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Anti-cancerous Effect of *Amomum subulatum* Against DMBA Induced Breast Cancer in Rats

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Abstract

The breast cancer incidences have increased many folds globally in the recent times. Female cancer patient around the world is more deceased than the male patients. Plethora of drugs have been discovered and by the advancement of medical treatment, it still remains associated with high mortality rate. *Amomum subulatum* (L.) has been extensively used in Indian medicine system Ayurveda, due to its various medicinal properties. It is the most common shrub found in the Indian subcontinent. However, there are very limited reports regarding its anticancer activity. Thus, the present study has been aimed to study the anticancer activity of *Amomum subulatum* seed extract on 7, 12-dimethylbenz(a)anthracene (DMBA) induced breast cancer in rats. Female Charles Foster rats, 55 – 60 days old weighing around (150±10 g) were used for the study and were induced DMBA (20 mg/mL dissolved in Olive oil) orally. After the development of breast tumours (about 0.5 cm), the rats were treated with *Amomum subulatum* ethanolic seed extract (150mg/Kg b.w./day) orally for 5 weeks and then volume of tumour was measured. *Amomum subulatum* treatment showed significantly reduced mammary tumour volume ($p < 0.05$), along with significant reduction ($p < 0.0001$) in the different serum biomarker such as TNF- α level and serum malondialdehyde (MDA) levels. Significant ($p < 0.0001$) improvement in both, the kidney and liver serum biomarker parameters were observed after the treatment with *Amomum subulatum* ethanolic seed extract. From the entire study, taking everything into account it can be interpreted that *Amomum subulatum* ethanolic seed extract possesses anti-cancerous activity by suppressing the progression of breast tumours in the rat model. The plant extract also possesses hepato-renal protective effect. Hence, it can be targeted as novel and safe anti-cancer drug against breast cancer.

Keywords: DMBA induced breast model; Tumour volume; TNF alpha; Seed extract of *Amomum subulatum* novel drug discovery.

Introduction

Cancer is the second leading cause of death in the globe. About one-sixth of all fatalities in the world are caused by cancer. The death rate from breast cancer is the highest of all female cancers. In 2020, about 11.7 percent of all new instances of cancer diagnosed among women will be breast cancer (2,261,419), and approximately 6.9 percent of all cancer-related deaths among women will be breast cancer (684,996). In 2018, this number is projected to rise to 2.1 million. According to the most recent statistics from the Global Cancer Report, breast cancer accounts for 14% of all new cancer cases in India (1,62,468 cases) [1-4]. Breast cancer risk factors include genetic and

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reproductive factors, long-term estrogen exposure, never breastfeeding, and other lifestyle choices. Environmental factors are also suspected of contributing to the development of breast cancer. Many diseases, including breast cancer, are on the rise, and this trend may have its roots in people's bad dietary habits and the low quality of the food they eat. Today's farmers increasingly depend on pesticides as a method of raising agricultural production. However, these herbicides and food preservatives include xenoestrogens. These synthetic xenoestrogens are interfering with the endocrine system, which increases the risk of breast cancer. Grilled, barbecued, and smoked meat all contain a carcinogen called polycyclic aromatic hydrocarbons (PAHs), which has been linked to an increased risk of breast cancer. The PAHs are pro-carcinogens that, after undergoing a series of processes mediated by cytochrome p450 enzymes in the body, become active carcinogens. [5-9]. Due to these variables and the ever-evolving lives of modern women, the incidence of breast cancer is on the rise. Rapid advances in contemporary medicine have allowed for better cancer treatment options such chemotherapy, radiation therapy, and the development of a wide range of innovative anticancer drugs. Although these therapies are excellent at prolonging the lives of cancer patients, they are not without their own set of side effects. The toxic effects of chemotherapy drugs are felt most acutely by the liver and the kidneys. Therefore, there is an urgent need to find and develop cancer therapies that are less expensive, more effective, and safer. Animal studies suggest that herbal medications and nutritional supplements may be very effective as chemo-preventive and anticancer therapies with tolerable side effects (Gharia et al; Miller et al; Li et al). *Amomum subulatum* or Black Cardamom is a spice crop cultivated in the central Himalayan and north-eastern area of India. It is a popular spice that has been utilized in Ayurvedic medicine from ancient times for its curative properties. The antioxidant properties of black cardamom aid in diabetes management by reducing blood glucose levels. Its antioxidant content also aids in decreasing cholesterol levels, which in turn reduces the risk of cardiovascular disease. Cough and cold symptoms are alleviated, and excess mucus in the airways is cleared. Black cardamom essential oil's anti-inflammatory qualities make it useful for treating a variety of inflammatory diseases. The anti-oxidative characteristics of the perennial plant black cardamom have been shown to have positive effects on many aspects of human health, including those of the skin, hair, and body [10-16]. Meager research has been carried out on breast cancer models; hence the present study aims to investigate the anti-cancerous effect of seed extract of *Amomum subulatum* on DMBA-induced breast cancer in rats.

