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RESEARCH ARTICLE
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ROLE OF PHYSALIS PERUVIANA IN COMBINATION WITH TAMOXIFEN IN DECREASING ANXIETY AND DEPRESSION IN RATS WITH DIMETHYLBENZ(A)ANTHRACENE (DMBA) INDUCED BREAST CANCER

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Abstract

Background: *Physalis Peruviana* has neuroprotective effects on anxiety and depression induced by Cancer. Psychosocial variables may influence breast cancer susceptibility and prognosis.
Objective: This research aims to evaluate the efficacy of *Physalis Peruviana* fruit extract in anxiety and depression due Breast cancer induced by DMBA in Albino rats.
Materials and Methods: The 60 female albino rats used in this study were divided into 05 groups and induced with tumor and treated for 10 weeks at Liaquat University of Medical and Health Sciences, Jamshoro.
Results: Rats in Group E demonstrated improving activity and reduction in depression and anxiety.
Conclusion: The *Physalis Peruviana* extract has neuroprotective effects on depression and anxiety.

Keywords: *Physalis Peruviana*; Breast Cancer; Tamoxifen resistance; Anxiety; Depression.

Introduction

Breast cancer prevalence in Asia is high, impacting 1 in 9 women, with 83,000 Pakistanis affected, and its incidence is rapidly increasing (Shoukat1, et al.2023). With an incidence rate of 50 cases per 100,000 people, affects 11% of women globally and causes over 40,000 deaths.(Hu 2021).
Tamoxifen, a widely used treatment for breast cancer, reduces overall mortality by 31%, but 50% of cases become resistant and 40% cease responding (Wang, Hu et al. 2019). The potential benefits of

modifying diets or incorporating supplements for cancer patients and enhancing current treatments are driving the investigation of dietary compounds Medicinal plants, which contain a diverse range of bioactive phytochemical compounds, present a promising alternative to conventional pharmaceuticals. These plants possess notable antioxidant, antibacterial, and anticancer effects, and have the potential to impact depression and anxiety (Peng, Setyawati et al. 2019). *Physalis Peruviana*, commonly referred to Golden Berry, is a botanical species native to South America, specifically originating regions of Pre-Columbian Peru (Patron, Mokhtar et al. 2022). Research on berries' health benefits has shown potential for their use in breast cancer prevention and treatment, particularly in individuals with depression and anxiety (Bessonneau and Rudel 2019). This study aims to examine the significant bioactive properties of extracts derived from *Physalis Peruviana* (Yen, Chiu et al. 2010). Psychosocial variables may influence breast cancer susceptibility and prognosis. Acute stress accelerates mammary tumor progression in rats, while chronic stress impedes or facilitates tumor formation (Suárez AI 2018). Learned helplessness, a depressive-like behavior, can exacerbate tumor growth in animals unable to evade chronic stress. There are various animal models that can be utilized to study depressive behavior (Rajpar F at al 2019).
The primary objective of development of these methods has been to assess the effectiveness of different chemicals in treating depression. This study was done to evaluate neuroprotective effects of *Physalis Peruviana* to improve the depression and anxiety caused by breast cancer in rats.

MATERIAL AND METHODS

Experimental Design: This experimental study was conducted in the Department of Anatomy, Liaquat University of Medical and Health Sciences (LUMHS), Jamshoro in collaboration with the Animal House of Department of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tando Jam, and Pathology Department (LUMHS) after ethical approval. A total of 60 adult healthy female rats of weight between 150-170 grams were used after one week of acclimatization. Animals were allocated randomly into 5 equal groups; A, B, C, D, and E, each containing 12 animals. Animals were kept in different cages according to their groups as under (Shaikh et al 2019).

Animal Groups and Treatments:
Group A: With normal diet and water.
Group B: - With Breast Cancer only not treated.
Group C: –With Breast cancer receiving tamoxifen.
Group D: –With Breast cancer receiving 4β-Hydroxywithanolide E.
Group E: – With Breast cancer receiving tamoxifen with 4β-Hydroxywithanolide E.

Tumor Induction: Dimethylbenz(a)anthracene (DMBA) is a chemical compound that is derived from phenanthrene with known immunosuppressive properties and is widely recognized as a highly potent carcinogen in laboratories.

Soy oil: purchased from the Departmental store (Gul* and 2023).

