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Original Research Article

Phytochemical and Antiproliferative Effect of *Indigofera stenophylla* Guil. And Perr. (Fabaceae) Against 7,12-Dimethylbenzanthracene induced Breast Tumor in Rats

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ABSTRACT

Breast cancer is a serious public health issue. Chemotherapy is clinically employed, and herbal medicines provide an alternative therapeutic option. *Indigofera stenophylla* is traditionally used for the management of breast cancer. Although it is yet to be validated, this study investigated the photochemistry and antiproliferative effect of methanol aerial parts extract of *Indigofera stenophylla* (MAEIS) against 7,12-dimethylbenzanthracene (DMBA)-induced breast tumors in rats. Phytochemical screening was conducted using standard procedures. Using Lorke's approach, the oral median lethal dose for MAEIS was determined. A single *intraperitoneal* injection of 20 mg/kg DMBA was used to establish a breast tumor in a female Wistar rats. Tumor growth, oxidative stress markers, inflammatory cytokines, and histological profiles were measured and evaluated. The MAEIS indicated the presence of alkaloids, steroids, triterpenes, flavonoids, carbohydrates, glycosides, and saponins. The oral median lethal dose was 2,154 mg/kg. MAEIS significantly (p<0.01) reduced tumor volume in a dose dependent manner. It also attenuates DMBA-induced increases in the malondialdehyde, tumor necrotic factor-alpha, and nitric oxide levels while enhancing antioxidant enzyme activities (p<0.01). Histology showed that MAEIS ameliorates breast tissue damage. Methanol aerial parts extract of *Indigofera stenophylla* contains phytochemicals with antiproliferative properties against breast tumors in rats. Mechanistically, it may possibly act via anti-oxidant and anti-inflammatory mechanisms, supporting its ethno-medicinal use for breast tumor treatment.

Keywords: Anti-inflammatory, anti-oxidant, anti-tumor, oxidative Stress, *Indigofera stenophylla*

Introduction

Cancer continues to be a significant public health challenge around the world because of its elevated rate of morbidity and mortality.[1] Breast cancer is the utmost recurrently encountered type of cancer and the foremost cause of cancer-related mortality among women worldwide. In 2020, it represents nearly 12% of all new cancer cases and 6.9% of all cancer deaths.[2] In developing countries, breast cancer incidence and mortality rates are rising rapidly due to lifestyle changes, increasing life expectancy, and a lack of awareness.[3] Inflammation and oxidative stress are recognized as key drivers of cancer initiation and progression.[4] Chronic inflammation fosters tumor development through the release of reactive species of oxygen (ROS) and nitrogen (RNS), which lead to DNA damage and mutations.[5]

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Cancer cells also experience elevated oxidative stress due to increased metabolic demands and oncogene activation,[6] which triggers a vicious cycle of inflammation, DNA damage, and further tumor growth.[7] Thus, addressing oxidative stress and inflammation is a viable approach to treat and prevent breast cancer.[8] In experimental models, some plant-derived substances with anti-inflammatory and antioxidant characteristics have shown chemopreventive actions against breast tumors.[9,10] African traditional medicine uses the plant *Indigofera stenophylla* (Fabaceae) to cure a variety of illnesses, including breast cancer ethnomedicinally.[11] However, its anti-tumor activity and mechanism have not been scientifically validated. Previous studies on other *Indigofera* species have reported antioxidant, anti-inflammatory, and cytotoxic activities attributed to their phytochemical constituents like flavonoids, alkaloids, and terpenoids.[12].This study aimed to characterize the phytochemicals in preliminary form and assess the chemopreventive ability of *Indigofera stenophylla* (MAEIS) methanol aerial parts extract against breast tumors induced by 7,12-dimethylbenzanthracene (DMBA) in rats. The MAEIS effect on tumor growth, oxidative stress markers, inflammatory cytokines, and histological alterations in the breast, liver, and kidney were investigated. The findings of this study will validate *Indigofera stenophylla*, in the management of breast cancer through the inhibition of oxidative and inflammatory pathways to support further development of effective and affordable alternative therapy.

Methods and Materials

Materials

The 7,12-dimethylbenzanthracene (Sigma-Aidrich, USA), methanol (Sigma-Aidrich, USA), Vanier caliper (Vemier, France), weighing balance (SF-400, USA), -and ELISA kits for NO, TNF-α (FineTest, China) were used.

