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EFFECT OF *ARGEMONE MEXICANA* Linn LEAVES EXTRACT ON DMBA-INDUCED MAMMARY TUMORS IN WISTER RATS.

Vennila Gurusamy[1], Santhini Arulselvi Kaliyaperumal[2], Dr.T.Sivakami[3], Dr.V.Shanmugapriya[4].

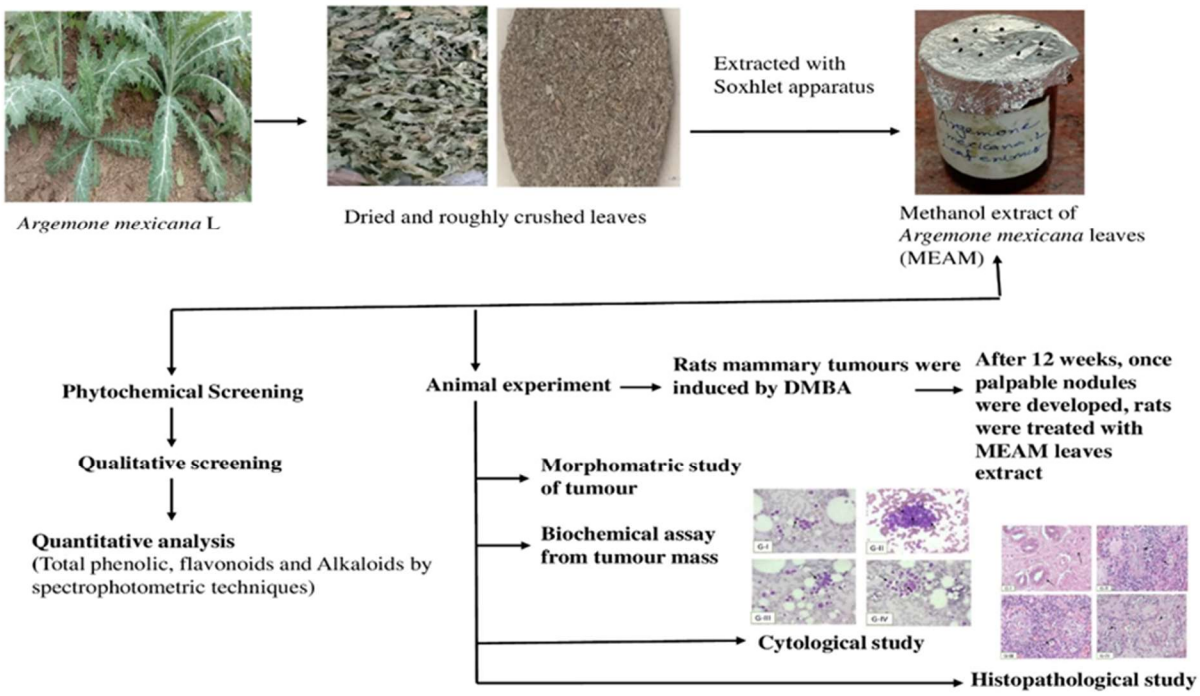
1. Department of Anatomy, Vinayaka Mission's Medical College, Vinayaka Mission's Research Foundation (Deemed to be University), Karaikal (Tamilnadu), India-609 609.
2. Department of Anatomy, Nandha Medical College and Hospital, Erode-Perundurai main road, Medukadai, Erode, Tamilnadu, India -638052.
3. Department of Pharmacology, JKKN Pharmacy College and Research Institutions, Namakkal Dist, Tamilnadu, India-638183.
4. Professor & HOD, Department of Anatomy, Thanjavur Medical college, Thanjavur.
5. Professor, Department of Biochemistry, Vinayaka mission medical College and hospital, Karaikal.

Corresponding author: Santhini Arulselvi Kaliyaperumal

ABSTRACT:

Breast cancer is a major threat worldwide, despite various therapeutic measures, it remains associated with high mortality rate. *Argemone mexicana*, traditionally used in several conditions, experimentally, plant extract showed anti-microbial, antioxidant, cytotoxic, and anti-cancer properties. However, there is lack of reports regarding its anticancer activity *in-vivo*. This study is aimed at investigating the anticancer properties of *Argemone mexicana* leaves (AML) extract on DMBA-induced mammary tumors. Female rats, 7-8 weeks old (100 ± 20 g) were used for the study and tumors were induced by DMBA (25 mg/kg/b.w/S.C). After the development of palpable nodule (10-12 weeks), the rats were treated with AML methanolic extract (200, 400 mg/kg b.w) and tamoxifen(TAM) at 10 mg/kg/b.w (standard drug) orally for 12 weeks. At the end of experiment, tumours were excised, measured weight and volume, then used for biochemical and histopathological studies. As a results, AML treatment showed significantly reduced mammary tumor weight and volume ($P < 0.01$), along with significant reduction ($P < 0.0001$) in tumour marker(CEA) and malondialdehyde (MDA) level. Significant ($P < 0.0001$) improvement in SOD, CAT, GSH levels were also observed after the treatment with AML extract. These effects contributed to the decrease the density of cancer cells in breast tumors, and the invasion of these cells into the tumor connective tissue. In conclusion, the extract of AML, contains anti-oxidants and anti-proliferative effects, it can be used as a therapeutic medicine against breast cancer.

Keywords: Breast cancer, *Argemone mexicana* leaves, DMBA (7,12-dimethylbenz(a)anthracene), antioxidant, anti-proliferative activities.



INTRODUCTION

Cancer is a group of conditions characterized by unrestrained growth and spread of abnormal cells. In India, cancer is one of the most common complications, and its incidence has been rising in the last 20 years, as in other developing countries. Lung and breast cancers were the leading locations of cancer in males and females [1]. Rapid urbanization, population aging, inactive and unhealthy lives, internal and out-of-door air pollution, including exposure to polycyclic aromatic hydrocarbons (PAH), etc., are new and confounding problems responsible for the arising cancer burden [2] . Among PAHs, DMBA (7,12-dimethylbenz (a) anthracene) is a carcinogen used to produce breast cancer in experimental rats; this model mimics the breast cancer of humans [3] . DMBA is an indirect carcinogen and has a longer latency period. Chemical carcinogens were deposited at the site of injection, observed, and activated primarily in the liver and all other tissues. Mammary glands are also responsible for DMBA through initiating cytochrome p450 (CYP) enzymes, and it is widely accepted that exposing PAHs prompts CYPs for detoxification and bio-activation [4] . Increased CYPs trigger DMBA into DMBA-3,4-diol-1,2-epoxide (DMBA-DE), which disrupts the redox cycle balance and causes oxidative stress in tissues. Created reactive oxygen species (ROS) causing damage to DNA or disturbing the cell cycle leads to unbridled proliferation of cells causing growths [5] . The treatment of mammary lumps depends on targeting the DNA damage, controlling unbridled proliferation by arresting the cell cycle, increasing programmed cell death, and reducing some molecular pathways that cause abnormal cell cycles [6] . Targeted therapy drugs specifically target cancer cells by interfering with specific molecules involved in their growth and survival. Side effects can vary depending on the specific drug used but may include skin rashes, diarrhea, liver problems, cardiovascular effects,

and blood clotting disorders. The side effects of targeted therapy are generally less severe than those of traditional chemotherapy [7] .