Plant Material: The fresh ripe fruits of *Physalis peruviana*, were purchased from the Botanical store of Karachi, Pakistan identified and verified by Department of Botany at Sindh University (Gul* et al 2023).

Aqueous Extract Preparation: One hundred grams (100gm) of dried fruit of *Physalis Peruviana* (PP) was crushed in mortar and pestle diluted with 1litre of distilled water boiled at 65℃ for 3 hours. The extract was filtered through filter paper. The filtrates were dried and powder was stored in an air tight vial, stored at -21℃ till use (Lodhi M 2019).

Methodology: Carcinoma was developed by a single dose of 20 mg DMBA in soy oil (5ml) given intra gastric by gavage method. Tumor was induced in four groups. Group A was left as a positive control. Physical examinations were performed weekly. Each rat's mammary gland was checked by inspection and palpation. At 6th week after DMBA administration, the tumor was confirmed by sacrificing one rat from each group. Tamoxifen was given at standard dose of 100l tamoxifen in soy oil intraperitoneal at each day for consecutive 05 days. The rats were quarantined for 24 hours following the last injection. Histological analysis was followed after 07days to monitored adverse reactions after tamoxifen injections. After induction and confirmation of tumor by histological analysis Group E rats were treated with 4β-Hydroxywithanolide E from *Physalis Peruviana* plant along with tamoxifen as used in previous groups for observation of their combined effects (Syna Pervaiz Singha 2022).

Assessment of behavioral changes:
The rats in all groups were assessed for behavioral changes by using different models for physical parameters of general behavior and different models for anxiety and depression.

1. Daily observations: were conducted to assess the physical parameters associated with general behavior, including sniffing, head down posture, nose poking, and rearing. The aim was to determine the frequency, duration, and sequential occurrence of these behaviors throughout the administration of the drug (Kumar 2018).

2. Anxiety was evaluated by light and dark apparatus and open field test.

3. **Depression** was assessed by tail suspension test.

Anxiety Test:
Open field test: This test is initial screening for anxiety-related behavior in mice by assessing general locomotors activity. In this experimental procedure, a mouse is positioned in the corner of the open field apparatus (Accuscan Instruments, Columbus) under an illumination level of 100 lux (Rajpar F at al 2019). The assessment of locomotion involved quantifying the number of floor units traversed, the frequency of rearing (defined as the number of instances an animal assumed an upright position on its hind limbs), and the duration of immobility (defined as the period during which an animal did not exhibit spontaneous movement). Handheld counters and stopwatches were utilized to accurately estimate the frequency of each of these behaviors. Each rat was placed in the center of the open-field arena for three minutes to observe behavioral parameters. Animals were alternated between control and experimental groups to minimize the potential effects of circadian changes on open field behavior. The test was conducted during morning timings, with control and experimental rats mixed together. Grading was done according to following criteria (table1 and 2).

Table1 : Grading system for movements of rat (Bano 2018)

Grade	Movement
Grade 0	No apparent insufficiency
Grade 1	Slightly atypical walk
Grade 2	Markedly atypical walk
Grade 3	Major difficulties in movement
Grade 4	No activity >24 hours

Table2 : Grading system for activity of rat (Bano 2018)

Grade	Activity
Grade 1	Active movement in the device
Grade 2	Decreased movement in the device
Grade 3	Slow activity
Grade 4	No activity > 24 hours

4. Weight Measurement: The weights of each rat were recorded on a weekly basis. In order to prevent escape, the animals were confined into bowls of known weight. The animals were individually weighed in grams and the measurements were documented (Gul* et al 2023).

