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ORIGINAL ARTICLE

Chemotherapeutic Action of Acetonic Leaf Extract of *Indigofera longiracemosa* Boivin ex Baill on DMBA-Induced Mammary Carcinoma in Sprague Dawley Rats

Shabna K[1*] and Arun Kumar G[2]
[1*] Ph. D. Scholar, Department of Microbiology, Hindusthan College of Arts & Science, Coimbatore, India, 641 028
ORCID ID: 0009-0009-3477-522X
[2]Assistant professor, Department of Microbiology, Hindusthan College of Arts & Science, Coimbatore, India, 641 028
ORCID ID: 0000-0002-5984-9899
***Corresponding Author:** Shabna. K
Email: shabnakalathil@gmail.com

ABSTRACT
This study aims to evaluate the therapeutic effects of the acetonic leaf extract of Indigofera longiracemosa Boivin ex Baill on DMBA-induced mammary carcinoma in Sprague-Dawley rats, given its unexplored anticancer potential and a rich phytochemical profile identified by GC-MS analysis. The purpose of this study is to examine the bioactive components and their therapeutic effects on biochemical parameters in the mammary tissues of DMBA-induced mammary carcinoma rat models over a 210-day experimental period. Female Sprague-Dawley rats were used in the study and divided into five groups; group I served as the control. Group II rats were induced with mammary carcinoma by administration of DMBA (25 mg/kg) by single gastric intubation. Group III received tamoxifen citrate (10 mg/ kg) orally. Group IV received a low dose of the acetonic extract of Indigofera longiracemosa (AEIL; 100 mg/Kg), and Group V rats received a high dose of AEIL (200 mg/Kg), both administered orally (by gavage) daily for 90 days after DMBA induction. Administration of AEIL restored liver function in DMBA-treated rats, with high dose (HD) achieving 90-100% normalization of SGOT, SGPT, ALP, and total bilirubin levels toward control values. HD treatment brought urea, uric acid, and creatinine back to 92%, 101%, and 155% of control levels, respectively. HD treatment restored total cholesterol, triglycerides, and LDL cholesterol, with an average of 93.3% of each returning to normal. DMBA causes oxidative stress, lowering SOD, catalase, GPX, GSH and raising LPO. AEIL restored antioxidant enzyme by 93% and reduced LPO, showing a 73% restoration of enzyme balance. DMBA disrupts glycolytic enzymes; HD treatment restores them to ~92-134% of control levels. The HD treatment restores mitochondrial enzyme activities to about 90% to 106% of normal levels. The AEIL demonstrates comprehensive therapeutic potential for breast cancer management, restoring metabolic homeostasis by rejuvenating glycolytic and citric acid cycle enzymes, enhancing antioxidant defenses, and normalizing lipid profiles. Concurrently, AEIL exhibits potent antitumor activity, as evidenced by microscopic observations. This multifaceted action underscores AEIL's therapeutic efficacy in mitigating breast cancer progression, positioning it as a promising adjuvant therapy.
Keywords: *Mammary carcinoma, DMBA, Tamoxifen, Indigofera longiracemosa Boivin ex Baill, Female Sprague Dawley rats, chemotherapy effects*

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INTRODUCTION

According to recent data, breast cancer accounts for almost one in six cancer-related deaths in women, or roughly 685,000 fatalities worldwide. Over 3 million more cases of breast cancer and 1 million deaths from the disease are predicted for 2040, mostly due to population growth and ageing.[1] In 2024, cancer will still be a significant health concern in India. Disruptions in the rules of cell-cycle regulation are the core cause of unchecked cancer cell growth.[2] However, the chemotherapeutic medicines used to treat

cancer can help patients live longer and provide temporary comfort.[3] Still, there are negative side effects from several anticancer medications.[4] Currently, both the scientific and commercial communities are highly interested in the ongoing development of innovative anticancer medications derived from natural products, as cancer is one of the most common diseases in humans.[5] Furthermore, breast cancer was the leading cause of death for all cancers, accounting for 15% of all female cancer deaths.[6] Chemotherapy and radiation therapy are commonly used to treat it, and they are recognized risk factors for an additional malignancy as well as cancer-related mortality.[7] A major issue for public health in both developed and underdeveloped countries, cancer is brought on by means of molecular alterations in cells. To cure cancer, the hunt for a novel medication derived from medicinal plants has advanced significantly. Numerous scientists are diligent in their search for artificial medications to treat breast cancer. [8-11] Due to toxicities that limit dosage and medication resistance, which might harm host cells, these medicines possess a minimal safety margin and the possibility of therapeutic failure. Therefore, using natural items to control and eradicate cancer is an alternative. Effective anticancer agents can be found in medicinal plants, and it's noteworthy that Plants and other natural sources account for 60% of the anticancer medications currently in use.[12] *Indigofera longiracemosa* Boivin ex Baill, a small tree or shrub that is native to Nepal, Sri Lanka, and India, is a member of the Fabaceae family. Its height ranges from 2 to 5 meters, and its crown is cylindrical. Both dry deciduous forests and scrublands are home to it. All snake venoms have been neutralized by using the roots, which demonstrate antidiabetic effects in rats [13] and exhibit antioxidant action as well.[14] The entire plant exhibits antipyretic and anti-inflammatory effects. [15-16] No research has been conducted on the anti-breast cancer properties of *I. longiracemosa.* This evidence led to the selection of *I. longiracemosa* for this study. This study aims to assess the chemotherapeutic impact of the acetone leaf extract of *Indigofera longiracemosa* Boivin ex Baill (AEIL) on female Sprague-Dawley rats who have been induced to develop mammary carcinoma by DMBA. By lowering oxidative stress damage to cellular components*, Indigofera* extract exhibits protective properties against DMBA-induced toxicity.[17] Because the extract helps restore liver enzyme and bilirubin levels to normal, it may influence cellular defense mechanisms by activating the Nrf2 pathway, which regulates the production of antioxidant genes.[18] The leaf extract of *Indigofera* shows protective effects against DMBA-induced toxicity, with reduced oxidative stress.[19] Extract may have a role in altering the cellular defense system, possibly via the Nrf2 pathway, which regulates the antioxidant genes, as proved by its capacity to regulate bilirubin and liver enzyme levels.[20] The current study aims to investigate the chemotherapeutic capacity of AEIL against DMBA-induced toxicity. To achieve this objective, a comprehensive analysis of various biochemical parameters was conducted. These include tumor analysis, liver function tests, kidney function tests, lipid profile studies, and antioxidant enzyme assays. Additionally, the activities of glycolytic enzymes and citric acid cycle enzymes were evaluated, and the total protein level was also assessed.

