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# EXPLORING THE THERAPEUTIC POTENTIAL OF *CAPSICUM ANNUUM* EXTRACT AGAINST BREAST CANCER IN SPRAGUE DAWLEY RATS

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## ABSTRACT

Ethnopharmacological relevancy: Many studies showed that various segments of Capsicum annuum L, has therapeutic and ameliorative potential against cancer which is one of the major contributor for causing oxidative stress, adduct formation in genetic material and cell proliferation, etc.

Aim of study: Current research was carried out to analyze anti-cancerous potential of Capsicum annuum extract in breast cancerous rats.

Material and methods: Capsicum annuum ethanolic extract was attained via employing Soxhlet apparatus. This obtained extract was further utilized for research trial on rats. For this purpose, 50 healthy female rats were chosen for study, the rats were segregated into five groups, 10 rats in each group; G0 (negative control group), G1 (positive control group), G2 (doxorubicin as a standard drug), G3 and G4 (rats receiving capsicum extracts). Breast cancer in rats was induced by administering the single dose of DMBA (50mg/kg) through gavage. Blood samples were investigated for breast cancer biomarkers and serum biomarkers to analyze the outcome of treatments. Furthermore, tumor weight, volume, burden and incidence was also noticed along with histopathological examination of tissues.

Results: The Capsicum annuum extracts prevented the rise in concentration of biomarkers of breast cancer in rats that are CA 15.3, CA 27.29 and CEA. Furthermore, the capsicum extracts also significantly improved the level of serum biomarkers such as TAC (Total antioxidant capacity) and TOS (Total oxidative stress) in breast cancerous rats. The tumor weight, volume, burden and incidence was significantly decreased in $G_2$, $G_3$ and $G_4$ groups treated with doxorubicin and Capsicum annuum extracts. Histopathological examination of breast tissues showed that tissue damage was reversed in groups G3 and G4 treated with capsicum extracts.

**Keywords:** Capsicum annuum, breast cancer, anti-cancerous, therapeutic potential, functional food

**List of abbreviations:**

DMBA= 7, 12 Dimethyl Benz (A) Anthracene
CA= Cancer antigen
CEA= Carcinoembryonic antigen
TAC= Total antioxidant capacity
TOS= Total oxidative stress
GC = Negative control
PC = Positive control
ABTS= (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
CRD= Completely randomized design

## 1. Introduction

Cancer as devastating human disease causes huge mortalities worldwide each year. Cancer produces myriad effects on metabolism and enables cells proliferation inappropriately in order to adapt to tumor generation (Schmidt *et al*., 2021). Previous mortalities and experiences describes the hallmarks of cancer as a multistep developmental process including dodging growth inhibitors, maintaining proliferative signaling, combating cell death, promoting angiogenesis, turning on metastasis and invasion, changing energy metabolism and avoiding immune devastation (Lu and Zhan, 2018). According to World Health Organization, cancer is currently the 2$^{nd}$ non-communicable cause of death worldwide. In 2020, there were 19.3 million new cases of cancer worldwide (18.1 million without skin cancer). Based on the statistics breast malignancy is the most common cancer in Pakistan having 23.1% cases particularly among women of middle age (Zaheer *et al*. 2019). Around two out of every five people today will have cancer at some point in their lives. Breast carcinoma is the major form of tumor that prevails in higher percentage among women particularly in Asian and African countries. It is divided into various types depending upon the hormones and expression of various genes that are involved in its progression (Laya *et al*., 2021). Cancer targets the surrounding cells and migrates to other body cells through lymphatic system (Hassanpour and Dehghani, 2017). In Pakistan the prevalence of cancer is 1.3% the cases reported on breast cancer in Pakistan are 2.09 million (Saleh *et al*., 2018). According to NCI mortality rates due to cancer are 8.2 billion particularly in South Asian and African countries. In 2020 an estimated death rate due to all type of cancers was 9.6 million (Siegel *et al*., 2020). Breast cancer is one of the most prevalent types of cancer in the world. Moreover, it is the major cause and primary killer of female cancer patients worldwide. Cancer can be induced by administering DMBA through oral route and it is suggested that the therapeutic effects of medicinal plants i.e. *Aegle marmelos*, *Capsicum annuum* and drugs i.e. simvastatin has some anti-cancerous effects which are moderately like that of tamoxifen in breast cancer (Akhouri *et al*., 2020; Karimi *et al*., 2019).

Owing to the expeditious rise in diseases and behavioral disorders, public health is becoming a major element in the existing society and human well-being. Health promoting foods are now considered as an important factor that can prevent and cure many diseases, especially organic foods. Natural foods are a rich source of components with health enhancing and promoting activities. Consequently plants remain the major source of active compounds and research concentrated on their activity is gaining popularity in the last few decades (Basharat *et al*., 2020). Diet rich in fruits and vegetables is a healthy way of preventing diseases like diabetes, cancer and obesity. Among fruits and vegetables capsicum, grapes, berries, onion, ginger and garlic are most commonly used as a therapeutic diet (Laya *et al*., 2021). Capsaicin in *Capsicum annuum* is a well-known cancer fighter, reduces the speed of cancer cell growth, inhibits DNA synthesis, vessel sprouting and chemotactic mobility of endothelial cells as well as acts as a mitotic inhibitor. In addition dihydrocapsaicin and nor-dihydrocapsaicin are the most abundant capsaicinoids in *Capsicum*

*annuum* having anti-cancerous, anti-mutagenic and immunomodulatory properties (Akhouri *et al*., 2020).

