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Anticancer Activity of *Argemone mexicana* L. Leaves Methanol Extract against Chemically Induced Mammary Tumours in Wister Rats

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ABSTRACT: Breast cancer is the most common malignancy in women, accounting for 31% of all female cancers around the world. Most of the anticancer drugs are associated with adverse side effects and development of multidrug resistance. Numerous studies have reported that plants extracts and phytochemicals isolated from plants are potent biological agents with anticancer properties. The whole plant of *Argemone mexicana* is traditionally used to treat the tumours, inflammation, skin conditions, jaundice, dysentery etc,. Being several phytochemicals have received interest of its antiproliferative and anti-tumour activities. In our study, we have investigated the anticancer activity of *Argemone mexicana* l. leaf (family: papaveraceae) against DMBA(7,12 dimethylbenz[a]anthracene) induced mammary tumors in wistar rats. We have used standard methods of soxhlet methanol extract of *Argemone mexicana* and phytochemicals analysis by GCMS. The drug was administrated orally to tumour induced rat in respective of 200mg, 400mg, which were compared with tumour control and standard drug (Temoxifen 10mg/kg/b.w) treated groups. At the end of experimental period rats were sacrificed with anesthesia, tumour tissues were collected and fixed in 10% buffered formalin for histopathological studies. The results were calculated as mean ± SD of six animals of all groups. The statistical analysis was done by using one-way ANOVA. P value was <0.05, considered as statistically significant. Histopathology, drug treated groups showed marked improvement in cellular architecture, when compared to untreated groups. 400mg/kg treated groups increased cellular morphology and mild pleomorphic changes like standard drug treated groups. From the results of study, we have concluded that methanol extract of *Argemone mexicana* might have anti-cancer effect by increasing apoptosis, preventing oxidative stress and proliferation, thus, the study needs further confirmation to be used as an alternative treatment for breast cancer. Definitely these herbal extract can be a novel contribution to the people against breast tumours.

Keywords: Breast cancer, *Argemone mexicana* L., DMBA, GCMS analysis.

INTRODUCTION

Cancer is the uncontrolled proliferation of abnormal cells due to lack of apoptosis and which spreads to other organs which is related to multifactorial genetic problem in developed and under developing countries in worldwide (Akhouri *et al.*, 2020). Cancer is the second common disease next to the cardiovascular and diabetes diseases. Particularly breast cancer is detected frequently in women even men can get too; accounting for more than one in ten new cancer diagnosis each year in females. As per WHO, in 2020 there was 2.3 million women diagnosed with breast cancer and 6,85,000 deaths globally (https://www.who.int/news-room/fact-sheets/detail/breast-cancer). Mammary gland is the organ present in superficial fascia of pectoral region, it has lactiferous ducts, fibrous tissues and fatty substance. The anatomical location cancer may various in terminal duct units like in-situ ductal carcinoma, accumulation of cancer cells within the ducts. Ductal forming invasive carcinomas are branching, cancer cells filled ducts like massive tumour developed. Next to this infiltrating lobar carcinoma, develops from surrounding structures of lactiferous ducts, which ma compress the ducts, but intra-ductal lining cells are normal cell (Tabár *et al.*, 2022).

The risk factors of breast cancer are age, genetics, estrogen exposure, lack of breastfeeding, hormone treatments, radiation exposure and other lifestyle modification increases the risk (Antony, 2018). Must include other environmental factors like smoking, synthetically used preservatives added to the food, pesticides reached the human through food chain and

smoke from automobile fuels, burning carbon based materials and industrial fumes are composed of polycyclic aromatic hydrocarbons (PAHs) (Plaza-Bolaños *et al.,* 2010) (Adeyeye *et al.,* 2020) (Sampaio *et al.,* 2021) From above PAHs can enter the human body through various routes, PAHs found to be carcinogenic, mutagenic and potent immunosuppressive effects (Rengarajan *et al.,* 2015). Dimethyl benzanthace (DMBA) is one among the PAHs group, commonly used carcinogenic in experimental animals. The breast cancer model in rats are similar to breast cancer in humans with histopathological features like estrogen dependent breast cancer and tumour progression (Rengarajan *et al.,* 2015; Karimi *et al.,* 2019; Hamza *et al.,* 2022).

In India, lot of medicinal plants are available with less toxicity and cheaper, it is called Botanical Garden of the world, taught many alternative medicines and meditation to the world (Krishnamoorthy & Sankaran 2016). The medicinal plants with its secondary metabolites like polyphenolic, alkaloids and flavonoids have been reported to have properties of anti-mutagenic, anti-proliferative effects. In our study we have investigated the anticancer effect of *Argemone mexicana* leaves against DMBA induced mammary tumous in Wister rats.

