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PHARMACOLOGICAL EVALUATIONS OF SOLID LIPID NANOPARTICLES (SLNs) CONTAINING DAIDZEIN IN WISTAR RATS.

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INTRODUCTION

Breast cancer is a malignant disease marked by uncontrolled growth of breast cells due to genetic mutations either inherited (e.g., BRCA1/BRCA2) or acquired that disrupt normal cell cycle regulation; these abnormal cells typically arise in ducts or lobules, forming tumors that may invade nearby tissues or metastasize. It is classified into non-invasive types like ductal carcinoma *in situ* and invasive types such as invasive ductal carcinoma and invasive lobular carcinoma , with subtypes defined by receptor status: hormone receptor-positive, HER2-positive, and triple-negative breast cancer (TNBC), each influencing prognosis and treatment. Clinical signs include lumps, changes in breast shape or skin texture, nipple discharge or retraction, redness, swelling, or peau d'orange appearance. chemotherapy, hormone therapy (tamoxifen, aromatase inhibitors), and targeted agents (trastuzumab for HER2-positive tumors).

In India, breast cancer has emerged as the most common cancer among women, with an estimated 192,000 to 200,000 new cases diagnosed annually during 2024–2025. This alarming rise is driven by lifestyle changes, urbanization, and limited awareness, especially in rural areas. Tragically, for every two women diagnosed, one loses her life to the disease, making breast cancer the leading cause of cancer-related deaths among Indian women. The burden is highest among women aged 30 to 69, and while urban centers offer better access to diagnostics and treatment, many in semi-urban and rural regions face delays due to inadequate healthcare infrastructure. With over half a million women currently living with breast cancer in India, early detection, public education, and equitable access to care are critical to reversing this trend.

KEYWORDS: Daidzein SLNs, Wistar Rats, RBC, WBC.

SIDE EFFECTS:

Cancer drugs produce hot flashes, joint pain, mood swings, and increased risk of thromboembolic events or osteoporosis, diarrhea and in rare cases heart dysfunction. neutropenia, fatigue, gastrointestinal disturbances, pneumonitis, colitis, hepatitis, or endocrine dysfunction.

SELECTION OF DRUGS (ACTIVE PHARMACEUTICAL INGREDIENTS)

Daidzein:

The drugs selected for the research is Daidzein are bioactive isoflavones found predominantly in soybeans. This compound has various health-promoting properties, including antioxidant, anti-inflammatory, and anticancer effects. The application in therapeutic treatments has gained interest, especially for conditions such as cancer, cardiovascular diseases, and menopausal symptoms.Daidzein is poorly water-soluble, which makes them difficult to absorb effectively in the gastrointestinal tract. Nanotechnology is used in this case to enhance their bioavailability and therapeutic efficacy. Daidzein was purchased from otto kemi,India and all other chemicals were analytical grade.

EXPERIMENTAL ANIMALS:

Female Wistar rats (n=25) weighing 80-100g and aged 40 days were obtained from Mass biotech, Chennai, India. All the experimental protocols used in this study were approved by the Annamalai University Animal Ethics Committee. The animals were caged group wise and maintained under constant environmental conditions with a room temperature of 23 ± 2 °C and relative humidity between 55-65% with 12h light/12 h dark cycle. Animals were provided with water and standard rat pellet diet ad libitum throughout the experimental period.

OBSERVATIONS AND MONITORING

A.Clinical Observations

Following dosing, the general behaviour of animals will be closely monitored at 1, 4, 8, and 24 hours, and subsequently every 24 hours for a period of 14 days. Observational parameters will include signs of lethargy or hyperactivity, tremors or convulsions, respiratory rate and difficulty, physical appearance such as changes in fur, skin, or eyes and any signs of distress including alterations in posture, gait or movement. In addition, daily monitoring of food and water intake will be conducted to assess overall health and well-being. Body weight

measurements will be recorded prior to dosing and then every two days throughout the study duration.

B. Mortality

The number of deaths will be recorded for each group. If any animals die, the time of death and any symptoms preceding death will be documented.

