Anti-tumor property of *Argemone mexicana* leaves methanol extracts against 7, 12-Dimethylbenz[a]anthracene (DMBA) induced mammary tumors in experimental rats

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Abstract

Background: Breast cancer is a complex and multistage process. Many natural compounds are available for an effective and efficient alternative method against breast cancer. This study focused on the anti-cancer activity of *Argemone mexicana* leaf extract on Dimethylbenz[a]anthracene (DMBA)-induced mammary tumors in experimental rats. **Materials and Methods:** *Argemone mexicana* Leaves (AML) were collected, extracted by soxhlet apparatus with methanol solvent, and analyzed by Gas Chromatography and Mass-Spectroscopy (GCMS). Healthy Female Sprague-Dawley rats (7-8 weeks and 100 ± 20 g) were divided in five groups and used to induce mammary tumors by DMBA [25 mg/kg/ Subcutaneous (s.c.)]. After developing a palpable nodule (10-12 weeks after DMBA), the rats were treated with AML extract [200 and 400 mg/kg/Body Weight (b.w.)] and Tamoxifen (TAM 10 mg/kg) orally for 12 weeks. Body weight, tumor weight and volume measurements, lipid peroxidation, antioxidant and tumor marker [Carcinoembryonic Antigen (CEA)] levels, and histological investigations were assessed. **Results:** Following AML treatment, there was a reduction in weight and volume ($P < 0.01$) of breast tumor, as well as a significant decrease in levels of lipid peroxidation and tumor marker CEA ($P < 0.001$) and improved Superoxide Dismutase (SOD), Reduced glutathione (GSH), and caspase levels ($P < 0.001$). In cytological results, AML improved the cellular architecture, and significant restoration in histopathological examinations revealed a tumor with minimal glandular epithelium proliferation, a decrease in mitotic figures, and nuclear alterations. **Conclusion:** The study suggests that AML extract has a chemoprotective effect against DMBA-induced mammary tumors in experimental animals, specifying its possibility as a therapeutic agent for breast cancer treatment.

Keywords: 7, 12-Dimethylbenz[a]anthracene, Mammary tumors, *Argemone mexicana,* GCMS, Anti-tumor activity, Morphology and Histopathology changes

Introduction

Avoiding 40–50% of cancer cases worldwide is possible, and specific prevention measures have proven economically feasible. Colorectal and breast cancers are expected to have the highest increases, rising to 4.7 million and 4.4 million cases annually by 2070 from 1.8 million and 2.1 million cases, respectively, in 2018. Breast carcinoma is the most common malignancy in women[(1)]. Surgery, radiation, chemotherapy, and hormone therapies are among the recently established cancer treatments that are not entirely effective in resolving the disease and may cause more harm than advantage to patients. Yet, many of natural agents have not been fully explored and may have fewer side effects. They can be used in combination with other treatment methods[(2)]. To explore the anti-cancer activity of *Argemone mexicana* Leaves (AML) herbal extract, an experimental rat model was used to identify valuable insights into mammary carcinogenesis development and evaluate the novel, natural therapeutic strategies.

A well-known chemical carcinogen, 7,12-Dimethylbenz [a] anthracene (DMBA) was used to induce carcinogenesis in experimental animals, which served as a well-established model to investigate mammary carcinoma. Many characteristics of human breast adenocarcinomas, including histological advancement, are seen in rat mammary carcinomas[(3)]. After metabolizing within the body, the DMBA forms DMBA-3,4-diol-1,2-epoxide (DMBA-DE) reactive intermediates[(4)], which causes DNA strand breaks. Base pair modifications may be by activation of Aryl hydrocarbon Receptor (AhR), and DMBA upregulates Notch receptors and ligands, which supports proliferation, survival, and the upkeep of cancer stem cell populations and Wnt/β-catenin dysregulation pathway[(5-7)].

A thorough review of the literature on plants used against cancer identifies approximately 1400 taxa, some of which have been utilized for generations to cure malignant disorders. *Argemone mexicana,* commonly known as Mexican prickly poppy or yellow thistle, belongs to the

Papaveraceae family. Ibrahim et al., reported that an aqueous extract of *A. mexicana* leaves, significantly reduced inflammation in rats with paw edema caused by carrageenan[8]. Kulshrestha et al. evaluated the cancer-preventive activity of Argemone mexicana leaves ethanol extract against DMBA-induced skin carcinoma in mice and studied the Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathways[9]. Extracts from *Argemone mexicana* had cytotoxic effects on immortalized cell lines such as Adenocarcinomic Human Alveolar Basal Epithelial Cells (A549), SiHa, and KB that were equivalent to those of berberine as conventional medication[10]. Methanolic leaf extract of *Argemone mexicana* has been reported to have anti-cancer properties against Michigan Cancer Foundation-7 (MCF-7) cancer cell lines by Varun and Sudha[11]. Several studies have been reported that *Argemone mexicana* leaves have anti-inflammatory and cytotoxic effects in in-vitro and in-vivo (skin cancer). However, no animal experiment has reported its anti-breast cancer properties in vivo. This study aimed to investigate the anti-cancer activity against DMBA-induced mammary carcinoma in rats.