Materials and Methods

Chemicals and reagents

The 7, 12-dimethylbenz(a)anthracene (DMBA) was procured from the Scientific chemical shop in Patna, Bihar, India; the product number was D3254-1G (CAS number: 57-97-6), and the lot number was PXLNG2901. The product code was 1009330344. The 99% purity of all other compounds and solvents used in this analysis was maintained.

Ethanolic extract preparation of *Amomum subulatum* seeds

Amomum subulatum seeds were gathered from a garden in Patna, Bihar, India. A well-known botanist in Patna, Bihar, India, was able to identify the seeds. The seeds were air dried at 37 degrees Celsius after being shade dried for three days. After that, the seeds were powdered and steeped in 100% ethanol for a whole day and night. Filter paper was used to remove any leftover particles from the ethanolic mixture of seeds. The filtrate was then placed in a rota vapor apparatus, where it was extracted using absolute ethanol. The dosage of ethanolic extract was determined after the LD50 value was calculated (2100 mg/Kg body weight), and the final dose was titrated to 150mg/Kg body weight.

Experimental Animals

Twenty-four female Charles Foster rats were provided by the Mahavir Cancer Sansthan and Research Centre's animal house in Patna, India (CPCSEA Registration no. 1129/PO/ReBi/S/07/CPCSEA). The research investigations were approved by the Institutional Animal Ethics Committee (IAEC) with protocol number 2021/1H-06/10/21. All of the animal experimentation methods were carried out in accordance with the regulations established by the Committee for the Protection and Control of Experiments on Animals (CPCSEA), New Delhi. Food and water were made available to the rats at ad libidum. Before beginning the research, the rats were acclimated for seven days. Two rats were housed in each standard polypropylene cage for the studies. The rats were randomly allocated to either a control or treatment group. The rats were housed in a room with a constant temperature (24 2°C) and a light/dark cycle of 12 hours.

Design of the Study

Female rats of the Charles Foster strain (n=24) were split into three groups of six individuals, based on their ages (55-60 days) and weights (150-20 g).

Group I- Control group.

Group II- DMBA group - DMBA induced rats only.

Group III- DMBA + *Amomum subulatum* group – DMBA-induced rats treated with *Amomum subulatum* ethanolic seed extract (150 mg/kg body weight per day) for 5 weeks after tumour development (about 0.6 cm).

At the completion of the treatment, the rats were made unconscious with ketamine and sacrificed during the diestrous phase of their estrous cycle. In order to collect blood for analysis, the orbital puncture method was used. Serum was

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analyzed biochemically, inflammatory markers, and lipid peroxidation were measured. Breast tissues were fixed in 10% formalin for histological examination.

Tumour induction

Mammary gland tumours were induced in female Charles Foster rats (150± 10 g). About 55 days of age, these rodents were utilized for the study. A single dose of 7,12-dimethylbenz(a)anthracene (DMBA) dissolved in olive oil at a concentration of 20 mg/mL was delivered intragastrically, as per the methodology of [17]. Twenty rats were palpated weekly beginning in the fourth week after DMBA injection to monitor tumour progression. After 20 weeks, every single one of the 18 DMBA-treated rats had developed tumours. At week 19, the first tumour was discovered.

Evaluation of mammary tumour volume

Breast tumour volumes were measured using a vernier caliper. Where L and B are the perpendicular tumour diameters in centimetres (cm), we were able to calculate the tumour's volume (V) using the formula V(cm3) = (L B2)/2.

Hematological parameters study

The obtained blood samples were examined using standard procedures to assess haematological parameters such as complete blood count, white blood cell count, platelet count, and haemoglobin percentage.

Biochemical assays

Biochemical analysis was performed using a UV - Vis spectrophotometer (UV-10, Thermo Scientific, USA) in accordance with the established kit method (Coral crest). Alkaline phosphatase (ALP) was measured using the method developed by [18], total bilirubin was measured using the method developed by [19], and Kidney biomarkers urea, creatinine, and uric acid were analyzed using various techniques [20-23].

Lipid peroxidation (LPO)

TBARS, a biomarker of LPO, was evaluated using the two-step heating approach [24], which relies on the spectrophotometric evaluation of color reproduction during the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA). A 10% trichloroacetic acid (TCA) solution was added to 0.5 mL of serum in a centrifuge tube, and the mixture was heated in a water bath at 90°C for 15 minutes. The combination was centrifuged at 3000 rpm for 10 minutes after chilling at ambient temperature, and the resultant 2 mL supernatant was mixed with 1 mL of 0.675% TBA solution in a test tube before being heated in a water bath at 90°C for 15 minutes. Then, we measured the spectrum at 532 nm using a UV-visible spectrophotometer (a Thermo Scientific UV-10 USA).