MATERIAL AND METHODS

***Argemone mexicana*.** *Argemone mexicana* (Papaveraceae family), common called as Mexican prickly poppy principally American origin, now it's available worldwide. In India, it's periodic plant, flowers from May-November and fruits from July-January of ever time, substantially in tropical and tropical areas. It's spinous in stem, leaves, seeds with unheroic lactiferous juice and yellowish candescent flowers. The whole plant of *Argemone mexicana* is traditionally used to treat the tumours, inflammation, skin conditions, jaundice, dysentery etc., (Simoes-Pires *et al.,* 2014; Pathak *et al.,* 2021; Arokiyaraj *et al.,* 2013; Orozco-Nunnelly *et al*., 2021; Monika *et al.,* 2020).

Alkaloids and Flavanoids are the major factors of this plants. Being several alkaloids, berberine is the main elements of *Argemone mexicana*, which converting autophagic vacuoles and accumulates in cellular cytoplasm. Which has been reported to contains anti-inflammatory, anti-cancer, anti-microbial, crack mending, hepatic-defensive, anti-anxiety, and anti-diabetic activities (Gali *et al.,* 2011; Elizondo-Luévano *et al.,* 2021; Habtemariam *et al.,* 2020; Desai *et al.,* 2014).

Collection and Identification of Plant: *Argemone mexicana* leaves were collected from townlets of Perundurai taluk, Erode quarter Tamilnadu in January to June in 2021 and 2022. The plant was authenticated by Dr. Sherif from botanical survey of India, Coimbatore. Survey no is BSI/ SRC/5/23/2021/ Tech/ 260 and plant material stored in institution natural herbarium.

Preparation of plant for extraction: Fresh and healthy *Argemone mexicana* L. plants leaves were collected and washed, dried on paper tissue at room temperature for a week; leaves come flexible and spines on under exterior of leaves were get soft. After drying, plant leaves were grounded individually to make crushed by using mortar and pestle techniques (AI-Shehri *et al.,* 2019).

Collection of extract from the plant was done by using the system followed by earlier studies (More *et al.,* 2016). Dried and coarsely grounded leaves were extracted by soxhlet apparatus with methanol. There after it was assayed by phytochemical screening and GC- MS with the dilution of 1mg/ ml of methanol.

Phytochemical analysis:

Primary screening tests. Standard Phytochemical screening tests were carried out using a traditional protocol and reagents on the methanolic extracts of *Argemone mexicana* leaves as described by Ugochukwu (Ugochukwu *et al.,* 2021).

Then we've followed for Alkaloids (Wagner's reagent), Carbohydrates (Molisch's test), Flavonoids (Alkaline reagent test), Phenols (Ferric chloride test), Amino acids and Proteins (1 ninhydrin result in acetone), Saponins (Foam test), Tannins (Braymer's test), Cardiac glycosides (Keller Kelliani's test). Testing for presence of phytochemicals in the plant extract would have been a substantiation for their eventuality as well as to do the coming stage of analysis.

Gas Chromatography Mass Spectroscopy (GCMS).

GC-MS analysis of these extracts was carried out by following the methodology of Hema *et al*. (2010) 26 on methanol extract. Chromatograph associated to a mass spectrometer (GC- MS – Perkin- Elmer) equipped with Elite-1, fused silica capillary column (30 m ×0.25 mm × 1 m df, composed of 100 Dimethyl poly siloxane). For GC/ MS finding, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999) was used as the carrier gas at constant inflow rate of 1 ml/ min and an injection volume of1.5 ml/ min was employed (Split rate of 101) injector temperature 250°C; ion- source temperature 280 °C. The microwave temperature was programmed from 110 °C (isothermal for 2 min) with an increase of 10 °C/ min to 2000 °C, likewise 5 °C/ min to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70eV; a check interval of 0.5 seconds and fractions from 45 to 450 Da. Total GC running time was 36 minutes. The relative chance quantity of each element was calculated by comparing its average peak area to the total areas, software espoused to handle mass spectra and

chromatograms was a Turbo mass (Mass spectra with inbuilt libraries NIST (National Institute of Standard and Technology-0.8 L database and wiley- 8 library).

