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The effects of 4β-hydroxy withanolide E extracted from *Physalis peruviana* on Complete Blood Count of Dimethylbenz(a)anthracene-induced Breast Cancer in Albino Rats

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Abstract

Background: Several studies reveal changes in blood parameters in patients with cancer. Complete blood count is a good predictor for many cancer diagnoses and prognoses. They are also helpful in assessing drug efficacy and toxicity. Cancer may be treated with bioactive and antioxidant compounds derived from plants. The effects of *Physalis peruviana* on the complete blood count of dimethylbenz(a)anthracene (DMBA)-induced breast cancer are not well characterized.

Methods: This study was conducted at Liaquat University of Medical and Health Sciences, Jamshoro, for six months on 60 female albino rats 5 equal groups (n = 12 per group). The effects of *Physalis peruviana* fruits and tamoxifen on complete blood count in dimethylbenz(a)anthracene induced breast cancer were assessed. Blood samples were collected in weeks 1, 5, 7, and 10 for hematological changes before, during, and after treatment.

Result: Rats of group E (Tumor-induced albino rats treated with the extracts of *Physalis Peruviana* and tamoxifen) gave the best results compared to other groups. In this group, increases in weight along with an improvement in hematological parameters were observed when compared with others.

Conclusion: The extract from *Physalis Peruviana* helps in the reversal of pancytopenia with anemia and thrombocytopenia caused by tumor-inducing drugs.

Introduction

In women, breast cancer is the most common malignancy and the second leading cause of cancer-related death worldwide. Approximately one in nine women develops breast cancer during her lifetime. In Pakistan, the incidence rate is about 50 per 100,000 women, with around 83,000 cases reported annually and more than 40,000 deaths [1]. There are many therapeutic methods including surgical removal, radiation and chemotherapy. This troubling condition challenges healthcare workers to search for a new cure and remedy. Among many others, the oldest anticancer Stainless steel cages were used for housing animals with 2 cm thick rice husk bedding in each cageremedies from extracts of several plants are drawing attention due to potential side effects and resistance of current therapies [2].

Physalis peruviana belongs to the Solanaceae family and the *Physalis* genus known as golden berry and Cape gooseberry [3]. The anti-proliferative effects of *Physalis peruviana* leaves, stems, and whole plants on various carcinomas including colon, chronic myeloid leukemia, and breast cancer are well recognized. Its bioactive compound, 4β-Hydroxy withanolide E generates reactive oxygen species (ROS) inducing apoptosis of cancer cells. The accumulation of this bioactive compound in tumor cells contributes to regulating Nrf2 and superoxide dismutase protein expression. The presence of catalase leads to decrease in tumor size [4]. The blood is a vital connective tissue; a basic medium of exchange for oxygen, carbon dioxide, nutrients, and metabolites essential for life [5]. Several studies report various side effects on blood parameters mostly red blood cells due to cancer therapy [6]. This study is intended to determine various hematological parameters of DMBA-induced breast cancer and to assess the effects of *Physalis peruviana* extract 4β-Hydroxy withanolide E on various hematological parameters.

Methods

Study place and duration

This study was conducted after institutional ethical approval for six months at the Liaquat University of Medical and Health Sciences, Jamshoro in Pakistan in collaboration with Sindh Agricultural University Tando Jam Animal House and the Medical Research Center Jamshoro, Pakistan.

Animals: For this study, 60 adults female Wistar albino rats were purchased from the National Institute of Health, Karachi. Healthy female rats weighing between 165-175 g were included in this study.

Experimental design: Animals were handled according to protocol of animal's care provided by guidelines of National Institute of Health Pakistan (NIH). Stainless steel cages were used for housing animals with 2 cm thick rice husk bedding in each cage; well-ventilated and had a 12-hour light-dark cycle alternating with temperature 22 ± 2 °C and relative humidity 55 ± 10% [7]. The environment of the cages and the surroundings were kept properly hygienic and adequately ventilated. Each cage contained four animals. food and water were available *ad libitum*. The standard chow was used as feed and tap water to drink. Animals were divided into five equal groups (n = 12 per group), each housed in separate cages. All animals were weighed and a blood sample was taken before, during and after experiment [8].

Animal Groups and Treatments:

Group A: Control (vehicle-treated normal animals).
Group B: Disease control (DMBA-induced, untreated).
Group C: TR1– DMBA Induced rats were treated with tamoxifen.
Group D: TR2– DMBA induced rats were treated with 4 β-Hydroxy withanolide E
Group E: TR3– DMBA induced rats were treated with tamoxifen and 4 β-Hydroxy withanolide E.