Tumour necrosis factor-alpha (TNF- α) assay

The ELISA method was used to determine serum TNF- levels. A rat TNF- ELISA kit (Cat. No. 872.010.001) was manufactured by the French firm Diaclone. The blood TNF- level was determined using a Merck ELISA reader in accordance with the manufacturer's instructions and the published literature [25].

Histopathology study

Breast tissue from rats was removed, sectioned, and preserved in 10% formalin for a full day. After being dehydrated in ethanol, the tissues were embedded in paraffin. Sections of tissue 5µm thick were cut and stained with haematoxylin and eosin for histological analysis.

Statistical analysis

The information is shown as a mean and its associated standard error of the mean (SEM). Tumour volume was compared between the DMBA and DMBA + *Amomum subulatum* groups using a two-way analysis of variance (ANOVA) with time and medication as the two factors. One-way analysis of variance (ANOVA) and Tukey's multiple comparison tests were used to evaluate the statistical significance of the differences between the groups with regards to biochemical, LPO, and hormonal testing. GraphPad Prism 5 (GraphPad Software, Inc., San Diego, USA) was used for the analysis, and a significance level of $p< 0.05$ was used for the statistical tests.

Results

Morbidity and mortality

There was tumour growth within mammary teats 1, 3, and 7 in all six DMBA-exposed animals. Tumour development in the remaining six rats from teats 1, 3, and 6 was greatly slowed in the DMBA + *Amomum subulatum* group. There were no reported fatalities in any of the groups. Graphical representation of the DMBA group and the DMBA group+ *Amomum subulatum* showed significant regression (Figure 1).

Evaluation of Tumour volume:

Both the DMBA and the DMBA + *Amomum subulatum* groups had their tumour volumes grow with time. Tumour volume in DMBA-induced rats was decreased by a statistically significant amount ($p< 0.005$) when *Amomum subulatum* ethanolic seed extract was administered with DMBA, as shown in Figure 2. The final tumour volume was reduced by 39% because of the *Amomum subulatum* seed extract.

Evaluation of malondialdehyde (MDA) level:

Malondialdehyde (MDA), a marker of lipid peroxidation, was found to be significantly higher in the DMBA group compared to the control group ($p <0.05$). However, the MDA

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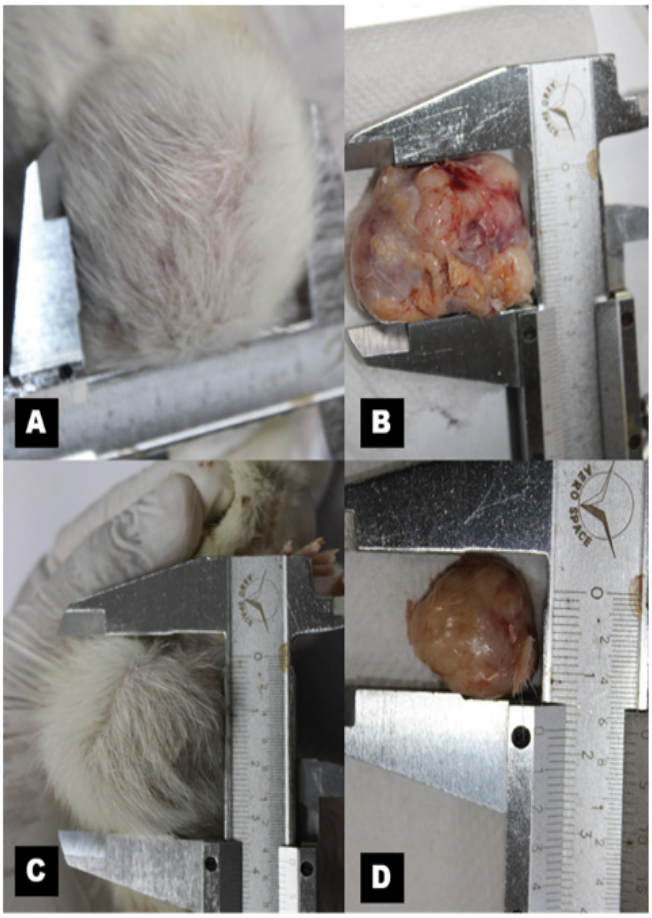

Figure 1: Images 1A and 1B show tumours in rats treated with DMBA alone (20 mg/mL in olive oil) whereas images 1C and 1D show tumours in rats treated with DMBA plus Amomum subulatum. After a tumour reached 0.6 cm in diameter, Amomum subulatum (at a dosage of 150 mg/Kg body weight each day) was given for 5 weeks.

level in the DMBA + *Amomum subulatum* group reduced significantly ($p < 0.05$) compared to the DMBA group (Table 1).