MATERIAL AND METHODS

Herbal extract and constituent separation

I. longiracemosa leaves were gathered from the Edayur north region of Malappuram district, Kerala (Latitude = 10.88035, Longitude = 76.09092) throughout October 2022. The plant's authenticity was confirmed by the Botanical Survey of India, Coimbatore, Tamil Nadu, India. The powdered leaf material was macerated with acetone at room temperature(25°C). The extract was examined using common screening techniques to determine whether it contained different phytochemical compounds. For the detection of terpenoids, phenols, steroids, alkaloids, tannins, flavonoids, glycosides, saponins, proteins, etc., a standard procedure was employed. [21]. The phytochemical profile of *I. longiracemosa* was analyzed using GC-MS [22] with a Shimadzu Nexis GC-2030 coupled to an AOC-30/20i auto sampler, equipped with an SH-I-5Sil MS column (30.0 m x 0.25 mm,0.25 µm film thickness). The identification of phytochemical constituents was carried out using GC-MS solutions software, with spectral matching against the NIST 20 library (Unpublished data).

Fig. 1 *Indigofera longiracemosa* Boivin ex Baill

Animals

Sprague-Dawley female rats were acquired from the Biogen animal facility located in Bangalore, India. They were 21 days old and weighed 70 grams at the start of the trial. There was a 12-hour light/12-hour dark cycle, and all rats were maintained at room temperature (22°C) after receiving approval from the Institutional Animal Ethics Committee (IAEC) with proposal No. KMCRET/ReRe/Ph. D/94/2024.

Design of experimental studies

Thirty female Sprague-Dawley rats were divided into five groups (n=6) to evaluate the potential anti-tumor effects of the acetonic leaf extract of *Indigofera. longiracemosa* versus tamoxifen (Table 1). The control group (Group I) received 1 ml of olive oil orally, while the other groups (Group II-V) were given a single dose of 7,12-dimethylbenz [a]anthracene (DMBA) at 25 mg/kg in 1 ml of olive oil to induce mammary tumors. After approximately four months, tumors developed, after which the rats were assigned to different treatments. Group II received no further treatment after tumor development. Group III was treated with tamoxifen (10 mg/kg) for 90 days. Groups IV and V received the acetonic plant leaf extract orally at 100 mg/kg (low dose) and 200 mg/kg (high dose), respectively, for 90 days. Treatments began at the fifth month after DMBA exposure, and all animals were monitored until day 210 post-DMBA exposure. The aim was to evaluate whether the plant extract can inhibit or reduce DMBA-induced tumor growth compared with tamoxifen in a model approved by the Institutional Animal Ethics Committee.

Preparation of tissue homogenate

The tissues were weighed, and a 0.025 M Tris-HCl buffer at pH 7.5 was used to create a 10% (w/v) tissue homogenate. Using the clear supernatant after centrifugation at 10,000 ×g for 10 minutes, the following biochemical characteristics were determined.[23]

Tumor analysis

Tumor weight, tumor incidence, and tumor volume were examined. [24-26]

Analysis of Biochemical and Enzymatic Parameters

Liver function test

In this group, the Jendrassik-Grof method was used to measure total bilirubin, whereas the Reitman-Frankel method was used to measure Serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT). Alkaline Phosphatase (ALP) using the Babson method [27].

Kidney function test

Urea, uric acid, and creatinine are among the biochemical measurements that can be used to evaluate kidney function. While the Berthelot method is used to estimate urea, the Caraway method can be used to estimate uric acid. Finally, one can use the Jaffe method to estimate creatinine [28].

Lipid profile analysis

As part of an extensive lipid profile analysis, total cholesterol, triglycerides, and low-density lipoprotein (LDL) cholesterol were calculated. The Kunkel method was used for triglyceride calculation. Total cholesterol was estimated using the Liebermann-Burchard reaction, and LDL cholesterol was calculated using the Friedewald equation [29].

Assay of antioxidant enzymes

Antioxidant enzyme activity was estimated using systematic methods. Superoxide dismutase (SOD) activity was evaluated using the method of Kakkar et al. Catalase (CAT) activity was measured using the Sinha method. Glutathione peroxidase (GPX) activity was measured using the Rotruck et al. method. Reduced glutathione (GSH) levels were estimated using the Ellman and Moron method, and the findings were confirmed using the Beutler et al. method. Lipid peroxidation (LPO) was evaluated using the thiobarbituric acid reactive substances (TBARS) assay [30].

Assay of Glycolytic Enzymes

The glycolytic enzyme activity was measured using known methods. The Branstrup method was used to determine how active hexokinase was. We used Salas et al.'s method to find out how much glucokinase there was. We used the Nordlie and Arion method to measure glucose-6-phosphatase activity. The Gancedo and Gancedo method was used to determine the activity of fructose-1,6-diphosphatase [30].

Enzyme assay for the citric acid cycle

Standardized techniques were used to estimate the citric acid cycle enzyme activity. The King method was used to measure isocitrate dehydrogenase activity. Slatter and Bonner's approach was used to estimate succinate dehydrogenase activity. The Mahler method was utilized to quantify the activity of malate dehydrogenase [18].

Total Protein Estimation

Using the Lowry et al. method, total protein was estimated to evaluate changes in protein metabolism associated with breast cancer [33].

Cytopathological techniques

Histopathology is the study of tissues under a microscope to look for abnormalities. In order to do this, diseased tissues from biopsy or necropsy must be collected, fixed, sectioned, stained, and examined under a microscope. Tissues exhibiting severe morbid alterations in addition to normal tissue were cut into thin pieces, each 3 to 5 mm thick. For 24 to 48 hours, the tissue was kept at room temperature in the fixative. The morphology of breast tissue was examined, and mammary cancer was diagnosed using the Haematoxylin and Eosin staining method and common fixatives, such as 10% formalin.

Statistical analysis

The values are shown as mean ± standard deviation (SD). A one-way ANOVA was used to determine statistical significance, and Dunnett's post-hoc test was used to compare each treated group to the control group. The results include 95% confidence intervals (CI) and *P*- values, where ns denotes non-significant and $P < 0.05$ indicates significance.

RESULTS

AEIL yielded a rich mixture of phytochemicals. Preliminary screening revealed the presence of terpenoids, triterpenoids, phenols, steroids, alkaloids, tannins, flavonoids, glycosides, saponins, proteins and amino acids. AEIL GC-MS analysis revealed a complex phytochemical profile, with notable compounds including alpha-tocopherol and beta-tocopherol. -D-mannoside, 3-Hydroxy-3-methyl-oxindole- Ac derivative, phytol and 9,12,15-Octadecatrienoic acid (Z, Z, Z), which are associated with antioxidant and anticancer activities. They are all of particular interest for their potential roles in mitigating DMBA-induced oxidative stress and tumorigenesis. The study shows that the DMBA+STD treatment restored nearly all of the lost body weight (93.65%), while the HD treatment went even further, achieving 114.29% recovery and surpassing the control group. Groups III and V also showed a clear reduction in tumor volume compared to the DMBA-only group. AEIL demonstrated strong anti-tumor effects against DMBA-induced mammary cancer, with marked decreases in tumor weight, incidence, and burden. In particular, HD treatment reduced tumor weight by 93.79%, tumor volume by 60.07%, and incidence by 46.60%, highlighting its restorative strength. Tamoxifen performed slightly better in some measures, with the highest tumor weight reduction (94.01%) and volume reduction (65.2%). Overall, HD consistently outperformed LD and came close to matching the standard treatment's effectiveness (Tables 1-3 and Figures 2-3).

