ISSN: 2456-2912
VET 2024; 9(1): 163-169

www.veterinarypaper.com
Received: 02-11-2023
Accepted: 13-12-2023

VS Karhale
Research Scholar, Department of Veterinary Pharmacology and Toxicology, MVC, Parel, Mumbai, Maharashtra, India

SS Sole
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, Mumbai Veterinary College (MVC), Parel, Mumbai, Maharashtra, India

SM Ghadigaonkar
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, Mumbai Veterinary College, Mumbai, Maharashtra, India

RV Gaikwad
Professor; Head, Department of Veterinary Clinical Medicine Mumbai Veterinary College, Mumbai, Maharashtra, India

PV Meshram
Assistant Professor, Department of Veterinary Pathology, Mumbai Veterinary College, Mumbai, Maharashtra, India

S Bharucha
Assistant Professor, Department of Veterinary Physiology, Mumbai Veterinary College, Mumbai, Maharashtra, India

VP Mote
Research Scholar, Department of Veterinary Pharmacology and Toxicology, MVC, Parel, Mumbai, Maharashtra, India

SY Shirale
Assistant Professor,
Department of Veterinary Pharmacology and Toxicology, Mumbai Veterinary College, Maharashtra, India

SS Chavan,
Animal Museum Manager,
Department of Veterinary Pharmacology and Toxicology, Mumbai
Veterinary College, Maharashtra, India

P Joshi
Research Scholar, Department of Veterinary Pharmacology and Toxicology, MVC, Parel, Mumbai, Maharashtra, India

SS Jadhav
Research Scholar, Department of Veterinary Pharmacology and Toxicology, MVC, Parel, Mumbai, Maharashtra, India

HY Palampalle
Professor, Department of Veterinary Parasitology, Mumbai Veterinary College, Mumbai, Maharashtra, India

Corresponding Author:
SS Sole
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, Mumbai Veterinary College (MVC), Parel, Mumbai, Maharashtra, India

Catechin enhances the antitumour activity of Bleomycin in DMBA-induced mammary carcinoma in rats

VS Karhale, SS Sole, SM Ghadigaonkar, RV Gaikwad, PV Meshram, S Bharucha, VP Mote, SY Shirale, SS Chavan, P Joshi, SS Jadhav and HY Palampalle

DOI: https://doi.org/10.22271/veterinary.2024.v9.i1c.869

Abstract
In present study, the therapeutic potential and synergistic effects of a combination of bleomycin and catechin were evaluated in mammary tumor animal models. Forty female Wistar rats were divided into five groups: normal control, disease control, bleomycin treatment, catechin treatment, and a combination of catechin and bleomycin treatment. Mammary tumors were induced using 7,12-dimethylbenz[a]-anthracene (DMBA) administered via intragastric tube. After 45-60 days, animals in tumor-induced groups exhibited clinical signs of tumorous tissue development, leading to decreased body weights on days 60 and 90 compared to the normal and treatment groups. However, significant improvements in serum estrogen levels and histopathological lesions were observed in the group receiving a combination of catechin and bleomycin, indicating a potential therapeutic synergy in treating mammary tumors. These findings suggest the promising efficacy of the combined treatment approach in addressing mammary tumors in experimental models.

Keywords: Cancer, DMBA, Wistar female rats, Bleomycin, Catechin, estrogen

Introduction
Cancer is a condition when a few of the body's cells grow out of control and spread to other bodily regions. The tumor gains independence as it grows and develops the capacity to spread to other tissues. Tumour growth is influenced by environmental variables such as nutrition, lifestyle, and exposure to carcinogens, as well as hereditary predisposition [1, 2].
After skin tumors, mammary gland tumors are the most prevalent neoplasms in dogs [3]. Roughly 50% of canine mammary tumors are malignant and either directly or indirectly result in the death of the affected animals. They account for 25 to 30% of all cancers in chèvre [4].
The breast is highly influenced by hormones and growth factors, which can either promote cell growth or differentiation, permanently changing the breast's structure and properties. Estrogens play a crucial role in both healthy and cancerous breast development. For cells to respond to estrogen, they must have estrogen receptor alpha (ER). The amount of ERα in a tissue indicates its sensitivity to estrogen [5].
The bulk of chemical carcinogens are polycyclic aromatic hydrocarbons (PAHs), which include the DMBA [6]. The PAHs must undergo biotransformation in the liver and mammary gland [7]. Petroleum and its derivatives and PAHs are common organic contaminants that enter the environment through oil spills and incomplete fossil fuel combustion. Because most PAHs are long-lasting in the background, bioaccumulation occurs, severely disrupting biological equilibrium and polluting the ecosystem [8]. No of the delivery route, PAH causes cancers in various animal organs. A multitude of significant behavioural abnormalities and physiological disease processes, including cancer and aging, are brought on by DMBA exposure [9].
Treating malignancies aims for curative, cytoreductive, or palliative results. While advanced therapies like hormone, immunotherapy, gene therapy, and targeted treatments are used, surgery, chemotherapy, and radiation are the most common modalities. Surgery remains a cornerstone, often curing more cancers than any other approach [10]. The primary goal of cancer therapy is to kill malignant cells while sparing healthy ones. Traditional cytotoxic drugs have a therapeutic ratio of 2:1 to 6:1 (cancer cells to normal cells). Innovative gene therapy can achieve ratios of 100:1 to 100,000:1, offering promising new methods for cancer treatment [11].