Evaluation of TNF- α levels

There was a statistically significant increase in serum TNF-alpha between the DMBA-treated group and the control group ($p<0.05$). The blood TNF-alpha level was decreased in the DMBA + *Amomum subulatum* group compared to the DMBA group ($p<0.05$) (Table 2).

Evaluation of haematological parameters

The haematological analysis revealed that the DMBA-treated rats had significantly lower levels of red blood cells, white blood cells, platelets, and haemoglobin percentage than the control group rats, whereas the *Amomum subulatum* seed extract-treated rats had significantly restored levels to normal ($p<0.05$) (Table 3).

Changes in liver and kidney serum biomarker parameters

In comparison to the control group, the DMBA group had significantly increased serum total bilirubin, ALT, and ALP levels ($p<0.05$). When compared to the DMBA group with the DMBA + *Amomum subulatum* group, total bilirubin, ALT, AST, and ALP levels in the serum were all significantly lower in the DMBA + *Amomum subulatum* group ($p<0.05$; Table 4). The DMBA group had substantially ($p<0.05$) greater serum creatinine, urea, and uric acid levels than the control group, indicating kidney impairment. In comparison to the DMBA group, serum urea and uric acid levels were significantly lower in the DMBA + *Amomum subulatum* group ($p<0.005$) (Table 4).

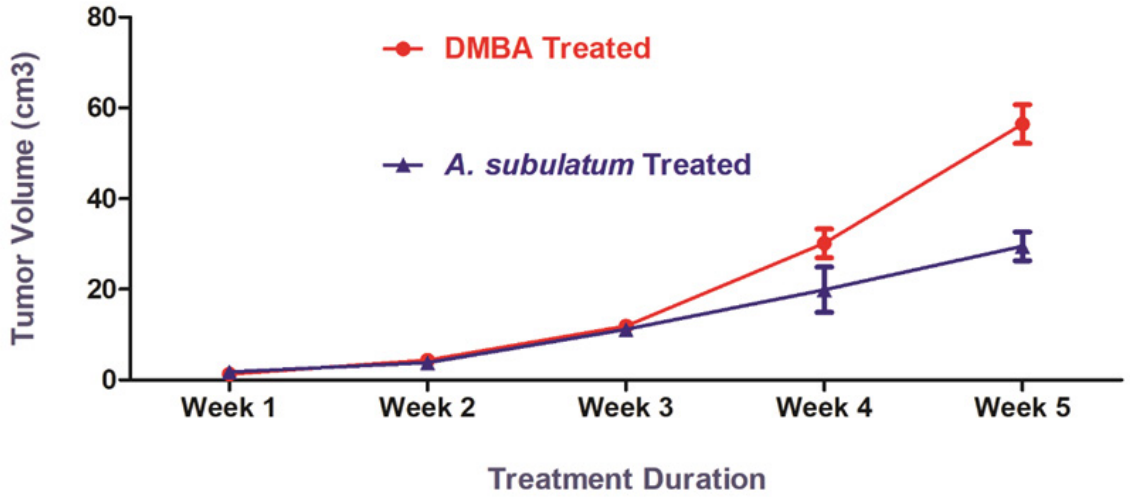

Figure 2: The effect the treatment had on tumour size reduction in the experimental groups. Amomum subulatum was administered at a dosage of 150 mg/Kg body weight per day for 5 weeks after about 0.6 cm tumour growth in both the DMBA group and the DMBA + Amomum subulatum group (Mean standard error of the mean, n=6).

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Table 1: Levels of Lipid Peroxidation in different treatment groups

Parameters	Control	DMBA Treated	*Amomum subulatum* Treated
Lipid Peroxidation (nmol/ml)	4.24 ± 0.65	210.52 ± 6.22	33.6 ± 4.23

The data are presented as mean ± S.E, n = 6, significance at p< 0.05.

Table 2: TNF alpha levels in different treatment groups

Parameters	Control	DMBA Treated	*Amomum subulatum* Treated
TNF alpha (pg/mL)	7.31 ± 0.59	106.25 ± 4.87	33.6 ± 4.23

The data are presented as mean ± S.E, n = 6, significance at p< 0.05.