Materials and Methods

Chemicals

Chemicals were used in analytical-grade purity. Used chemicals were ethyl alcohol (7 liters of 100% ethanol dissolved in 3 liters of distilled water, used to disinfect leaves), methanol 90%, 0.9% normal saline, 0.05% acetic acid, distilled water for preparation of reagents and drugs, and Dimethyl Sulfoxide (DMSO), DMBA, 100mg Carboxymethylcellulose (CMC), Formalin, Disodium Hydrogen Phosphate (Na_2HPO_4), Sodium Dihydrogen Phosphate (NaH_2PO_4), and Sodium Chloride (NaCl). They all were purchased from Sigma-Alrich, Bangalore.

Preparation of plant extract

Fresh green AMLs were collected and authenticated from the Botanical Survey of India, Tamil Nadu. They were thoroughly cleaned with tap and distilled water to remove soil particles and then dried in the shade. Five hundred grams of dried leaves were coarsely powdered by mortar and pestle and extracted with 70% methanol using the cold percolation method. Three days later, the extract was dehydrated over a boiling water bath after being filtered with No. 1 Whatman filter paper. The extract was stored at -4°C in the refrigerator and used for additional analysis. The methanolic extract's LD50 dose was determined to be 2000 mg/kg of body weight. A final dose of 200, 400 mg/kg Body Weight (b.w.) was determined by comparing the 1/10th and 1/5th doses of LD50.

Chromatography and Mass-Spectroscopy (GC-MS)

Methanol extract of AML has been investigated by GC-MS using the protocol outlined by Singh and Yerramilli[12]. GC-MS, PerkinElmer mass spectrometer interfaced with a chromatograph and coupled with silica capillary column (30 m, 0.25 mm, 1 µm df, mainly comprised of dimethyl polysiloxane). A split ratio of 10:1 was used with an injection volume of 1.5 mL/min, and helium gas (99.999%) was utilized as the carrier gas at a constant flow rate of 1 mL/min. The ion source was 280°C, and the injector was 250°C. Oven heat was scheduled according to protocol, starting at 110°C (isothermal for two minutes). The detected compounds ranged from 50 to 550 amu, with 36 minutes being GC's overall running time. The relative amount of each element was ascertained by comparing the mean area of the peak to the total area. Mass spectra from the Wiley-8 library and the NIST-0.8 L database, as well as chromatograms, were handled by Turbo Mass.

Animal Experiment

The Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) commandments were followed to maintain female Sprague-Dawley (SD) rats. The Institutional Animal Ethical Committee accepted the experimental research design. The rats were properly acclimated for 14 days.The experimental animals were placed in polypropylene cages. The animals were housed at 22 ± 3°C with a 12-hour light/dark cycle and with enough food and water.

Experimental design

Healthy female SD rats, aged 6-7 weeks old, weighing 100 ± 20 g, were divided into five groups (n=6) at random and the research was carried out for six months (24 weeks).

Group I: Control rats were administered with 2 ml/kg b.w. Saline p.o.

Group II, III, IV, and V animals were induced with a single subcutaneous injection of 7,12- dimethylbenz[a]anthracene at 25 mg/kg/b.w[13]. Animals were palpated twice a week after four weeks of DMBA; once a palpable nodule had developed (approximately 10–12 weeks after DMBA, about 0.5 cm), animals were treated with AML extract (200 and 400 mg/kg) orally for 12 weeks (weekly trice). The experimental doses were determined from an acute toxicity study; a single dose of 2000mg/kg was used to test the toxicity as OECD 423. We selected the extract-safe doses of 1/10 and 1/5 from the acute toxicity results for our experiment[14].

Group II: Rats induced with a single dose of DMBA (25 mg/kg/b.w/s.c) and let it for the full course of 24 weeks as control group (Tumor Control, No treatment).

Group III: Treated with AML (200 mg/kg/b.w/p.o)

Group IV: Treated with AML (400 mg/kg/b.w/p.o)

Group V: Treated with Tamoxifen (TAM,10 mg/kg/b.w/p.o)[15]

At the end of 24 weeks, under isoflurane anesthesia, vacuum tubes were used to extract blood from the retro-orbital venous plexus, collect serum, and analyze it for several tumor marker assays. Tumors were excised from experimental rats. A group of cells was scraped on the cut edges of the mammary tumor mass by spatula, placed on microscopic slides for cytological studies[16], and stored in liquid nitrogen (N_2) at 70°C for the biochemical assay. The remaining portion was preserved in formalin 10% buffer for histology investigations.

Tumor weight and volume

All experimental animals were observed during the experimental period (six months), and body weight, water, and food intake were recorded every week until rats were sacrificed. Control and tumor-bearing animals' final mean body weight and tumor incidence were compared. Excised tumors were weighted (g), and volumes (V) were calculated from vernier caliper measurements in two different dimensions. Calculation of volume[17]: Tumor width (W) and tumor length (L) are represented by the formula $V = (W^2 \times L)/2$. The mean ± standard deviation value of tumor volume isdenoted as cm^3.

Biochemical Assay

Preparation of tissue homogenate: A 100mg of fresh mammary tissues, smashed into tissue pits, 10% (w/v) of tissue homogenate was prepared in ice-cold 0.1M Tris-base HCL with 5 M KCL (pH-7.4) by using a tissue grinder, a Potter Elvehjem homogenizer. Later, the tissue homogenates were centrifuged for 30 minutes at 4ºC with 12,000 rpm, and the supernatant was frozen at -20ºc for biochemical assay.