Many natural compounds are responsible for an effective and efficient alternative method for chemoprevention and therapeutic treatment against breast cancer [8] . The natural agents can be used in combination with other treatment methods, such as chemotherapy and radiation, to prevent their side effects [9] . Still, there are a lot of natural agents present but not completely explored to treat conditions like cancer. Further, various explored natural herb derivatives, including vinblastin, etoposide, and paclitaxel medicines, are now combined with varied nano-carriers that have been developed for compounding their conditioning against breast cancer [10] . From the review of literature and ethno-pharmacological studies, present investigation aimed to identify the anti-cancer effect of *Argemone mexicana* leaves (AML) extract against DMBA (7,12-dimethylbenz (a) anthracene)-induced mammary tumors in rats.

Fig 1: *Argemone mexicana* L. (plant)

Argemone *mexicana* Linn (Family: Papaveraceae) is an annual plant, grows throughout the semitropical and tropical regions, occurs in practically every region of India, and generally occurs on roadsides [11] . Which is used in several traditional drugs to treat varied conditions like dropsy, jaundice, ophthalmia, scabies, and cutaneous affections. Experimentally, the plant extract showed

anti-microbial, antioxidant, cytotoxic, and anti-cancer properties [12,13] . Alkaloids like berberine and tetrahydroberberine, protopine, and benzophenanthridines have been isolated from the plant (14) . Sarkar et al. reported that the ethanolic extract of *Argemone mexicana* leaves was reported to have significant anti-inflammatory and analgesic activity [15]. Sourabie et al. investigated the anti-hepatotoxic activity of an aqueous extract and a crude leaf powder suspension of *Argemone mexicana* against CCl4 induced hepatitis in rats [16]. Above the review of literature, AML might possess effective biological activity against various experiments.

MATERIALS AND METHODS:

Preparation of *Argemone mexicana* leaves methanol extract:

Fresh green leaves of AML were collected from the Botanical Survey of India, Southern Regional Centre Coimbatore, Tamil Nadu, and were authenticated by taxonomists. They were rinsed clearly with tap and distilled water and shade dried to evacuate the soil particles. 500 grams of shaded dried leaves were powdered coarsely with the help of a mortar and pestle and extracted with 1.2 liters of 70% methanol by the cold percolation procedure. After 3 days, the contents were filtered using No. 1 Whatman filter paper and distilled over a boiling water bath. The obtained extract was stored in the refrigerator at −4 °C and utilized for further analyses. The LD50 dose of the methanolic extract was found to be 2000 mg/kg body weight. The final dose, $1/10^{th}$ and $1/5^{th}$ doses of LD50, was calculated and titrated to 200,400 mg/kg body weight.

Experimental Animals:

Female SD rats were maintained as per the regulations of the Committee for Control and Supervision of Experiments on Animals (CPCSEA, New Delhi, India), and the experimental research design was approved by the Institutional Animal Ethical Committee, JKKN Educational Institution, Kumarapalayam, Namakkal district (JKKN/IAEC/Ph.D./04/2021). After proper acclimatization of rats for 14 days, they were divided into five groups with six rats each, and the study was carried out for 6 months with adequate food and water. The experimental animals were placed in the polypropylene cages, with six animals in each cage, and they were randomly distributed into control and treated groups. The temperature of the animal house was maintained at 22±3 °C for the rats with a 12-hour light/dark cycle.

Experimental design:

Animals, aged 6-7 weeks old, weighing 100 ± 20 g, were classified into 5 groups of 6 animals each, and the research was carried out for 6 months (24 weeks).

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

Group II, III, IV, and V rats were administered a single subcutaneous injection of 7,12-dimethylbenz[a]anthracene (DMBA) at 25 mg/kg/b.w. Animals were palpated twice a week after 4 weeks of DMBA; once a palpable nodule had developed (approximately 10–12 weeks after DMBA, about 0.5 cm), animals were treated orally for 12 weeks (weekly trice).

Group II: Rats were treated a single dose of DMBA (25 mg/kg/b.w/s.c) and let it for the full course of 24 weeks.
Group III: Rats were treated with Tamoxifen (TAM,10 mg/kg/b.w/p.o).
Group IV: Rats were treated with AML extract (200 mg/kg/b.w/p.o).
Group V: Rats were treated with AML extract (400 mg/kg/b.w/p.o).
At the end of 24 weeks, blood was collected from the retro-orbital venous plexus in vacuum tubes under isoflurane anesthesia. The same anesthesia was administered to euthanize the animals. For ten minutes, the blood was centrifuged at 3500 rpm. Serum was collected, and analysis was done for different tumor marker assays. Each analysis was carried out at Chennai, India's Biogen Laboratory Private Limited, a NABL-accredited laboratory. The mammary tumors were removed and washed with cold PBS, calculated the volume and weight of the tumors in each group. A group of cells were scraped on cut edges of mammary tumor mass by spatula [17] was placed on microscopic slides for cytological studies and stored in liquid nitrogen (N2) at 70°C for biochemical assay, remaining portion was stored in 10% buffered formalin for histopathological studies.

Tumor induction:

Freshly prepared single dose of 25 mg of DMBA (7,12-dimethylbenz(a)anthracene) dissolved in 1 ml of oil emulsion (0.5 mL of sunflower oil and 0.5ml of saline) was administered through subcutaneous mode using a 2 ml syringe with a 26-gauge needle at the space between the 3rd and 4th mammary papillae [18]. Animals were palpated for tumor nodules weekly starting from the 4th week after the administration of DMBA. The initial tumor appeared around the 10th week after administration of DMBA, while by the 12th week, a tumor appeared in all six rats.

Chemicals and reagents:

All chemicals and solvents used in the research were of analytical grade and procured from SISCO Research Laboratories Private Limited, D.K. Enterprises, India; Sigma Aldrich, Argutus, Dublin, Ireland; Dako, Carpinteria, CA, USA; or Louis, MO, USA.