Animal experiment. Healthy female Sprague Dawley (SD) rats (5-6 weeks old) weighing 80-100g were used for this study. The study was approved by Institutional Animal Ethical Committee (IAEC) of JKK Nataraja Educational Institution, Kumarapalayam, Namakkal district and proposal number is JKKN/IAEC/Ph.D./04/2021. After getting the ethical clearance certificate we procured the rats from biogen Laboratory Animal Facility (CPCSEA Reg No: 971/PO/RcBt/S/2006/CPCSEA), Bangalore-562107. After two weeks of accustom, all the acute toxicity procedures and animal care were followed as in guideline of OECD-423.

EXPERIMENTAL DESIGN

Animals were divided into five groups (6 animals / group = 30). Sample size is calculated by based on ANOVA, E (the degree of freedom which must be between 10 and 20) = Total number of animals – Total number of groups (Ilyas *et al.,* 2017).

Group I: Control- received only normal saline (no DMBA & treatment)

Group II: Tumor Control- Tumor induced rats (Rats with a single subcutaneous injection of 7,12 - Dimethylbenz (a) antharacene (DMBA) 25mg/kg dissolved in 1ml of (0.5ml of sunflower oil and 0.5ml of saline) given at the space between 3-4th mammary papillae.

Group III: Treatment group– Rats treated with DMBA after the palpable tumour were developed then treated with *Argemone mexicana* L. leaf extract 200 mg/kg/b.w for 12 weeks administered orally.

Group IV: Treatment group – Rats treated with DMBA after the palpable tumour were developed then treated with 400mg/kg/b.w of *Argemone mexicana* L. leaf for 12 weeks administrated orally.

Group V: Treatment group – Rats treated with standard drug Tamoxifen at 10 mg/kg/b.w for 12 weeks administered orally.

At the end of the experimental period, all the rats were sacrificed by cervical dislocation, tumorous tissues were removed, measured the weight and calculated the volume later stored in 10% buffered formalin for histopathological study.

Morphological changes: Mammary tumours were excised from the rats after the experimental period, removed the of debris, weighed in electronic balance, and tumours were measured in two dimensional diameter by Vernier caliber and calculated the volume of the tumour (Xia *et al.,* 2020).

Histopathology study: Formalin fixed tissues were dehydrated with graded alcohol from 50, 70, 100% then embedded in paraffin wax. Sections were cut 7µm thickness in microtome and followed the route H&E staining procedure as mention in (Suvarna *et al.,* 2018) investigated under light microscope.

Statistical analysis: The results were calculated as mean ± SD of six animals of all groups. Statistical analysis done by following one-way ANOVA. P value was <0.05, considered as statistically significant.

RESULTS

Identification of various phytochemical from methanol extract of *Argemone mexicana* leaves was done by standard procedures of different tests for Alkaloids, Carbohydrates, Flavonoid, Phenols, Amino acids and Proteins, Saponins, Tannins, Cardiac glycosides. Table 1 showed that presence and absence of different compounds from the extract of *Argemone mexicana*. It was found that positive of alkaloids, flavonoids, phenols, protein and aminoacids, saponins and tannins and found negative for carbohydrates, Cardiac glycosides, sterols, terpenoids. Khan reported that total quantification of flavonoids and phenolic compounds were higher in leaves of *Argemone mexicana*, which may primary responsible for its biological activities (Khan and Bhadauria 2019).

Compounds identified by GCMS: The Gas Chromatography Mass Spectrometer (GCMS) analysis of methanol extract of *Argemone mexicana* leaves was done by interpretation with the similarity of NIST (National Institute Standard and Technology) database. The highest similarity of database is identified as final compound, based on molecular weight, molecular formula, concentration and retention time. Fig. 1 shown peaks in GCMS analysis of *A. mexicana* leaves reveals that presence of several compounds. In Table 2 based on review of literature, we have listed the major compounds of *A. mexicana* are 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, n-Hexadecanoic acid(palmitic acid), Phytol, 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-, Hexadecanoic acid, methyl ester which have been reported with antimicrobial, antioxidant, anti-cancer and cytotoxic against MCF-7 cell lines and its other biological activities (Kiran *et al.,* 2017; Ponnusamy *et al.,* 2018; George and Mohammed 2018).

Treatment impact on Morphology of tumour: After the administration DMBA, the first palpable tumour has developed at 9th week for 4 rats, 2 weeks later remaining animal had developed palpable tumours. Tumour incidence has increased with time, at the end of the experiment almost 28 rats developed (93%) mammary tumours. The tumour location as in where the carcinogen had injected at inguinal region, and histology of mammary tumours reveals that 100% mammary tumour development and from the developed

mammary tumours were identified as fibro-adenoma (5/28) and invasive carcinoma (23/28) with ulcerated skin, invading tumours contained large area of necrosis and haemorrhage.