Lipid peroxidation levels in tissues were assayed by the methods of Tsikas[18]. The levels of Thiobarbituric Acid Reactive Substance (TBARS) were expressed in nmoles of Mass Drug Administration (MDA) liberated per mg of protein. Peskin and Winterbourn approach was utilized to evaluate the activity of superoxide dismutase (SOD)[19]. Reduced glutathione (GSH) levels of experimental rats was estimated by the methods of Cheng[20]. Protein concentrations were determined by Mæhre et al.[21]. A 100 milligrams of protein were diluted with lysis buffer (50µl), and then incubated in microtiter 96-well plates added 5 µl of the 4 mM p-nitroaniline Caspase (pNA-Caspase 3),activity assessed by cleaved-free pNA. In a microtiter plate reader, absorbance at 405 nm was used to measure free pNA (cleaved substrates)[22]. Carcinoembryonic Antigen (CEA) levels (ngm/ml) for CA-15-3 were analyzed in the serum after 12 weeks of palpable mammary tumors by the solid-phase ELISA kit based on the direct sand switch technique. The absorbance was read at 450nm[23].

Cytological study [Hematoxylin and Eosin (H&E)]

Fixing the cytological wet smears in 95% ethanol for 15 minutes, then the slides were then fixed in 70% and 50% alcohol for each one minute and gently rinsed with tap water, and the procedure steps were followed Dey[24]. The slides were stained with a hematoxylin solution for 15-20 minutes, rinsed with 1% acid alcohol for one minute, and rinsed in tap water for the blueing process. Then, the slides were counterstained with eosin for 2-3 dips and twice in each graded alcohol (95% and 100%) for one minute. Finally, it was dipped in xylene, mounted on a coverslip with DPX, and examined under 40X magnification of a light microscope (Olympus CH20i Tr).

Histopathological study (H&E)

Formalin (10%) fixed tumors were hydrated, and gradual dehydration was done by graded alcohol. Then, tissues were cleared (xylene) and embedded in a paraffin wax block (56–58°C). Using a rotatory microtome, 3–4 µm thickness sections were obtained, and sections were stained with the gold-standard histological dyes hematoxylin and eosin[25]. The stained sections were observed under 40X magnification of a simple microscope (CH20ITR, Olympus Microscope) for analyzing lesions in tumor tissue.

Statistical Analysis

A one-way ANOVA method was used to examine the data from the experimental investigation, and then the Turkey Honest Significant Difference (HSD) test to compare the groups. The data were statistically significant ($P < 0.01$) and displayed as mean ± SD.