Plant Material and Extract Preparation: *Physalis peruviana* was obtained from Botanical store Karachi, Pakistan, certified by the Department of Botany of Sindh University Jamshoro. One hundred grams of Physalis peruviana fruit was boiled in 1 liter of distilled water (infusion method). The extract was filtered through lyophilization and dried for 3 hours and stored at -21°C until use.

Tumor Induction: DMBA (Dimethylbenz(a)Anthracene) is a yellowish green, odorless chemical derivative of phenanthrene; an immunosuppressant and a potent laboratory carcinogen purchased from Sigma Aldrich.
Soy oil: purchased from the Departmental store.

A single dose of DMBA (20 mg kg^{-1}) in soy oil (5 ml) given by intra-gastric gavage is sufficient to induce tumor in the mammary gland. Each rat was weighed and examined for tumor in the mammary gland by gross inspection, palpation weekly. The tumor was confirmed by biopsy on the 6th week after an administration of DMBA [9].

***Physalis peruviana* administration**: Extracted dried *Physalis peruviana* 100 mg/kg/week administered with diet.

Tamoxifen administration: Tamoxifen (Sigma-Aldrich) was dissolved in soy oil at 20 mg·mL^{-1}. A dose of 75

mg·kg^{-1} body weight was administered by intraperitoneal injection.

Blood sampling: Day 0 was designated as the time of DMBA administration. Blood was collected at baseline (prior to DMBA induction, Day 0), and subsequently at weeks 1, 5, 7, and 10. Treatments with Physalis peruviana extract commenced at week 4, and thus weeks 5, 7, and 10 represent during-treatment intervals. The blood samples were collected before tumor induction and at weeks 1, 5, 7, and 10 after anesthetizing rats with ketamine injection. The blood (1ml) was collected by retro-orbital technique via the orbital sinus in heparinized micro-crit capillary tubes and transferred to the vacutainer blood collection tube [10].

Statistical analysis:

The data were analyzed using SPSS version 26.0. Numerical parameters (weight, hemoglobin, platelets, red blood cells, and white blood cells) were presented as mean ± standard deviation (SD). Group means were compared using one-way ANOVA. When ANOVA indicated significance, post-hoc comparisons between groups were performed using Tukey's test. For planned pairwise comparisons, Student's t-test with appropriate correction was applied. The value of $p < 0.05$ was considered statistically significant. Data outliers and unusually large standard deviations were retained in the analysis; however, these are noted in Table 2 for transparency.

Results

We assessed the difference in body weight measurement (table 1) and hematological parameters; Hemoglobin (Hb), Red Blood Cells (RBCs), White Blood Cells (WBCs) and platelets with Mindray BC-2300 hematology analyzer (Table 2).

Figure 1: The administration of *Physalis peruviana* and collection of the blood sample.

Weight measurement

The mean body weight was compared among the five groups using one-way ANOVA, which showed a significant increase in the weight of rats in Group E compared with Group B ($p < 0.001$). Rats in the Negative Control group (Group B) experienced a consistent trend of weight loss through week 7. However, the mean weight recorded at week 10 (261.66±76.46 g) is anomalously high and contradicts the overall observation of declining health in this group. This figure, along with its very large standard deviation, suggests a potential error in data recording.

Hematological parameters

By applying for the ANOVA test, a significant decrease in RBC and hemoglobin in both Groups B and C ($p < 0.001$) was observed. At week 5, RBC differences between groups were not significant ($p = 0.867$), but by weeks 7 and 10, significant declines were seen in Groups B and C compared with controls ($p < 0.001$). There was a significant increase in RBCs and hemoglobin in Group E. Hemoglobin and platelet counts in Group B showed large standard deviations at weeks 7 and 10, reflecting high inter-animal variability. WBC and platelets increased to near-normal levels after treatment with Physalis peruviana. WBC counts increased markedly in Groups B–E at week 7, indicating leukocytosis, but declined in Group B at week 10 (2.01 ± 1.88 $\times 10^9$/L), consistent with terminal immune suppression. We also found a significant decrease in WBC and platelets in Groups B and D ($p < 0.001$). A significant increase in WBC and platelets in Group E ($p < 0.001$) was observed after 10 weeks. There were no major variations between Group A and Group E. After treatment, Group E revealed gross improvement in body weight and all hematological parameters.