In experimental trials, many carcinogenic chemicals are used for the induction of breast cancer. 7, 12-dimethylbenz[a]anthracene (DMBA) owing to its carcinogenic and immunosuppressive properties it is most commonly employed to induce mammary cancer. The DMBA oxidative metabolism leads to the generation of free radicals which in turn disrupts redox balance. The free radicals bind to the cell level macromolecules via nucleophilic sites resulting in carcinogenic reactions. Zingue *et al*. (2016) and Gueyo *et al*. (2020) used DMBA and observed 100 % breast cancer in rats. Depending on the above mentioned evidences, this research work is designed to evaluate the anticancer characteristics of *C. annuum* against breast cancer induced by DMBA in an animal modeling.

## 2. Material and Methods

The current study was conducted at Faculty of Food Nutrition and Home Sciences (FFNHS) in National Institute of Food Science and Technology (NIFSAT), University of Agriculture Faisalabad (UAF), Pakistan.

### *2.1. Sample preparation*

Fresh *Capsicum annuum* fruits were purchased from the local market of Faisalabad, Pakistan. Fruits were rigorously washed with distilled or purified water, exposed to sun drying and transformed into a powder. A dehydrated capsicum samples were extracted by employing 95% ethanol via Soxhlet apparatus and later on solvent was eliminated by using rotary evaporator by adopting the method as described by Ahmad *et al*. (2022). *Capsicum annuum* fruits produced 22.13% extract.

### *2.2. Induction of breast cancer*

Breast cancer in Sprague dawley rats was induced by gavage using 50 mg per kg body weight of the chemical DMBA (7, 12 Dimethyl Benz (A) Anthracene) mixed with 1ml of corn oil by using the method as described by Anna *et al*. (2021).

### *2.3. Efficacy trial plan*

Defatted prepared ethanolic extract of *Capsicum annuum* was used for research study to analyze its anti-cancerous characteristics. The welfare and care of rats was affirmed by following the guidelines and protocols of ethical committee along with persistent monitoring throughout the study. For efficacy trial, 50 rats (n=10) weighing 250 grams were chosen randomly from the animal room located at National Institute of Food Science and Technology (NIFSAT), UAF. Rats were acclimatized for the period of 1 week and were being provided with ordinary drink and food. Temperature range in animal room was in the range of 20 to 35 $^{o}$C and humidity level was also set up to 75%. Rats were further segregated into five groups with ten rats (n=10) in every group depending on the type of feed they were being administered, G0 (negative control group) receiving normal diet; G1 (positive control group) diseases rats administered normal diet; G2 (rats receiving 0.55mg/kg doxorubicin as a standard drug via intravenous route); G3 and G4 received two different concentrations of *Capsicum annuum* extract including 500 and 1000mg/kg, correspondingly (Kim *et al*. (2019) and Kurnijasanti *et al*. (2017). Table 1 depicts the grouping of rats and feed they were being provided.

### *2.4. In vivo examination*

On the 45$^{th}$ day, the rats were anesthetized, blood was assembled through the coagulant and anti-coagulant vials via heart puncture. The blood was exposed to clotting for thirty minutes; centrifuged for 15 minutes at 3000 rpm to separate serum. The serum acquired was utilized for the analysis of breast cancer biomarkers and serum biomarkers by following the method as described by Shao et al. (2015). The enzyme linked solid phase immunosorbent assay and radioimmunoassay were used for analysis. The reagents used were namely acetate buffer and ABTS (2, 2 azinobis 3

benzoethylthiazoline sulfonic acid). Initially 220µl of reagent 1 was assorted with 0.5 µl of serum sample and was incubated for 30 seconds; absorbance was recorded at 425 nm. Later on 20 µl of reagent 2 was mixed and added with the samples and absorbance was recorded at 425 nm Oh *et al*. (2015).

### *2.5. Tumor variables*

The tumor appearance endpoints such as tumor weight, tumor number, latency to first number and tumor incidence were measured with the help of Vernier caliper and screw gauge by following the method as described by Saleh *et al*. (2018). The above mentioned formula was used to calculate the tumor mass volume:

Tumor volume ($mm^3$) = $4\ \pi\ (A/2)^2 \times (B/2)$

Where: *A*= negligible tumor axis

*B*= substantial tumor axis

### *2.6. Histopathological examination*

Breast tissues were excised out and fixed in formalin buffered solution (10%), thoroughly washed with alcohol and purified with xylene in order to help the histopathological examination of the breast tissues. Breast tissues were impregnated with paraffin wax and 5µm tissue sections were anchored on the slides and dewaxed by using the xylene and remoistened while using the alcohol by means of a rotating microtome (Leica GmbH RM22545 Microsystems, Wetzlar) by following the method of Ashraf *et al*. (2021).

**Table 1** Treatment plan for various groups of rats with *Capsicum annuum* extract

| Groups | Treatments |
|---|---|
| G0 | Healthy rats fed on normal feed |
| G1 | Diseased rats fed on normal feed |
| G2 | Normal diet + 0.55mg/kg doxorubicin |
| G3 | Normal diet + 500mg/kg *Capsicum annuum* extract |
| G4 | Normal diet + 1000mg/kg *Capsicum annuum* extract |

G0= Negative control; G1= Positive control; G2= Group 02; G3= Group 03; G4= Group 04

### *2.7. Statistical analysis*

All the obtained data and parameters (n=10) were subjected to appropriate statistical analysis (Statistix 8.1 software) through one way ANOVA and one way factorial under CRD. Results were indicated as Mean ± SD for every parameter including tumor variables, breast cancer biomarkers and serum biomarkers. The treatment means were compared by applying the HSD test (Tukey test) where the significance level was $P \leq 0.05$ (Montgomery, 2017).