Collection, identification, and preparation of plant materials

The plant was collected in October 2023, and the aerial parts of *Indigofera stenophylla* were sorted. In the herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria, the plant was authenticated and issued a voucher specimen number of 0763. The plant was dried, finely powdered, and stored at 25ºC.[13]

Extraction

About 1500 g of the fine powder was extracted with 10L of 70% v/v methanol. Using a rotary evaporator, the solvent was subsequently evaporated to obtain a solid methanol aerial parts extract of *Indigofera stenophylla* at 45ºC.[13]

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the methanol was performed using standard procedure.[14]

Experimental animals

A total of 50 female Wistar rats were purchased from the Department of Pharmacology and Therapeutics, Ahmadu Bello University animal house. The animals were made to acclimatize at ambient temperature and were administered a laboratory diet obtained from Olan poultry feed and water *ad libitum.*

Experimental Study Design

The oral median lethal dose (LD_{50}) of the MAEIS was determined using the Lorke's approach.[15] It involves two stages. Nine rats were divided into three groups of three at random in phase one. The MAEIS was given at the dose of 10, 100, and 1000 mg/kg body wright to groups 1, 2, and 3 respectively. According to the results of phase 1, three (3) groups, each with one rat, received 1600, 2900, and 5000 mg/kg body weight of the were administered MAEIS in phase 2. The value of the LD_{50} was calculated as the square root of the minimum lethal dose and maximum nonlethal dose that is $LD_{50} = \sqrt{Minimal\ lethal\ dose \times Maximal\ survival\ dose}$

The graded doses of MAEIS for this study were extrapolated using 30% of the LD_{50}. The highest dose of MAEIS selected for the study was 600 mg/kg. The study ensured that the highest dose of MAEIS was below 30% of the LD_{50}.[16]

Breast tumor induction

The induction of the breast tumor in fifty rats was initiated using 20 mg/kg single dose of 7,12-dimethylbenzanthracene (DMBA) *intraperitoneally* beneath the mammary gland. The stock concentration of 20 mg/mL was prepared by dissolving 20 mg in 1 mL of olive oil. Eight weeks after induction, the rats were palpated for tumor and thirty were selected based on the presence of palpable tumor mass.[17]

Experimental design

Thirty-six rats total were divided into six groups, each with six rats, in order to screen for cancer. Normal saline (10 mL/kg) was administered *intraperitoneally* as a normal control to Group 1, which was not originally pretreated with DMBA for tumors induction. The second group was likewise given normal saline (10 mL/kg) *intraperitoneally*. This group was similarly evaluated for the existence of a palpable tumor mass after tumor induction with DMBA as a negative control. After DMBA was used to induce a tumor, groups three, four, and five were first examined for the presence of a palpable tumor mass. They were then given oral doses of 150, 300, and 600 mg/kg of the methanol aerial parts extract of *Indigofera stenophylla*, respectively. Following tumor induction with DMBA as a positive control, group six was also first examined for the existence of a palpable tumor mass and was administered *intraperitoneally* with 10 mg/kg of cyclophosphamide. The course of therapy lasted four weeks.[17]

Organismic examination

The tumor diameter was taken weekly using Vanier Caliper and the body weight of the rats were taken weekly using a weighing balance. After 28 days treatment, the weight of the rats and the weight of the kidney, liver, spleen, brain and the heart were taken and recorded using weighing balance and used to estimate the relative organ weight using the formular, $Relative\ Organ\ Weight\ (ROW) = (Organ\ weight \div weight\ of\ the\ animal) x\ 100$ and the tumor volume was calculated from the tumor diameter using the formula ($V = 4/3\pi r^3$) after being sacrificed under chloroform anesthesia where r represent the radius of the tumor diameter, v represent volume, 4/3 and π are constant value.[17]

Hematological investigation

The blood sample was collected using EDTA containers through cardiac puncture at the end of the treatment for the hematological investigations.[17]

Biochemical investigation

The blood sample was collected using plain containers through cardiac puncture at the end of the treatment for biochemical investigations.[17] ELISA kits were used for NO and TNF-α proinflammatory markers investigation according to the manufacturer's instruction while spectroscopic analysis was used for SOD, CAT and GSH stress markers. The technique for the determination of malondialdehyde (MDA) concentration described by a method adopted by Wisudanti *et al*.[18] Serum catalase (CAT) activity was measured using sinha's method adopted by Umar *et al*.,[19] Superoxide dismutase (SOD) activity was assessed using the method described by Sun *et al*, adopted by Umar *et al*.,[19] Reduced glutathione (GSH) levels were determined using modified Tietez's method adopted by Norman *et al*.,[20] were adopted.