Results

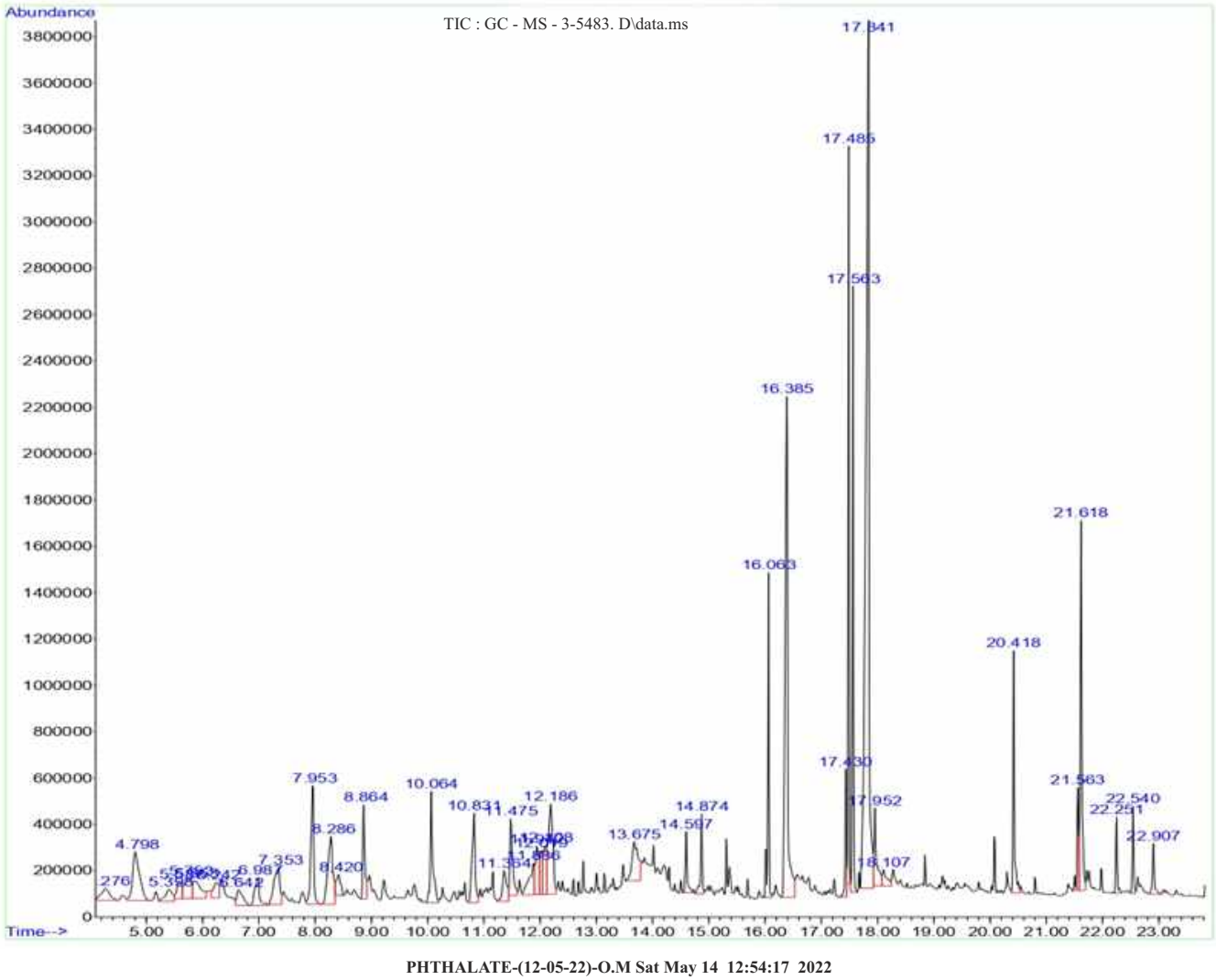
Compounds identified from GC-MS results

Figure 1 shows GC–MS identified bioactive components indicated by various peaks. Identification and characterization of compounds were done by comparing the spectrum of mass fractions with the Wiley and NIST libraries. A total of 41 elements were detected in the crude AML extract.

Data Path : D:\MSDCHEM\1\DATA\2022\MAY-22\13-05-22/
Data File : GC-MS-3-5483.D
Acq On : 14 May 2022 8 : 30
Operator :
Sample : T-17183-ARGEMONE-PURITY
Misc :
ALS Vial : 37 Sample Multiplier : 1

Search Libraries : C:\Database\NIST08.L Minimim Quality : 0

Unknown Spectrum : Apex
Integration Events : RTE Integrator - rteint.p



PHTHALATE-(12-05-22)-O.M Sat May 14 12:54:17 2022

Figure 1: GCMS analysis of *Argemone mexicana* leaves (Library Search Report)

Figure 1 shows peaks of GCMS chromatogram (Chromopark Life Science). The National Institute of Standards and Technology's mass spectral library contains spectra of reference compounds, which are used to identify the retention time, peak area, chemical formula, and molecular weight of the compounds on the list of chemicals found (NIST 14).

Identified compounds included 9,12,15-Octadecatrienoic acid (Z,Z,Z) (22.28%); n-Hexadecanoic acid (palmitic acid, 10.55%); 9,12,15-Octadecatrienoic acid methyl ester (Z,Z,Z) (6.3%); Phytol (5.15%); 9,12,15-Octadecatrien-1-ol (Z,Z,Z) (4.48%); Piperidine, 1-methyl- (3.19%); Hexadecanoic acid methyl ester (3.33%); 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl; and Benzoic acid (2.7%). Additionally, Hexadecanoic acid 2,3-dihydroxypropyl ester (2.68%); N-Ethyl-2-carbomethoxyazetidine (2.22%); 2-Methoxy-4-vinylpheno (2.16%); Isosorbide Dinitrate (1.94%); Butanedioic acid monomethyl ester (1.75%); 3H-Cyclopenta[c]pyridazin-3-one 2,5,6,7-tetrahydro (1.62%); Benzofuran 2,3-dihydro (1.64%); 9,12-Octadecadienoic acid (Z,Z)- methyl ester, 10,13-Octadecadienoic acid methyl ester

(1.45%); Octadecanoic acid (stearic acid) (1.42%); and 9,12-Octadecadienoic acid (Z,Z)- 2-hydroxy-1-(hydroxymethyl)ethyl ester (1.34%) were represented based on Retention Time (RT) and area of peak. This analysis identified the active elements containing reported and non-reported phytochemical compounds. But the isolation of compounds needed advanced chromatography like High Performance Liquid Chromatography (HPLC). Furthermore, a few compounds were tabulated based on retention time and previously reported biological activities[26-35] (Table 1).

Table 1: Vital Compounds Identified by GC-MS Analysis of AML Extract Along with Their Biological Activity

Name of the Compound (Name of Peaks	Retention Time (Min)	Area of Peak (%)	Molecular Formula	Biological Activity
Benzoic acid	8.286	2.7	C_6H_5COOH	Antifungal activity[26].
2-Methoxy-4-vinylphenol	10.064	2.16	$C_9H_{10}O_2$	Inhibition of proliferative cell nuclear antigen[27]
Piperidine, 1-methyl-	12.186	3.19	$C_6H_{14}CIN$	Cytotoxic potential against tumor cells[28]
Hexadecanoic acid, methyl ester	16.063	3.33	$C_{18}H_{36}O_2$	Antioxidant, anti-inflammatory activity, and antimicrobial properties[29]
n-Hexadecanoic acid (palmitic acid)	16.385	10.55	$C_{16}H_{32}O_2$	Cytotoxic potential by inhibiting DNA topoisomerase-I[30]. Also found that the substance has antimicrobial, anticancer, and antioxidant properties[31]
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	17.430	1.43	$C_{19}H_{34}O_2$	Anti-inflammatory and anti-microbial activity[32]
Phytol	17.485	6.3	$C_{19}H_{32}O_2$	Cytotoxic activity against MCF-7 cell lines[33]
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	17.563	5.15	$C_{20}H_{40}O$	Anti-inflammatory and cancer preventive[29]
Octadecanoic acid (stearic acid)	17.841	22.28	$C_{18}H_{30}O_2$	Antitumor activity, antimicrobial activity, antioxidant[34]
9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	21.618	4.48	$C_{18}H_{32}O$	Anti-inflammatory and anxiolytic activity[35]

Morphological changes

From the mean value of the initial and final body weight of rats, weight gain had occurred in all the experimental groups. Compared to the DMBA alone-induced non-treated group, AML (200, 400 mg/kg) and TAM treated rats increased their body weight gain (Table 2). Compared to mean values of tumor weight and volume from the control groups, TAM, AML (200,400 mg/kg) treated groups were significantly reduced. Results were statistically significant ($P<0.01$) (Figure 2).