## 3. Results

### *3.1. Breast cancer biomarkers*

#### *3.1.1. Cancer antigen 15.3 (CA 15.3)*

The potential effect of *Capsicum annuum* extract on CA 15-3 level is depicted in Table 2. The results clarify that CA 15-3 levels were considerably altered with respect to each group. The CA 15-3 levels in the breast tissues assorted from 28.45±4.87 U/mL to 25.67±4.39 U/mL in the treatment groups ($G_3$ and $G_4$). The maximum value (35.69±4.25 U/mL) for CA 15-3 levels was noticed in $G_1$ (positive control group) receiving DMBA and routine feed while the lowest value (11.21±1.92 U/mL) was found in $G_0$ (negative control group). It can be clearly seen from the Table 2 that there is a significant reduction in CA 15-3 levels (21.39±3.66 U/mL) in $G_2$ (standard drug group) whereas a substantial decrease in CA 15-3 levels (28.45±4.87 U/mL, 25.67±4.39 U/mL) was also observed in $G_3$ and $G_4$ groups which were being administered with 500mg/kg and 1000mg/kg of *Capsicum annuum* extract throughout the study.

### *3.1.2. Cancer antigen 27.29 (CA 27.29)*

The therapeutic effect of *Capsicum annuum* extract on CA 27-29 level is presented in Table 2. It is clear from the results that levels of CA 27-29 were substantially altered with respect to each group. The CA 27-29 levels in the breast tissues assorted from 34.7±4.80 U/mL to 31.8±4.94 U/mL in the treatment groups ($G_3$ and $G_4$). The maximum value (44.6±4.56 U/mL) for CA 27-29 levels was noticed in $G_1$ (positive control group) receiving routine feed and DMBA while the lowest value (22.4±3.83 U/mL) was found in $G_0$ (negative control group). It can be clearly seen from the Table 2 that there is a substantial reduction in CA 27-29 levels (29.4±3.95 U/mL) in $G_2$ (standard drug group) whereas a substantial decrease in CA 27-29 levels (34.7±4.80 U/mL, 31.8±4.94 U/mL) was also observed in $G_3$ and $G_4$ groups which were being administered with 500mg/kg and 1000mg/kg of *Capsicum annuum* extract throughout the study.

### *3.1.3. Carcinoembryonic antigen (CEA)*

The therapeutic potential of *Capsicum annuum* extract on CEA level is shown in Table 2. It is clear from the results that levels of CEA were substantially altered with respect to each group. The CEA levels in the breast tissues assorted from 2.32±0.39 ng/mL to 1.98±0.33 ng/mL in the treatment groups ($G_3$ and $G_4$). The greatest value (8.36±1.43 ng/mL) for CEA levels was noticed in $G_1$ (positive control group) receiving DMBA and routine feed while the lowest value (0.12±0.02 ng/mL) was found in $G_0$ (negative control group). It can be clearly seen from the Table 2 that there is a significant reduction in CEA levels (1.42±0.24 ng/mL) in $G_2$ (standard drug group) whereas a substantial decrease in CEA levels (2.32±0.39 ng/mL, 1.98±0.33 ng/mL) was also observed in $G_3$ and $G_4$ groups which were being administered with 500mg/kg and 1000mg/kg of *Capsicum annuum* extract throughout the study.

## *3.2. Serum biomarkers*

### *3.2.1. Total antioxidant capacity (TAC)*

The therapeutic potential of *Capsicum annuum* extract on TAC levels is depicted in Table 3 and Figure 1. It is clear from the findings that serum levels of TAC were significantly altered with respect to each group. The TAC levels in the breast tissues varied from 1.81±0.31 mmol/L to 1.92±0.32 mmol/L in the treatment groups ($G_3$ and $G_4$). The lowest values for serum TAC (1.44±0.26 mmol/L) were recorded in $G_1$ group (Positive control) as compared to other groups. The maximum value (1.99±0.34 mmol/L) for TAC levels was noticed in $G_0$ group (negative control) receiving routine feed. It can be clearly seen from the Table 3 that there is a considerable increase in serum TAC levels (1.92±0.32 mmol/L) in $G_4$ group whereas a substantial increase in serum TAC levels (1.68±0.28 mmol/L, 1.81±0.31 mmol/L) was also observed in $G_2$ and $G_3$ groups which were being administered with doxorubicin cyclophosphamide and 500mg/kg of *Capsicum annuum* extract throughout the study. The minimum increase in $G_2$ group may be linked with the oxidative stress induced by drug.

### *3.2.2. Total oxidative stress (TOS)*

The therapeutic outcome of *Capsicum annuum* extract on serum TOS level is shown in Table 3 and Figure 1. The results elucidate that serum TOS levels were extensively improved with respect to each group. The serum TOS levels in the breast tissues varied from 9.23±1.58 µmol/L to 7.13±1.22 µmol/L in the treatment groups ($G_3$ and $G_4$). The maximum value (16.08±2.75 µmol/L) for serum TOS levels was noticed in $G_1$ group (positive control) receiving DMBA and routine feed while the lowest value (7.13±1.22 µmol/L) was found in $G_0$ group (negative control). It can be clearly seen from the Table 3 that there is a significant reduction in serum TOS levels (7.13±1.22 µmol/L) in $G_4$ group whereas a substantial reduction in serum TOS levels (11.6±1.98 µmol/L, 9.23±1.58 µmol/L) was also noticed in $G_2$ and $G_3$ groups which were being administered with doxorubicin cyclophosphamide and 500mg/kg of *Capsicum annuum* extract throughout the study.