Preliminary Phytochemical Analysis:

The phytochemical experiment was done to screen the different substances present in the AML extract based on the standard methods suggested by Ugochukwu [19]. The total phenolic content of crude AML extract was estimated by Folin-Ciocalteu methods. After incubation time, absorbance was measured at a constant wavelength of 750 nm with a spectrophotometer. The total phenol content was expressed in an equal volume of gallic acid (mg of GAE/g of AML extract) [20]. Total flavonoids estimation by aluminum trichloride methods [21]. Quercetin was used as a reference; the diluted extract and quercetin were kept at room temperature for 39 min; and the absorbance of the admixture was measured at 510 nm using a spectrophotometer. The total flavonoid content was expressed in the same quantum of quercetin (mg of QCE/g of AML extract). The total alkaloid content of the methanol extract of AML was determined by the techniques of [22]. In this technique, concentrated ammonium hydroxide was added drop by drop to the crude

leaf extract until complete precipitousness had formed. Filtered precipitate was washed with ammonium hydroxide. The final content was alkaloids, which were dried and weighted in an electronic balance.

Morphological study:

All experimental animals were weighed at the beginning of the experiment. During the course of the experimental period (24 weeks), food intake, water intake, and body weight were monitored every week until rats were sacrificed. Observed the difference between initial and final mean body weight of control and tumor-bearing animals. Excised tumors were weighted (g) and volumes (V) were calculated from vernier caliper measurements in two different dimensions. In 1995, Bousquet et al proposed a formula: V = (W2×L)/2, where W is the tumor width and L is the tumor length. Here is the mean ± standard deviation value of tumor volume, denoted in cm^3 .[23]

Biochemical Assay:

Preparation of tissue homogenate: 100mg of fresh mammary tissues, cut into small pieces, and homogenates (10% w/v) were prepared in ice-cold 0.1M Tris-base HCL buffer with 5 M KCL (pH-7.4) by using a tissue grinder, a Potter Elvehjem homogenizer. Tissue homogenates were centrifuged at 12000 rpm for 30 min at 4 oC.The supernatant was separated and frozen at -20ºc for biochemical assay.

Lipid peroxidion levels in tissues were assayed by the methods of [24]. The levels of TBARS were expressed in nmoles of MDA liberated per mg of protein. The superoxide dismutase (SOD) activity was assessed by the method of Misra and Fridovich [25]. The catalase (CAT) activity was determined in the tissue homogenate by the method of Sinha *et al*. [26]. Reduced glutathione (GSH) in the mammary tissues of the experimental groups was estimated by the methods of Beutler [27]. Carcinoembryonic antigen (CEA) levels (ngm/ml) 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 [28].

Microscopic study:

Cytological study (H&E)

Fixed the cytological wet smears in 95% ethanol for 15 min, then the slides were fixed in 70% and 50% alcohol for each 1 min and gently rinsed with tap water, and the procedure steps were as follows [29]. The first slides were stained with a hematoxylin solution for 15-20 minutes, rinsed with 1% acid alcohol for 1 minute, and rinsed in tap water for the blueing process. Then the slides were counterstained with eosin for 2-3 dips and two changes in each graded alcohol (95% and 100%) for 1 minute. Finally, it was dipped in xylene, mounted on a cover slip with DPX, and examined under 40X magnification of light microscope (Olympus CH20i Tr).

Histopathological study (H&E)

Excised part of tumors fixed in 10% formalin for 24 hours. Later, they were dehydrated gradually using different grades of alcohol, cleared in xylene, and embedded in paraffin wax with a melting point of 56–58 °C. Serial sections were taken with 3−4 µm thickness with a rotatory microtome

(INCO MRM-1120) and stained with gold standard histological dyes hematoxylin and eosin [30]. The sections were examined under 40X magnification of light microscope (CH20ITR, Olympus Microscope) for analyzing lesions in tumor tissue.

S.No	Phytochemical Test	Methanol extract of *Argemone mexicana.* L(a)
1	Test for Alkaloids (Wagner's reagent):	++
2	Test for Carbohydrates (Molisch's test):	_
3	Test for Flavonoids (Alkaline reagent test):	+
4	Test for Phenols (Ferric chloride test):	+
5	Test for Amino acids and Proteins (1% ninhydrin solution in acetone):	+
6	Test for Cardiac glycosides (Keller Kelliani's test):	-
7	Test for Saponins (Foam test):	++
8	Test for Tannins (Braymer's test):	+
9	Test for Sterols (Liebermann-Burchard test):	_
10	Test for Terpenoids (Salkowki's test)	-

Statistical Analysis:

The data obtained in the experimental study were analyzed using a one-way ANOVA, followed by the test Turkey HSD for comparison between the groups. The results were expressed as mean ± SEM, and values with $P < 0.01$ were considered as statistically significant.

RESULTS:

Table 1: Results showing phytochemical screening of *Argemone mexicana* leaves:

++, highly present; +, present; and −, absent

Table 2: Results of total Flavonoids, Alkaloids, Phenols components of *Argemone mexicana* leaves:

Analysis	Extract		
	Water	Methanol	Ethanol
Total Flavonoids (mg QCE/g)	12.19±0.43	20.34±0.04	11.28±0.04
Total Phenols (mg GAE/g)	10.85±0.22	11.88±0.09	8.83±0.07
Total Alkaloids (mg/g)	153.7±1.57	154±1.76	149.3±1.06

All the values are mean ± SD (n=3). Mean values of alkaloids and phenols values were given in the column are not significantly different, whereas flavonoids values are significant ($P < 0.05$). QCE=Quercetin equivalent, GAE= Gallic acid equivalent.

Table 3: Effect of *Argemone mexicana* leaves extract on body weight (g) compared between control and experimental groups:

Experimental Groups	Parameter				
	Initial body weight (g)	Final body weight (g)	Weight gain(g)	Mean tumor weight (g)	Mean volume (cm^3)
Control	115.83±3.43	167.5±4.57	51.66±4.49	0	0
DMBA alone	120±3.82	165.16±3.23	45.16±3.13*	4.35±2.26	4.75±0.64
DMBA+TAM (10mg/kg/bw)	118.33±3.72	191.5±3.40	81.5±4.27[a]	2.16±0.72 [a]	1.95±0.18 [a]
DMBA+AML(200 mg/kg/bw)	118.16±4.48	199.6±2.62	74.83±3.93[b]	3.21±1.26 [b]	3.62±0.32 [b]
DMBA+AML(400 mg/kg/bw)	119.83±3.89	199.5±3.30	79.66±3.09 [a]	2.98±1.43 [a]	2.66±0.45 [a]

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

Table 4: Shows levels of lipid peroxidation, anti-oxidant and tumour marker in control and experimental animals.