High cancer treatment morbidity and mortality rates, coupled with limited effectiveness of therapies, highlight the need for innovative cancer management [12]. Chemoprevention offers various interventions, utilizing pharmacological, nutritional, phytochemical, and whole plant extracts to prevent, halt, or reverse carcinogenesis processes [13].

Bleomycin is essential for treating lymphoma, squamous cell carcinomas, germ cell tumors, and malignant pleural effusion. It disrupts tumor cell cycles by breaking DNA strands. Chelation of metal ions forms a pseudo-enzyme, which generates free radicals like superoxide and hydroxide, damaging DNA [14].

Bleomycin side effects include fever, chills, skin changes, nail problems, hair loss, nausea, vomiting, mouth sores, and lung issues. It can also result in serious effects like lung fibrosis, allergies, low blood pressure, and vascular complications that may lead to heart attacks [15].

Phytochemicals from medicinal plants are of interest for treating various human and animal disorders, including cancer [16]. Plant extracts are extensively studied as potential cancer preventive agents due to their ability to halt the carcinogenic process. They offer bioactive compounds that can lead to new cancer-fighting strategies. Combining these plant metabolites in chemoprevention regimens can have synergistic effects, increasing effectiveness through multiple intervention points [17].

There is a long history of using plants to treat cancer.[18] Clinically effective anticancer drugs often incorporate plant-derived components, as they provide access to complex molecular structures hard to replicate in the lab. Medicinal plants contribute to anticancer effects through various mechanisms, including DNA topoisomerase inhibition, impact on cytoskeletal proteins, antiprotease and antioxidant actions, and immune system stimulation [19-20].

Cancer chemotherapy has faced drug resistance and adverse side effects. Multi-drug chemotherapy is increasingly favored over mono-substance therapy, using drug combinations for better therapeutic outcomes. Adding green tea catechins to multi-drug treatment regimens is believed to enhance effectiveness and minimize side effects [21].

Among the many advantages of catechins is their ability to stop or lessen skin damage. The key component of tea leaves, catechins, has powerful physiological antioxidant and therapeutic properties. They are a subset of the polyphenol chemicals present in many medicinal plants. *Camellia assumica* and *Camellia sinensis* are the primary sources of catechins.

Extensive research has examined green tea catechins' cancer-preventive abilities. (-)-Epigallocatechin-3-gallate, a type of green tea catechin, is known for its strong antioxidants and its impact on cancer-related signal pathways. These catechins inhibit tumor metastasis, angiogenesis, and reduce cancer cell proliferation, contributing to their anticancer actions.[20]

Materials and Methods

The present study was designed to study the anti-neoplastic potential of bleomycin and catechin combination in tumors induced by DMBA in Wistar female rats. The current research was conducted at the central lab animal house, Department of Veterinary Pharmacology and Toxicology, Mumbai Veterinary College (MVC), Parel, Mumbai (India).

Chemicals and Drugs

DMBA (7-12-Dimethylbenzanthracene) (purity >95%) were purchased from Sigma Aldrich Chemicals Pvt. Ltd., Bleomycin (BLM) and Catechin hydrate (C0705-1G (+)-Catechin Hydrate, 97.0%) from Tokyo Chemical Industry (TCI) pharma, India was purchased.

Experimental Animals

In the present study, adult female Wistar rats weighing 120-135gms were procured from the central lab animal house, Department of Veterinary Pharmacology and Toxicology, Mumbai Veterinary College (MVC), Parel, Mumbai (India) and kept in the same department. This lab animal facility is approved by CPCSEA and the Institutional Animal Ethics Committee (IAEC) and the CPCSEA Registration Number is MVC/IAEC/02/July/2020.

Total no of animals – 40 (As per approved)

Experimental design

Animals shall be randomized into five groups of eight animals each. The groups are termed as numbers I, II, III, IV, and V (Table 1).