Table 3: Haematological parameters

Parameters	Control	DMBA Treated	*Amomum subulatum* Treated
RBC Count (× 10^6 mm^{-3})	7.9 ± 2.1	2.1 ± 0.84	5.8 ± 1.44
WBC Count (mm^{-3})	8500 ± 8.4	17500 ± 12.9	10000 ± 3.52
Platelets counts (×10^6 mm^{-3})	2.3 ± 0.95	0.66 ± 0.97	2.1 ± 0.66
Haemoglobin (g/mL)	14.1 ± 1.88	8.1 ± 1.77	11.9 ± 0.44

The data are presented as mean ± S.E, n = 6, significance at p< 0.05.

Table 4: Biochemical Parameters Study

Parameters	Control	DMBA Treated	*Amomum subutalum* Treated
SGPT(U/mL)	28.56 ± 1.7	210.86 ± 4.55	60.56 ± 3.78
SGOT(U/mL)	31.25 ± 2.54	246.93 ± 8.41	56.78 ± 2.34
ALP(KA units)	3.67 ± 0.98	55.92 ± 3.83	12.45 ± 4.53
Urea (mg/dL)	32.54 ± 2.51	80.59 ± 7.90	50.23 ± 2.29
Uric acid(mg/dL)	3.94 ± 1.66	20.59 ± 2.75	11.34 ± 1.85
Creatinine(mg/dL)	0.65 ± 0.82	3.84 ± 1.98	1.45 ± 0.64

The data are presented as mean ± S.E, n = 6, significance at p< 0.05.

Histopathological findings

In the present histopathological examination Figure 3A, the mammary tissue section of the control rat, shows normal architecture of mammary tissue. The DMBA group rat shows a mammary tumour section with normal arrangement of lobules with adipocytes Figure 3B. Patches of embryonic mesenchymal cells and papillary projections in the mammary tissue of the DMBA group rat validate the diagnosis of tubulo-papillary cancer of the breast in rats. The DMBA + *Amomum subulatum* group rat shows a mammary tumour section in Figure 3C. In these sections, there is significant regression in the tumour cells.

Discussion

Metabolic activation by the cytochrome p450 enzyme transforms DMBA into the more potent carcinogen DMBA-3,4-dihydrodiol-1,2-epoxide (DMBA-DE). Multiple reactive oxygen species (ROS) are generated during metabolic activity, which upsets the redox balance of tissues. Malondialdehyde (MDA), a consequence of lipid peroxidation (LPO), is facilitated by these reactive species. High levels of MDA have been generally recognized as an indication of oxidative stress and antioxidant status in both animal models of cancer and human cancer patients. In the present study, serum MDA levels were shown to be significantly higher in the DMBA

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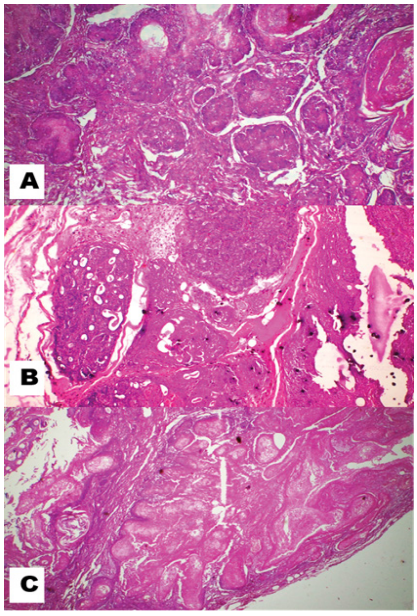

Figure 3: Microphotograph of rat mammary tissue stained with haematoxylin and eosin. (A) Section of control rat mammary tissue showing the normal arrangement of lobules with adipocytes x200. (B) Patches of embryonic mesenchymal cells and papillary projections in the mammary tissue of the DMBA group rat validates the diagnosis of tubulo-papillary cancer of the breast x500. (C) The mammary tissue section of DMBA + *Amomum subulatum* seed -treated group rat showing significant maturity in the fibrous tissues indicating the significant tumour regression process x500.

group compared to the control group. However, compared to the DMBA group, serum MDA levels reduced considerably in the DMBA+ *Amomum subulatum* group. Antioxidant potential of *Amomum subulatum* ethanolic seed extract may be established through its ability to reduce malondialdehyde (MDA) levels. The essential oils included in *Amomum subulatum* seeds range from 2-3%. The primary component of the essential oils is the oxygenated monoterpene 'eucalyptol' or 1,8-ceniole (65%-80%), the content of which varies among cultivars and geographical circumstances of growth. According to [26], the primary components of essential oils from the Indian (Sikkim) varieties are 1,8-cineole, alpha- and beta-pinene, and alpha-terpineol, but according to Satyal et al.1,8-cineole, alpha- and beta-pinene, and alpha-terpineol are the primary components from the Nepalese types. [27] found the following compounds in the essential oil of Himachal Pradesh (Indian) cultivars: 1,8-cineole, -terpineol, limonene, nerolidol,4-terpineol, -terpineol, -3-carene, -myrcene, and germacrene.5 Shrestha found that the essential oils of Nepalese cultivars largely included -terpineol, terpine-4-ol, pinocarvone, nerolidol, and pinocarveol, which is quite different from the terpene profile reported by the essential oil community at large [28-32].