Animal Group	TBARS (mM/100g of tissue)	SOD (U/mg of protein)	**CAT (U/mg of protein)**	**GSH (mg/g of tissues)**	CEA (ng/ml of serum)
Control	1.33± 0.06	15.22 ± 0.20	54.77 ± 1.31	15.77 ± 0.36	1.997 ± 0.10
DMBA alone	2.76± 0.17 [a]	9.74 ± 0.09[a]	28.29 ± 0.80 [a]	8.29 ± 0.48 [a]	8.200 ± 0.17 [a]
DMBA+TAM (10mg/kg/bw)	1.34± 0.01 [b]	13.81 ± 0.09[c]	**55.29 ± 0.16 [c]**	**15.12 ± 0.3 [c]**	2.277 ± 0.17 [b]
DMBA+AML(200 mg/kg/bw)	2.24± 0.08	10.83± 0.29[b]	**40.63 ± 2.57 [c]**	**10.71 ± 0.69**	3.400 ±0.23 [b]
DMBA+AML(400 mg/kg/bw)	1.57± 0.08 [b]	12.97 ± 0.15[c]	**52.68 ± 0.28 [c]**	**13.35 ± 0.80 [b]**	2.867 ± 0.14 [b]

Values are expressed as mean ± SEM (n=6). [a]P<0.001 Vs Control [b]P<0.01; [c]P<0.001 Vs DMBA alone. Data were analyzed by oneway ANOVA followed by Tukey HSD multiple comparison test.

Cytology results:

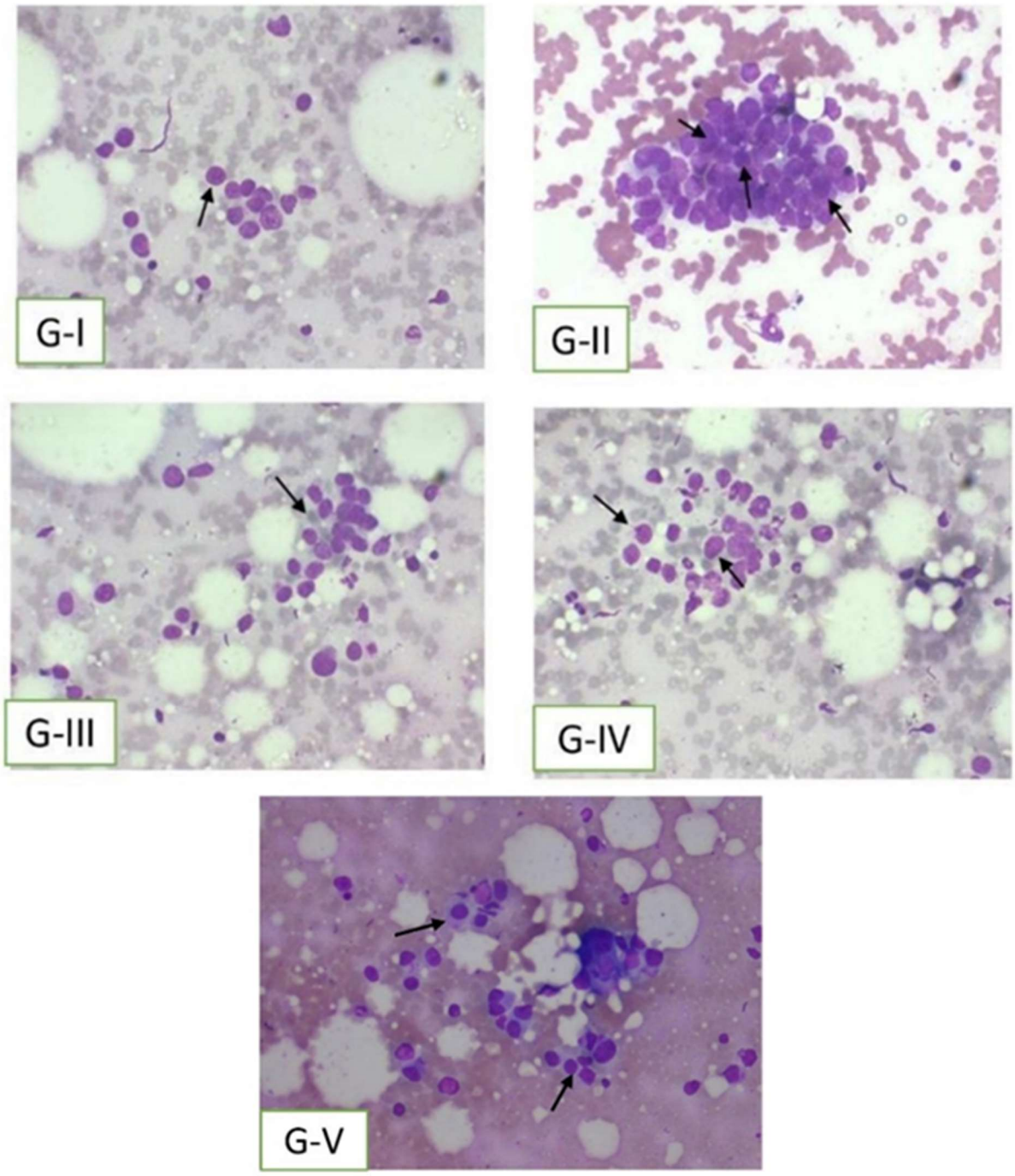


Fig 2: G-I (normal control) shows normally appearing scattered epithelial cells, G-II (DMBA alone) cluster of tumour cells with hyperchromatic nucleus and severe nuclear atypia, G-III (DMBA+ TEM 10mg) moderate cluster of tumour cells with normally appeared hyperchromatic nuclei. G-IV (DMBA+250mg of AM) cluster of ductal epithelial cells with hyperchromatic, pleomorphic nuclei, G-V (DMBA+500mg of AM) mild cluster of ductal epithelial cells with benign bare nuclei.