Abbreviations: PC, positive control (Normal); NC, negative control (Induced with DMBA, no treatment) – Control experiment; TR1, DMBA induced rats treated with tamoxifen; TR2, DMBA induced with 4β-hydroxy withanolideE; TR3, DMBA induced with tamoxifen and 4β-hydroxy withanolide E

Discussion

The phytochemicals found in leaves, fruits, and other parts of several plants have therapeutic potential for treating various diseases including cancers [11]. The incidence of breast cancer is increasing in developing countries [12]. Several factors contribute to cancer development, including inflammation [13]. In breast tissue, DMBA causes DNA damage and promotes cell proliferation [14]. Vitamin C& E, polyphenols, carotenoid compounds, lutein, and lycopene are antioxidants present in many plant extracts, helps in tumor suppression [15].

The complete blood count reflects the response of cellular immunity in a patient with cancer. [16]. In this study, after induction with DMBA (carcinogen), blood sampling at weeks 1, 5, 7, and 10 revealed drastic variations among Group A (Positive Control) and Group

B (Negative control (Induced with DMBA no treatment). We found significant improvement in all parameters of Group E (DMBA induced rats treated with tamoxifen and 4β-hydroxy withanolideE) compared to Group B (Negative control (Induced with DMBA no treatment).These observations are line with Akuru [17] who reported indices of DMBA administered albino rats, given aqueous concentrate of different plants. For RBC and Hb concentrations, there was a significant increase ($p \leqslant 0.05$) in all the treated groups when contrasted with the control and DMBA groups ($p \leqslant 0.05$) [17].

In our results, there was a significant decrease in RBC and hemoglobin in all four groups induced by DMBA compared to the Group A. This contrasts with Akuru et al, who showed contrary results and reported that there was an additional increase in the RBC level in all groups when compared with the control and DMBA untreated group, however not significant ($p \leqslant 0.05$)[17]. Low Hb and RBC counts observed may be consistent with anemia associated with cancer; however, survival or prognostic implications were not assessed in this animal study [18,19]. The unusually large variation in hemoglobin values in Group B (week 7 and 10) suggests that some animals developed severe anemia, while others maintained near-normal levels. This heterogeneity should be considered when interpreting the group averages. There is a high incidence of anemia observed in cancer patients, which may be caused by bleeding, nutritional deficiencies, damage to the bone marrow, or tumor infiltration [20].

Different plants have been shown to possess anti-anemic properties. Zingue et al. observed ethanoic extracts of plants were useful in increasing multiple biochemical markers of DMBA-induced mammary tumors, including MCV, MCHC, MCH, RBC, PCV, and hemoglobin concentrations, compared to DMBA-untreated animals [21].

White Blood Cells have increased to normal levels after treatment with *physalis peruviana* and tamoxifen in group E (DMBA induced rats treated with tamoxifen and 4 β-Hydroxywithanolide E) versus Group C (DMBA induced rats treated with tamoxifen) and D (DMBA induced with 4β-hydroxy withanolideE). This was similar to the study conducted by Akuru et al, who revealed the white blood cells of DMBA administered to albino rats given different plant extracts. There was a significant increase ($p \leqslant 0.05$) in the white blood cell level of animals treated with 1000mg/kg of a plant when compared with the control and the DMBA-untreated group. The DMBA group had the most reduced white blood cell level(not significant, $p \leqslant 0.05$) [17]. When the WBC count is below the normal level, infection is a serious risk, whereas higher counts increase the chance of developing invasive breast cancer. This is also consistent with the findings of Chen et al. [22], who found that plant extracts could reduce the levels of WBCs, neutrophils, and lymphocytes in DMBA-induced mammary cancer. In a study, Akuru et al (2019) measured blood platelet concentrations of

Weight	Week	Groups					*p-value*
		A: PC	B: NC	C: TR1	D: TR2	E: TR3	
(grams, mean ± SD)	1	190.83±5.14	194.58±23.39	201.25±21.43	198.75±19.67	212.08±12.33	*0.054*
	5	209.5±11.5	176.25±26.89	178.75±26.80	175.41±25.71	199.16±12.58	*<0.0001**
	7	230.41±14.91	159.16±32.53	191.66±20.92	187.91±21.15	211.66±11.14	*<0.0001**
	10	276.25±11.30	261.66±76.46	207.91±17.76	207.08±23.68	233.33±15.71	*<0.0001**

* *p*-value is statistically significant calculated by One Way ANOVA

Table 1: Comparison of weight at week 1,5,7 and 10 in different groups (n=12) of Albino rats