Table 1: Experimental design

Sr. No.	Groups	Number of animals	Dose rate and route of administration
1	Control group / without treatment	8	No treatment
2	Positive control Group /DMBA treatment	8	DMBA @80 mg/kg Gastro-gavaged
3	DMBA treatment (Mammary tumor) + Bleomycin	8	DMBA @80 mg/kg Gastro-gavaged, BLM @ 60mg/kg IP
4	DMBA treatment (Mammary tumor)+ Catechin	8	DMBA @80 mg/kg Gastro-gavaged, Catechin @ 20mg/kg PO
5	DMBA treatment (Mammary tumor)+ Catechin + Bleomycin	8	DMBA @80 mg/kg Gastro-gavaged, Catechin @20mg/kg PO, BLM @ 30mg/kg IP

Administration of Drugs

DMBA

Animals administered a single dose of 0.5 ml DMBA (80 mg/kg) in sesame oil via intragastric route. 80 mg/kg of DMBA can cause 100% tumor incidence [22]. DMBA takes 8–10 weeks approximately to induce tumors in female Wistar albino rats. Animals were sacrificed after the study period i.e., after 16 weeks of DMBA administration to collect-blood and mammary tissues for further analysis [23].

The treatment was started at 60 days post DMBA administration and three doses of Bleomycin and Catechin were given at weekly interval.

Bleomycin: Bleomycin @ 60mg/kg IP, which is marketed for clinical use.

Catechin: Catechin @ 20mg/kg PO [24].

Collection of blood: Blood was collected from the retro-orbital plexus with the help of a capillary tube [25], on 0th, 30th, 60th and 90th before sacrificing the animals. The blood was collected in K3-EDTA anticoagulant vials for hematological examination and plain vials for serum biochemical examination. Both blood and serum samples were processed within 8 hours of post-collection. A thin blood smear was prepared for differential leukocyte count.

Estimation of serum estrogen

Serum estrogen was analyzed using Radioimmunoassay (RIA) using kits by IMMUNOTECH S.R.O. Radiova, Prague, Czech Republic (Catalogue no. A21854, HSN code-28443090, Batch no. 221205D) according to the manufacturer's guidelines.

Radioimmunoassay

The method of performing radioimmunoassay is as follows. First, an antibody that is highly specific for the hormone to be measured is produced. Second, a small quantity of this antibody is (1) mixed with a quantity of fluid from the animal containing the hormone to be measured and (2) mixed simultaneously with an appropriate amount of purified standard hormone that has been tagged with a radioactive isotope. However, one specific condition must be met, there must be too little antibody to bind completely both the radioactively tagged hormone and the hormone in the fluid to be assayed. Therefore, the natural hormone in the assay fluid and the radioactive standard hormone compete for the binding sites of the antibody. In the process of competing, the quantity of each of the two hormones, the natural and the radioactive, that binds is proportional to its concentration in the assay fluid. Third, after binding has reached equilibrium, the antibody-hormone complex is separated from the remainder of the solution, and the quantity of radioactive hormone bound in this complex is measured by radioactive counting techniques. If a large amount of radioactive hormone has bound with the antibody, it is clear that there was only a small amount of natural hormone to compete with the radioactive hormone, and therefore the concentration of the natural hormone in the assayed fluid was small. Conversely, if only a small amount of radioactive hormone has bound, it is clear that there was a large amount of natural hormone to compete for the binding sites. Fourth, to make the assay highly quantitative, the radioimmunoassay procedure is also performed for "standard" solutions of untagged hormone at several concentration levels.

Histopathology

The tissue samples of mammary tissues were collected in 10% neutral buffered formalin (NBF) and processed by routine paraffin embedding method. The tissue sections of 4 microns were stained with standard Hematoxylin (H) and Eosin (E) protocol suggested by Chauhan (1995).

Statistical analysis

The data generated during the experiment were statistically analyzed by using One-way analysis of variance (ANOVA). The comparisons between the groups and between the days were tested by the Tukey test. The values were represented as mean, standard error mean and (p<0.05) considered significant. Mean values and standard error of mean were calculated and all the values were expressed as mean ± SEM (Graph Pad Prism 5 Software, 2007).

Results

In this study, 40 female Wistar rats were used, divided into five groups (A to E) with eight animals in each group. Group A was the normal control, while Group B was the positive control with induced tumor using the chemical carcinogen DMBA. Group C received DMBA-induced chemotherapy treatment with bleomycin, Group D was treated with the herbal constituent catechin, and Group E received a combination of catechin and bleomycin. After the administration of DMBA, the animals in Groups B, C, D, and E developed clinical signs of tumorous tissue growth on the abdominal and peripheral skin tissues and on the back of the animals. The tumors were observed to be soft, rubbery, and adherent to the skin. Some rats exhibited multiple tumor growths at various sites on the body. The DMBA-induced tumors proliferated but did not metastasize without treatment.

Body weight of rats

Table 2: Mean body weight

Group No.	Intervals of Study			
	0 Day	30 Day	60 Day	90 Day
A	137.50 ± 6.80	170.00± 12.10	197.00±9.21	228.00±10.16
B	133.33± 5.57	151.83± 7.14	142.50±5.86*	135.83±5.16*
C	131.66± 6.00	155.50±7.51	141.33±5.23*	161.67±4.01*
D	138.33± 8.33	161.00±8.13	150.33±7.62*	158.50±8.32*
E	130.00 ± 7.30	153.16±8.83	147.83±7.80*	178.66±5.78*

A- Normal Control, B- Positive (tumour) Control, C- Treatment with Bleomycin
D- Treatment with Catechin and E- Treatment with Catechin and Bleomycin
*- Indicates statistically significant difference (p≤0.001)
The significant reduction in body weight gain in animals of group B, C, D and E were noticed on 30th and 60th day as compared to control group A.