Table 1: Experimental group design and treatment protocol

Group	TREATMENT
Group I	Control rats given only Saline orally
Group II	Rats given DMBA (25 mg/ kg) were induced by single gastric intubation of 1ml olive oil.
Group III	Rats treated with DMBA (25 mg/ kg) were induced by single gastric intubation of 1ml Olive oil + Tamoxifen Citrate 10mg/kg orally
Group IV	Rats treated with DMBA (25 mg/ kg) were induced by a single gastric intubation of 1 ml of olive oil + AEIL 100 mg/kg.
Group V	Rats treated with DMBA (25 mg/ kg) were induced by single gastric intubation of 1ml olive oil + AEIL 200 mg/kg.

Table 2: Initial and final body weights of control and experimental groups

Group	Control	Only DMBA	DMBA+STD	DMBA+L.D	DMBA+H.D
Initial Body Weight (g)	126±1.91	126±0.79	125±2.68	119±2.17	130±2.76
Final Body Weight (g)	280±5.11	217±9.57	276±5.09	226±4.88	289±2.89

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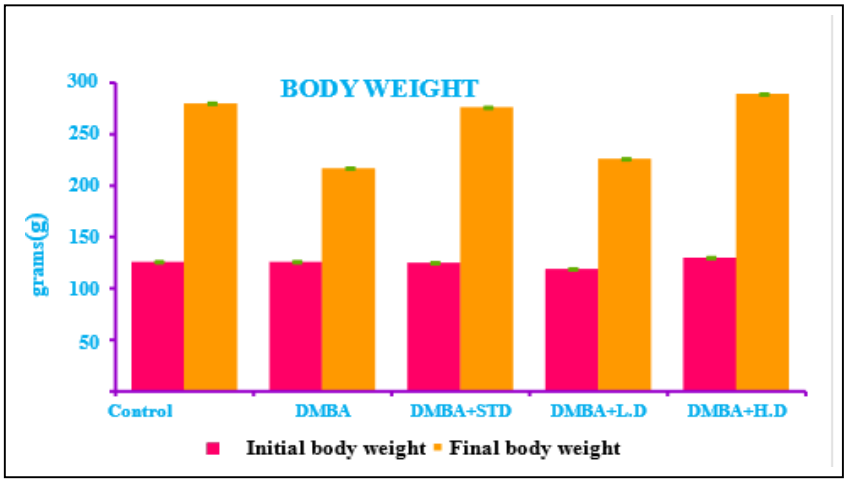

Fig. 2: Initial and final body weights of the control and experimental group

Table 3: Tumor analysis in DMBA- Treated experimental groups

Group	Only DMBA	DMBA+STD	DMBA+L.D	DMBA+H.D
Tumor Weight (mg/g)	113±12.2	6.77±1.23***	19.2±3.96***	7.01±1.04***
Tumor Volume (cm^3)	0.839±0.05	0.292±0.05**	0.565±0.17	0.335±0.04*
Tumor Burden (g)	7.67±0.88	4.33±0.88	5.33±0.88	4±0.57
Tumor Incidence (%)	6.33±0.33	2.67±0.33**	4.67±0.88^{ns}	3.38±0.28*