Tissue processing for histopathological examination

The liver, kidney, and mammary gland tissues were fixed in a 10% formalin solution and subjected to dehydration through a series of graded alcohols. After dehydration, the tissues were cleared with xylene, which was inserted in paraffin and then fixed on slides. They were subsequently stained with a reagents hematoxylin and eosin for virtualization before being examined and photographed under microscope.[21]

Statistical analysis

Data were presented as Mean ± SEM (Standard Error of Mean). The effect of the MAEIS on organ weight, oxidative stress and proinflammatory markers were analyzed using one-way Analysis of Variance (ANOVA) while the effect of MAEIS on body weight and tumor volume using repeated measure ANOVA followed by Bonferroni post-hoc test for multiple comparisons. Differences were considered significant at $p<0.05$ and $p<0.001$ using IBM Statistical Package for Social Sciences (SPSS) software, version 23

Results and discussion

Phytochemical characterization of the methanol aerial parts extract of Indigofera stenophylla

Preliminary phytochemical analysis of the *Indigofera stenophylla* methanol aerial parts extract showed the presence of alkaloids, tannins, glycosides, carbohydrates, flavonoids, and saponins in addition to steroids and triterpenes, as seen in Table 1. Numerous secondary metabolites, including triterpenes, alkaloids, flavonoids, and others, have been shown to have antitumor action.[22,23,24] The observed activity might be attributed to the metabolites found in the MAEIS.

Median lethal dose of the methanol aerial parts extract of Indigofera stenophylla

Table 1: Phytochemical Constituents of the Methanol Aerial Parts Extract of *Indigofera stenophylla*

Constituent	Test	MAEIS
Carbohydrates	Molisch	+
Anthraquinones	Bontrager	-
Glycosides	Keller-Kiliani	+
Steroids/Terpenes	Liebermann-Burchard Salkowski	+
Tannins	Lead acetate	+
Flavonoids	Shinoda	+
	Ferric chloride	+
Alkaloids	Dragendorff	+
	Wagner	+
Saponins	Frothing	+

Key: + = present, - = absent, MAEIS = Methanol aerial parts extract of *Indigofera stenophylla*

The oral median lethal dose of the MAEIS was found to be 2,154 mg/kg in rats. The rats showed no sign of toxicity. The eyes, skin, and mucous membrane appeared normal. There was neither a sign of neurological, psychological, nor gastrointestinal abnormalities such as trembling, behavioral changes, coma, diarrhea, and falling of the hair from the rat's skin.[15]

Effect of the methanol aerial parts extract of Indigofera stenophylla on relative organ weight and tumor volume in DMBA-induced breast tumors in Wistar rats

Table 2 showed that the kidney, liver, and spleen significantly changes (p<0.001) for cyclophosphamide, whereas the liver alone showed significant changes in relative body weight (p<0.05) at a dosage of 600 mg/kg of the MAEIS when compared to the negative control. When compared to the diseased DMBA-induced tumor rats, the tumor volume was significantly (p<0.05) lower in the MAEIS (150, 300, and 600 mg/kg) and cyclophosphamide (10 mg/kg) groups through weeks two, three, and four, as seen in Figure 1. DMBA and its metabolites trigger the formation of breast cancer either through DNA adduct or chronic inflammatory mechanism,[25] or through the ductal elements of the mammary gland by increasing the substantial oxidative stress,[17] and induced DNA mutation.[26]

Table 2: Effect of Methanol Aerial Parts Extract of *Indigofera stenophylla* Oral Administration on Relative Organ Weight in DMBA Induced Breast Tumor in Wistar Rats for 4 Weeks