Serum estrogen

Table 3: Mean values of total serum Estrogen (Pg/ ml) in female Wistar rats (day-wise)

Group no.	Intervals of Study			
	0 Day	30 Day	60 Day	90 Day
A	45.50± 1.83	45.83±2.52	45.50±1.76	45.50±1.38
B	47.83±2.00	63.66±1.72*	76.16±3.36*	79.83±2.67*
C	45.16±1.35	66.33±3.55*	75.5±2.09*	49.00±1.39*
D	44.00±2.40	69.00±1.50*	78.66±2.99*	56.50±4.69*
E	45.00±1.63	72.00±1.91*	80.33±1.78*	47.16±2.92*

A- Normal Control, B- Positive (tumor) Control, C- Treatment with Bleomycin
D- Treatment with Catechin and E- Treatment with Catechin and Bleomycin
*- Indicates statistically significant difference (p≤0.001)

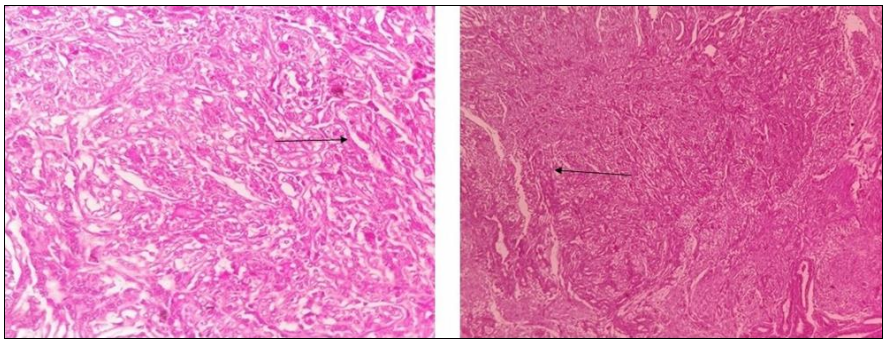

Fig 1: The skin with tumour issue showing proliferating epithelial tubules no papillary compaonent (adenocarcinoma) of mammaru gland H&E stain. X 100

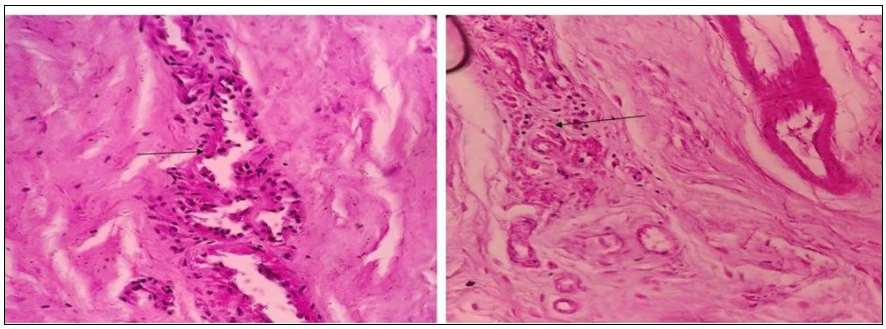

Fig 2: The tumour tissue mass showing degenerative and necraotic change of neoplastic cells in fibrosarcoma. H&E stain. X100

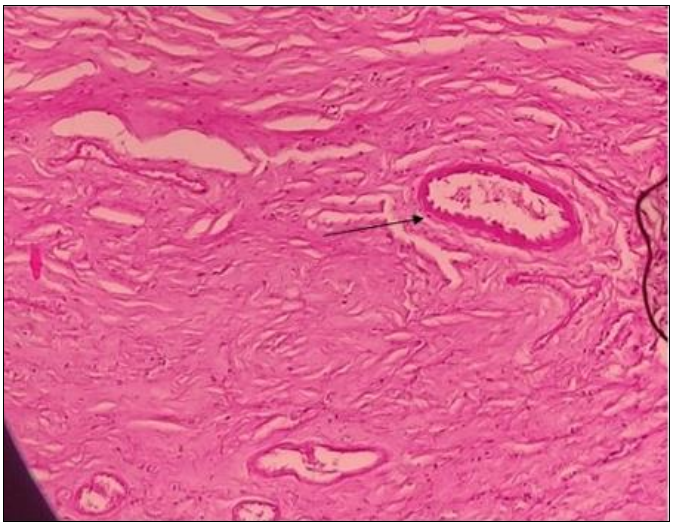

Fig 3: Normal mammary gland showing secretory lobes, lobules and intralobular ducts. H&E stain x100