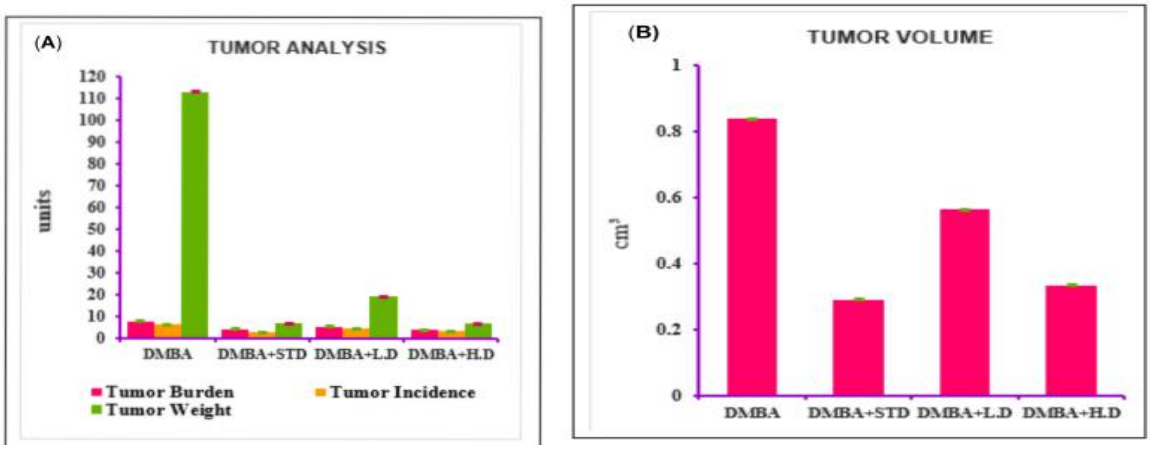

Fig. 3 Tumor analysis of Experimental groups

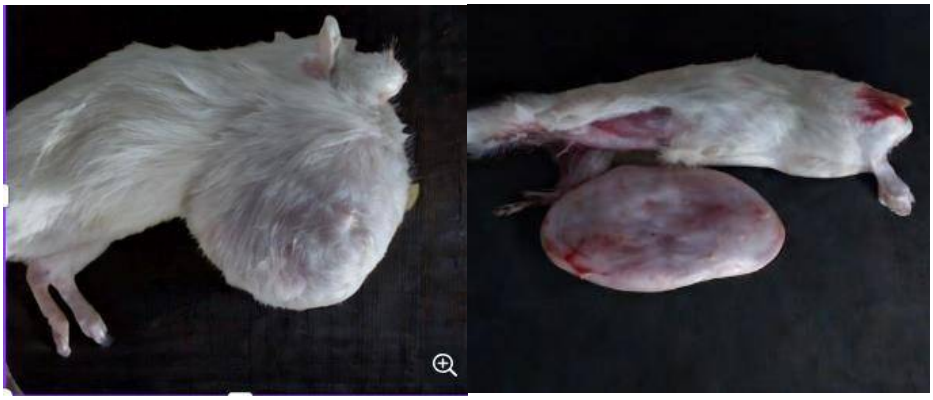

Fig. 3 C(i): DMBA (25 mg/ kg) Only

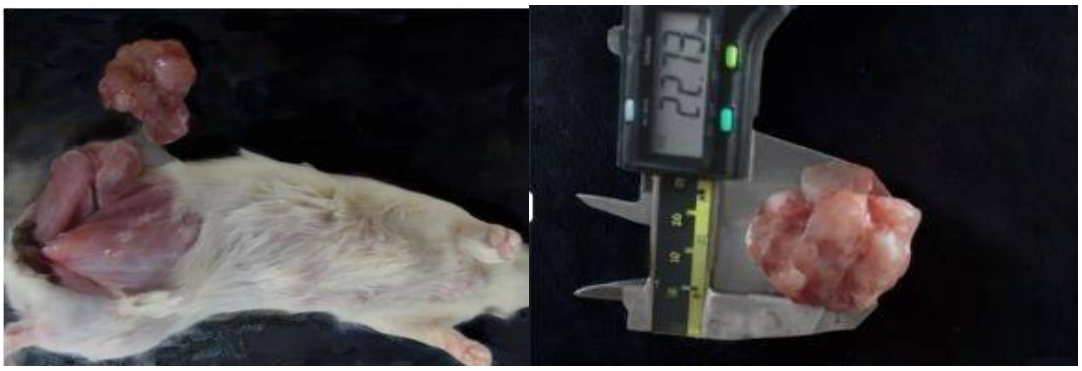

Fig. 3 C(ii) DMBA (25 mg/ kg) +STD

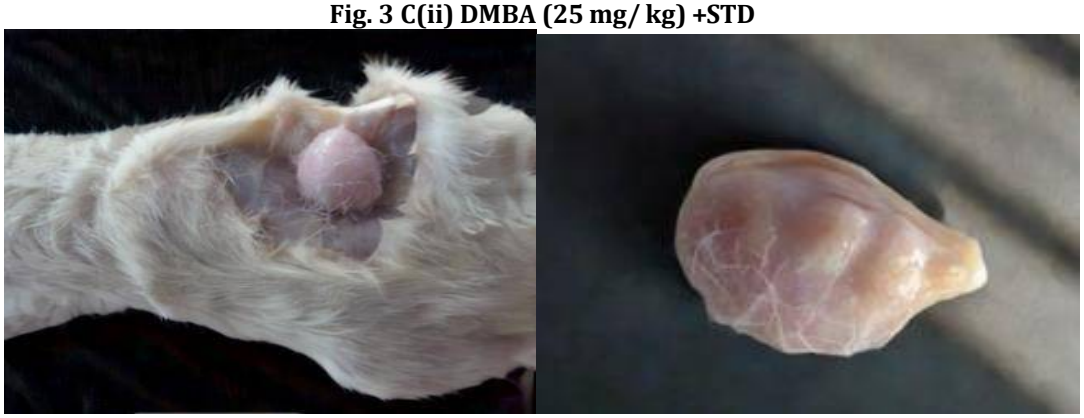

Fig. 3 C(iii) DMBA (25 mg/ kg) + L.D 100 mg/kg

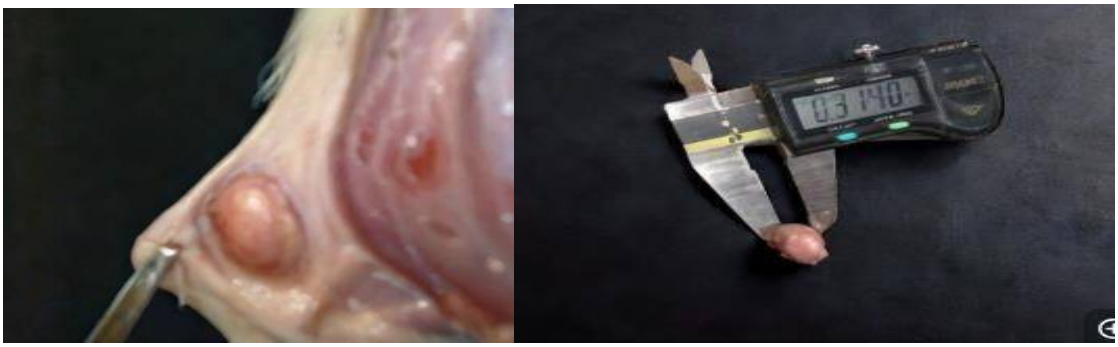

Fig. 3 C(iv) DMBA (25 mg/ kg/ kg) + H.D 200 mg/kg

When DMBA was given, liver enzymes (SGOT, SGPT, ALP) rose sharply, signaling liver damage. Treatment with the HD acetonic leaf extract and tamoxifen brought these levels down significantly, while Group IV showed moderate improvement (Table 4, Fig. 4A). Both treatments also reduced total bilirubin close to normal, with Group IV showing a notable drop (Table 4, Fig. 4B). For kidney function, DMBA increased

urea, uric acid, and creatinine, pointing to impaired renal health. These findings are outlined in Table 5 and Fig. 5.

Table 4. Effect of DMBA and treatment interventions on liver function parameters

Group	Control	only DMBA	DMBA+STD	DMBA+ L. D	DMBA+H.D
SGOT (IU/L)	39±2.89	98.6±1.73***	37±1.73*	44.7±0.86**	39.2±4.04*
SGPT (IU/L)	33.3±2.6	117.4±5.2***	34.7±1^{ns}	45.31±6.06***	35.82±4.04^{ns}
ALP (IU/L)	116±4.04	224.8±3.12***	118± 2.31^{ns}	144±13.6*	127± 2.31^{ns}
Total Bilirubin(mg/dL)	0.5±0.08	1.25±0.02**	0.49±0.02^{ns}	0.96±0.13***	0.48±0.03***

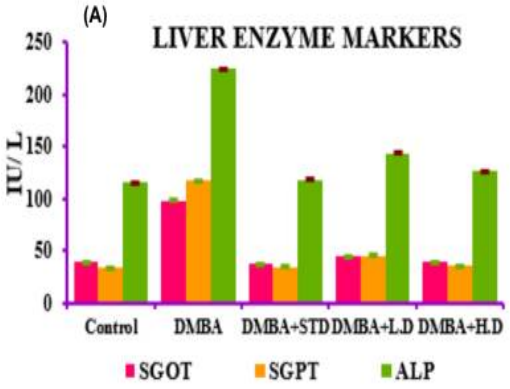

Fig. 4A Liver Enzyme Markers

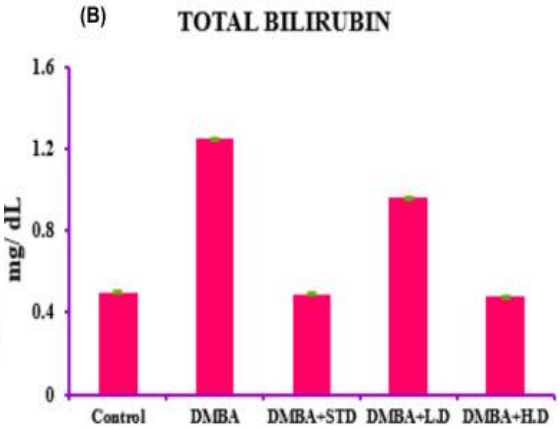

Fig. 4B Total Bilirubin

Table 5. Effect of DMBA and treatment regimens on kidney function parameters

Group	Control	only DMBA	DMBA+STD	DMBA+L.D	DMBA+H.D
Urea (mg/dL)	19.8±1.67	106±4.07***	18.61±3.18**	20.74±3.41***	18.02±0.52*
Uric acid (mg/dL)	3.65±0.26	14.2±0.35***	3.8±0.23^{ns}	8.05±0.15***	3.7±0.44^{ns}
Creatinine (mg/dL)	1.2±0.15	8.3±0.52***	1.4±0.23^{ns}	5.2±0.35***	1.86±0.41***