Histopathology results:

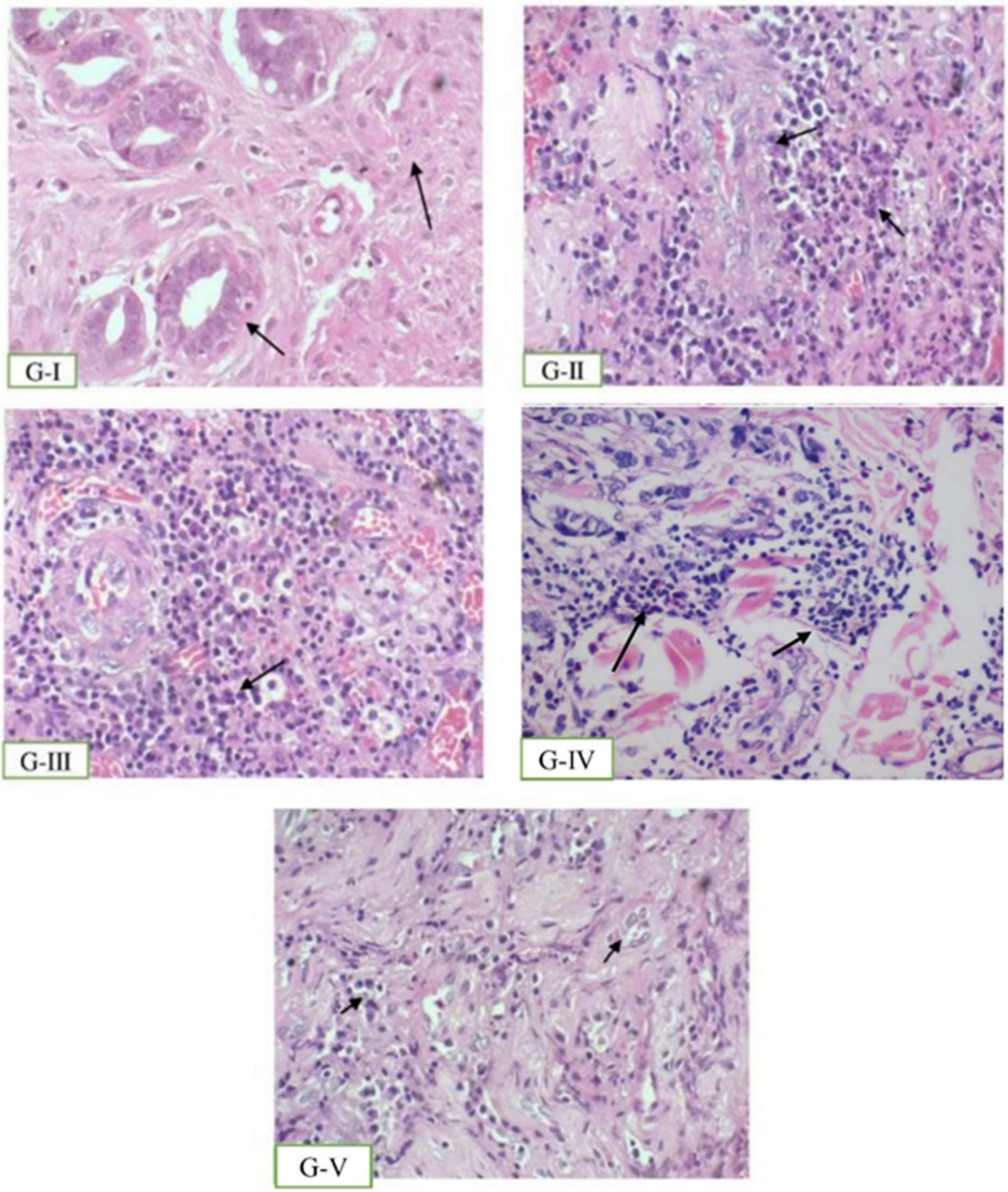


Fig 3: G-I (normal control) appeared normal architecture of breast parenchyma with ducts, G-II (DMBA alone) annumerously proliferated epithelial cells; entering into ductal lumen and increased mitosis, G-III (DMBA+ TEM 10mg) enlarged epithelial cells with mild hyperplasia; less nuclear pleomorphic mostly round to oval. G-IV (DMBA+250mg 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.

Phytochemical screening of AML extract:

AML extract phytochemical screening was carried out with methanol, and the results revealed the presence of various secondary metabolic constituents such as alkaloids, carbohydrates, flavonoids, phenols, amino acids and proteins, glycosides, saponins, tannins, steroids, and terpenoids (Table 1). The phytochemical screening of AML was done quantitatively in the three different extracts, namely, aqueous, methanol, and ethanol, respectively. Comparatively, methanol extract indicated a significant amount of active metabolites such as flavonoids (20.34±0.04 mg QCE/g), phenols (11.88±0.09 mg GAE/g), and alkaloids (154±1.76 mg/g), followed by aqueous and ethanol extracts, respectively (Table 2).

Effect of 400 mg/kg of AML against DMBA-induced breast tumor morphology:

From the mean value of the initial and final body weight of rats, weight gain has occurred in all the experimental groups; comparatively, the DMBA alone-treated group gained 45 grams; DMBA+TAM, DMBA+AML (400, 200 mg/kg) treated rats increased in weight respectively. Compare to the mean values of tumor weight and volume from the experimental groups, there was increased weight in DMBA alone treated rats (4.3g and 4.75 cm3), DMBA + TAM, DMBA + AML (200,400 mg/kg) treated groups were significantly reduced the tumour volume and weight. Results were statistically significant ($P<0.01$). Increased tumor weight and volume in DMBA alone-treated rats were compared to TAM and AML (400 mg/kg)-treated rats, which showed a decrease in tumor weight and volume compared to AML (200 mg/kg)-treated rats (table 3).

Effect of 400 mg/kg of AML against DMBA-induced breast tumors on antioxidant levels in the mammary tissue

Compare to the mean values of SOD, CAT, and GSH levels, there was a decrease in SOD, CAT, and GSH in the DMBA alone treated group (Table 4). Simultaneous treatment with TAM 10 mg/kg as well as with AML 400 mg/kg upsurges in the antioxidant levels equivalent to those of the control group (Table 4) compared to AML (200 mg/kg)-treated rats. These levels were statistically significant ($P < 0.01$).

Effect of 400 mg/kg of AML against DMBA-induced breast tumors on lipid peroxidation in the mammary tissue

Table 4 depicts the levels of TBARS in mammary tissues. These results were statistically significant ($P < 0.01$). The mean levels of TBARS, compared to the DMBA alone group, there was a significant downregulation in TBARS levels in the AML 400 mg/kg b.w. group compared to the AML 200 mg/kg b.w. group. The TAM 10 mg/kg b.w. group showed a remarkable decrease of TBARS compared to the DMBA alone group.