	Kidney (g)	**Liver (g)**	**Spleen (g)**	**Brain (g)**	**Heart (g)**
NORMAL SALINE	0.38	4.33	0.58	1.26	0.36
DMBA 20 mg/kg	0.44±0.16	5.09±0.14	0.57±0.22	1.05±0.23	0.34±0.13
DMBA + MAEIS 150 mg/kg	0.42±0.02	4.39±0.38	0.55±0.25	1.13±0.11	0.41±0.30
DMBA + MAEIS 300 mg/kg	0.39±0.32	4.50±0.22	0.73±0.23	1.13±0.38	0.36±0.32
DMBA + MAEIS 600 mg/kg	0.36±0.24	3.75^{ab}±0.19	0.60±0.33	0.96±0.38	0.28±0.34
DMBA + CYCL 10 mg/kg	0.34±0.33 ^{ab}	3.33±0.11 ^{ab}	1.12±0.28 ^{ab}	1.11±0.40	0.39±0.38

Key: Data were presented as Mean ± Standard Error of Mean (SEM). Data were analyzed using ONE-WAY ANOVA, followed by Bonferroni post-hoc test, a (p<0.05), b (p < 0.001), n = 6, MAEIS (Methanol aerial part extract *of Indigofera stenophylla)*, CYCL (Cyclophosphamide), DMBA (7,12-dimethylbenzanthracene).

Table 3: Effect of the Methanol Aerial Parts Extract of *Indigofera stenophylla* Oral Administration on Oxidative Stress Markers in DMBA Induced Breast Tumor in Wistar rats

Treatment	**Dose (mg/kg)**	**SOD (U/mL) ±SEM**	**GSH (U/mL) ±SEM**	**CAT (U/mL) ±SEM**	**MDA (nmol/mL) ± SEM**
N/S	10 mL/kg	16.48±1.12	8.58±0.45	11.08±0.33	53.72±2.70
DMBA	20	6.98±0.23**	5.08±0.28**	3.53±0.13**	299.83±17.25**
DMBA + MAEIS	150	7.68±0.86^{ab}	5.77±0.20^{a}	7.02±0.35 ^{a}	208.87±10.47^{ab}
DMBA + MAEIS	300	10.20±3.90^{a}	7.67±0.21	4.47±0.10^{ab}	140.53±6.24^{ab}
DMBA + MAEIS	600	13.42±3.21	7.98±1.01	4.05±0.22^{ab}	223.85±1.02^{ab}
DMBA + CYCL	10	10.25±1.73^{a}	6.35±0.58^{a}	3.82±0.33^{ab}	110.67±4.29^{ab}

Key: Data were presented as Mean ± SEM. Data were analyzed using ONE-WAY ANOVA followed by Bonferroni post-hoc test, n = 6 in each treatment group and, SOD (Superoxide dismutase), GSH (Glutathione peroxidase), CAT (Catalase), MDA (Malondialdehyde), DMBA (7,12-dimethylbenzanthracene), MAEIS (Methanol aerial part extract *of Indigofera stenophylla)*, CYCL (Cyclophosphamide), * (Significant difference between groups at p<0.05 relative to normal control), ** (Significant difference between groups at p<0.001 relative to normal control), ^{a} (Significant difference between groups at p<0.05 relative to DMBA) and ^{b} (Significant difference between groups at p<0.001 relative to DMBA).

Table 4: Effect of the methanol aerial parts Extract of *Indigofera stenophylla* oral administration on tumor necrotic factor alpha and nitric oxide in DMBA induced breast tumor in Wistar rats

Treatment	**Dose (mg/kg)**	**TNF α (pg/mL) ±SEM**	**NO (pg/mL) ±SEM**
Normal Saline	10 mL/kg	147.99±3.29**	176.57±1.67**
DMBA	20	210.71±4.54	271.15±7.45
DMBA + MAEIS	150	169.12±1.18^{ab}	194.60±4.00^{ab}
DMBA + MAEIS	300	185.44±1.82^{ab}	187.16±1.98^{ab}
DMBA + MAEIS	600	155.91±0.62^{ab}	210.26±3.37^{ab}
DMBA + CYCL	10	151.29±2.92^{ab}	180.50±1.03^{ab}