At the end of the 14-day observation period, or if any animals show severe signs of distress:

At the conclusion of the study, all animals will be humanely euthanized in accordance with ethical guidelines, either through an overdose of anesthesia such as isoflurane or by cervical dislocation. Following necropsy, major organs including the liver, kidneys, heart, and lungs will be inspected for visible abnormalities. Tissue samples from the liver and kidneys will be carefully collected and preserved for subsequent histopathological examination.

HISTOPATHOLOGICAL EXAMINATION

Tissue samples will be fixed in 10% formalin and subsequently processed for histological examination. Standard hematoxylin and eosin (H&E) staining will be employed to assess cellular architecture and detect any abnormalities or tissue damage. The stained slides will then be examined under a light microscope, with any observed lesions or pathological changes carefully documented and photographed for further analysis.

ANTICARCINOGENIC STUDIES:

Group 1 - Normal control rats	Fed with normal pellet diet and water.
Group 2 - Cancer control	DMBA 25 mg/kg s.c once a week for four weeks.
Group 3 - Reference Standard	DMBA 25 mg/kg s.c once a week for four weeks, and Tamoxifen (50 mg/kg/day, orally) once a week for four weeks.
Group 4 - Test drug **Daidzein SLN**	DMBA 25 mg/kg s.c once a week for four weeks, and **Daidzein SLN** 25mg/kg orally daily for 16 weeks.

Table 1. Treatment protocol in various experimental groups (n=4)

After DMBA administration, rats were monitored daily for tumor development, location, and size, with 80% developing mammary tumors by day 90 and selected for further analysis. Tumor latency, volume, burden, and count were recorded, along with weekly body weights. Blood samples were collected for hematological and biochemical assessments, including markers like TSA, glucose, cholesterol, BUN, creatinine, ALT, AST, and ALP. Antioxidant levels (SOD, GSH) and lipid peroxidation (TBARS) were measured in various tissues and plasma. Mammary tissues underwent histopathological, electron microscopic, and immunohistochemical analysis to evaluate cellular changes and treatment effects

TUMOR LATENCY PERIOD STUDY DESIGN:

This study investigates how long it takes for tumors to develop in rats after being treated with DMBA to induce breast cancer, followed by either Tamoxifen or daidzein SLN. DMBA was administered subcutaneously at 25 mg/kg once a week for four weeks. Tumor growth was checked every three days starting one week after the first dose, and the time taken for a tumor of about 5 mm or more to appear was recorded to determine the latency period.

HEMATOLOGICAL PARAMETERS:

To assess the hematological parameters in the breast cancer study model described (where rats are treated with DMBA and the test drug: Daidzein SLN), the following hematological parameters will be measured for each group.

Hematological Parameters to be measured:

1. WBC count (x 10^3/µL)
2. RBC count (x 10^6/µL)
3. Hemoglobin (g/dL)
4. MCV (Mean Corpuscular Volume, fL)
5. MCH (Mean Corpuscular Hemoglobin, pg)
6. MCHC (Mean Corpuscular Hemoglobin Concentration, %)
7. Hematocrit (HT, %)
8. Lymphocytes (%)
9. Monocytes (%)
10. Heterophils (%)
11. Bleeding time (sec.)
12. Clotting time (sec.)

HEMATOLOGICAL PARAMETERS ANALYSIS

The following hematological parameters will be analyzed using standard laboratory equipment:

A. Complete Blood Count (CBC):

WBC (White Blood Cell count, x 10^3/µL)**:** Measured using an auto-analyzer or a hemocytometer. RBC (Red Blood Cell count, x 10^6/µL): Measured using a cell counter or hemocytometer. Hemoglobin (Hb, g/dL): Measured by the cyanmethemoglobin method. MCV (Mean Corpuscular Volume, fL): Calculated using the formula: RBC count (million/µL) \times 10. MCH (Mean Corpuscular Hemoglobin, pg): Calculated using the formula: MCH=RBC count (million/µL) Hemoglobin (g/dL)×10. MCHC (Mean Corpuscular Hemoglobin Concentration, %): Calculated using the formula: {Hemoglobin (g/dL)\ 100. Hematocrit (HT, %): Measured using a microcentrifuge for blood separation into plasma and cellular components.