### *3.3. Tumor variables*

The effect of *Capsicum annuum* extract on tumor variables has been depicted in Table 4 in the form of mean values. The tumor weight (121.7±5.27 mg), tumor burden (7.81±1.41 n), tumor volume (0.79±0.14 $mm^3$) and tumor incidence (5.98±1.14 %) was higher in $G_1$ positive control group which was treated with DMBA as compared to other treatment groups. A significant reduction was noticed in tumor weight (33.00±3.65 mg), burden (3.82±0.68 n), volume (0.25±0.04 $mm^3$) and incidence (2.65±0.47 %) in $G_2$ group which was receiving doxorubicin as a standard drug. The $G_4$ group treated with 1000mg/kg of *Capsicum annuum* extract showed the rapid reduction in tumor weight (53.67±2.64 mg) and tumor volume (0.43±0.07 $mm^3$) as compared to the $G_3$ group (65.00±3.49 mg, 0.45±0.08 $mm^3$) which was treated with 500mg/kg of the extract. However, the tumor burden (4.00±0.72 n) and tumor incidence (4.00±0.72 %) was significantly reduced in $G_3$ group as compared to the $G_4$ group (4.56±0.82 n, 4.80±0.86 %).

### *3.4. Histopathological examination*

In the histopathological analysis the tissue section obtained from breast of normal rats showed no sign or indication of malignant tumor Figure 2 (A). DMBA induced tumor rats who were only DMBA treated they showed a neoplastic infiltrating composition of cells aligned in glands as it can be seen it Figure 2 (B). Individual tissues or cells were oval to round shaped having average eosinophilic cytoplasm and oval round shaped vesicular nucleus with several depicting nucleoli. Areas of blackish lump with necrosis were also recognized Figure 2 (B). The breast tissue sections of DMBA induced Sprague Dawley rats treated with doxorubicin affirmed a circumscribed damage with areas of fibrosis and adenosis. No indication or evidence of malignant tumor was noticed Figure 2 (C). In next group of Sprague Dawley rats, DMBA induced breast tumor tissues were treated with 500mg/kg of ethanolic extract of *Capsicum annuum*, as a consequence, ductal hyperplasia (adenosis) isolated by collagenous fibro stroma was observed. No sign or evidence of malignant tumor was examined Figure 2 (D). A higher dose of ethanolic extract (1000 mg/kg) of *Capsicum annuum* showed even more significant results with tissues of abscessed lesion comprised of diffused neutrophils infiltration. Stroma manifested swarmed vessels and collagenous fibrous tissue. No sign or evidence of malignant tumor was observed Figure 2 (E). Overall breast tissues obtained from positive control group ($G_1$) exhibited tissue infiltration in lobules, proliferated ducts and fibrous stroma. Necrosis was also observed in large areas (Figure 2 B). However, the tissue sections obtained from the breast of rats administered with doxorubicin cyclophosphamide depicted fewer infiltration and necrosis (Figure 2 C); likewise the rats which were being provided with 500 mg/kg and 1000 mg/kg ethanolic extract of *Capsicum annuum* also revealed less infiltration and necrosis. The treatment groups $G_3$ and $G_4$ were capable to repair some of the damaged breast tissues towards normal (Figure 2 D; Figure 2 E).

**Table 2** Effect of *Capsicum annuum* extract on biomarkers of breast cancer in blood of various groups of rats

| Treatments | Cancer antigen 15-3 (U/mL) | Carcinoembryonic antigen (ng/mL) | Cancer antigen 27-29 (U/mL) |
|---|---|---|---|
| G0 | $11.21±1.92^{d}$ | $0.12±0.02^{c}$ | $22.4±3.83^{c}$ |
| G1 | $35.69±4.25^{a}$ | $8.36±1.43^{a}$ | $44.6±4.56^{a}$ |
| G2 | $21.39±3.66^{c}$ | $1.42±0.24^{b}$ | $29.4±5.03^{bc}$ |
| G3 | $28.45±4.87^{b}$ | $2.32±0.39^{b}$ | $34.7±4.80^{b}$ |
| G4 | $25.67±4.39^{bc}$ | $1.98±0.33^{b}$ | $31.8±4.94^{b}$ |

Values indicated are means ± SD of ten values

Mean values within a row, having distinct superscript are considerably different from each other

**Table 3** Effect of *Capsicum annuum* extract on serum biomarkers in blood of various groups of rats

| Treatments | TAC (mmol/L) | TOS (µmol/L) |
|---|---|---|
| G0 | $1.99\pm0.34^{a}$ | $7.16\pm1.22^{c}$ |
| G1 | $1.44\pm0.26^{b}$ | $16.08\pm2.75^{a}$ |
| G2 | $1.68\pm0.28^{ab}$ | $11.6\pm1.98^{b}$ |
| G3 | $1.81\pm0.31^{ab}$ | $9.23\pm1.58^{bc}$ |
| G4 | $1.92\pm0.32^{a}$ | $7.13\pm1.22^{c}$ |