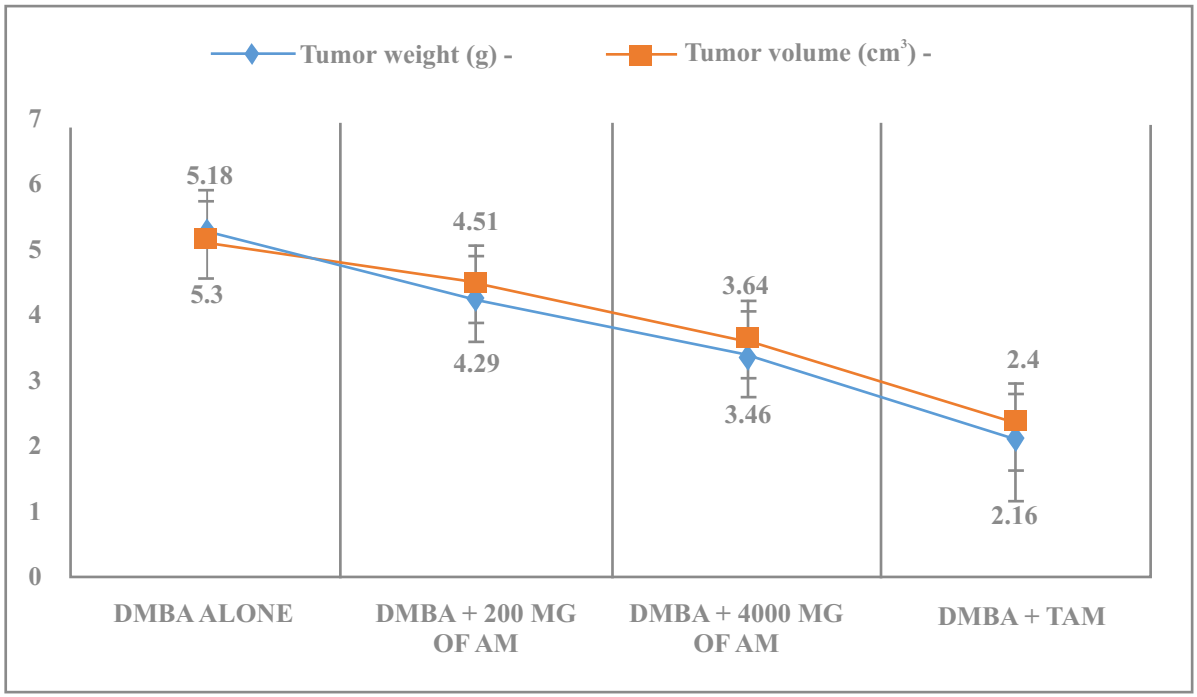


Figure 2: Effect of *Argemone mexicana* leaves extract on tumor weight (g) and tumor volume (cm^3) against DMBA induced Mammary tumors

Values are expressed as mean ± SEM (n=6). Data were analyzed by One Way ANOVA followed by Dunnett's test post hoc analysis. [a]P<0.01; [b]P<0.001 Vs Tumor Control.

Biochemical results

Significant decrease in mean levels of Thiobarbituric Aid Reactive Substances and tumor marker (CEA-CA 15-3), in AML 400 mg/kg/b.w. treated group compared to the AML 200 mg/kg/b.w. treated rats and TAM 10 mg/kg/b.w. treated rats was observed. Mean values of Superoxide Dismutase (SOD), GSH, and caspase levels had decreased in DMBA alone-induced non-treated group, compared to other treatment groups. Results were ($P < 0.01$) statistically significant (Figure 3).