B. Immune Profile:

1. Lymphocyte, Monocyte, and Heterophil Percentages: Differential WBC count is performed by preparing a blood smear and counting at least 100 cells under a microscope. The percentages of lymphocytes**,** monocytes, and heterophils are calculated.

C. Coagulation Parameters:

To measure bleeding time, a small cut is made on the rat's tail and the time taken for bleeding to stop is recorded. For clotting time, a drop of blood is placed on a slide and observed until a clot forms, noting the time required for clotting.

Statistical Analysis

Data Analysis: For all hematological parameters, the results will be expressed as mean ± standard deviation (SD) for each experimental group. Statistical significance will be determined using appropriate tests, with a p-value less than 0.05 considered indicative of a significant difference

SERUM BIOCHEMICAL PARAMETERS IN RATS:

The study of serum biochemical parameters involves assessing various biochemical markers in the blood that can provide insight into organ function**,** metabolic disturbances, and toxicity. These parameters are measured to evaluate the effects of treatments or disease models, such as the DMBA-induced breast cancer model with treatments using Daidzein SLN.

Blood Sample Collection:

At the end of the study, blood samples will be collected from each animal via the retro-orbital sinus or tail vein under light anesthesia using ketamine and xylazine. Approximately 1–2 mL of blood will be drawn per animal for serum separation. The collected blood will be allowed to clot at room temperature for 30 minutes, followed by centrifugation at 3000 rpm for 10 minutes to obtain the serum.

SERUM BIOCHEMICAL PARAMETERS

Serum biochemical parameters in this study are assessed using standard laboratory techniques or automated analyzers. Glucose is measured by the glucose oxidase-peroxidase method (70–100 mg/dL), cholesterol via enzymatic oxidase method (50–100 mg/dL), and creatinine using the Jaffe method (0.5–1.5 mg/dL). Urea is analyzed by the urease method (10–50 mg/dL), uric acid by the uricase method (2–7 mg/dL), and triglycerides by the glycerol kinase method (40–150 mg/dL). Total bilirubin is measured by the diazo method (0.1–1.2 mg/dL), total protein by the biuret method (6.0–8.0 g/dL), and albumin by the bromcresol green method (3.5–5.0 g/dL). Enzyme markers include ALP (50–150 U/L), GGT (5–30 U/L), SGOT and SGPT (10–40 U/L each), and LDH (100–200 U/L), using respective spectrophotometric or colorimetric assays to evaluate organ function and metabolic status.

ACUTE TOXICITY OF DAIDZEIN

Acute toxicity studies:

This study aimed to assess the short-term toxicity of daidzein in rats by estimating its LD50 and observing clinical and pathological effects. Healthy Wistar rats, aged 8–10 weeks, were acclimatized for seven days under controlled conditions and divided into groups: a control group receiving distilled water and experimental groups receiving daidzein at low (50 mg/kg), medium (200 mg/kg), and high (500 mg/kg) doses via oral gavage. Each dose was freshly prepared and administered based on individual body weight. The rats were monitored for 14 days for behavioral changes, signs of toxicity, and overall health, following ethical guidelines to ensure humane treatment.

In this study, the acute toxicity of Daidzein is evaluated by administering various doses to healthy adult rats. The animals were observed for a 14-day period post-treatment.

The following results were obtained:

The control group showed no signs of toxicity, and all animals remained healthy with stable body weight. At the low dose (50 mg/kg), no toxicity or behavioral changes were observed. The medium dose (200 mg/kg) caused mild lethargy and reduced food intake, with a slight 5% drop in body weight but no deaths. The high dose (500 mg/kg) led to more noticeable toxicity, including severe lethargy, dehydration, and a 10% weight loss, though no mortality occurred. The estimated LD50 for daidzein is above 500 mg/kg, indicating low acute toxicity but increased risk at higher doses. Necropsy revealed no major organ damage at low and medium doses, while mild liver and kidney cell enlargement was seen at the highest dose, suggesting minor tissue stress.