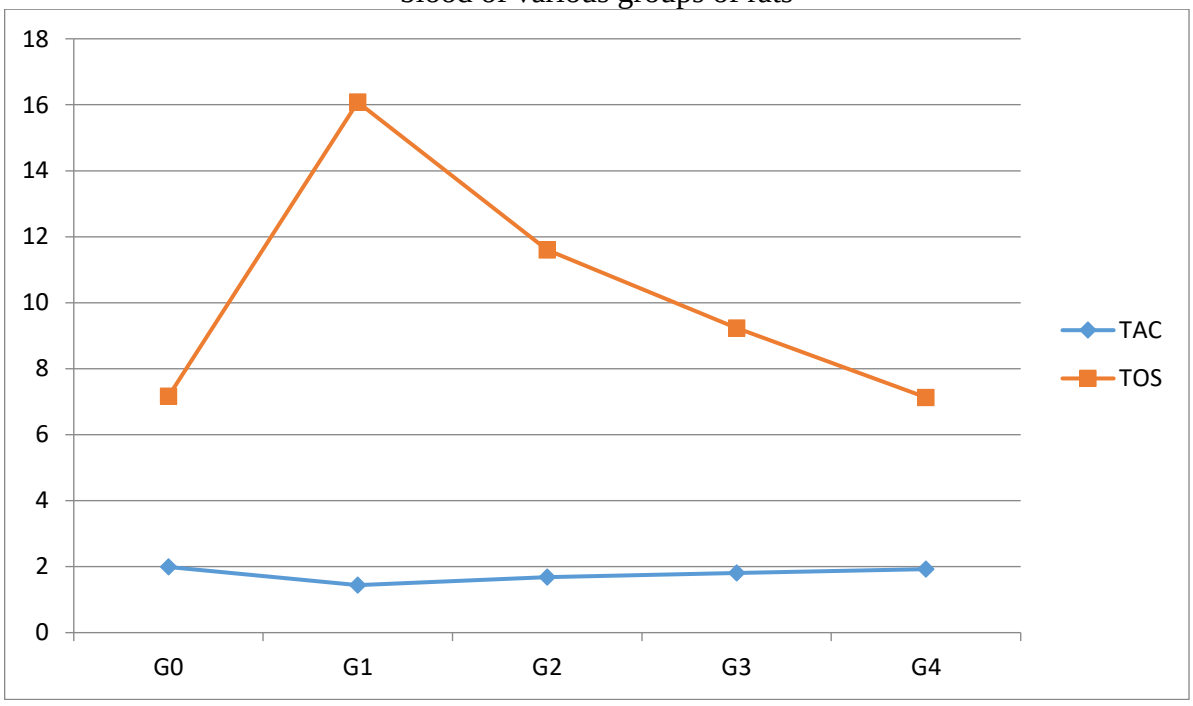
Values indicated are means ± SD of ten values
Mean values within a row, having distinct superscript are considerably different from each other

**Table 4** Effect of *Capsicum annuum* extract on tumor variables in various groups of rats

| Treatments | Tumor weight (mg) | Tumor burden (*n*) | Tumor volume ($mm^3$) | Tumor incidence (%) |
|---|---|---|---|---|
| G0 | $0.00\pm0.00^{d}$ | $0.00\pm0.00^{c}$ | $0.00\pm0.00^{d}$ | $0.00\pm0.00^{d}$ |
| G1 | $121.7\pm5.27^{a}$ | $7.81\pm1.41^{a}$ | $0.79\pm0.14^{a}$ | $5.98\pm1.14^{a}$ |
| G2 | $33.00\pm3.65^{c}$ | $3.82\pm0.68^{b}$ | $0.25\pm0.04^{c}$ | $2.65\pm0.47^{c}$ |
| G3 | $65.00\pm3.49^{b}$ | $4.00\pm0.72^{b}$ | $0.45\pm0.08^{b}$ | $4.00\pm0.72^{b}$ |
| G4 | $53.67\pm2.64^{b}$ | $4.56\pm0.82^{b}$ | $0.43\pm0.07^{b}$ | $4.80\pm0.86^{b}$ |

**n* denotes the no. of tumors per rat; values are indicated as mean ± SD
Mean values within a row, having distinct superscript are considerably different from each other

**Figure 1** Graphical representation of effect of *Capsicum annuum* extract on serum biomarkers in blood of various groups of rats