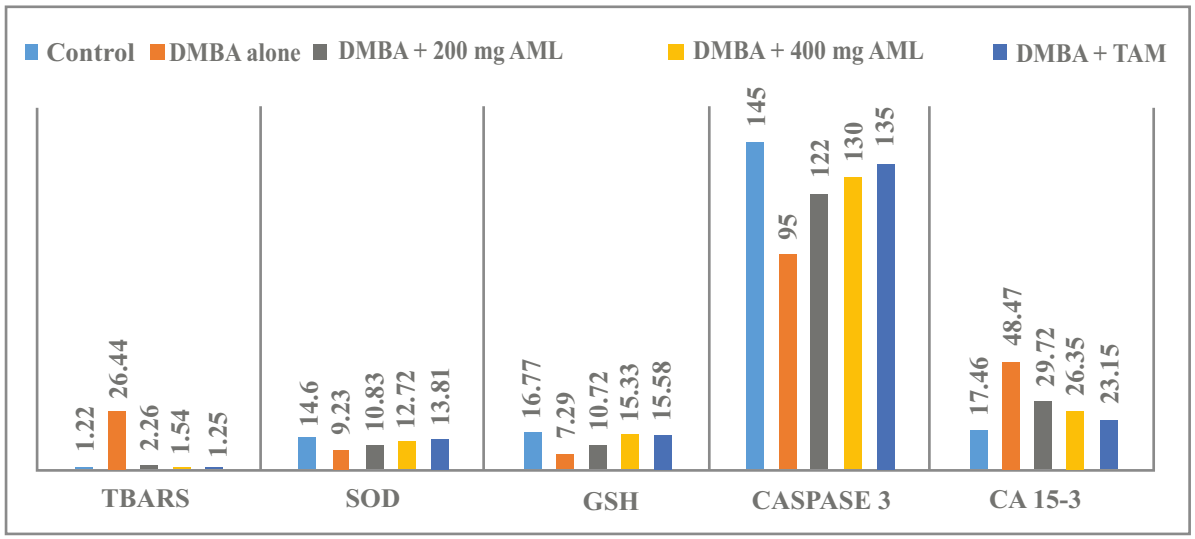


Figure 3: Effect of *Argemone mexicana* leaves on biochemical assay including lipid peroxidase, enzymatic and non-enzymatic anti-oxidant, tumor marker levels

Values are expressed as mean ± SEM(n=6). P<0.001 Vs Control, P<0.01; P<0.001 Vs DMBA alone. Data were analyzed by one-way ANOVA followed by Turkey HSD multiple comparison test. Units: TBARS (mM/100g of tissue); SOD (U/mg of protein); GSH (mg/g of tissues); Caspase (Conc. µg/ml); CA 15-3 (ng/ml of serum).

Cytological changes

Figure 4 shows that the control group appeared to have normally scattered ductal epithelial cells; the DMBA alone-induced and non-treated group showed a cluster of tumor cells with a hyperchromatic nucleus and severe nuclear atypia. The AML (200 mg/kg) group seemed to have a smaller cluster of ductal epithelial cells with hyperchromatic, pleomorphic nuclei; the AML (400 mg/kg)-treated group revealed a milder cluster of ductal epithelial cells with benign bare nuclei. The TAM-treated group appeared as a moderate cluster of tumor cells with normally appearing hyperchromatic nuclei. Compared to normal control, DMBA alone-induced non-treated animals observed severe malignancy changes. In treated groups, AML 200 mg showed moderate changes; like a lower amount of hyperchromatic and nuclear pleomorphic, TAM and AML (400mg) treated rats group appeared to have more or less the same cellular pattern and highly improved cellular architecture.

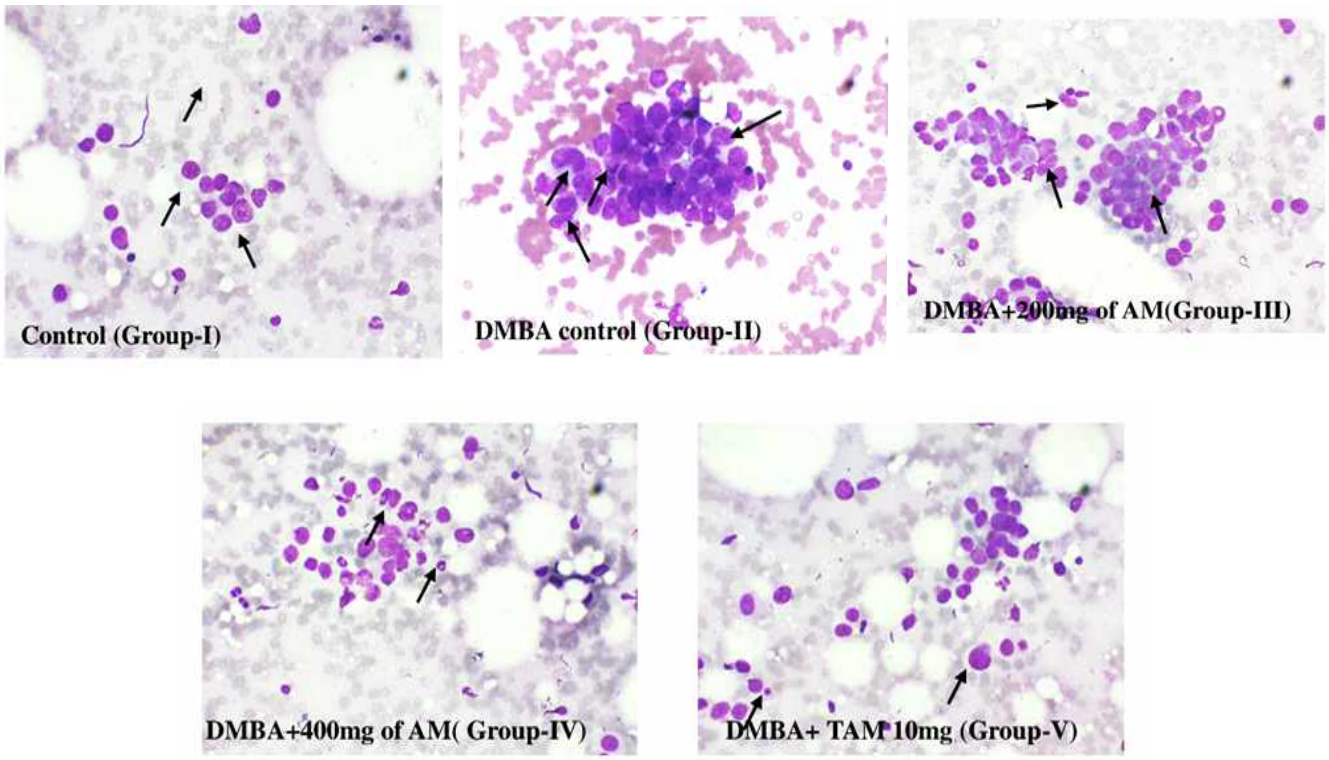


Figure 4: Cytological observation of DMBA induced mammary tumors and effect of *Argemone mexicana* leaves methanol extract