Effect of 400 mg/kg of AML on tumor markers in DMBA-induced breast cancer

The serum level of the CEA biomarker was significantly ($P < 0.01$) increased in the DMBA alone group compared to the control group, while in the DMBA-protected groups, DMBA + TAM, AML groups, the CEA level significantly ($P < 0.01$) decreased compared to the DMBA alone group.

Simultaneous administration of TAM (10 mg/kg) and AML at a dose of 400 mg/kg showed equal protection compare to the control group (Table 4).

Effect of AML on mammary tumor cytology based on haematoxylin and eosin (H&E) staining:

Fig. 2 shows, the control group appears normally scattered ductal epithelial cells; the DMBA alone-treated group showed a cluster of tumor cells with a hyperchromatic nucleus and severe nuclear atypia; the DMBA+-TAM-treated group appeared as a moderate cluster of tumor cells with normally appearing hyperchromatic nuclei. The DMBA+AML (200 mg/kg) group seemed to have a smaller cluster of ductal epithelial cells with hyperchromatic, pleomorphic nuclei; the DMBA+AML (400 mg/kg)-treated group revealed a milder cluster of ductal epithelial cells with benign bare nuclei. Compared to the control group, the DMBA alone-treated group exposed severe malignancy changes. In treated groups, DMBA+ AML 200 mg was given moderate changes, like a lower amount of hyperchromatic and nuclear pleomorphic, TAM and AML (400mg) treated rats appeared to have more or less the same cellular pattern and highly improved cellular architecture.

Effect of AML on mammary gland histopathology based on hematoxylin and eosin (H&E) staining

In histopathological sections showed, control group appeared normal cytoarchitecture in the epithelium and ductal system. However, the DMBA alone-treated group showed abnormal changes in the mammary tissue epithelium with moderate to severe anaplastic transformation of the glandular epithelium. There was increased cancer cells' proliferation of alveolar and ductal epithelium with hyperchromatic nuclei that showed multiple mitotic cells. Solid tumor masses invaded the mammary tissue with extensive areas of necrosis along with acute inflammation. The breast tissues showed a reduction of neoplastic tissue, inflammation, and necrosis in the DMBA+AML administered with near-normal cyto-architecture. However, the DMBA + TAM group showed significant restoration of the histological architecture in tumor tissue with minimum proliferation of the glandular epithelium and reduction in mitotic figures and nuclear changes.

DISCUSSION:

The most prevalent cause of fatalities related to cancer among women is breast cancer. The exposition to environmental pollutants and chemicals induced mutation, DNA damage, oncogene activation, and cancer genesis causes the formation of reactive oxygen species (ROS) [31]. ROS alters the expression of tumor suppressor genes involved in apoptosis, interferes with the membrane integrity of the cell by reacting with polyunsaturated fatty acid (PUFA) ultimately leading to the formation of malondialdehyde (MDA) [32]. It is widely accepted that MDA levels found in cancer patients are important indicators of damage from oxidative stress and status as antioxidants. In agreement with previous studies on DMBA model, mammary tissues of rats treated with DMBA alone were characteristics with inducing several markers of oxidative stress including MDA and diminution of endogenous antioxidants such as SOD, CAT and GSH [33]. According to this, rats receiving DMBA have had inadequate antioxidant levels. Administration of AML prevented the oxidative damage to protein and lipids (MDA) and restored the SOD, CAT and GSH activities in mammary tissues of DMBA-treated rats. The protective effect of AML

against oxidative damage in DMBA-treated rats indicates that the chemo preventive efficacy of AML is related to its antioxidant effects. Several reports suggest that the level of CEA to be significantly elevated from normal levels in breast cancer conditions [34]. Carcino-embryonic antigen (CEA) was elevated in DMBA administered rats which were declined when treating with *AML* extract. Significant lowering effect of MDA and CEA suggests that AML could be used as a complementary anti-cancer agent.

Results of phytochemical screening methods of *Argemone mexicana* leaves (AML) extract showed the presence of alkaloids, phenols, flavonoids, tannins, saponins, amino acids, fatty acids, and other components (table 1), which are known to have the medicinal and physiological properties. In agreement with this study, Sarkar et al [15] found the similar results of phytochemical screening and pharmacological activities of *Argemone mexicana* aerial parts. The quantitative phytochemical screening of selected phytochemicals present in the methanol extract of Argemone mexicana is shown in (Table 2). In line with published data, Gali et al [35] reported alkaloids and flavonoids, the main bioactive agents in AML extract have also been found in high amount in this study. The relatively high amount of flavonoid (an antioxidants/free radical scavenger) could possibly be responsible inhibition of tumorigenesis in rats, also found the nature of cytotoxic activity is found to be apoptotic rather than necrosis by Hoechst test on Hela and MCF cell lines.

From the results of this study, administration of AML(400mg/kg/bw) extract for 12 weeks, significantly reduced the tumour weight, volume and enhanced body weight, similar to the administration of TAM (standard drug) compare to DMBA alone treated rats. Manna and Holz [36] was reported TAM induce the apoptosis of cancer cells through the increase in the activity of protein phosphatase (tumor suppressor protein) and the inhibition of TGFβ-Akt pathway (an oncogenic protein). Tumor growth inhibition was also observed with the methanol extract of *Argemone mexicana* leaves(AML) is suggests that AML extract would have used the same signaling pathway as that of tamoxifen (TAM) to inhibit tumor growth.

The histopathological study also confirms the anti-proliferative nature of AML extract which is evident from the difference between the degree of severity between AML treated group and DMBA treated group. In female rats treated with DMBA, the early morphological pre-neoplastic alterations that occur throughout the development of breast cancer were identified, which mainly induce the formation of lesions in the terminal ducts and induce hyperplasticity of the ductal epithelial cells, atypical hyperplasticity, and canceration of the ducts [37]. In addition to benign and malignant tumor formation, the mammary glands of rats received DMBA exhibited a wide range of preneoplastic stages as well. Treatment with AML extract (400mg/kg/bw) showed the presence of thick fibrous to collagen tissue reaction in tumor mass which is the result of desmoplastic reaction also supports the protective and anti-proliferative nature of the AML extract.

Earlier studies have documented that AML extract contain benzylisoquinoline alkaloids like, benzophenanthridines, sanguarine, rotoberberines and protopines, protomexicine, mexitin

dehydrocorydalmine, jatrorrhizine, columbamine, dl-tetrahydrocoptisine and dihydrocoptisine. The presence of phenolic substances in the AML extract, notably rutin, syringic acid, and gallic acid, may be responsible for its significant anticancer and apoptotic effects as well as its antioxidant properties, which were confirmed in these experiments. Kulshrestha et al [38] administered the ethanol extract of AML to experimental animals with induced skin cancer. The AML extract treated rats showed decreased TNF-α concentration in the serum, which indicated the anti-inflammatory potential of the extract and accounts for its anticancerous potential. Same study also confirms the blocking of the NF-κB inflammatory pathway by reduced concentration of p65 subunit. From above findings where cancer was prevented maximally by AML extract in experimental animals.