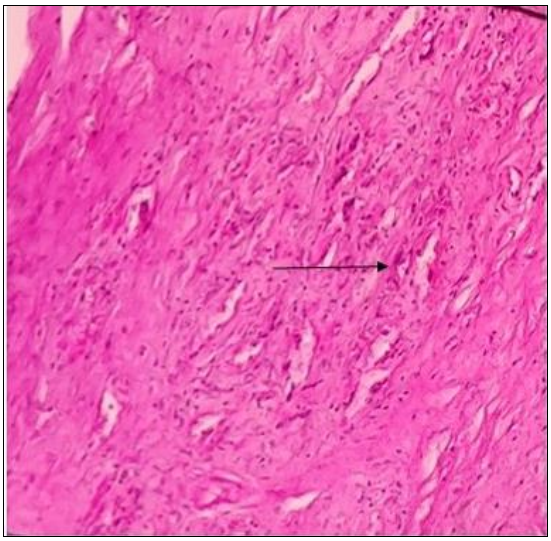

Fig 5: Showing areas of celluar debris of eosinophilic nature with degenerated cell and foci of necrosis. H&E stain x100

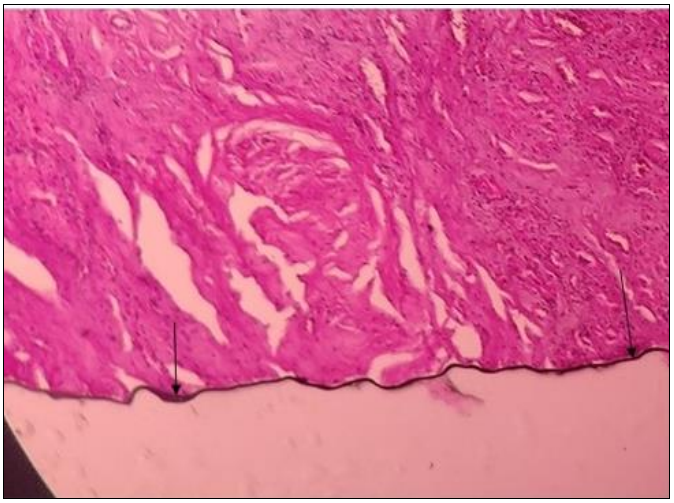

Fig 4: Normal histomorphological features of skin showing dermis and epodermis and subcutaneous layer. H&E stain x100

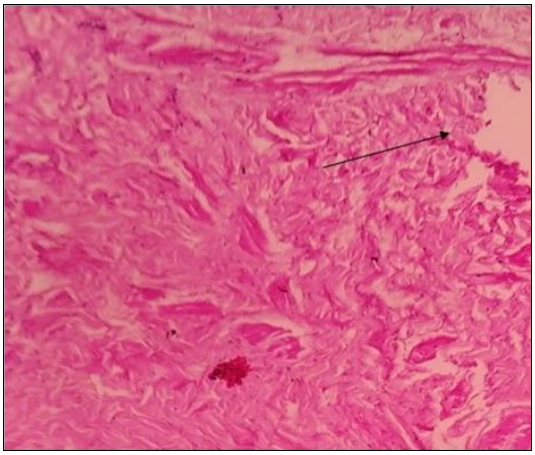

Fig 6: Showing presence of proliferative tumour cells as well as absence of significant degenerative changes in tumour calls. H&E stain x100

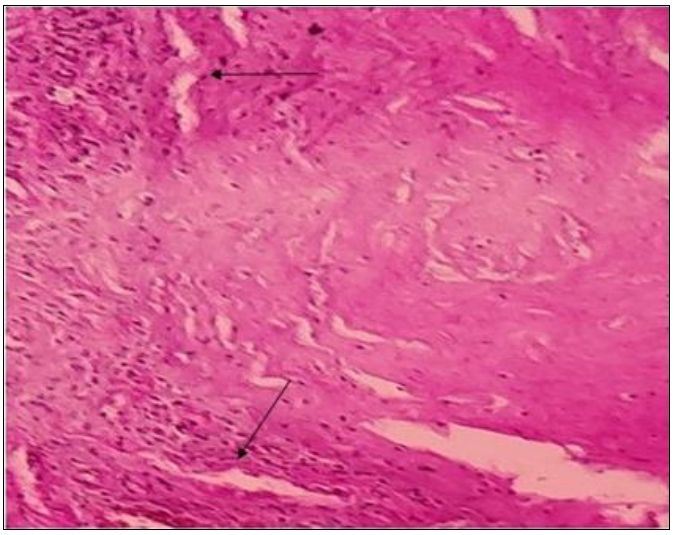

Fig 7: Showing areas of celluar debris of eosinophilic nature with degenerated cell and foci of necrosis. H&E stain x100