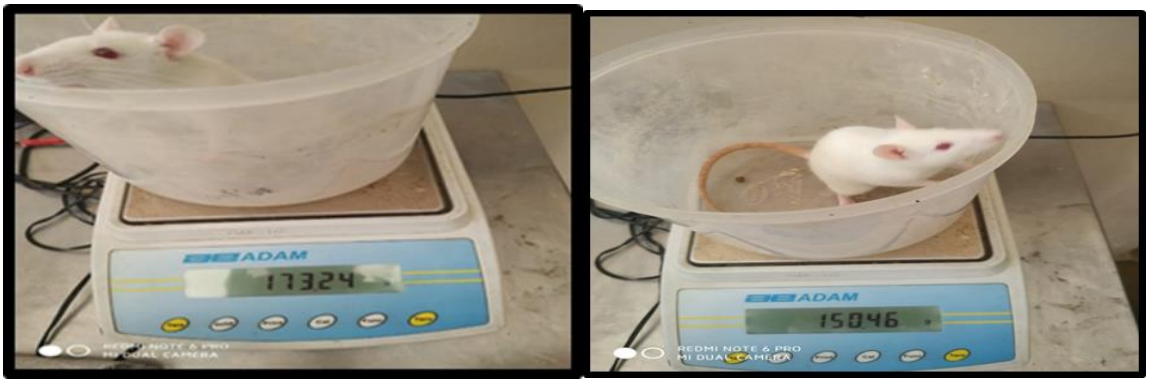


Figure: 1 Weight measurement of rats

Statistical Analysis

The mean values with their standard error of the mean (SEM) were computed using the SPSS statistical software package version 26. One-way analysis of variance (ANOVA) was followed by a post hoc Dennett test at significance levels of $P < 0.5$ and $P < 0.1$.

RESULT

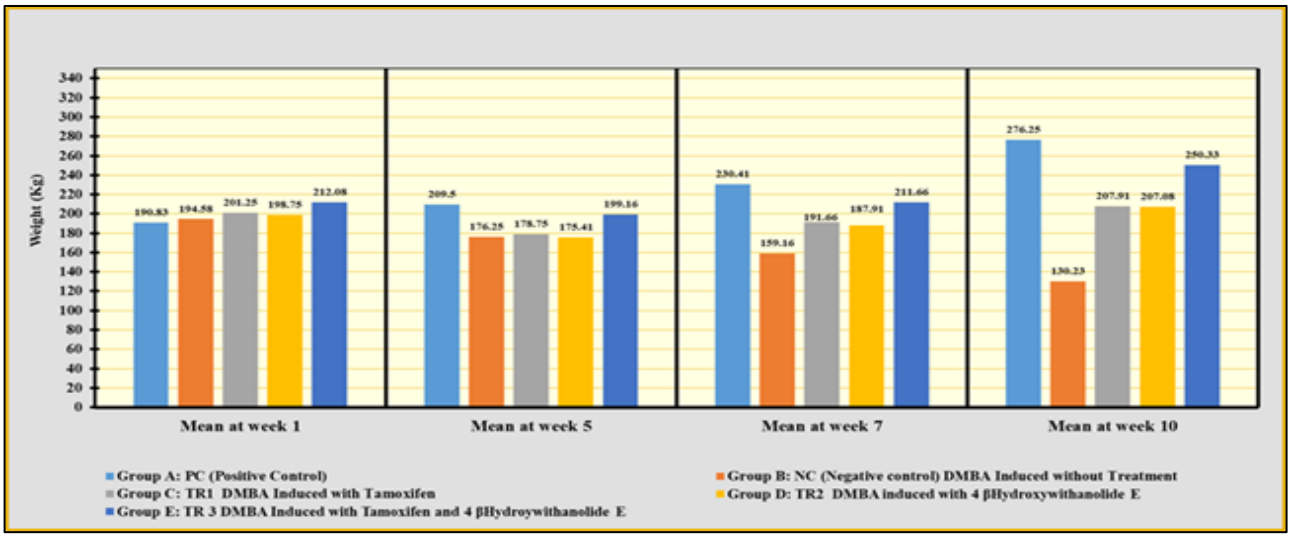
Effect on Weight Table 1 shows the effect of DMBA induced only and treated with tamoxifen, physalis peruviana alone and both in combination on weight variation in rats. The administration of extracts from Physalis Peruviana at a dosage of 100mg/kg resulted in a signifi cantand noteworthy enhancement in weight when compared to the subjects' original weights on the 10^{th} week. This improvement was observed at both doses, with a reduction in weight by 11%. The study analyzed the weight of rats in different groups, with no significant difference observed in Group E compared to Group A and B. However, the weight of rats in anti-tumour groups (Group C and Group D) did not show a significant decline compared to the healthy control group. In week 5, the analysis revealed a significant disparity in the weight of rats between Group E and Group D, with a notable decline in the control group. However, the weight of rats in Group E exhibited a substantial rise compared to Group B. In week 7, the study revealed a significant disparity in the body weight of rats between Group E and Group B, with a notable reduction in the control group. However, no significant change was observed in Group C and Group D at week 10. (Gul* and 2023)

Behavior Changes:

Effect on Anxiety and depression

Open Field Test: The study involved 60 rats, with 100.0% exhibiting activity in the first week. In the fifth week, all rats in group A showed signs of activity. Graph reveals the effects of *Physalis Peruviana* extract on anxiety by OFT. *Physalis Peruviana* extract 100mg/kg showed a greatly substantial rise in the total distance on the 5^{th} week significant and greatly substantial rise in center entries on the 7^{th} and 10^{th} weeks respectively; significant and greatly significant increment in the duration of rearing's at the7^{th} and 10^{th} weeks in comparison with control, respectively (Table 3)100

and 200 mg/kg showed an extremely substantial increment in the total distance on the 8th day; greatly significant increment in center entries, center time; number, and duration of rearing at the7th and 10th weeks in comparison with control. In the 7th week, 40.0% of the rats showed a drop in activity levels, with 10 rats experiencing a significant decrease. In the third week, 16.7% of the rats exhibited delayed movement, with 83.3% belonging to Group B and 66.6% to Group C. In the 7th week, all rats in group A showed activity (Shaikh P 2019). In the7th week, 19.7% of the rats exhibited a grade 2 drop in activity and behavior alterations. The study found that Group D showed significant improvements in motor skills and behavioral modifications, while Group A showed a higher percentage of improvements. In the tenth week, all rats in group A exhibited activity. In the tenth week, all rats in group B exhibited Grade 4 behavior alterations exclusively, with 11.7% of the total sample size dying during the trial. Group E showed great improvement in activity and movements (Farhana Rajpar 2021).



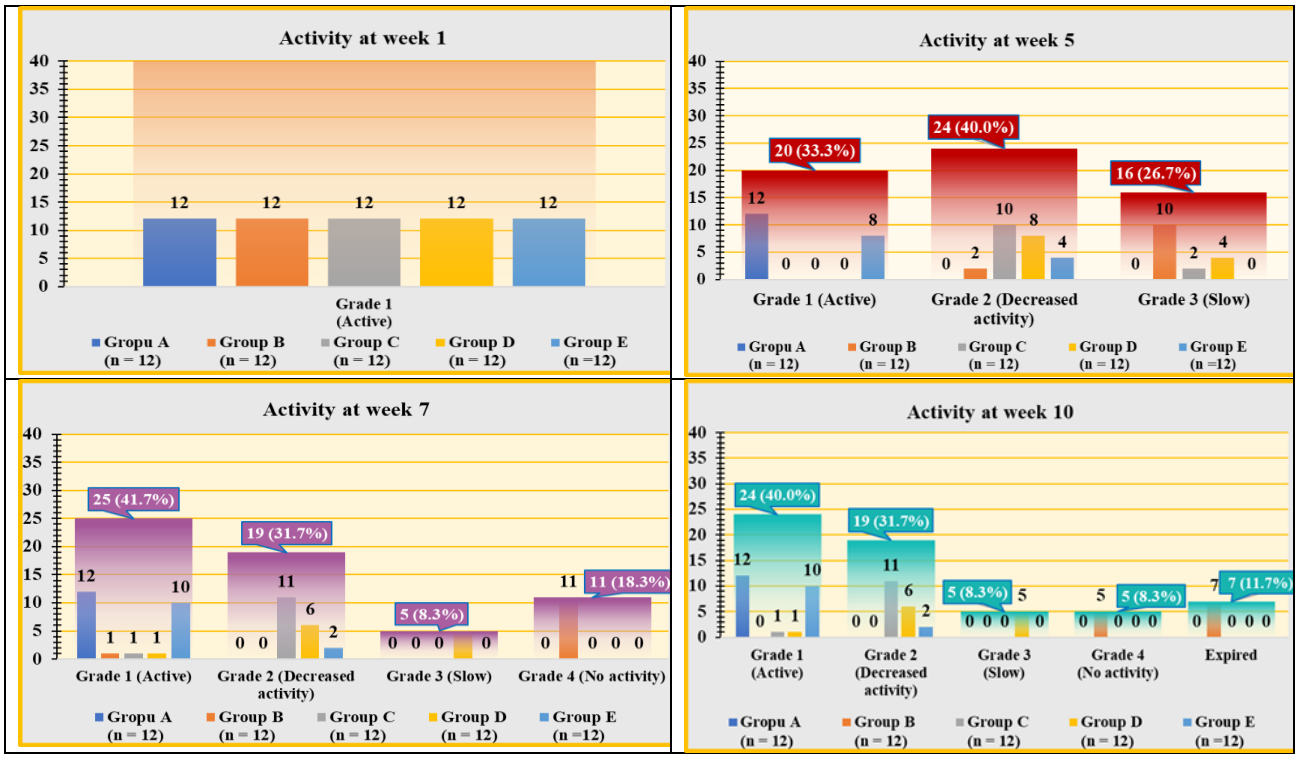
Graph: 1 Comparison of Weight measurements in different groups at week 1, 5,7 & 10