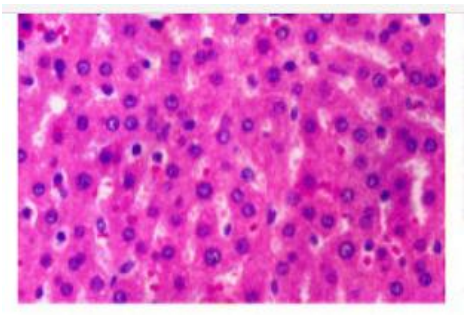

Fig: 1 Daidzein 500 mg/kg

Groups	Dose (mg/kg)	Clinical Signs	Body Weight Changes	Mortality	Organ Pathology Findings
Control	-	No toxicity signs, normal activity and behaviour	No significant changes	0%	No abnormalities in organs
Daidzein (Low Dose)	50	No observable toxicity, normal activity, no lethargy	No significant changes	0%	No abnormalities in organs
Daidzein (Medium Dose)	200	Mild lethargy, slight reduction in food intake	No significant changes	0%	No abnormalities in organs
Daidzein (High Dose)	500	Severe lethargy, reduced food intake, slight dehydration	No significant changes	0%	No abnormalities in organs

Table 2: Observations of Clinical Signs and Body Weight Changes in Rats Administered Daidzein

Discussion:

In the acute toxicity assessment of daidzein, no mortality was observed across all dose groups, indicating a relatively safe profile for both compounds. At higher doses (200 mg/kg and

500 mg/kg), mild clinical symptoms such as lethargy, reduced activity, and weight loss were noted; however, these effects were reversible shortly after exposure. The estimated LD50 for daidzein exceeded 500 mg/kg, while. Additionally, mild hypertrophy of liver and kidney cells was observed at the highest doses, pointing to minimal organ stress without any signs of overt damage or necrosis.

ANTI CANCER ACTIVITY:

Group	**Tumor Latency Period (Days)**	**Tumor Appearance (First Visible Tumor)**	**Tumor Growth Inhibition (%)**
Group 1: Normal Control	0 days (No tumor induction)	No tumors observed	-
Group 2: Cancer Control (DMBA)	16 ± 2 days	16 ± 2 days	-
Group 3: Reference Standard (Tamoxifen)	22 ± 3 days	22 ± 3 days	38%
Group 4: Test Drug Daidzein SLN	26 ± 4 days	26 ± 4 days	50%

Table 3: Tumor Latency Period Observations

Results and Discussion:

Tumor Latency Period:

In the experimental study, the normal control group (Group 1) did not develop any tumors, confirming that cancer induction does not occur without the administration of DMBA. In contrast, the cancer control group (Group 2) exhibited tumor development at 16 ± 2 days, establishing a baseline for tumor latency following DMBA exposure. Tamoxifen treatment (Group 3) significantly delayed tumor onset, with a latency period of 22 ± 3 days, consistent with its known estrogen receptor-modulating effects. Notably, daidzein SLN (Group 4) extended tumor latency to 26 ± 4 days, indicating potential anticancer activity, particularly when delivered via solid lipid nanoparticles (SLNs) to improve bioavailability and targeting, underscoring its promise in breast cancer prevention or therapy.

HISTOPATHOLOGY:

After treatment, animals were humanely sacrificed and their tumor tissues and major organs were collected, cleaned with saline, and fixed in formalin. The samples were dehydrated with ethanol, cleared in xylene, and embedded in wax. Thin slices were cut and placed on slides, then stained with hematoxylin and eosin to highlight cell structures. Under the microscope, signs of tissue damage such as cell death, nuclear changes, and cell division were examined to assess the effects of treatment.

ANIMAL STUDY RESULTS:

Histopathology Findings

Group 1: Normal Control (Healthy Rats)

Histopathological analysis of the mammary glands showed healthy tissue structure with no signs of abnormal growth, damage, or inflammation. The epithelial cells appeared intact and well-organized. Additionally, organs like the liver, kidney, spleen, and lungs showed no signs of damage, confirming that the treatment caused no harmful effects and was safe for the animals.