Histopathology

The histopathological examination of mammary glands in this study unveiled the impact of DMBA-induced tumors on metabolic and systemic stress in groups B, C, D, and E animals. Group A, the control, displayed a low-grade pattern with a typical mammary gland structure featuring well-defined ducts and epithelial layers (Figure 3). Conversely, in Group B, cellular abnormalities such as fibrosis, lipoma, necrosis, and congested vascular tissue indicated a higher-grade pattern, reflective of more aggressive cancer characteristics (Figure 1 and 2). Notably, Groups C (Figure 5) and E (Figure 7) demonstrated potential for reducing the grade of mammary tumors, as their tissue showed mild to moderate degenerative changes, including areas of cellular debris, degenerated cells, and foci of necrosis. These observations suggested a shift towards lower-grade characteristics due to the treatments administered. In contrast, Group D, treated with catechin alone, failed to exhibit a decrease in tumor grade, maintaining a higher-grade pattern characterized by proliferative tumor cells and the absence of significant degenerative changes. These findings highlight the capacity of specific treatments, particularly those in Groups C and E, to influence the histological grade of mammary tumors, potentially bearing implications for cancer management and treatment decisions. Understanding the ability of treatments to impact tumor grades can be crucial in tailoring therapeutic strategies for mammary cancer.

Discussion

Cancer remains a prominent global health concern, exerting a significant impact on both economically developed and less developed regions. This burden is poised to escalate worldwide, driven by demographic shifts, notably in less developed nations, which house approximately 82% of the global population. The etiological factors contributing to this increase encompass evolving lifestyles, encompassing smoking habits, dietary patterns, sedentary behaviors, and shifts in reproductive choices, such as delayed childbearing and reduced parity. These factors collectively augment the susceptibility to cancer and warrant comprehensive scientific exploration.

Furthermore, breast cancer stands as the most prevalent neoplastic disease among women, significantly contributing to cancer-related mortality. Intriguingly, among dogs, mammary gland tumors rank second only to cutaneous neoplasms. This cross-species parallel offers a promising avenue for gaining insights into shared risk factors and genetic profiles. It is worth noting that domestic dogs and cats exhibit an elevated prevalence of mammary tumors, with feline cases predominantly presenting as aggressive malignancies. This unique interplay between human and animal cases holds the potential for valuable research endeavors.

This experimental study was designed to validate the efficacy of catechin, a natural plant constituent, in combating cancer. Catechin's anti-cancer properties were assessed using a female Wistar rat model with DMBA-induced tumors. The study juxtaposed catechin with the conventional chemotherapeutic antibiotic drug, bleomycin, derived from *Streptomyces verticillus*, which was evaluated in a separate group of rats with induced tumors. Additionally, a third group of tumor-induced rats received a combination of both drugs.

To affirm anticancer potential of catechin, the study employed various methods, including radioimmunoassay for serum estrogen estimation and histopathology. These findings were then compared across distinct groups, including a normal control group, a tumor-positive control group, and the treatment groups. The results of study are presented and critically discussed in the following sections, shedding light on the promising role of catechin in cancer therapy.

The investigation revealed a substantial decrease in body weight gain among animals in groups B, C, D, and E on the 30th and 60th days in comparison to the control group A. This phenomenon may be attributed to tumor development, giving rise to a para-neoplastic syndrome. This, in turn, perturbed metabolic processes, resulting in reduced food intake and subsequently decreased body weight in tumor-bearing animals. However, after the 60th day, animals in the treated groups (C, D, and E) exhibited a less pronounced reduction in mean body weight compared to the positive control group B. This could signify a potential reversal of the wasting often associated with cancer.

These observations align with the findings of previous studies by Perumal *et al*. (2005) and Padmavathi *et al*. (2006). Notably, in the initial stages of the study, there was no significant change in body weight for both control and experimental rats [26, 27]. However, as the study progressed, a considerable (p<0.001) decrease in body weight was evident in the DMBA-induced tumor-bearing female SD rats by the end of the 90th day. In contrast, Al-Saeedi's study (2014) reported that the body weight of rats did not exhibit significant differences between normal and tumor-bearing rats, indicating the absence of side effects that could lead to weight loss in their experimental model [23].

Furthermore, Janssens *et al*. (2016) suggested that catechins, such as those found in catechin, play a beneficial role in managing body weight. They promote sustained energy expenditure, fat oxidation, and preservation of fat-free body mass, particularly following energy restriction-induced weight loss, as often seen in cancer patients [28]. However, Hursel *et al*. (2009) found a more modest positive effect of catechins, including epigallocatechin gallate (EGCG), on body weight [29].

The serum estrogen values indicate a significant role of catechin in exerting antiproliferative and pro-apoptotic effects, potentially through the reduction of elevated estrogen levels in rats with mammary tumors. This suggests that catechin's impact on hormonal regulation is closely linked to its anticancer properties. These findings are consistent with the observations made by Fukuda *et al*. (1985), who reported

elevated serum estrogen levels in DMBA-induced rat mammary tumors [30]. Elevated estrogen levels are known to contribute to the growth and development of capillary endothelial cells, thereby promoting angiogenesis, which, in turn, leads to the formation of tumor masses.