Parameters	Week	Groups					*p-value*
		A: PC	B: NC	C: TR1	D: TR2	E: TR3	
RBC ($x10^{12}/L$)	1	9.15±0.65	9.50±0.92	9.49±0.76	7.99±0.76	7.79±0.76	*0.390*
	5	8.45±0.65	8.80±0.92	8.79±0.76	7.29±0.76	7.09±0.76	*0.867*
	7	9.65±0.61	6.60±1.79	6.55±2.06	7.29±0.76	6.09±0.76	*<0.0001**
	10	9.37±0.96	7.93 ±2.65	4.59±0.80	5.09±0.76	9.05±0.76	*<0.0001**
Hemoglobin (g/dL)	1	14.42±1.64	15.17±1.15	14.17±1.15	12.97±1.15	11.67±1.15	*<0.0001**
	5	13.42±1.64	14.17±1.15	13.17±1.15	11.97±1.15	10.67±1.15	*<0.0001**
	7	15.73±1.82	11.15±1.69	14.48±0.91	13.48±0.91	12.98±0.91	*<0.0001**
	10	15.65±1.71	6.30±5.23	14.20 ± 1.51	11.98±0.91	11.48±0.91	*<0.0001**
WBC ($x10^9/L$)	1	8.01±1.38	7.61±0.98	6.99±1.43	7.21±1.38	6.71±1.38	*0.144*
	5	7.71±1.38	7.31±0.98	6.69±1.43	6.91± 0.91	6.41±1.38	*0.154*
	7	7.54±0.91	13.66±4.57	14.95±4.04	14.45±4.04	13.95±4.04	*<0.0001**
	10	7.01±1.15	2.10±1.88	7.35±2.59	6.95±2.59	13.45±4.04	*<0.0001**
Platelet ($\times10^9/L$)	1	387.83±82.6	370.16±67.63	366.33±23.56	367.83±53.40	693.75±254.5	*0.132*
	5	387.33±82.6	369.66± 76.3	365.83±23.56	367.33±53.40	693.25±254.5	*<0.0001**
	7	369.50±62.7	781.66±259.62	666.91±151.29	469.50±62.73	781.66±74.94	*<0.0001**
	10	415.33±144.2	65.5 ± 48.5	449.62±161.29	367.50±62.73	779.66±259.62	*<0.0001**

* *p*-value is statistically significant calculated by One Way ANOVA

Values flagged indicate unusually high variability; these should be verified against raw data. Large SDs likely reflect inter-animal variation due to disease severity

Table 2: Comparison of Hematological parameters at week 1,5,7 and 10 in different groups(n=12) of Albino rats

group showed a significant increase ($p \leq 0.05$) when compared to the control group and the DMBA STD group. The DMBA-untreated group had lower values than the various treated groups (although not significantly, $p \leq 0.05$). According to Rochet, Markovic [23] because platelets release a variety of growth factors and cytokines that promote angiogenesis, high platelet counts are associated with poorer prognosis for cancer, which is crucial to breast cancer metastasis [24]. This study reveals that this extract reduced blood platelets and hence angiogenesis, as evidenced by the lower values of platelet count. However, platelet counts in Group B at week 10 displayed extreme variability ($655.5 \pm 485.5 \times 10^9$/L), likely reflecting heterogeneous disease progression. Similar is reported by Zingue et al [21], that ethanolic extracts of the plant reduced platelet concentration in tumors induced by DMBA compared to the group not exposed to DMBA.

This study concludes *Physalis peruviana* aqueous extract was found to enhance all hematological parameters of DMBA-induced breast cancer in albino rats. It increases the red blood cells and platelet count and stabilizes white blood cells in their function to reduce the anemic burden caused by cancer. It decreases the risk of thrombocytopenia. Compared with different adverse effects and contraindications of traditional drugs, cultural values, efficacy, low cost, and easy availability encourage the use of medicinal herbs. The use of *Physalis peruviana* in breast cancer treatment has significant potential, especially the adjunct to tamoxifen. To evaluate these effects, further research is required, particularly clinical studies.

Competing Interests

The authors declare that there is no conflict of interest.

Author Contributions

Fahmida Gul: Designed the experiment, data collection and analyzes the study and drafting of the manuscript. Corresponding author

SamreenMemon: Conceived the idea,was the in-charge of data collection, final approval of the manuscript.

Ikramuddin Ujjan; Brought new ideas, helped intechnical issues.

PushpaGoswami:Helped indata collectionand drafting of manuscript.

Kanwal Abbas:Helped in statistics fordataanalysis and final drafting of manuscript

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