Key: Data were presented as Mean ± Standard Error of Mean (SEM). Data was analyzed using One-way ANOVA followed by Bonferroni post-hoc test, n = 6 in each treatment group and, DMBA (7,12-dimethylbenzanthracene), MAEIS (Methanol aerial part extract *of Indigofera stenophylla)*, CYCL (Cyclophosphamide), TNF-α (Tumor necrotic factor alpha), and NO (Nitric oxide), * (Significant difference between groups at p<0.05 relative to

normal control) **(Significant difference between groups at $p<0.001$ relative to normal control), [a] (Significant difference between groups at $p<0.05$ relative to DMBA) and [b] (Significant difference between groups at $p<0.001$ relative to DMBA).

Table 5: Effect of Methanol Aerial Parts Extract of *Indigofera stenophylla* on Hematological Parameters in DMBA Induced Breast Tumor Rats

	N/S (10mL/kg)	DMBA (20 mg/kg)	DMBA + MAEIS 150 mg/kg	DMBA + MAEIS 300 mg/kg	DMBA + MAEIS 600 mg/kg	DMBA + CYCL 10 mg/kg
WBC (10^3/uL)	3.77±0.07*	6.17±0.105	5.30±0.263	4.33±0.243	4.87±0.08*	3.93±0.203*
LYMPH (10^3/uL)	5.23±0.092	6.13±0.243	5.90±0.132	5.85±0.28	5.70±0.474	5.47±0.462
MID (10^3/uL)	0.32±0.031	0.40±0.037	0.40±0.037	0.70±.073	0.73±0.021	0.53±0.042
GRAN (10^3/uL)	2.00±0.190	2.73±0.201	2.53±0.173	2.58±0.17	2.77±0.067	3.03±0.056
RBC (10^6/uL)	6.15±0.38	5.67±0.211	6.07±0.076	6.10±0.037	5.92±0.083	6.10±0.073
HGB (g/dL)	13.12±0.46	12.60±0.329	13.60±0.132	14.13±0.165	12.10±0.490	12.53±0.532
PLT (10^3/uL)	151.35±6.86**	122.67±5.420	167.67±7.315**	168.67±1.308**	174.17±1.833**	176.67±4.387**

Key: Data were presented as Mean ± Standard Error of Mean (SEM). Data was analyzed using One-way ANOVA followed by Bonferroni post-hoc test, n = 6 in each treatment group and, DMBA (7,12-dimethylbenzanthracene), MAEIS (Methanol aerial part extract *of Indigofera stenophylla)*, CYCL (Cyclophosphamide) and * (Significant difference between groups at $p<0.05$ relative to normal control) **(Significant difference between groups at $p < 0.001$ relative to normal control) [a] (Significant difference between groups at $p<0.05$ relative to DMBA) and [b] (Significant difference between groups at $p<0.001$ relative to DMBA). WBC (White blood cell), PLT (Platelet count), HGB (Hemoglobin Concentra ration), GRAN (Neutrophil), MID (Mean index of distribution

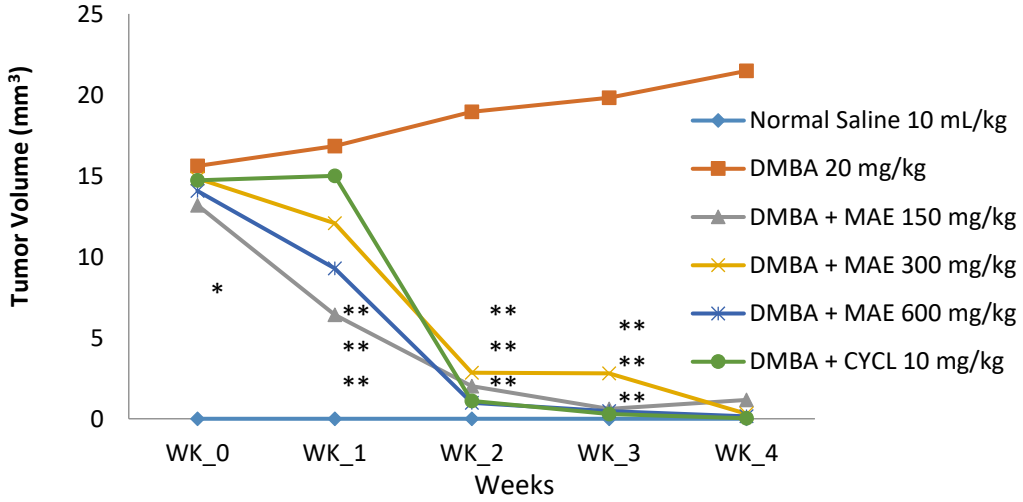

Figure 1: Effect of 4 Week Oral Administration of Methanol Aerial Parts Extract of *Indigofera stenophylla* on Tumor Volume in DMBA Induced Breast Tumor in Wistar Rats