Table 3 Distribution of rats according to the comparison of% of Activity at week at 1, 5, 7 & 10 in Groups

Activity	A	B	C	D	E	Total	P-value
Week 1 Grade 1	12(100.0%)	12(100%)	12(100%)	12(100%)	12(100%)	60(100%)	0.60
Week 5 Grade 1 Grade 2 Grade 3	12(100.0%) 0 0	0 2(16.7%) 10(83.3%)	0 10(83.3%) 2(16.7%)	0 8(66.7%) 4(33.3%)	8(66.7%) 4(33.3%) 0	20(33.3%) 24(40.0%) 16(26.7%)	0.005
Week 7 Grade 1 Grade 2 Grade 3 Grade 4	12(100.0%) 0 0 0	1(8.3%) 0 0 11(91.7%)	1(8.3%) 11(91.7%) 0 0	1(8.3%) 6(50.0%) 5(41.7%) 0	10(83.3%) 2(16.7%) 0 0	25(41.7%) 19(31.7%) 5(8.3%) 11(18.3%)	0.004
Week 10 Grade 1 Grade 2 Grade 3 Grade 4 **Expired**	12(100.0%) 0 0 0 0	0 0 0 5(41.7%0 7(58.3%)	1(8.3%) 11(91.7%) 0 0 0	1(8.3%) 6(50.0%) 5(41.7%) 0 0	10(83.3%) 2(16.7%) 0 0 0	24(40.0%) 19(31.7%) 5(8.3%) 5(8.3%) 7(11.7%)	0.000

* Mean ± Standard Deviation 0*P-value <0.0001 is highly significant calculated by ANOVA

Graph 2 Activity of Rats at 1, 5, 7 &10 week



DISCUSSION:

Effect on Weight: Significant variations in the weight of rats were seen in Group B and Group E. The rats belonging to Group E exhibited a statistically significant rise in weight compared to the other groups. However, this gain was not as pronounced as that observed in typical rats. Conversely, the rats in Group B saw a substantial loss in weight ($p < 0.0001$). Tamoxifen has demonstrated efficacy in suppressing tumour growth in animal studies as well (Wang, Yue et al. 2021). Several investigations have shown that Physalis Peruviana exhibits anti-tumour capabilities (Kaur, Makanjuola et al. 2017). It is noteworthy that the negative control group, which was administered with DMBA but did not receive any form of treatment, had a substantial reduction in weight. This observation aligns with prior research demonstrating that exposure to DMBA has the potential to induce weight reduction in rats (Yuan, Cai et al. 2022).

Behavior Changes:

Effect on Anxiety and Depression: The results of the study demonstrated that Group E exhibited a statistically significant improvement of 75.0% (p-value =<0.0001) in activity and behavior changes compared to Group C. In contrast, Group D showed a total of 5 rats (8.3%, n = 60) with Grade 3, while Group B had 5 rats (8.3%, n = 6) with Grade 4. The findings of this study indicate that the administration of tamoxifen in combination with Physalis Peruviana may result in notable enhancements in behaviour modifications when compared to the use of tamoxifen alone. There was improvement in depression and anxiety in the rats of Group E. This result is consistent with a recent study by (Siddique (2021) reveals Physalis Peruviana exhibits neuroprotective properties and has the potential to enhance cognitive function in rats by another investigation conducted by (Kasali, Tuyiringire and colleagues (2021) as well. Moreover, extant literature has demonstrated that the administration of tamoxifen in isolation might lead to deleterious consequences on cognitive abilities, such as memory deterioration (Gregorowitsch, Ghedri et al., 2012)

CONCLUSION: Results revealed that Physalis peruviana has significant effects in reversing toxic effects of DMBA and improves depression and anxiety, which suggest its neuroprotective effects. Further studies are required evaluate its efficacy.

Conflict of Interest: The authors declare no conflict of any interest.

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