Table 1: Phytochemical Screening of Methanol Extract of *Argemone mexicana* L. Leaves.

Sr. No.	Phytochemical Test	Methanol extract of *Argemone mexicana. L*
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)	-

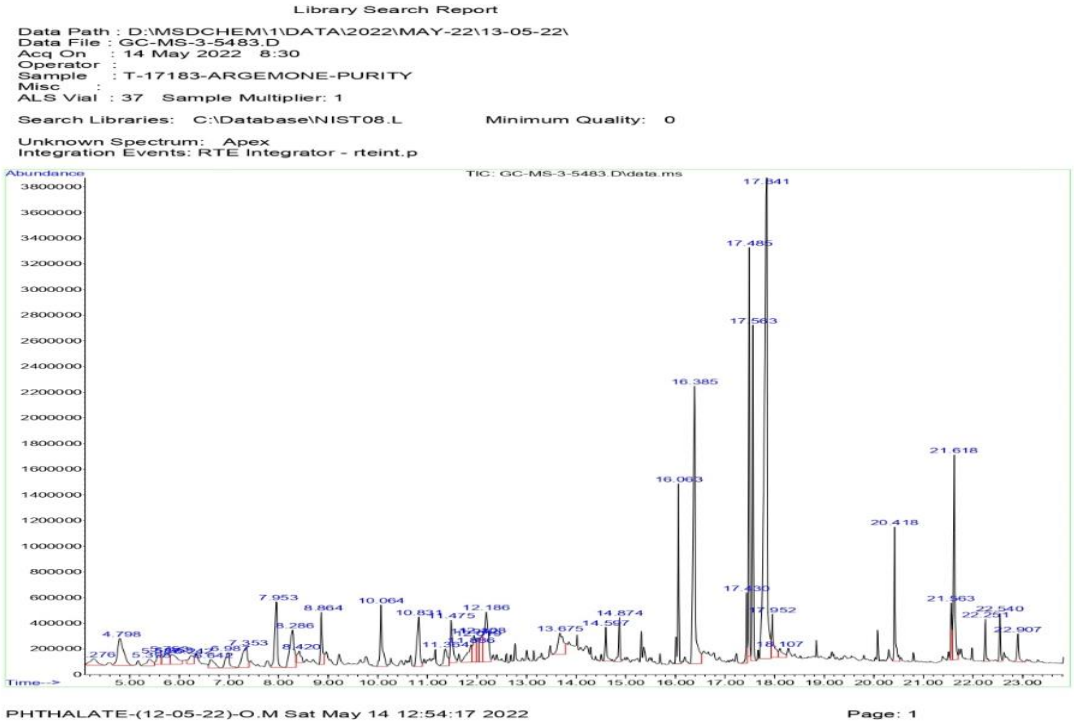

Fig. 1. GCMS results of Methanol Extract of *Argemone mexicana* L. Leaves.

Table 2: List of vital components and its biological activity from results of GCMS.

Sr. No.	Retention Time (Min)	Name of the Compound (Name of Peaks)	Molecular formula	Molecular weight (g/mol)	Area of peak (%)	Biological activity
1.	4.798	3-Thietanol	C_3H_6OS	90.15	2.64	Antifungal activity against dermatophytes
2.	7.353	Butanedioic acid, monomethyl ester	$C_5H_8O_4$	132.1	1.75	Antihyperlipidemic property and Antiperoxidative effect
3.	7.953	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144.1	2.7	Antioxidant, Ameliorative effect on reproductive system
4.	8.286	Benzoic acid	C_6H_5COOH	122.1	2.7	Antifungal activity, Inhibiting cancer