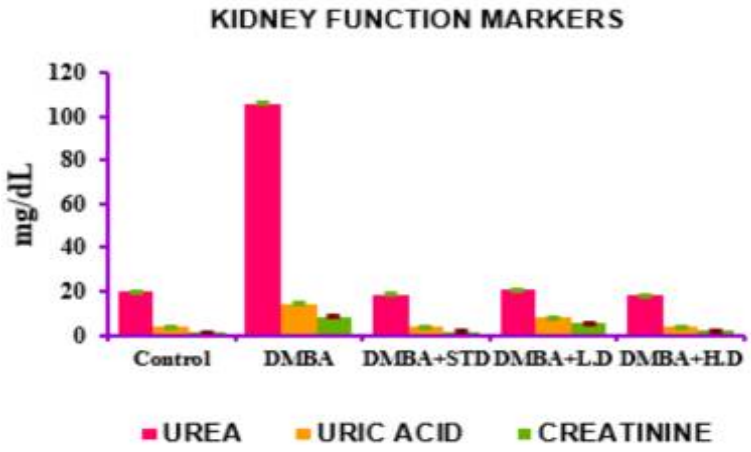

Fig. 5 Kidney Function Markers

Compared with the control group, the DMBA-treated group exhibited notable increases in total cholesterol and LDL cholesterol. The DMBA+STD and DMBA+HD treatments restore lipid parameters by ~93-97%, bringing total cholesterol, triglycerides, and LDL close to control levels. High-dose and tamoxifen interventions provide near-complete recovery of the lipid profile. A summary of these results may be found in Table 6 and Figure 6. When DMBA causes breast cancer, oxidative stress is usually present. Antioxidant enzymes such as SOD, CAT, and GPX are crucial for reducing oxidative damage.

Remarkably, the activity of these antioxidant enzymes decreased by DMBA treatment, increasing the susceptibility of cells to oxidative stress. Group III and V treatments restore antioxidant parameters to ~ 100-217% of control levels, indicating near-complete or even enhanced recovery of SOD, catalase, GPX, GSH, and reduction of LPO (Table 7, Figure 7a and 7b).

Table 6: Lipid profile in control and DMBA- Treatment Groups

Group	Control	Only DMBA	DMBA+STD	DMBA+L.D	DMBA+H.D
Total Cholesterol (mg/dL)	158±1.44	300±8.66***	162.4±5.77^{ns}	181.3±14.4**	167.9±2.89^{ns}
Triglycerides (mg/dL)	95.3±1.47	183±3.7***	98.57±1.36^{ns}	126.5±3.72***	101±4.73^{ns}
LDL-Cholesterol (mg/dL)	92.6±1.67	145.7±3.49***	94.8±0.64^{ns}	114.4±2.14***	96.17±0.32^{ns}

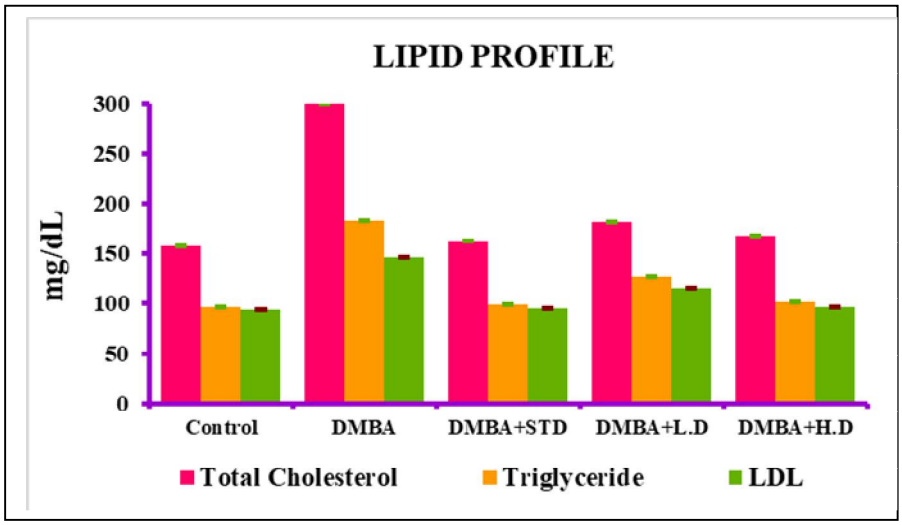

Fig. 6: Lipid profile

Table 7: Oxidative stress and antioxidant profile across experimental groups

Group	Control	Only DMBA	DMBA+STD	DMBA+ L. D	DMBA+H.D
SOD (U/mg protein)	13.34±0.02	2.11±0.04	14.81±0.02^{ns}	10.16±0.05^{ns}	13.57±0.02^{ns}
CATALASE (KU/mg protein)	67±0.02	18.1±0.06***	124.74±0.07^{ns}	97.2±0.06***	118.91±0.02^{ns}
GPX (MU/ mg protein)	18.42±0.08	3.4±0.01***	31.47±0.01^{ns}	22.96±0.01*	29.14±0.01^{ns}
GSH (nmol /mg)	19.72±0.02	2.318±0.02^{ns}	28.61±0.02^{ns}	17.42±0.01^{ns}	23.76±0.02*
LPO (nmol /mg)	0.67±0.02	2.36±0.02***	0.54±0.01^{ns}	0.38±0.04^{ns}	0.49±0.03^{ns}

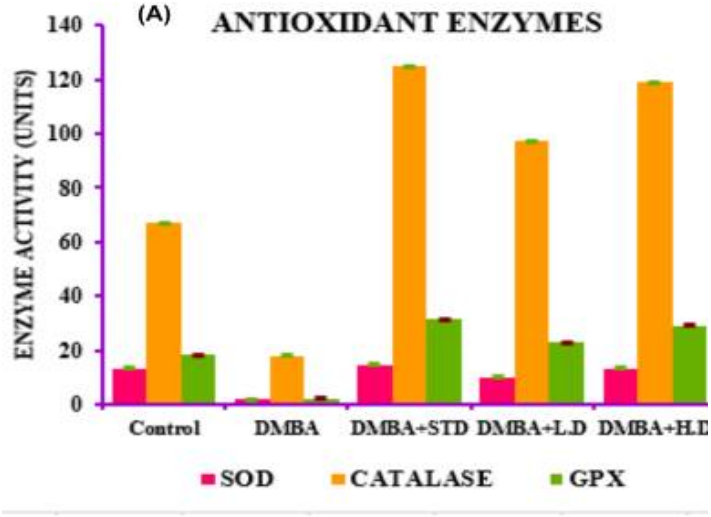

Fig. 7A Antioxidant Enzymes

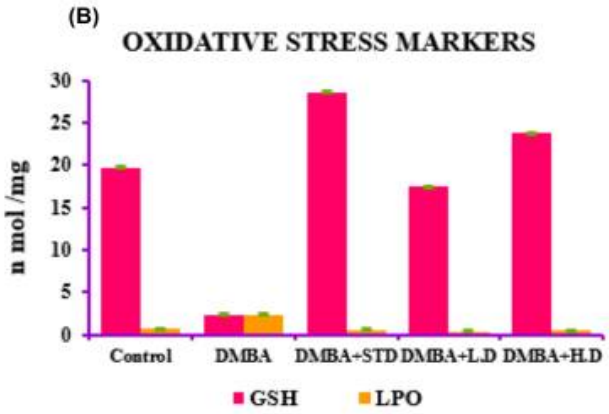

Fig. 7B Oxidative Stress Markers

DMBA treatment increases hexokinase to ~152% and F1,6-diphosphatase to ~219% of control, while suppressing glucokinase to ~41% and glucose-6-phosphatase to ~32%, thereby shifting metabolism toward glycolysis. The AEIL significantly reduced hexokinase activity in a dose-dependent manner, suggesting anti-cancer effects by at least partially inhibiting glycolysis, a metabolic feature of cancer cells (see Table 8 and Figures 8a and 8b for specific results).

