenesis in Charles Foster rats results in significant alterations in plasma hematological parameters. Administration of *Calotropis gigantea* effectively ameliorated these changes through its antioxidants and immunomodulatory properties. The finding indicates that *Calotropis gigantea* may serve as a promising natural therapeutic agent for the management of breast cancer associated hematological complications. However, further studies are required to elucidate its molecular mechanism and clinical applications.

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