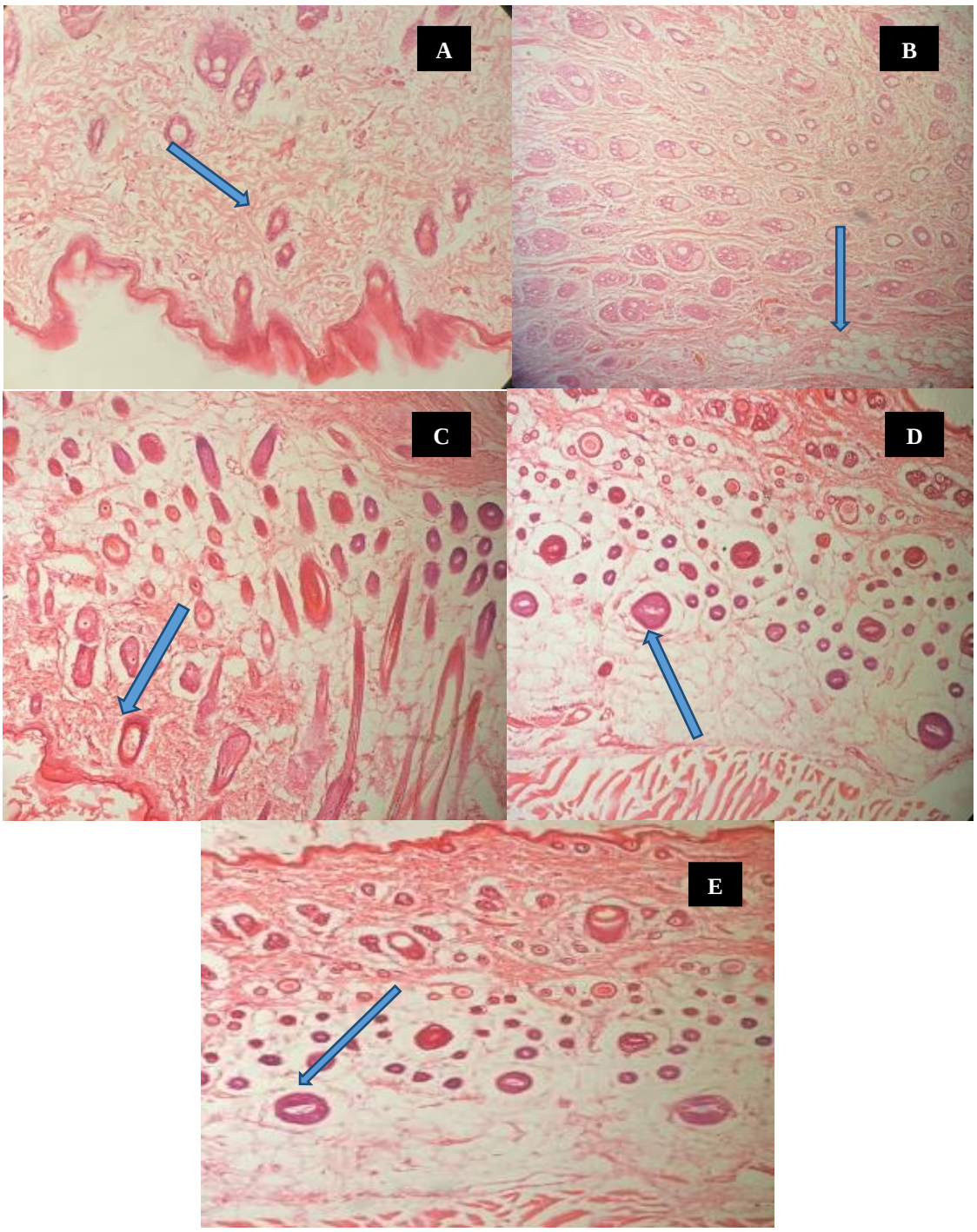



**Figure 2** Histopathological images of breast tissues

Figure 2(A) shows the image of normal breast tissues having no indication or sign of malignant cancer. Figure 2(B) shows the areas of blackish lump with necrosis. Figure 2(C) shows the image of breast tissues with areas of adenosis and fibrosis. Figure 2(D) shows the image of breast tissues with adenosis isolated by collagenous fibro stroma. Figure 2(E) exhibits the image of breast tissues with stroma manifested swarmed vessels and collagenous fibrous tissue.

## 4. Discussion

Findings of the present research showed that ethanolic extract of *Capsicum annuum* had a substantial effect on breast cancer. Significant reduction in levels of breast cancer biomarkers including CA 15.3, CA 27.29 and CEA was noticed. The end points of tumorigenesis such as tumor weight, volume, burden and incidence were significantly decreased in groups treated with ethanolic extract of *Capsicum annuum* as compared to the DMBA treated group. There was also a significant

effect of extract on serum biomarkers ($P \leq 0.05$). The histopathological analysis of breast tissues which were being provided with capsicum extract showed no evidence or sign of hyperplasia and malignant tumor. The results of current study are in accordance to the findings of previous studies. Lin *et al*. (2017) conducted an efficacy study to associate serum levels of CA 15.3 and healing response in patients with advanced breast carcinoma. It was also reported that change in levels of CA 15.3 showed the healing response and it functions as an important marker principally in patients with unobtainable abrasions. Coppola *et al*. (2021) determined the levels of CA 27.29 in the women after 2.5 years of cancer diagnosis and it was examined that higher levels of CA 27.29 were associated with disease progression. Hall *et al*. (2019) conducted a trial to evaluate the anti-cancer potential of taxol against breast carcinoma. It was found that levels of CEA were increased in rats which were receiving no dose. Furthermore, drug and taxol treated rats exhibited lower CEA levels. The CEA value in rats administered with taxol was 4.9±0.84 ng/mL in comparison to the positive control rats (9.6±1.8 ng/mL). Elwan *et al*. (2020) determined the effect of *Capsicum annuum* dietary supplementation on blood biochemistry, hematology and antioxidant capacity in rats. The findings of this study showed greater total antioxidant capacity after consumption of 200g of *Capsicum annuum* in treatment groups. Higher levels of TAC are linked with the absorption of capsaicinoids and phytochemicals in bloodstream. It was also found that oxidative damage was reduced to a significant level owing to decrease in oxidation. . Capsaicin is a well-known substance found in capsicum and prevents the initiation of reactive oxygen species (ROS) which are generated by oxidative damage Luo *et al*. (2021). The results exhibited maximum improvement in serum levels of TAC and TOS. Markedly, 32% reduction in oxidative stress was observed after capsaicin treatment. Henry *et al*. (2020) examined the tumor variables in various groups of rats. The tumor weight, incidence, volume and burden were higher in rats treated with DMBA as 122.7 ± 3.01 mg, 6.00 ± 0.89 %, 0.83 ± 0.07 $mm^3$ and 7.83 ± 0.75 n correspondingly. The significant decrease in these tumor variables was noticed in rats which were being administered with *T. Chebula ethanolic* extract as 54.67 ± 2.58 mg, 4.83 ± 0.75 %, 0.44 ± 0.01 $mm^3$ and 4.66 ± 1.36 n. Deepalakshmi and Mirunalini (2013) also evaluated the potential of *Ganoderma lucidum* on mammary tumor incidence. Breast cancer in rats was induced by a single injection of DMBA (30mg) subcutaneously. In a positive control group, the breast cancer incidence was 100% whereas it was 70% in *G. lucidum* treated groups. The tumor volume was also less in a group treated with *Ganoderma lucidum* extract as compared to the DMBA group.