Increased expression of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-) is a hallmark of the carcinogenic process triggered by DMBA, and TNF-mediated upregulation of the transcription factor NF-kB (nuclear factor-kB) is critical for the survival and proliferation of neoplastic cells. Increased levels of TNF- are a key factor in breast cancer's progression. The present study indicated that serum TNF- levels were considerably higher in the DMBA group compared to the control group. However, the blood TNF- level in the DMBA+ *Amomum subulatum* group was significantly lower than in the DMBA group. Blood TNF- levels are reduced, demonstrating the anti-inflammatory properties of an ethanolic seed extract from *Amomum subulatum*. The antioxidant activity of *Amomum subulatum* ethanolic extract also has proliferation-regulating properties, including its anti-proliferative effects on T cells [33, 34]. Although the DMBA + *Amomum subulatum* group showed a tendency toward lower breast tumour volume than the DMBA group at the conclusion of the research, the difference was still statistically significant. Furthermore, a maximum of 39% tumour growth inhibition was also identified in the penultimate week of therapy, and it is extremely probable that there would have been a significant decline in the mammary tumour volume of the medicinal plant-treated group if treatment could have been prolonged for a longer duration. There are a number of effective anticancer drugs available today, but many of them come with serious side effects that may have an impact on many different parts of the body. The impact of *Amomum subulatum* seed extract on vital organs including the liver and the kidneys must be assessed, thus. Detoxification-related processing of xenobiotic compounds like DMBA mostly occurs in the liver. The chemical carcinogen's metabolism led to both liver damage and oxidative stress. The DMBA group had greater levels of ALT, AST, and ALP in their serum than the other two groups. An increased serum hepatitis biomarker is a sign of liver damage. Blood levels of total bilirubin, ALT, AST, and ALP were all significantly lower in the DMBA + *Amomum subulatum* group compared to the DMBA group. Serum levels of liver biomarker measures were lower in the DMBA + *Amomum subulatum* group, suggesting hepatoprotective effects of the ethanolic seed extract of *Amomum subulatum*. Extensive documentation of related research on different models is available [35, 36].

The kidney is a key organ that not only removes toxic byproducts of metabolism but also creates necessary substances. As a result, renal impairment may impede the excretion and metabolism of chemotherapeutic medicines, leading to increased systemic toxicity. Urine, creatinine, and uric acid are all indices of renal function, and all were shown to be considerably higher in the DMBA group's serum. The elevated level of renal biomarker shows that DMBA has nephrotoxic effects. Reduced serum levels of urea, creatinine, and uric acid were more pronounced in the DMBA + *Amomum subulatum* group compared to the DMBA group alone. The quick recovery of serum kidney biomarker levels is another

evidence of the protective effects of *Amomum subulatum* seed extract against DMBA-induced renal injury in rats. Extensive documentation of related research on different models is available [37-39]. Histopathological analysis confirms anti-proliferative properties of ethanolic seed extract from *Amomum subulatum*. Breast tissue sections from the DMBA group showed papillary projections, cystic dilatation, cellular sheet development, pleomorphic, and patches of embryonic mesenchymal cells, all of which are consistent with tubular-papillary carcinoma of the breast and a more rapid tumour growth rate. In the DMBA + *Amomum subulatum* group, most of the fibrous structures are developed, suggesting a slower pace of tumour formation. The eventual tumour volume of the two-treatment group was assessed, further demonstrating the antiproliferative features. The antiproliferative contents present in the seeds of *Amomum subulatum* are the flavonoids (Apigenin, catechin, epicatechin, daidzein, epigallocatechin, genistein, hesperetin, kaempferol, luteolin, myrietin, naringenin, rutin, alpinetin, cardamonin) which play the vital role in the regression of the tumour. In the present study there had been 39% tumour regression which is a significant finding of the study. Similar, studies on other models have been well documented by [40-46].

Conclusion

In light of these findings, it is conceivable that *Amomum subulatum* ethanolic seed extract possesses antitumorigenic qualities, especially with regards to its capacity to neutralize free radicals. The plant extract also assists in maintaining the kidneys and liver functioning. As a result, it is plausible to assume that the plant extract both prevents and treats breast cancer in rats that has been developed from DMBA. An ethanolic extract of *Amomum subulatum* seeds has shown potential as a chemotherapeutic agent for the treatment of breast cancer, and this area of study continues to advance. In addition, additional research is needed to determine the molecular mechanism and mode of action of *Amomum subulatum* seed extract. But, the current research shows promising in preventing breast cancer in DMBA-treated rats.