Key: Data were presented as mean ± SEM. Data were analyzed using Repeated Measure Analysis of Variance, followed by Bonferroni post-hoc test, * ($p<0.05$), ** ($p<0.001$), n = 6 and, MAEIS (Methanol aerial part extract *of Indigofera stenophylla)*, CYCL (Cyclophosphamide) DMBA (7,12-dimethylbenzanthracene).

The DNA metabolite DMBA-DE forms a DNA adduct in P53 and H-Ras genes to initiate carcinogenesis.[27] The tumors induced with DMBA and NMU (N-nitroso-N-methylurea) are morphologically and histologically similar to human estrogen-dependent breast cancer.[28] Studies have shown that MDA, SOD, CAT, GSH, TNF-α, and NO are implicated in breast cancer evaluation.[29,30,31,32]

Effect of the methanol aerial parts extract of Indigofera stenophylla on oxidative stress markers in DMBA-induced breast tumors in Wistar rats

The MAEIS showed significant increased ($p<0.01$) in the levels of superoxide dismutase (SOD), reduce glutathione (GSH) in a dose-dependent, and catalase (CAT) in a dose-independent manner, while the malondialdehyde (MDA) level decreased in a dose-independent compared to the DMBA-induced breast tumor group. Similarly, cyclophosphamide at the dose of 10 mg/kg significantly increased the level of SOD, GSH, and CAT while MDA decreased (Table 3). The decrease in the level of MDA in this study is in conformity with that reported in the literature.[26]Malondialdehyde (MDA) is one of the most significant and liquid peroxidation aldehyde, and it promotes tumors by altering the structure and function of proteins, DNA, and other macromolecules. MDA level measurement in tissue and plasma has been widely employed in cancer assessment, particularly breast cancer. Reducing MDA levels has been linked to a lower risk of developing breast cancer due to antioxidant action.[33] MAEIS antioxidant activity is validated with the potential of reducing breast cancer progression.
The increased in the enzymatic activity of SOD in this study is in conformity with that reported by literature,[30,34,35,36] Superoxide dismutase (SOD), an enzyme that catalyzes the conversion of

superoxide radicals into hydrogen peroxide and oxygen as intermediate byproducts of normal oxidative phosphorylation in the mitochondria has been used extensively as an oxidative stress biomarker.[10] Catalase (CAT), an enzyme that catalyzes the conversion of hydrogen peroxide radicals into water as intermediate byproducts of normal oxidative phosphorylation in the mitochondria and has been used extensively as oxidative stress biomarker. The increment in the level of SOD and CAT in this study confirmed the antioxidant activity of MAEIS thereby attenuating the tumor cell proliferation. This corroborates with the work reported in the literature.[10] Reduced Glutathione (GSH), is a critical antioxidant that control the conversion of hydroxyl radicals into water as byproducts of normal oxidative phosphorylation in the mitochondria and has also been used as an oxidative stress biomarker. Literature has shown that decreased GSH levels have been implicated pathophysiological conditions.[31] Oxidative stress is an essential index in cancer development and evaluation with clinical biomarkers that are critical in the evaluation of the body tissue and blood as identifier of breast cancer.[30] The increment in the level of GSH exhibited by the MAEIS further confirmed the antioxidant activity of the extract associated with breast tumor, also in conformity with that reported in the-literature.[31]

Effect of the methanol aerial parts extract of Indigofera stenophylla on inflammatory cytokines in DMBA-induced breast tumor in Wistar rats