## 5. Conclusion

Herbal and traditional medicinal approaches have been used for many years throughout the world at household level but not adapted at industrial and research level which needs to be extensively studied in animals for the sake of their use in pharmacological based medicines appropriate for the treatment of human beings without any harmful effect. Extracts and herbal medicines have been used in several efficacy trials but there was biasness owing to other biologically active substances in extracts which can interrelate with the biological role of any specific substance of interest and depicts the biased consequences. The phytochemicals present in plants are majorly recognized for their defensive actions. Among them flavonoids and phenolics are the most important ones having anticancer, antimicrobial, antioxidant and anti-inflammatory properties. They employ their therapeutic effects by stimulating the immune system, slowing the growth of cancerous cells and preventing the DNA damage which leads to the cancer progression and various other ailments. In this perspective, green *Capsicum annuum* known as “Shimla mirch” contains a variety of biological substances in abundant amounts with anti-cancerous and immune enhancing activities. The present research was planned to explore the anti-cancerous and anti-tumor effects of green *Capsicum annuum* against breast cancer. Results of the present research showed that ethanolic extract of *Capsicum annuum* had a significant effect on breast cancer. Significant reduction in levels of breast cancer biomarkers including CA 15.3, CA 27.29 and CEA was noticed. The end points of tumorigenesis such as tumor weight, volume, burden and incidence were significantly decreased in groups treated with ethanolic extract of *Capsicum annuum* as compared to the DMBA treated group.

There was also a significant effect of extract on serum biomarkers ($P \leq 0.05$). The histopathological analysis of breast tissues which were being provided with capsicum extract showed no evidence or sign of hyperplasia and malignant tumor. Our study findings reported that *Capsicum annuum* ethanolic extract might be useful in the prevention of breast cancer. Furthermore, keeping in view the above mentioned results, *Capsicum annuum* should be utilized as a viable replacement for nutritive and synthetic sweeteners in various foodstuffs to avoid the occurrence of numerous physiological diseases.

## References


1. Ahmad, R., A. Alqathama, M. Aldholmi, M. Riaz, A.N. Abdalla, A. Mostafa, H.M. Al-Said, A.M. Alqarni, R. Ullah, S.S. Asgher and M. Amir. 2022. Gas Chromatography-Mass Spectrometry (GC-MS) Metabolites Profiling and Biological Activities of Various Capsicum annum cultivars. Plants. 11: 102-114.
2. Akhouri, V., Kumari, M., & Kumar, A. 2020. Therapeutic effect of Aegle marmelos fruit extract against DMBA induced breast cancer in rats. Scientific Reports, 10(1), 1-12.
3. Anna, N. and W.P. Olszewski. 2021. The role of stromal immune microenvironment in the progression of ductal carcinoma in situ (DCIS) to invasive breast cancer. Breast Cancer Research. 23: 106-118.
4. Ashraf, M., V. Kalaichelvan, R. Ragunathan and V. Venkatachalam. 2021. Antiproliferative potential of ethyl acetate extract of *Clerodendrum thomsoniae* Balf. F. on DMBA-induced breast cancer in female Sprague Dawley rats. Indian J. Pharmaceutical Educ. Res. 55: 1-10.
5. Basharat, S., S.A. Gilani, F. Iftikhara, M.A. Murtazac, A. Basharatd, A. Sattare, M.M. Qamarf and M. Alig. 2020. Capsaicin: Plants of the Genus Capsicum and Positive Effect of Oriental Spice on Skin Health. Skin. Pharmacol. Physio. 2: 1-11.
6. Coppola, L., A. Cianflone, K. Pane, M. Franzese, P. Mirabelli and M. Salvatore. 2021. The impact of different preanalytical methods related to CA 15-3 determination in frozen human blood samples: a systematic review. Syst. Rev. 10: 102-111.
7. Deepalakshmi, K. and S. Mirunalini. 2013. Modulatory effect of Ganoderma lucidum on expression of xenobiotic enzymes, oxidant-antioxidant and hormonal status in 7,12-dimethylbenz (a) anthracene-induced mammary carcinoma in rats. Pharmacogn. Mag. 9: 159-167.
8. Elwan, M., M. Abdelhakeam, S. El-Shafei, A.A. El-Rahman, Z. Ismail, A. Zanouny, E. Shaker 2, S. S. Al-Rejaie, M. Mohany and S. Elnesr. 2020. Efficacy of Dietary Supplementation with Capsicum Annum L on Performance, Hematology, Blood Biochemistry and Hepatic Antioxidant Status of Growing Rabbits. Anim. Sci. J. 10: 2-14.
9. Gueyo, T. N., Zingue, S., Mvondo, M. A., Mmutlane, E., Ndinteh, D. T., Pieme, C. A and Njamen, D. 2021. Cytotoxic and cancer chemopreventive potentials of the Anthonotha macrophylla P. Beauv aqueous extract on 7, 12-dimethylbenz [a] anthracene-induced breast cancer in rats. Biologia, 76(2), 729–739.
10. Hall, C., L. Clarke, A. Pal, P. Buchwald, T. Eglinton, C. Wakeman and F. Frizelle. 2019. A Review of the Role of Carcinoembryonic Antigen in Clinical Practice**.** Ann. Coloprotocol. 35: 294-305.
11. Hassanpour, S.H. and M. Dehghani. 2017. Review of cancer from perspective of molecular. J. Cancer. Res. Clin. Oncol. 4: 127-129.
12. Henry, D.P.J., J. Ranjan, R.K. Murugan, A. Sivanantham and M. Alagumuthu. 2020. Exploration of anti-breast effects of Terminalia chebula extract on DMBA induced mammary carcinoma in Sprague dawley rats. Future J. Pharm. Sci. 6: 1-13.
13. Karimi, B., Ashrafi, M., Shomali, T., & Yektaseresht, A. 2019. Therapeutic effect of simvastatin on DMBA-induced breast cancer in mice. Fundamental & Clinical Pharmacology, 33(1), 84-93.
14. Kim, E.H., S.Y. Lee, D.Y. Baek, S.Y. Park, S.G. Lee, T.H. Ryu, S.K. Lee, H.J. Kang, O.H. Kwon, M. Kil and S.W. Oh. 2019. A comparison of the nutrient composition and statistical