Table 8: Modulation of key glycolytic enzymes in experimental groups

Group	Control	Only DMBA	DMBA+STD	DMBA+L.D	DMBA+H.D
Hexokinase (nmoles of glucose 6 phosphate /min/mg protein)	3.2±0.3	8.07±0.11***	3.16±0.16^{ns}	5.3±0.24***	3±0.33^{ns}
Glucokinase (nmoles of glyceraldehyde formed /min/mg protein)	0.514±0.013	0.21±0.02	0.798±0.00693^{ns}	0.554±0.01*	0.687±0.013^{ns}
Glucose-6- Phosphatase (nmoles of pi liberated /min/mg protein)	63.4±5.86	20.5±2.08**	83±5.06^{ns}	77±6.57^{ns}	80.1±9.87^{ns}
Fructose 1-6- Diphosphatase (nmoles of pi liberated /min/mg protein)	35.2±4.7	77.1±5.75***	33.3±5.45^{ns}	36.3±6.32^{ns}	32.3±4.89^{ns}

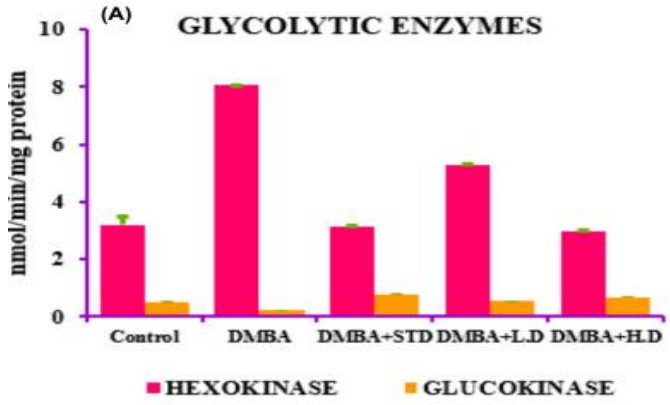

Fig. 8A Glycolytic Enzymes

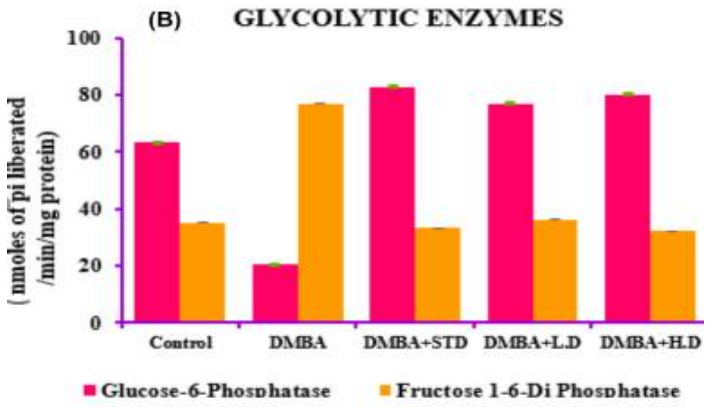

Fig. 8B Glycolytic Enzymes

Therapy groups showed changes in isocitrate dehydrogenase (ICDH) activity. Compared to the DMBA-treated group, the control group's ICDH activity was considerably higher. According to these results, the therapies may have an impact on the citric acid cycle, which could enhance their anti-cancer properties. High doses of *Indigofera longiracemosa* extract and tamoxifen increased succinate dehydrogenase activity similarly. MDH activity varied among the five groups and can be restored by specific therapies (Table 9 and Figure 9). The estimation of total protein showed notable differences across the five groups. Additional information is provided in Figure 10 and Table 10.

Table 9: Citric Acid Cycle Enzymes

Group	Control	only DMBA	DMBA+STD	DMBA+ L. D	DMBA+H.D
ICDH (nmol of Alpha- Keto Glutarate /min/mg protein)	36±.23	15±.16***	35.7±.88**	30.71±.68***	34.9±.63***
SDH (nmol of succinate oxidized/ min/mg protein)	13.3±0.2	5.71±0.30***	14.6±0.20**	10.92±0.93***	13.8±0.64 ns
MDH (nmol of NADH oxidized/ min/mg protein)	125±1.5	192.6±3.52***	127±1.83**	144.3±18.5*	131.9±3.18 ns

Table 10: Total Protein Estimation

Group	Control	only DMBA	DMBA+STD	DMBA+L.D	DMBA+ H. D
Total Protein (mg/dL)	6.32±0.20	3.41±0.32***	7.61±0.72ns	5.80±0.26***	7.32±0.20ns