The MAEIS reduced significantly ($p<0.01$) dose-dependent in both NO and tumor necrotic factor alpha (TNF-α) in comparison to the group that had breast tumors produced by DMBA. Likewise, Table 4 illustrates that cyclophosphamide (10 mg/kg) markedly reduced the levels of TNF-α and NO. The observed reduction in the expression of TNF-α attributed to MAEIS in this study aligns with findings reported in the literature.[37] Elevated levels of TNF-α in breast tumors have been shown to correlate with lymph node metastasis. Furthermore, a strong association exists between high serum levels of TNF-α in breast cancer and poor prognosis.[37] It has been proposed that TNF-α can induce either cell death or proliferation, depending on the biological condition.[38] NF-α is recognized as one of the major pro-inflammatory cytokines serving as mediator of cancer-related inflammation within the tumor microenvironment.[29] Several studies have explored the relationship between TNF-α and breast cancer. This highly versatile inflammatory cytokine is produced by various cell types, including smooth muscle cells, Kupffer cells, neutrophils, macrophages, natural killer cells, fibroblasts, T and B cells, keratinocytes and astrocytes.[38] additionally, cancer cells secrete TNF-α, which can act as an inherent promoter of tumors. Research indicates that TNF-α plays a significant role in mediating inflammation and utilizes various signaling pathways to regulate the expression of growth factors and other cytokines.[37] The reduction of TNF-α by MAEIS indicate its anti-inflammatory activity associated with breast tumor.

Soluble guanylate cyclase's (sGC) principal activator is nitric oxide (NO). It is produced by the enzyme nitric oxide synthase (NOS), which then diffuses through cell membranes to interact with the heme cofactor of sGC. This binding causes the enzyme to become activated and causes a notable rise in the levels of cGMP. Furthermore, sGC may be successfully activated by NO donors. Critical physiological processes including platelet aggregation, vasodilation, and neurotransmission are regulated by the second messenger, cGMP, through direct effects on ion channels, cGMP-phosphodiesterases, and cGMP dependent protein kinases (PKG).[39] Lowering of nitric oxide (NO) levels in cancer cells can inhibit certain signaling pathways that are crucial for cell proliferation and survival. Reduced NO production may suppress the activation of the PI3K/Akt pathways, which is often dysregulated in cancer cell and is vital for supporting cell growth, survival and angiogenesis.[40] Decreased in the level of NO is also associated with the downregulation of inducible nitric oxide synthase (iNOS), the enzyme responsible for the production of NO in the cancer cells. The reduction in the NO level due to MAEIS indicates that the plant may have direct inhibitory effect on iNOS expression. The reduction of NO by MAEIS confirmed its antitumor activity via suppression of P13K/Akt pathway activation in breast tumor. This study agrees with that reported in the literature.[40]

Effect of the methanol aerial parts extract of Indigofera stenophylla on hematological parameters, breast tissue, liver, and kidney histopathology in DMBA-induced breast tumor in Wistar rats

The MAEIS significantly increased ($p<0.001$) the platelet count, and white blood cell count in a dose dependent manner compared to the DMBA induced breast tumor group as seen in Table 5. The mean index of distribution, Lymphocyte and Neutrophil, Red Blood Cells, and Hemoglobin showed no significant changes.

The negative control treated with DMBA revealed the presence of neoplastic cells, glandular hardening, ductal dilation, inflammation, and hyperplasia of the fibrous connective tissues as seen in Plate I. Comparing the treatments groups at 150, 300 and 600 mg/kg relative to the negative control, showed mild to moderate neoplastic cells, moderate glandular hardening, slight ductal dilation, mild to moderate inflammation and hyperplasia of the fibrous connective tissues with the presence of apoptotic cells as observed in Plate I. The histopathology of the liver showed mild to moderate necrosis of the hepatocytes and congestion of the central vein while the sinusoids appeared normal for the MAEIS at 150, 300, 600 mg/kg, negative and positive control as seen in Plate II. The histopathological structure of the kidney tissues in all the treatment groups appeared normal for glomerulus, bowman capsule and the tubules as seen in Plate III.