Fig 4: shows G-I (normal control) shows normally appearing scattered epithelial cells, G-II (DMBA alone-induced non-treated control) cluster of tumor cells with hyperchromatic nucleus and severe nuclear atypia, G-III (DMBA+200mg of AM) cluster of ductal epithelial cells with hyperchromatic, pleomorphic nuclei, G-IV (DMBA+400mg of AM) mild cluster of ductal epithelial cells with benign bare nuclei. G-V (DMBA+ TAM 10mg) moderate cluster of tumor cells with normally appeared hyperchromatic nuclei.

Histopathological changes

In histopathological sections (figure 5), the control group appeared to have normal architecture of the epithelium and ductal system. On the other hand, the group that was induced with DMBA alone displayed aberrant glandular epithelium with frequent and sufficient anaplastic alterations and numerous mitotic cells with hyperchromatic nuclei. The breast tissues showed decreased neoplastic cells, inflammation, and necrosis in the AML (200,400 mg/kg/b.w.) treated groups. They restored the histological architecture with reduced mitotic figures, nuclear alterations, and less epithelial proliferation. However, the TAM administered group showed near-normal cyto-architecture with mild inflammatory changes observed.

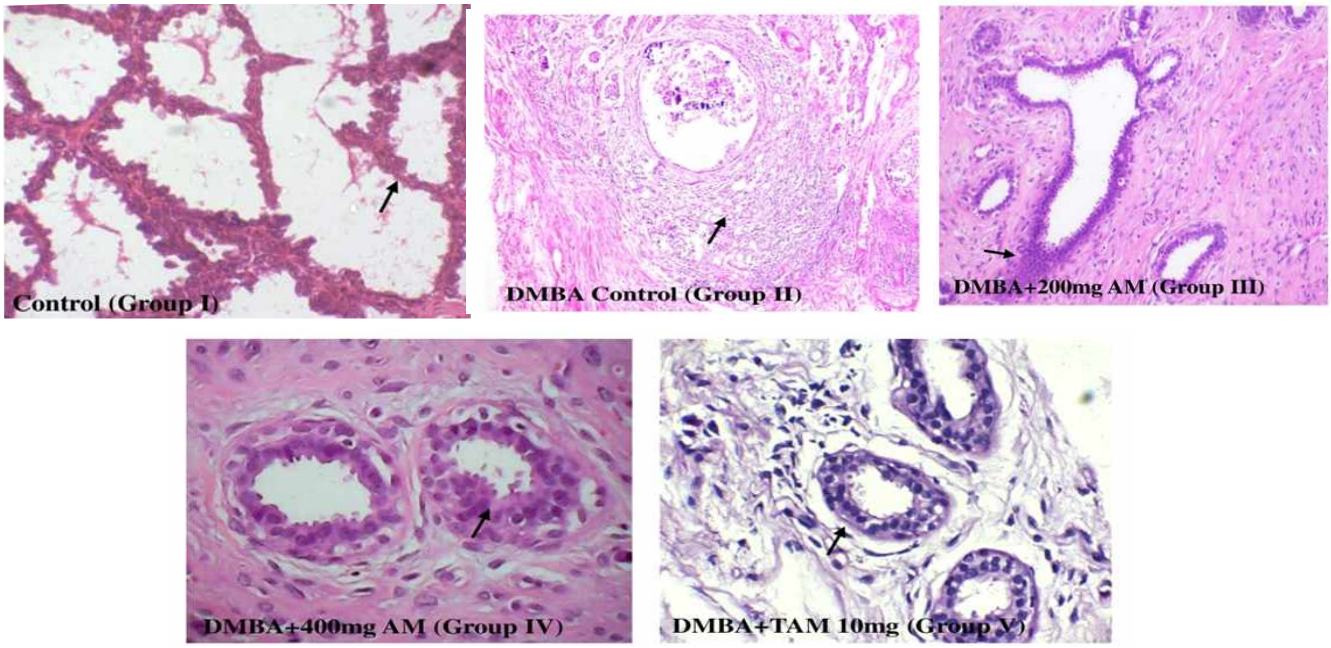


Figure 5: Histopathological observation of DMBA induced mammary tumors and effect of *Argemone mexicana* leaves methanol extract

Fig. 5: G-I (normal control) appeared normal architecture of breast parenchyma with ducts, G II(DMBA alone-induced non-treated control) annumerously proliferated epithelial cells; entering into ductal lumen and increased mitosis, G-III (DMBA+200mg of AM) proliferation of epithelial cells with local epithelial hyperplasia, G-V (DMBA+500mg of AM) moderate proliferation of epithelial cells with mild epithelial hyperplasia. G-V (DMBA+ TAM 10mg) enlarged epithelial cells with mild hyperplasia; less nuclear pleomorphic mostly round to oval.