CONCLUSION:

The experiments included in this study demonstrated the cancer-preventive effect of Argemone mexicana Linn leaves against DMBA induced mammary tumours. Due to its synergistic effects, the crude methanolic extract was chosen for the investigation instead of the isolated phytoconstituents, as it is known to produce better outcomes. It could be concluded that the best dose at which the anticancerous activity was found is 400 mg/kg/bw of AML extract. According to the overall results of morphometric, biochemical, and microscopic examinations, AML extract might have antitumor or anticancer activity on mammary tumors in rats.

FUTURE SCOPE:

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

Conflict of interest: none

REFERENCE:

1. Sathishkumar K, Chaturvedi M, Das P, Stephen S, Mathur P. Cancer incidence estimates for 2022 & projection for 2025: Result from National Cancer Registry Programme, India. Indian J Med Res [Internet]. 2022 Oct 1 [cited 2024 Mar 9];156(4&5):598–607. Available from: https://pubmed.ncbi.nlm.nih.gov/36510887/
2. Prescott SL, Wegienka G, Kort R, Nelson DH, Gabrysch S, Hancock T, et al. Project earthrise: Proceedings of the ninth annual conference of in vivo planetary health. Int J Environ Res Public Health [Internet]. 2021 Oct 2 [cited 2024 Mar 9];18(20):10654. Available from: https://research-repository.uwa.edu.au/en/publications/project-earthrise-proceedings-of-the-ninth-annual-conference-of-i
3. Mvondo MA, Wego Kamgaing MT, Wansi Ngnokam SL. Aqueous Extract of Dacryodes edulis (Burseraceae) Leaves Inhibited Tumor Growth in Female Wistar Rats with 7,12-Dimethylbenz[a]anthracene-Induced Breast Cancer. Evid Based Complement Alternat Med [Internet]. 2021 [cited 2024 Mar 9];2021. Available from: https://pubmed.ncbi.nlm.nih.gov/34367310/
4. Stoddard EG, Nag S, Martin J, Tyrrell KJ, Gibbins T, Anderson KA, et al. Exposure to an

Environmental Mixture of Polycyclic Aromatic Hydrocarbons Induces Hepatic Cytochrome P450 Enzymes in Mice. Chem Res Toxicol [Internet]. 2021 Sep 20 [cited 2024 Mar 9];34(9):2145–56. Available from: https://pubmed.ncbi.nlm.nih.gov/34472326/

5. Akhouri V, Kumari M, Kumar A. Therapeutic effect of Aegle marmelos fruit extract against DMBA induced breast cancer in rats. Sci Rep [Internet]. 2020 Dec 1 [cited 2024 Mar 9];10(1). Available from: https://pubmed.ncbi.nlm.nih.gov/33093498/
6. Otto T, Sicinski P. Cell cycle proteins as promising targets in cancer therapy. Nat Rev Cancer [Internet]. 2017 Jan 27 [cited 2024 Mar 9];17(2):93–115. Available from: https://pubmed.ncbi.nlm.nih.gov/28127048/
7. Deutsch A, Leboeuf NR, Lacouture ME, McLellan BN. Dermatologic Adverse Events of Systemic Anticancer Therapies: Cytotoxic Chemotherapy, Targeted Therapy, and Immunotherapy. Am Soc Clin Oncol Educ book Am Soc Clin Oncol Annu Meet [Internet]. 2020 May [cited 2024 Mar 9];40(40):485–500. Available from: https://pubmed.ncbi.nlm.nih.gov/32421446/
8. Laskar YB, Lourembam RM, Mazumder PB, Laskar YB, Lourembam RM, Mazumder PB. Herbal Remedies for Breast Cancer Prevention and Treatment. Med Plants - Use Prev Treat Dis [Internet]. 2020 Mar 25 [cited 2024 Mar 9]; Available from: https://www.intechopen.com/chapters/70593
9. Liu YQ, Wang XL, He DH, Cheng YX. Protection against chemotherapy- and radiotherapy-induced side effects: A review based on the mechanisms and therapeutic opportunities of phytochemicals. Phytomedicine [Internet]. 2021 Jan 1 [cited 2024 Mar 9];80. Available from: https://pubmed.ncbi.nlm.nih.gov/33203590/
10. Mathur P, Rawal S, Patel B, Patel MM. Oral Delivery of Anticancer Agents Using Nanoparticulate Drug Delivery System. Curr Drug Metab [Internet]. 2019 Oct 7 [cited 2024 Mar 9];20(14):1132–40. Available from: https://pubmed.ncbi.nlm.nih.gov/31589119/
11. Morey PR, Wankhade AM, Vyas J V., Paithankar V V. A Review on Argemone maxicana Linn. Res J Pharmacogn Phytochem [Internet]. 2023 May 20 [cited 2024 Mar 9];15(2):149–54. Available from: https://rjpponline.org/AbstractView.aspx?PID=2023-15-2-9
12. Saravanan R, Senthilkumar K, Dhachinamoorthi D, Uma Mahesh P, Vamsi Krishna G, Venkata Pavani K. Evaluation of Wound Healing and Anti Microbial Activity of the Argemone mexicana Linn (Papavraceae). Res J Pharm Technol [Internet]. 2017 Mar 28 [cited 2024 Mar 9];10(3):852–6. Available from: https://rjptonline.org/AbstractView.aspx?PID=2017-10-3-37
13. Kulshrestha S, Goel A. A PANORAMIC VIEW ON ARGEMONE MEXICANA: ITS MEDICINAL IMPORTANCE AND PHYTOCHEMICAL POTENTIALS. PLANT Arch. 2021 Apr 19;21(no 1).
14. Telkar SN, T V, Kuppast IJ, Dcosta D. Evaluation of Diuretic Activity of Argemone mexicana. Res J Pharmacol Pharmacodyn [Internet]. 2018 Sep 22 [cited 2024 Mar 9];10(3):105–8. Available from: https://rjppd.org/AbstractView.aspx?PID=2018-10-3-4
15. Sarkar KK, Mitra T, Acharyya RN, Sadhu SK. Phytochemical screening and evaluation of

the pharmacological activities of ethanolic extract of Argemone mexicana Linn. aerial parts. Orient Pharm Exp Med. 2019 Mar 14;19(1):91–106.