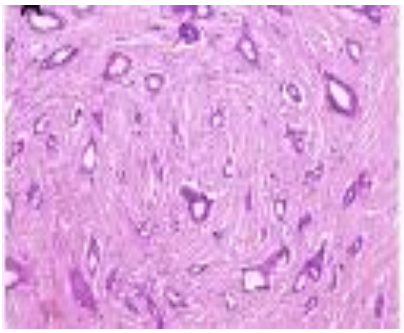

Fig: 2 Normal Control (Healthy Rats)

Group 2: Cancer Control (DMBA-Induced Tumor)

Histopathological examination of the mammary glands showed aggressive tumor growth with abnormal cell structures, high cell division, and poorly defined boundaries. Increased blood vessel formation supported the tumor's growth, while areas of cell death and inflammation indicated tissue damage. The liver and kidneys showed mild degeneration, suggesting some systemic toxicity likely due to cancer progression or exposure to the carcinogen.

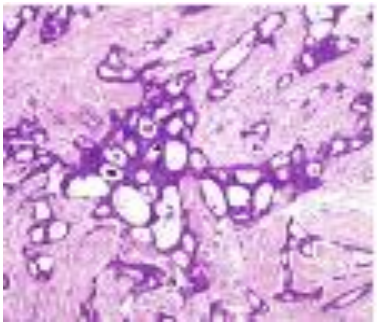

Fig:3 Cancer Control (DMBA-Induced Tumor)

Group 3: Reference Standard (DMBA + Tamoxifen)

Histopathological analysis after treatment showed partial tumor shrinkage, with fewer dividing cells and signs of active cell death like apoptosis and moderate necrosis. Blood vessel formation was reduced, indicating weaker support for tumor growth. Vital organs such as the liver, kidney, spleen, and lungs remained healthy, confirming that the treatment was safe and did not cause harmful side effects.

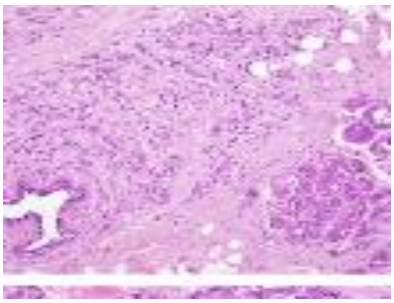

Fig: 4 Reference Standard (DMBA + Tamoxifen)

Group 4: Test Drug Daidzein SLN (DMBA + Daidzein SLN)

Histological analysis showed a clear reduction in tumor size compared to the cancer control group, with increased cell death marked by nuclear changes and moderate necrosis. Blood vessel formation was reduced, limiting tumor growth. Surrounding healthy tissues remained intact, and no damage was seen in major organs like the liver, kidney, spleen, or lungs, confirming the treatment's safety and targeted effectiveness.

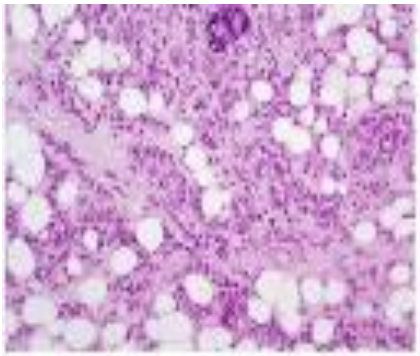

Fig: 5 Test Drug Daidzein SLN (DMBA + Daidzein SLN)

CONCLUSION:

This study evaluated the formulation, safety and anticancer efficacy of daidzein-loaded solid lipid nanoparticles (SLNs) in a DMBA-induced breast cancer model using Wistar rats. Acute toxicity assessments revealed no mortality across doses up to 500 mg/kg with only mild, reversible symptoms at higher concentrations and minimal organ stress. Daidzein SLNs significantly delayed tumor onset (latency: 26 ± 4 days) and achieved 50% tumor growth inhibition, outperforming Tamoxifen (38%). Hematological and serum biochemical parameters remained within normal ranges, indicating systemic safety. Histopathological analysis confirmed reduced angiogenesis, increased apoptosis, and preserved organ architecture in Daidzein-treated groups, highlighting its potential as a safe and effective anticancer agent with enhanced bioavailability through nanocarrier delivery.

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