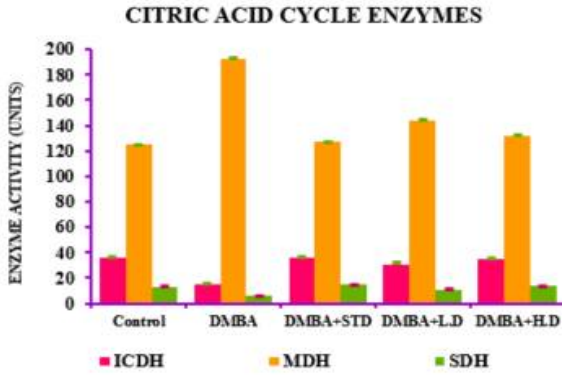

Fig. 9: Citric Acid Cycle Enzymes

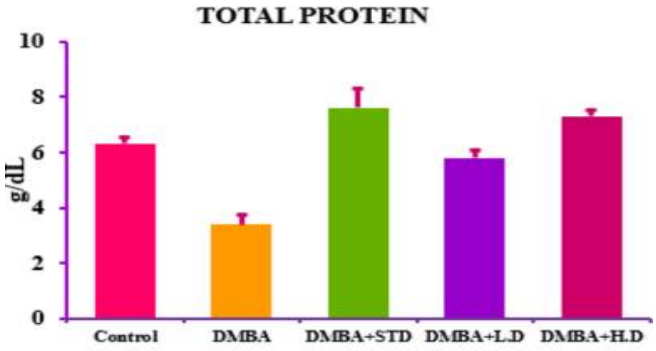

Fig. 10 Total protein

Haematoxylin and Eosin (H&E) staining was used to assess histological changes in breast tissues of control and treated Sprague-Dawley rats. DMBA treatment caused marked alterations in mammary tissue morphology (Figure 11). In contrast, rats treated with DMBA + Tamoxifen showed reduced tumour burden and cellular atypia. Cells appeared more uniform, with decreased nuclear pleomorphism, lower glandular complexity, and reduced cellular density. Mitotic activity was minimal (<0.01 mitoses/HPF), accompanied by decreased angiogenesis and enhanced stromal fibrosis, indicating a therapeutic response to tamoxifen (Figure 12). Tissue sections from rats treated with DMBA were administered a low dose of AEIL and underwent microscopic analysis, displaying prominent histopathological changes. Intense inflammatory cell infiltrates made up of lymphocytes and neutrophils were perceptible in the investigated area, indicating an immunological response. Coagulative necrotic conditions were also observed, which may reveal the death of tumour cells. For further details, please refer to Figure 13. Microscopic analysis of tissue slices from Group V revealed severe inflammatory cell infiltration, widespread coagulative necrosis, and a significant decrease in tumour cellularity. Both neutrophils and lymphocytes were clearly visible, with the latter showing signs of activation. Cytoplasmic eosinophilia, karyorrhexis, and severe nuclear pyknosis were observed in the necrotic regions. There was less angiogenesis and more stromal fibrosis in the surrounding tissue. Additional information can be found in Figure 14.

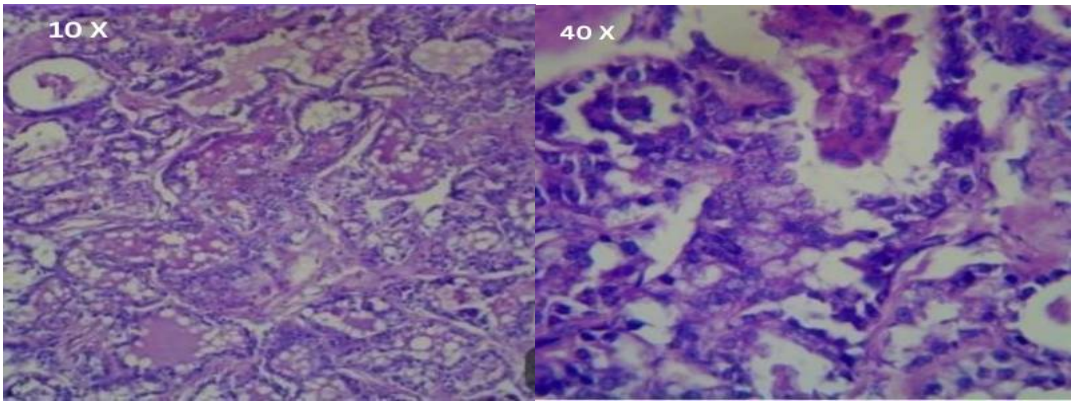

Fig. 11 Histological alterations in the mammary gland of DMBA-treated rats

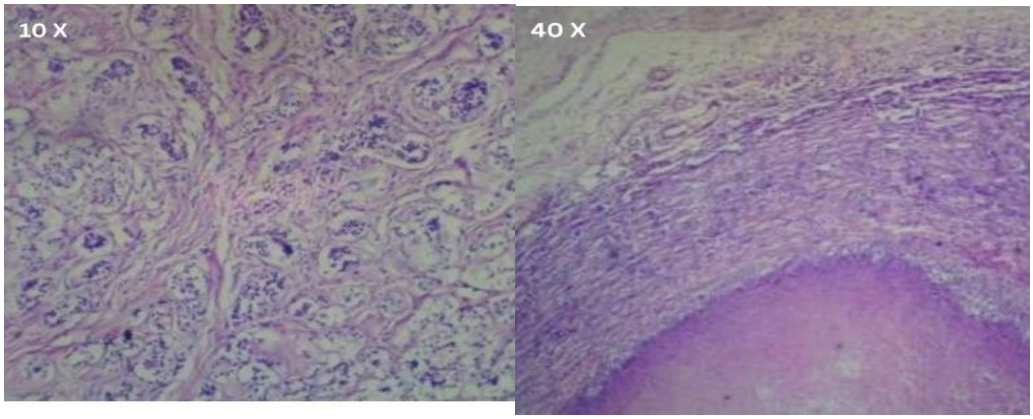

Fig.12.Histological alterations in the mammary gland of DMBA + Tamoxifen-treated rat