16. Sourabie TS, Ouedraogo N, Sawadogo WR, Nikiema JB, Guissou IP. # Biological evaluation of anti-inflammatory and analgesic activities of Argemone mexicana Linn . (Papaveraceae) aqueous leaf extract #. Int J Pharma Sci Res [Internet]. 2012;3(9):451–8. Available from: http://www.ijpsr.info/docs/IJPSR12-03-09-006.pdf
17. Sheaff MT, Singh N. Cytopathology: An introduction. Cytopathol An Introd. 2013 Jan 1;1–478.
18. Dhamodharan K, Vengaimaran M, Sankaran M. Chemomodulatory Effect of Capsaicin Encapsulated Chitosan Nanoparticles on Lipids, Lipoproteins and Glycoprotein Components in 7,12-Dimethylbenz[a]anthracene (DMBA) Induced Mammary Carcinogenesis in Sprague-Dawley Rats. 2021 [cited 2024 Mar 9]; Available from: www.ejmo.org
19. Ugochukwu S, Uche A, Ifeanyi O. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of Dennetia tripetala G. Baker. Asian J Plant Sci Res. 2013;
20. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colometric methods. J Food Drug Anal [Internet]. 2020 Jul 14 [cited 2024 Mar 9];10(3):3. Available from: https://www.jfda-online.com/journal/vol10/iss3/3
21. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, et al. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. J food drug Anal [Internet]. 2014 [cited 2024 Mar 9];22(3):296–302. Available from: https://pubmed.ncbi.nlm.nih.gov/28911418/
22. Fahal EM, Babitha AM, Aklakur R, Chanu TI, Saharan N. Qualitative and Quantitative Phytochemical Analysis of Moringa oleifera (Lam) Pods. IntJCurrMicrobiolAppSci [Internet]. 2018 [cited 2024 Mar 9];7(5):657–65. Available from: https://doi.org/10.20546/ijcmas.2018.705.080
23. P.F. B, M.F. B, D. C, K.M. F, D. P, C. C, et al. Preclinical evaluation of LU 79553: A novel bis-naphthalimide with potent antitumor activity. Cancer Res [Internet]. 1995 [cited 2024 Mar 9];55(5):1176–80. Available from: https://www.researchgate.net/publication/15332232_Preclinical_evaluation_of_LU_79553_A_novel_bis-naphthalimide_with_potent_antitumor_activity
24. Devasagayam TPA, Tarachand U. Decreased lipid peroxidation in the rat kidney during gestation. Biochem Biophys Res Commun [Internet]. 1987 May 29 [cited 2024 Mar 9];145(1):134–8. Available from: https://pubmed.ncbi.nlm.nih.gov/3593335/
25. Misra HP, Fridovich I. The Purification and Properties of Superoxide Dismutase from Neurospora crassa*. J Biol Chem. 1972;247(11).
26. Sinha AK. Colorimetric assay of catalase. Anal Biochem [Internet]. 1972 [cited 2024 Mar 9];47(2):389–94. Available from: https://pubmed.ncbi.nlm.nih.gov/4556490/
27. BEUTLER E, DURON O, KELLY BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963 May;61:882–8.

28. Dharmendra V, Palanivelu S, Panchanadham S. Immunomodulatory effect of Kalpaamruthaa on 7,12-dimethyl benz(a)anthracene-induced mammary carcinoma studied in rats. Comp Clin Path. 2014;23(4):1087–94.
29. Suryalakshmi, S, P, Thanhamani, S, Ravi, I, Vijay Sathish Kumar, Premalatha, Sasikala, K, R M. Comparison of Leishman Staining With H&E Staining Technique in the Study of FNAC Smears. IOSR J Dent Med Sci [Internet]. 2016 [cited 2024 Mar 9];15(4):2279–861. Available from: www.iosrjournals.org
30. Bancroft JD, Cook HC (Harry C, Stirling RW. Manual of histological techniques and their diagnostic application. 1994;457.
31. Sahoo BM, Banik BK, Borah P, Jain A. Reactive Oxygen Species (ROS): Key Components in Cancer Therapies. Anticancer Agents Med Chem [Internet]. 2022 Jun 9 [cited 2024 Mar 9];22(2):215–22. Available from: https://pubmed.ncbi.nlm.nih.gov/34102991/
32. Su LJ, Zhang JH, Gomez H, Murugan R, Hong X, Xu D, et al. Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis. Oxid Med Cell Longev [Internet]. 2019 [cited 2024 Mar 9];2019. Available from: https://pubmed.ncbi.nlm.nih.gov/31737171/
33. Wang Z, Zhang X. Chemopreventive Activity of Honokiol against 7, 12 - Dimethylbenz[a]anthracene-Induced Mammary Cancer in Female Sprague Dawley Rats. Front Pharmacol [Internet]. 2017 May 31 [cited 2024 Mar 9];8(MAY):320. Available from: /pmc/articles/PMC5450001/
34. Hao C, Zhang G, Zhang L. Serum CEA levels in 49 different types of cancer and noncancer diseases. Prog Mol Biol Transl Sci [Internet]. 2019 Jan 1 [cited 2024 Mar 9];162:213–27. Available from: https://pubmed.ncbi.nlm.nih.gov/30905451/
35. Gali Kiranmayi, G. Ramakrishnan, R. Kothai and BJ. In-vitro anti-cancer activity of the methanolic extract of the leaves of Argemone mexicana Linn. Int J PharmTech Res [Internet]. 2011;3(3):1329–1333. Available from: https://www.cabidigitallibrary.org/doi/full/10.5555/20113280498
36. Manna S, Holz MK. Tamoxifen Action in ER-Negative Breast Cancer. Sign Transduct Insights [Internet]. 2016 Feb 2 [cited 2024 Mar 9];5:1. Available from: /pmc/articles/PMC4792287/
37. Karnam KC, Ellutla M, Bodduluru LN, Kasala ER, Uppulapu SK, Kalyankumarraju M, et al. Preventive effect of berberine against DMBA-induced breast cancer in female Sprague Dawley rats. Biomed Pharmacother [Internet]. 2017 Aug 1 [cited 2024 Mar 9];92:207–14. Available from: https://pubmed.ncbi.nlm.nih.gov/28544934/
38. Kulshrestha S, Goel A, Siddiqi NJ, Fatima S, Sharma B. Cancer-Preventive Activity of Argemone mexicana Linn Leaves and Its Effect on TNF-α and NF-κB Signalling. Cancers (Basel) [Internet]. 2023 Dec 1 [cited 2024 Mar 9];15(23). Available from: /pmc/articles/PMC10705811/