The histopathological assessment of DMBA-induced mammary tumors in various treatment groups revealed distinct tumor grades. Group A, serving as the control, exhibited a low-grade pattern, indicating a relatively normal mammary gland structure. In contrast, Group B showed higher-grade characteristics, signifying aggressive cancer features, including fibrosis, lipoma, necrosis, and vascular congestion. Notably, Groups C and E displayed potential for lowering tumor grades, with evidence of degenerative changes and necrotic areas, suggesting a shift toward lower-grade characteristics. In contrast, Group D, treated with catechin alone, did not exhibit a reduced tumor grade, maintaining a higher-grade pattern. These findings emphasize the capacity of certain treatments, particularly in Groups C and E, to influence tumor grade, with potential implications for cancer management and treatment customization. Understanding this impact on tumor grades is vital for tailoring effective therapeutic strategies for mammary cancer.

The current study's results also align with the research conducted by Jang *et al*. (2013) and Ruch *et al*. (2020), highlighting the potent antiangiogenic effects of tea catechins. This antiangiogenic activity is attributed to the manifestation of antiproliferative and pro-apoptotic activities in breast cancer cells, which serves to alleviate carcinogen-induced reactive oxygen species stress [31, 32]. Furthermore, it can be inferred that catechin's potential to enhance antiproliferative and pro-apoptotic activities may be attributed to its role in reducing elevated estrogen levels in rats with mammary tumors.

Collectively, these findings underscore the multifaceted role of catechin in the context of estrogen regulation and its far-reaching implications in combating mammary tumors, shedding light on the intricate interplay between catechin's hormonal effects and its anti-cancer mechanisms. Such insights hold promise for the development of novel therapeutic strategies that target both hormonal regulation and tumor progression.

Conclusion

The present study was conducted to evaluate and validate the reduction in dose of bleomycin in combination with catechin in addition to anticancer properties. The serum estrogen values showed that the antiproliferative and pro-apoptotic activities might have resulted from catechin by reducing elevated estrogen levels in rats with mammary tumors. The histopathological examination of the mammary gland revealed the metabolic and systemic stress caused by the progression of the tumors by DMBA in groups B, C, D, and E animals. The treatment of catechin in combination with bleomycin has significantly improved parameters such as histopathology and serum estrogen levels, which suggest some synergistic activity in the molecules, which has brought about efficacy compared to less productive pure catechin. Therefore, the mechanistic study of catechin in combination with bleomycin can further be conducted as dose rate reduction of bleomycin was significantly proved in the present study in DMBA-induced rats.

Acknowledgement

The Authors are highly thankful to the Ministry of Information of Technology, Government of India, and SAMEER, an offset of TIFR for financial support.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

References

1. World Health Organization. 2020. Oral cancer facts; c2020. www.oralcancerfoundation.org
2. National Cancer Institute; c2000. https://www.cancer.gov.
3. Moulton JE, Rosenblatt LS, Goldman M. Mammary tumors in a colony of beagle dogs. Veterinary pathology. 1986 Nov;23(6):741-9.
4. Bloom HJ. The influence of delay on the natural history and prognosis of breast cancer: A study of cases followed for five to twenty years. Br. J. Cancer: British journal of cancer. 1965 Jun;19(2):228.
5. Carl PL, Chakravarty PK, Katzenellenbogen JA, Weber MJ. Protease-activated prodrugs for cancer chemotherapy. PNAS: Proceedings of the National Academy of Sciences. 1980 Apr;77(4):2224-8.
6. Currier N, Solomon SE, Demicco EG, Chang DL, Farago M, Ying H, *et al*. Oncogenic signaling pathways activated in DMBA-induced mouse mammary tumors. Toxicologic pathology. 2005 Oct;33(6):726-37.
7. Tamulski TS, Morreal CE, Dao TL. Comparative metabolism of 7, 12-dimethylbenz (a) anthracene in liver and mammary tissue. Cancer Research. 1973 Dec 1;33(12):3117-22.
8. Demir C, Gultekin SH, Yener B. Augmented cell-graphs for automated cancer diagnosis. Bioinformatics. 2005 Jan 1;21(suppl_2):7-12.
9. Das RK, Bhattacharya S. Inhibition of DMBA-croton oil two-stage mouse skin carcinogenesis by diphenylmethyl selenocyanate through modulation of cutaneous oxidative stress and inhibition of nitric oxide production. APJCP: Asian Pacific Journal of Cancer Prevention. 2004 Apr 1;5(2):151-8.
10. Bacon NJ, Dernell WS, Ehrhart N, Powers BE, Withrow SJ. Evaluation of primary re-excision after recent inadequate resection of soft tissue sarcomas in dogs: 41 cases (1999–2004). JAVMA: Journal of the American Veterinary Medical Association. 2007 Feb 15;230(4):548-54.
11. Bischoff JR, Kirn DH, Williams A, Heise C, Horn S, Muna M, *et al*. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. Science. 1996 Oct 18;274(5286):373-6.
12. Yu Y, Xu F, Peng H, Fang X, Zhao S, Li Y, *et al*. NOEY2 (ARHI), an imprinted putative tumor suppressor gene in ovarian and breast carcinomas. PNAS: Proceedings of the National Academy of Sciences. 1999 Jan 5;96(1):214-9.
13. Mehta RG, Murillo G, Naithani R, Peng X. Cancer chemoprevention by natural products: how far have we come?. Pharmaceutical research. 2010 Jun;27:950-61.
14. Claussen CA, Long EC. Nucleic acid recognition by metal complexes of bleomycin. Chemical reviews. 1999 Aug 17;99(9):2797-816.
15. Arafat S, Abdelmabood AA, Mohamed W, El-Tagy G, El-Swify A. Maxillofacial Vascular Anomalies in Pediatric Patients: A Review Article.
16. Desai AG, Qazi GN, Ganju RK, El-Tamer M, Singh J, Saxena AK, *et al*. Medicinal plants and cancer