						cell growth, Benzoic acid derived protocatechuic acid inhibit the breast cancer cell.
5.	8.864	Benzofuran, 2,3-dihydro	C_8H_8O	120.1	1.64	Anti-inflammatory, Anti-hypertensive agent, Anti-HIV activity
6.	10.064	2-Methoxy-4-vinylpheno	$C_9H_{10}O_2$	150.1	2.16	Potent Anti-inflammatory, Anti-cancer effect by inhibiting proliferative cell nuclear antigen, suppress the NF-kB, anti-microbial properties.
7.	10.831	N-Ethyl-2-carbomethoxyazetidine	$C_9H_{17}NO_2$	171.2	2.2	Potential Anti-oxidant property, cytotoxic activity
8.	11.475	3H-Cyclopenta[c]pyridazin-3-one, 2,5,6,7-tetrahydro	$C_7H_8N_2O$	136.1	1.62	Anti-tumour activity, cytotoxicity against cancer cells
9.	12.186	Piperidine, 1-methyl-	$C_6H_{14}CIN$	135.6	3.19	Cytotoxic potential against tuomur cells
10.	13.675	Isosorbide Dinitrate	$C_6H_8N_2O_8$	236.1	1.94	Effective on controlled severe pulmonary edema, vasodilator improving blood flow to the tumour
11.	16.063	Hexadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	284.5	3.33	Antioxidant, anti-inflammatory activity and antimicroial property, cytotoxic activity
12.	16.385	n-Hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$	256.4	10.55	Cytotoxic potential by inhibiting DNA topoisomerase-I, antimicrobial, anticancer, antioxident, cancer preventive.
13.	17.430	9,12-Octadecadienoic acid (Z,Z)-, methyl ester, 10,13-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.5	1.43	Antitumour activity, hepatoprotective
14.	17.485	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.5	6.3	Anti-inflammatory, anti-microbial activity
15.	17.563	Phytol	$C_{20}H_{40}O$	296.5	5.15	Cytotoxic activity against MCF-7 cell lines, antinociceptive effect, Anticonvulsant effect,
16.	17.841	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278.4	22.28	Anti-inflammatory, cancer preventive, antimicrobial activity
17.	17.952	Octadecanoic acid(stearic acid)	$C_{18}H_{36}O_2$	284.5	1.42	Antitumour activity, antimicrobial activity, anti-oxidant
18.	20.418	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	330.5	2.68	Antioxidant, antibacterial activity
19.	21.563	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{38}O_4$	354.5	1.34	Hepatoprotective, cancer preventive, Anti-inflammatory involved in lipid peroxidation processes
20.	21.618	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$	264.4	4.48	Antioxidant, anticancer activity, antimicrobial, anti-inflammatory and anxiolytic activity

Table 3: Mean values of animal weight, tumour number, weight and volume.

Experimental Groups	Changes in Animal body weight			Parameter				
	Initial body weight	Final body weight	Weight gain	Number of animals with tumour/n	Number of tumours/ groups	Tumour incidence (%)	Mean tumour weight (g)	Mean volume (cm^3)
Group I	94.16±9.17	158.33±6.83	64.17±2.34	0/6	0	0	0	0
Group II	120.5±10.32	165.83±14.63	45.33±4.31	7/8	15	87.5	4.35±2.26	4.75±0.64
Group III	115.83±8.7	185.83±12.11	70±3.41	8/10	10	80	3.21±1.26	3.62±0.32
Group IV	118±10.80	193.33±13.66	75.33±2.86	7/9	8	77.7	2.98±1.43	2.66±0.45
Group V	119±11.83	195.16±12.51	76.16±0.68	6/8	6	75	2.16±0.72	1.95±0.18

Table 3 shown in the mean body weight of animal and tumour number, weight, volume. At the end of experiment, rats were sacrificed and weighed above the measurements. Body weight of the rats compare to the normal control (Group-I), tumour control (Group-II) animal weight has decreased, but compare to the treated groups (Group-III, IV) which had increased the animal weight nearly standard drug treated groups (Group – V).

Next compare to the tumour control (group-II) with treated groups, it has been reported that increases of tumour incidence, weight and volume of the tumour, there was a decrease in tumour incidence, weight and volume of treated groups.

Histological results of mammary tumours: Fig. 2: shows histological changes: A. Goup-I, several lobules within the dense connective tissue with adipose tissue of the normal mammary gland. B. Group-II, nests of invasive ductal carcinoma cells invading to stroma and irregular cords of cells. C. Group-III & IV, Mild epithelial hyperplasia and an increased fibrous stroma. D. Group-IV, moderately cellular stroma, and benign looking glandular elements lined by cuboidal to low columnar epithelium with round to oval nuclei.