Discussion

The most frequent cause of cancer-related mortality among females is breast cancer. Reactive Oxygen Species (ROS) are created after exposure to chemicals and contaminants in the environment, resulting in mutation, DNA damage, activation of oncogenes, and cancer genesis[36]. Tumor suppressor genes implicated in apoptosis are expressed differently by ROS, which also compromises the integrity of the cell membrane by interacting with Polyunsaturated Fatty Acids (PUFA) and forming malondialdehyde (MDA)[37]. It is widely accepted that MDA levels found in cancer patients are essential indicators of damage from oxidative stress.

According to this study, rats that received DMBA alone-induced non-treated (Group II) (from review, standard carcinogen for animal model) have increased lipid peroxidase activity (TBARS), tumor markers (CEA), and inadequate antioxidant levels (SOD, GSH, Caspase).

In treatment groups, after administration of AML extract, restored levels of antioxidant SOD and GSH, enhanced caspase activities, and reduced oxidative damage was observed. Compared to the 200mg/kg AML extract treated group, the 400 mg/kg AML extract and TAM treated groups significantly returned antioxidant levels similar to the control group. Tumor markers were also significantly reduced in 400mg of AML and TAM-treated rats compared to tumor control (DMBA alone-induced non-treated).

The correlation between AML's antioxidant properties and its chemopreventive efficacy is demonstrated by the defensive effect of AML extract on oxidative damage in rats induced with DMBA.

GC-MS analysis of AML extract demonstrated many compounds with anti-tumor and anti-cancer activity in several cell lines. Rejinthala et al. reported that Piperidine suppresses triple-negative cancer of the breast cells by altering their viability and inhibiting their cell cycle in K562 cells at the M2/G phase[38]. Hexadecanoic acid (palmitic acid) induces cell apoptosis in tumor cells by enhanced invention of intracellular Reactive Oxygen Species, facilitates the mitochondrial pathway[39]. Hexadecanoic acid and phytol extracted from microalgae prevent the proliferation of MCF7 cells in vitro[40]. Reza et al. studied *Achyranthes ferruginea* chloroform fraction via GC-MS and identified various forms of octadecanoic acid methyl esters[41]. This compound has demonstrated potent cytotoxic activity against HeLa cells. An in-silico study further supports that octadecanoic

compounds are significantly associated with the target cancer cells. Plant-based anti-cancer and cytotoxic compounds have enormous therapeutic potential as they can achieve the desired results with fewer side effects. Further exploration of plant-derived therapeutics is needed today.

Morphological study showed reduced tumor reduced tumor weight and volume in treatment rats. Cytological observation in treatment group showed moderate cluster of cells with hyperchromatic benign bare nuclei. While histopathological H and E stain revealed improved cellular pattern in treatment groups.

According to Manna et al., TAM causes cancer cells to undergo apoptosis by inhibiting the TGFβ-Akt pathway and increasing the activity of a tumor suppressor protein[42]. *Argemone mexicana* leaves methanol extract was shown to be similar to TAM and reduced the tumor incidence, weight, and volume, which implies that AML extract would have inhibited tumor growth by using the same signaling mechanism as tamoxifen. The anti-proliferative properties of AML extract are further supported by cytology and histological evaluations; this is shown by the severity difference between the groups treated with DMBA and AML.

The mammary glands of rats given DMBA showed a broad spectrum of preneoplastic phases, contributing to the production of benign and malignant tumors. Administration of 400 mg/kg/bw of AML extract resulted in a dense contractile to fibrous tissue response in the tumor mass. This desmoplastic activity further reinforces the anti-proliferative and protective properties of the AML extract.

Previous research has shown that benzylisoquinoline alkaloids such as dihydrocoptisine, dl-tetrahydrocoptisine, sanguarine, rotoberberines, protopines, protomexicine, mexitindehydrocorydalmine, jatrorrhizine, and columbamine are present in AML extract. The presence of phenolic substances in the AML extract, notably rutin, syringic acid, and gallic acid, may be responsible for its significant anti-cancer and apoptotic effects, as well as its antioxidant properties, which were confirmed in these experiments.

Conclusion

The crude extract of *Argemone mexicana* leaves has several biologically active compounds, which might be responsible for its anti-tumor/anti-cancer properties against breast tumors caused by DMBA. The crude methanolic extract was used for the study over phytoconstituents isolated because of its synergistic effects, which are known to yield better results. Further, study reports that AML extract has shown reduced lipid peroxidase and tumor markers levels and improved antioxidant levels. Based on the dose-dependent manner, we conclude that 400 mg/kg/bw of AML extract is the optimal dose, which reduces the tumor incidence, weight, and volume. According to the overall results of morphometric, biochemical, and microscopic examinations, AML extract might have anti-tumor/anti-cancer potential in DMBA-induced rat mammary tumors.

Future scope

The study provides an important basis for further investigation into the isolation of components, characterization, and mechanisms of cytotoxic components in the extract. The molecular level of diagnosis can be continued for further confirmation.

Conflict of Interest: Nil

Source of Support: Nil

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Ethical consideration

This study was approved by Institutional Animal Ethical Committee and facilities provided by JKKN Educational Pharmacy College and Research Institute, Namakkal district, Tamil Nadu, India. Date: April 4th, 2021. Survey No: JKKN/IAEC/Ph.D./04/2021.

Authors' Contribution

VG: Designing the experiment, data analysis, draft writing, revising and approving the final draft of manuscript, SK: Designing the experiment, data analysis, draft writing, revising and approving the final draft of manuscript

Data Availability Statement

Data will be available with corresponding author on request.

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