profile in red pepper fruits (Capsicums annuum L.) based on genetic and environmental factors. Appl. Biol. Chem. 1: 1-13.
15. Kurnijasanti, R., A.I.P. Edward, Y. Kristijanto, D. Mahendra and N.I. Hermanda. 2017. Potential of green chili extract (*Capsicum annuum*) on myeloma cells death. Adv Health Sci Educ. 5: 1-3.
16. Laya, A., Koubala, B. B., Pathak, K. P., & Bueno, V. 2021. Vitamins and Provitamins Intake as New Insights to Prevent and/or to Treat breast Cancer: A Systemic Review. Int J Cancer Clin Res, 8, 162.
17. Lin, Y.T., H.C. Wang, Y.C. Hsu, C.L. Cho, M.Y. Yang and C.Y. Chien. 2017. Capsaicin Induces Autophagy and Apoptosis in Human Nasopharyngeal Carcinoma Cells by Downregulating the PI3K/AKT/mTOR Pathway. Int. J. Mol. Sci. 18: 1-16.
18. Lu, M., & Zhan, X. 2018. The crucial role of multiomic approach in cancer research and clinically relevant outcomes. EPMA Journal, 9(1), 77-102.
19. Luo, X. S. Yang, Y. Luo, H. Qiu, T. Li, J. Li, X. Chen, X. Zheng, J. Zhang, Z. Zhang and C. Qin. 2021. Molecular Characterization and Expression Analysis of the $Na^+/H^+$ Exchanger Gene Family in *Capsicum annuum* L. Plant Genomics. 12: 47-61.
20. Montgomery, D.C., 2017. In: Design and Analysis of Experiments, ninth ed. John Wiley and Sons. Inc., Hoboken, NJ, USA, pp. 162-264.
21. Oh, J., Giallongo, F. Frederick, T. Pate, J. Walusimbi, S. Elias, R.J. Wall, E.H. Bravo and A.N. Hristov. 2015. Effects of dietary Capsicum oleoresin on productivity and immune responses in lactating dairy cows. J. Dairy Sci. 98: 6327-6339.
22. Saleh, A., H.M. ElFayoumi, M. Youns, and W. Barakat. 2018. Rutin and orlistat produce antitumor effects via antioxidant and apoptotic actions. Naunyn-Schmiedeb. Arch. Phar. 2: 1-11.
23. Schmidt, D. R., Patel, R., Kirsch, D. G., Lewis, C. A., Vander Heiden, M. G., & Locasale, J. W. 2021. Metabolomics in cancer research and emerging applications in clinical oncology. CA: A Cancer Journal for Clinicians, 71(4), 333-358.
24. Shao, Y., X. Sun, Y. He, C. Liu and H. Liu. 2015. Elevated levels of serum tumor markers CEA and CA15-3 are prognostic parameters for different molecular subtypes of breast cancer. PLoS One. 7: 133-140.
25. Siegel, R.L., K.D. Miller and A. Jemal. 2020. Cancer statistics. CA: Cancer J. Clin. 70: 7-30.
26. Zingue, S., Cisilotto, J., Tueche, A. B., Bishayee, A., Mefegue, F. A., Sandjo, L. P., Magne Nde, C. B., Winter, E., Michel, T., Ndinteh, D. T., Awounfack, C. F., Silihe, K. K., Melachio Tanekou, T. T., Creczynski-Pasa, T. B., & Njamen, D. (2016). Crateva adansonii DC, an African ethnomedicinal plant, exerts cytotoxicity in vitro and prevents experimental mammary tumorigenesis in vivo. Journal of Ethnopharmacology, 190, 183–199. https://doi.org/10.1016/j.jep.2016.06.004.
27. Zaheer, S., N. Shah, S.A. Maqbool and N.M. Soomro. 2019. Estimates of past and future time trends in age-specific breast cancer incidence among women in Karachi, Pakistan: 2004–2025. BMC Public Health. 19: 1-9.