chemoprevention. Current drug metabolism. 2008 Sep 1;9(7):581-91.
17. Francy-Guilford J, Pezzuto JM. Mechanisms of cancer chemopreventive agents: A perspective. Planta medica. 2008 Oct;74(13):1644-50.
18. Madhuri S, Pandey G. Some anticancer medicinal plants of foreign origin. Curr. Drug Metab: Current science. 2009 Mar 25:779-83.
19. Yang AK, He SM, Liu L, Liu JP, Qian Wei M, Zhou SF, *et al*. Herbal interactions with anticancer drugs: mechanistic and clinical considerations. Curr.Med.Chem: Current medicinal chemistry. 2010 Jun 1;17(16):1635-78.
20. Yang CS, Wang H, Chen JX, Zhang J. Effects of tea catechins on cancer signaling pathways. The Enzymes. 2014 Jan 1;36:195-221.
21. Cao J, Han J, Xiao H, Qiao J, Han M. Effect of tea polyphenol compounds on anticancer drugs in terms of anti-tumor activity, toxicology, and pharmacokinetics. Nutrients. 2016 Dec 14;8(12):762.
22. Fackenthal JD, Olopade OI. Breast cancer risk associated with BRCA1 and BRCA2 in diverse populations. Nat.Rev.Cancer: Nature Reviews Cancer. 2007 Dec;7(12):937-48.
23. Al-Saeedi FJ. Study of the cytotoxicity of asiaticoside on rats and tumor cells. BMc cancer. 2014 Dec;14:1-3.
24. Crespy V, Williamson G. A review of the health effects of green tea catechins in *in vivo* animal models. J Nutr: The Journal of Nutrition. 2004 Dec 1;134(12):3431S-40S.
25. Sorg DA, Buckner B. A simple method of obtaining venous blood from small laboratory animals. Proc.Soc.Exp.Biol.Med: Proceedings of the Society for Experimental Biology and Medicine. 1964 Apr;115(4):1131-2.
26. Perumal BS, Sakharkar KR, Chow VT, Pandjassarame K, Sakharkar MK. Intron position conservation across eukaryotic lineages in tubulin genes. Front.Biosci.(Landmark Ed): Frontiers in Bioscience-Landmark. 2005 Sep 1;10(3):2412-9.
27. Padmavathi R, Senthilnathan P, Chodon D, Sakthisekaran D. Therapeutic effect of paclitaxel and propolis on lipid peroxidation and antioxidant system in 7, 12 dimethyl benz (a) anthracene-induced breast cancer in female Sprague Dawley rats. Life sciences. 2006 May 8;78(24):2820-5.
28. Janssens PL, Hursel R, Westerterp-Plantenga MS. Nutraceuticals for body-weight management: The role of green tea catechins. Physiology & behavior. 2016 Aug 1;162:83-7.
29. Hursel R, Viechtbauer W, Westerterp-Plantenga MS. The effects of green tea on weight loss and weight maintenance: a meta-analysis. Int J Obes: International journal of obesity. 2009 Sep;33(9):956-61.
30. Fukuda M, Maekawa J, Hosokawa Y, Urata Y, Sugihara H, Hattori T, *et al*. Hormone-dependent changes of blood vessels in DMBA-induced rat mammary carcinoma and its regression studied by 3H-thymidine autoradiography. BASIC APPL HISTOCHEM: Basic and Applied Histochemistry. 1985 Jan 1;29(1):21-43.
31. Jang JY, Lee JK, Jeon YK, Kim CW. Exosome derived from epigallocatechin gallate treated breast cancer cells suppresses tumor growth by inhibiting tumor-associated macrophage infiltration and M2 polarization. BMC cancer. 2013 Dec;13(1):1-2.
32. Ruch R. Gap junctions and connexins in cancer formation, progression, and therapy. Cancers. 2020 Nov 9;12(11):3307.