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Author Contributions

Design of experiments and preparation of manuscript (Jyoti Prakash, Chandrajeet Kumar, Arun Kumar). Conducted laboratory studies (Jyoti Prakash, Aparna Jyoti Kujur and Arun Kumar). Microphotography and interpretation were done by S.K. Proofreading of the manuscript (Jyoti Prakash., Chandrajeet Kumar., Sanjiv Kumar., Aparna Jyoti Kujur., Arun Kumar). All authors read and approved the final manuscript.

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Declaration of conflicting interests

The authors declare that they have no conflict of interest.

References

1. GLOBOCAN (2020).
2. IARC (International Agency for Research on Cancer) (2020).
3. WHO cancer Fact sheet (2018).
4. Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer 144 (2019): 1941-1953.
5. Paterni I, Granchi C, Minutolo F. Risks and benefits related to alimentary exposure to xenoestrogens. Crit Rev Food Sci Nutr 57 (2017): 3384-3404.
6. Fernandez SV, Russo J. Estrogen and xenoestrogens in breast cancer. Toxicol Pathol 1 (2010): 110-122.
7. Kim JH, Lee J, Jung SY, et al. Dietary factors and female breast cancer risk: A Prospective Cohort Study. Nutrients 9 (2017): e1331.
8. Parada H Jr, Steck SE, Bradshaw PT, et al. Grilled, barbecued, and smoked meat intake and survival following breast cancer. J Natl Cancer Inst 109 (2017): 299.
9. Rengarajan T, Rajendran P, Nandakumar N, et al. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pac J Trop Biomed 5 (2015): 182-189.
10. Makhija P, Handral HK, Mahadevan G, et al. Black cardamom (Amomum subulatum Roxb.) fruit extracts exhibit apoptotic activity against lung cancer cells. Journal of ethnopharmacology 287 (2022): 114953.
11. Drishya S, Dhanisha SS, Guruvayoorappan C. Antioxidant-rich fraction of Amomum subulatum fruits mitigates experimental methotrexate-induced oxidative stress by regulating TNF-α, IL-1β, and IL-6 proinflammatory cytokines. Journal of food biochemistry 46 (2022): e13855.
12. Drishya S, Dhanisha SS, Raghukumar P, et al. Amomum subulatum mitigates experimental thoracic radiation-

induced lung injury by regulating antioxidant status and inflammatory responses. Food & function 14 (2023): 1545-1559.