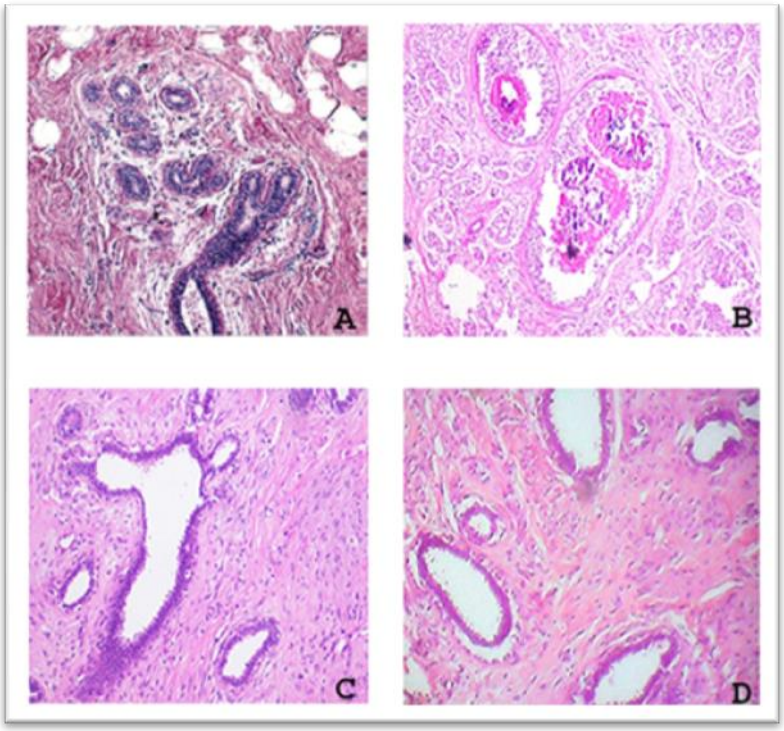

Fig. 2. Histological results of mammary tumours (H&E,40X).

DISCUSSION

Results of *Argemone mexicana* L. leaves methanol extract phytochemical screening showed that positive and negative compounds in different color changing and precipitation tests, which reveals presence of biologically active secondary metabolites like alkaloids, flavonoids, phenols, protein and amino acids, saponins and tannins, which might be a primary reason for considered as *A. mexicana* is a novel medicinal plant. The peaks of GCMS results shown that 41-active vital compounds were present in *A. mexicana* leaves extract, the reported major compounds are 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, n-Hexadecanoic acid(palmitic acid), Phytol, 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-, Hexadecanoic acid, methyl ester which have been already reported with antimicrobial, antioxidant, anti-cancer and cytotoxic against MCF-7 cell lines in different plants including anti-cancer and cancer preventive (Jabbar *et al.*, 2022). From the results methanol extract of *Argemone mexicana* might have strong productive and pharmaceutical properties.

Morphological changes of *Argemone mexicana* extract treated group (III&IV) reveals that ulcerated skin with no undergrown tumours, compared with tumour control (group-II) which shows bulky underneath tumour in the ulcerated skin. There was in marked decrease in tumour weight and volume in the *A. mexicana* crude extract treated groups compare to tumour control DMBA alone treated animals. *A. meicana* crude extract treatment started at palpable tumour had developed after 8-10 weeks of DMBA. The results of morphological changes reveals that *A. mexicana* affects the DMBA induced tumours, in both the doses level, which was 400mg/kg/b.w showed better improvement compare to

200mg/kg dose, nearly similar to standard drug (tamoxifen 10mg/kg/b.w). H&E stained histopathological results of mammary tumours from experimental animals shown that DMBA alone treated group increased pleomorphic cells with invasive ductal carcinoma when compare to normal control group (Arivazhagan *et al.*, 2014). *Argemone mexicana* L leaves treated group compared with tumour control, improving cellular morphology, mild pleomorphic nuclei, stroma with inflammatory cells. It reports that similar to Group IV (treated with standard drug) shows that improvement in glandular elements with round to oval nuclei (Perry *et al.*, 2016).

CONCLUSION

In conclusion, methanol extract of *Argemone mexicana* leaves consist of several active compounds with anti-microbial and anticancer effects. Because of its medicinal properties, *A. mexicana* leaves extract treated animals may shows that decreased tumour volume and weight, morphologically less proliferating tumours, no invade growth and ulcer on the skin. Extract treated rats mammary tumus with cellular improvement in the histopathological study compare to tumour control. From our study, *Argemone mexicana* leaves extract in low cost, as well as ability to regress the tumour growth and improve the cellular morphology almost similar to standard drug. Therefore, *Argemone mexicana* leaves may have pharmaceutical properties and medicinal values but the therapeutic effect and mechanism of action needed further investigations.

FUTURE SCOPE

In future, this study, can be continued in experimental view for isolation of its individual compounds, mechanism of action and molecular docking like additional research necessary for its effect on tumour cells.

Conflict of interest. None

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