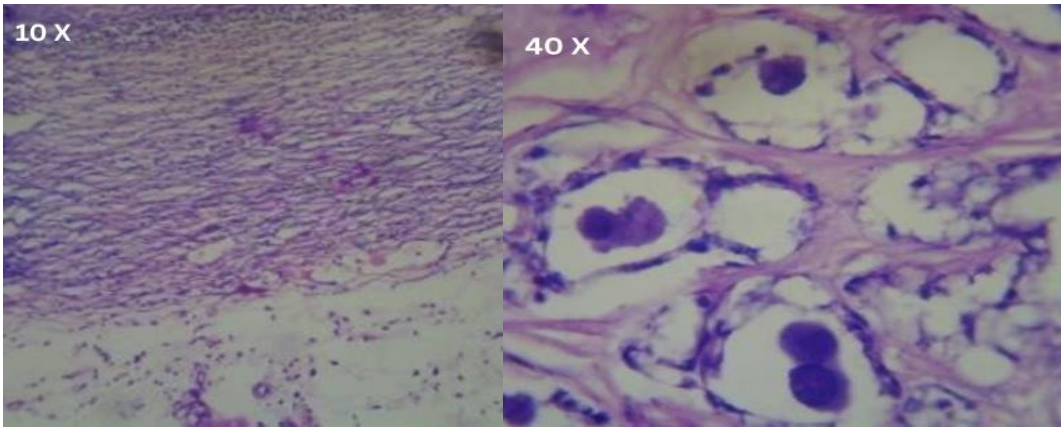

Fig. 13. Histological alterations in the mammary gland of DMBA+L.D-treated rats

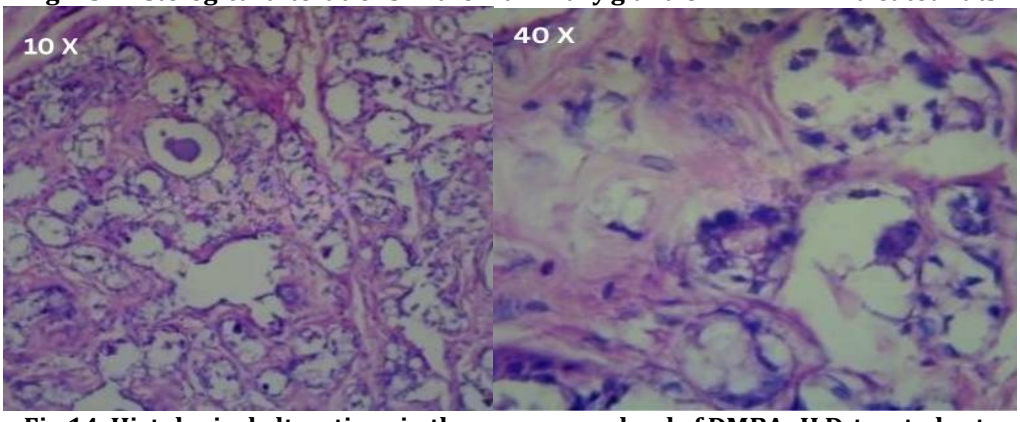

Fig.14. Histological alterations in the mammary gland of DMBA+H.D-treated rats

DISCUSSION

Alpha, -Tocopherol- beta. -D-mannoside is a vitamin E derivative that has strong antioxidant properties and may induce apoptosis in cancer cells, enhance chemosensitivity, and reduce tumor growth.26 3-Hydroxy-3-methyl-oxindole-3-ac derivative induces apoptosis, inhibits cell proliferation, and modulates oxidative stress pathways.[27] Phytol exhibits antiproliferative effects, induces apoptosis, and possesses anti-inflammatory properties.[28] 9,12,15-Octadecatrienoic acid (α-Linolenic acid) has shown potential anticancer effects. That has been studied for its protective effects against breast cancer in preclinical models, partly due to its anti-inflammatory and antioxidant actions.[29] A detailed discussion of these findings is beyond the scope of this manuscript and will be presented in a separate publication focusing on phytochemical profiling. AEIL showed encouraging results in fighting DMBA-induced mammary cancer. The high-dose treatment not only helped restore body weight beyond normal levels but also led to clear reductions in tumor size, weight, and incidence. Although tamoxifen performed slightly better in some measures, the high-dose AEIL came very close and was much stronger than the low-dose group. Overall, these findings suggest that AEIL, especially at higher doses, could be a promising option for recovery and tumor control. DMBA exposure caused clear liver and kidney damage, but treatment with the HD acetonic leaf extract and tamoxifen showed strong therapeutic benefits. Both helped restore enzyme and bilirubin levels toward normal, while also improving kidney function markers. Group IV showed moderate recovery, suggesting partial protection. These results highlight the extract's potential as a therapeutic agent with hepatoprotective and nephroprotective effects, comparable in some aspects to tamoxifen. DMBA treatment significantly elevated cholesterol and LDL levels, while combined interventions with Tamoxifen or H.D restored lipid parameters to near-control values. After treatment with tamoxifen and high-dose acetonic leaf extract, there was a therapeutic effect against DMBA-induced breast cancer through the restoration of antioxidant enzyme activity. Tamoxifen and the acetonic extract demonstrate a therapeutic effect against oxidative damage by restoring GSH levels and lowering LPO levels, which may slow the evolution of breast cancer. DMBA treatment shifts metabolism towards glycolysis, a hallmark of cancer cells, while AEIL counteracts this by reducing hexokinase activity and showing therapeutic potential. The extract's influence on cholesterol metabolism may further support its anti-cancer role. Its negative correlation with F1,6BP and tamoxifen highlights a promising glycolytic-targeted strategy. Researchers have found that AEIL shows promising anti-breast-cancer effects by helping cells return to a healthier metabolic state. Normally, cancer cells rely heavily on glycolysis even when oxygen is available, a phenomenon known as the Warburg effect. But AEIL seems to reverse this, nudging cells back toward oxidative phosphorylation, the more efficient energy pathway used by normal cells.[30] AEIL (Acetonic Extract of *Indigofera longiracemosa*) shows promise in fighting breast cancer by targeting enzymes that regulate cellular metabolism. Normally, exposure to DMBA, a known carcinogen, disrupts mitochondrial function, leading to reduced activity of critical citric acid cycle enzymes such as isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), and malate dehydrogenase (MDH). This breakdown in energy production fuels cancer cell survival. AEIL treatment, however, helps restore the activity of these enzymes, effectively rebalancing mitochondrial energy metabolism (Table 9 and Figure 9). By cutting off the energy supply that cancer cells depend on, AEIL may weaken their ability to grow and spread.[31] AEIL appears to work by restoring balance in metabolic enzymes, which can push malignant cells toward apoptosis and slow down their uncontrolled growth, two major drivers of cancer progression. Its effects are dose-dependent, with higher concentrations showing greater improvements in enzyme activity and protein stability, pointing to the possibility of an optimal therapeutic range for breast cancer treatment. By influencing metabolic pathways, offering antioxidant protection, and helping stabilize key proteins, AEIL demonstrates a multi-faceted anti-cancer potential that makes it a promising candidate for future breast cancer therapies. [32] Under a microscope, tissue slices showed an infiltrating neoplasm with glandular cells, each with a vesicular nucleus, mild eosinophilic cytoplasm, and round to oval morphologies. Nucleoli were seen in certain cells, indicating nuclear pleomorphism. A mitotic count of 0.1 mitoses/HPF indicated the presence of mitotic activity. The formation of mammary adenocarcinomas in response to DMBA treatment is consistent with these findings, demonstrating the effectiveness of this model in understanding the pathophysiology of breast cancer. Using Haematoxylin and Eosin (H&E) staining, DMBA treatment induced mammary adenocarcinomas with nuclear pleomorphism and mitotic activity, confirming its utility as a breast cancer model. Tamoxifen co-treatment markedly reduced tumour burden, atypia, and angiogenesis, demonstrating therapeutic efficacy. AEIL administration further enhanced anti-tumour responses, with necrosis, immune infiltration, and stromal remodelling, indicating strong immunomodulatory and cytotoxic effects.

CONCLUSION

Chemotherapeutic medicines have high side effects. In contrast, plant extracts are considered to be an attractive alternative because of their achievable anticancer qualities, lower toxicity, and improved bioavailability for cancer prevention and treatment. The investigation unveils the acetonic leaf extract of *Indigofera longiracemosa* Boivin ex Baill (AEIL) as a potent natural alternative for treating DMBA-induced mammary carcinoma, rewriting the rules of cancer therapy. AEIL dismantles tumour burden, disrupts abnormal cell morphology, and inhibits angiogenesis in rats, showcasing anti-tumour efficacy that outshines conventional treatments. GCMS analysis reveals a treasure trove of bioactive compounds with antioxidant and anticancer properties, positioning AEIL as a game-changer in oncology. Notably, AEIL modulates key metabolic enzymes, restoring balance to disrupted pathways: it normalizes hexokinase and glucose-6-phosphatase levels, curtails glycolysis, and boosts citric acid cycle activity via isocitrate dehydrogenase and succinate dehydrogenase upregulation, shifting energy metabolism away from cancer-driven Warburg effect. Additionally, AEIL enhances antioxidant defenses by upregulating superoxide dismutase, catalase, and glutathione peroxidase, mitigating oxidative stress. In conclusion, AEIL emerges as a potent natural alternative for treating DMBA-induced mammary carcinoma, demonstrating substantial antitumour efficacy superior to conventional treatments. GC-MS analysis revealed bioactive compounds with known antioxidant and anticancer properties. The acetonic leaf extract of *Indigofera longiracemosa* Boivin ex Baill may be a valuable candidate for further development as a therapeutic agent. Future studies will focus on optimizing a pharmaceutical formulation, evaluating its bioavailability, and exploring synergies with existing chemotherapeutics to develop a safe and effective adjuvant therapy for breast cancer. Thus, AEIL emerges as a beacon of hope in breast cancer therapy, illuminating pathways to safer, potent treatment.

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