13. Sudarsanan D, Suresh Sulekha D, Chandrasekharan G. Amomum subulatum induces apoptosis in tumour cells and reduces tumour burden in experimental animals via modulating pro-inflammatory cytokines. Cancer investigation 39 (2021): 333-348.
14. Rastogi S, Pandey MM, Rawat AKS. Spices: Therapeutic potential in cardiovascular health. Current pharmaceutical design 23 (2017): 989-998.
15. Drishya S, Dhanisha SS, Guruvayoorappan C. Anti-inflammatory potential exhibited by amomum subulatum fruits mitigates experimentally induced acute and chronic inflammation in mice: evaluation of antioxidant parameters, pro-inflammatory mediators and ho-1 pathway. Journal of the American College of Nutrition 40 (2021): 551-561.
16. Kumar G, Chauhan B, Ali M. Isolation and identification of new phytoconstituents from the fruit extract of Amomum subulatum Roxb. Natural product research 28 (2014): 127-133.
17. Liu G, Wang H, Zhang F, et al. The effect of VPA on increasing radiosensitivity in osteosarcoma cells and primary-culture cells from chemical carcinogen-induced breast cancer in rats. Int J Mol Sci 18 (2017): 1027.
18. Kind PRH, King EJ. Determination of Alkaline Phosphatase activity in serum. J Clin Pathol 7 (1954): 322.
19. Jendrassik GF, Grofs BM. Quantitative colorimetric determination of bilirubin in serum or plasma. Clin. Chim. Acta 27 (1938): 9.
20. Berthelot MPE. Berthelot's reaction mechanism. Report de Chimie Applique (1859): 2884.
21. Fawcett JK, Scott J. A rapid and precise method for the determination of urea. J Clin Pathol 13 (1960): 156-159.
22. Bones RW, Tausky HH. Colorimetric determination of creatinine by the Jaffe reaction. J Biol Chem 158 (1945): 581-591.
23. Fossati P, Prencipe L. Enzymatic colorimetric method of the determination of uric acid in serum. Clin Chem 26 (1980): 227.
24. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 186 (1990): 421-431.
25. Beutler B, Cerami A. Cachectin (tumour necrosis factor): a macrophage hormone governing cellular metabolism and inflammatory response. Endo Rev 9 (1988): 57-66.
26. Kaskoos RA, Mir SR, Kapoor R, et al. Essential oil composition of the fruits of Amomum subulatum Roxb. J Essent Oil-Bear Plants 11 (2008): 184-187.
27. Joshi R, Sharma P, Sharma V, et al. Analysis of the essential oil of large cardamom (Amomum subulatum Roxb.) growing in different agro-climatic zones of Himachal Pradesh, India. J Sci Food Agric 93 (2013): 1303-1309.
28. Agnihotri S, Wakode S. Antimicrobial activity of essential oil and various extracts of fruits of greater cardamom. Indian J Pharmaceut Sci 72 (2010): 657-659.
29. Satyal P, Dosoky NS, Kincer BL, et al. Chemical compositions and biological activities of Amomum subulatum essential oils from Nepal. Nat Prod Commun 7 (2012): 1233-1236.
30. Shrestha RL. GC-MS analysis, antibacterial and antioxidant study of Amomum subulatum Roxb. J Med Plants Stud 5 (2017): 126-129.
31. Rout PK, Sahoo D, Jena KS, et al. Analysis of the oil of large cardamom (Amomum subulatum Roxb.,) growing in Sikkim. J Essent Oil Res 15 (2003): 265-266.
32. Alam A, Majumdar RS. Antioxidant activity of essential oil of three cultivars of Amomum subulatum and standardization of high-performance thin layer chromatography (HPTLC) method for the estimation of 1,8-cineole. Afr J Biotechnol 17 (2018): 1129-1137.
33. Drishya S, Dhanisha SS, Guruvayoorappan C. Antioxidant-rich fraction of Amomum subulatum fruits mitigates experimental methotrexate-induced oxidative stress by regulating TNF-α, IL-1β, and IL-6 proinflammatory cytokines. Journal of food biochemistry 46 (2022): e13855.
34. Drishya S, Dhanisha SS, Raghukumar P, et al. Amomum subulatum mitigates total body irradiation-induced oxidative stress and its associated inflammatory response by enhancing the antioxidant status and regulating the pro-inflammatory cytokines. The Journal of nutritional biochemistry 107 (2022): 109064.
35. Parmar MY, Shah PA, Gao J, et al. Hepatoprotection through regulation of voltage dependent anion channel expression by Amomum subulatum Roxb seeds extract. Indian journal of pharmacology 43 (2011): 671-675.
36. Kumar G, Chauhan B, Ali M. Isolation and identification of new phytoconstituents from the fruit extract of Amomum subulatum Roxb. Natural product research 28 (2014): 127-133.
37. Yadav AS, Bhatnagar D. Modulatory effect of spice extracts on iron-induced lipid peroxidation in rat liver. BioFactors (Oxford, England) 29 (2007a): 147-157.

38. Yadav AS, Bhatnagar D. Free radical scavenging activity, metal chelation and antioxidant power of some of the Indian spices. BioFactors (Oxford, England) 31 (2007b): 219-227.

39. Hasanah NU, Tafrihani AS, Zulfin UM, et al. Nephroprotective effects of cardamom essential oil (Amomum compactum Soland. Ex Maton) on kidney cells. Indonesian Journal of Biotechnology 28 (2023): 86-93.

40. Dhakal R, Dihingia A, Ahmed RS, et al. Prophylactic and therapeutic potential of active phytoconstituents from Amomum subulatum Roxb. Food Frontiers 4 (2023): 60-84.

41. Dhir, Sarika, Rohit Dutt, et al. "Amomum subulatum Fruit Extract Mediated Green Synthesis of Silver and Copper Oxide Nanoparticles: Synthesis, Characterization, Antibacterial and Anticancer Activities" Processes 11 (2023): 2698.

42. Sethi J, Kumar C, Kumar A. Anticancerous Effect of Fruit pulp of Aegle marmelos against Benzo [A] pyrene Induced Lung Tumours in Rats. Int J Drug Dev Res J 15 (2023): 1001.

43. Akhouri V, Kumar A, Kumari M. Antitumour Property of Pterocarpus santalinus Seeds Against DMBA-Induced Breast Cancer in Rats. Breast cancer: basic and clinical research 14 (2020a): 1178223420951193.

44. Akhouri V, Kumari M, Kumar A. The therapeutic effect of Aegle marmelos fruit extract against DMBA-induced breast cancer in rats. Scientific reports 10 (2020b): 18016.

45. Ali S, Noreen A, Qamar A, et al. Amomum subulatum: A treasure trove of anti-cancer compounds targeting TP53 protein using in vitro and in silico techniques. Frontiers in chemistry 11 (2023): 1174363.

46. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28 (1957): 56-63.