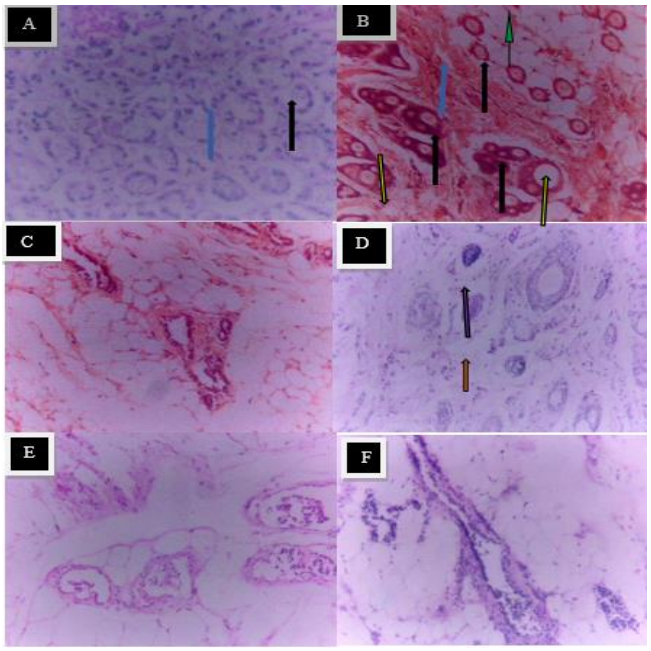

Plate I: Photomicrograph of a Section of Breast following 28 days Oral Administration of Methanol Aerial Parts Extract of *Indigofera stenophylla* on DMBA Induced Breast Tumor in Wistar Rats (H and E, 100X Magnification)

Key: A (Normal control), B (Negative control), C (150 mg/kg MAEIS treated group), D (300 mg/kg MAEIS treated group), E (600 mg/kg MAEIS treated group), F (Positive control), Red Arrow (Gland), Gold Arrow (Duct), Green Arrow (stroma), blue Arrow (fibrous connective)

The decreased in the WBC count is in conformity with that reported in the literature.[41,42] Increase in PLT count is associated to an extract that has antioxidant and inflammatory properties implicated in the management of thrombocytopenia and platelet related disorders.[42] This effect indicates its favorable toxicological profiles and selective antitumor activity.[43] This lack of hematological toxicity is an important consideration for the potential therapeutic development of an extract with antitumor agent.[44,45] The maintenance of normal red blood cell parameters, such as mean intermediate density cells, red blood cell count, and hemoglobin concentration, indicates that is not causing

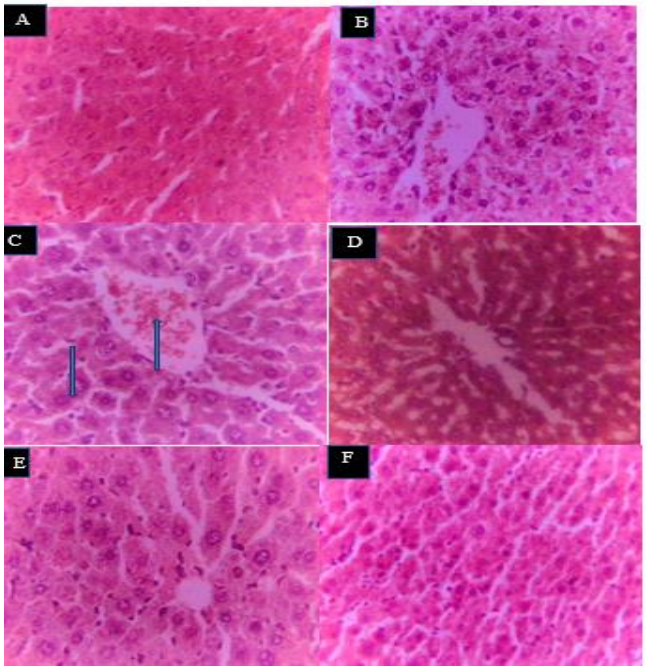

Plate II: Photomicrograph of a Section of Liver following 28 Days Oral Administration of Methanol Aerial Parts Extract of *Indigofera stenophylla* on DMBA Induced Breast Tumor in Rats (H and E, 400X Magnification)

Key: A (Normal control), B (Negative control), C (150 mg/kg MAEIS treated group), D (300 mg/kg MAEIS treated group), E (600 mg/kg MAEIS treated group), F (Positive control), Black (hepatocytes), blue (Central vein) and white colour (sinusoids).

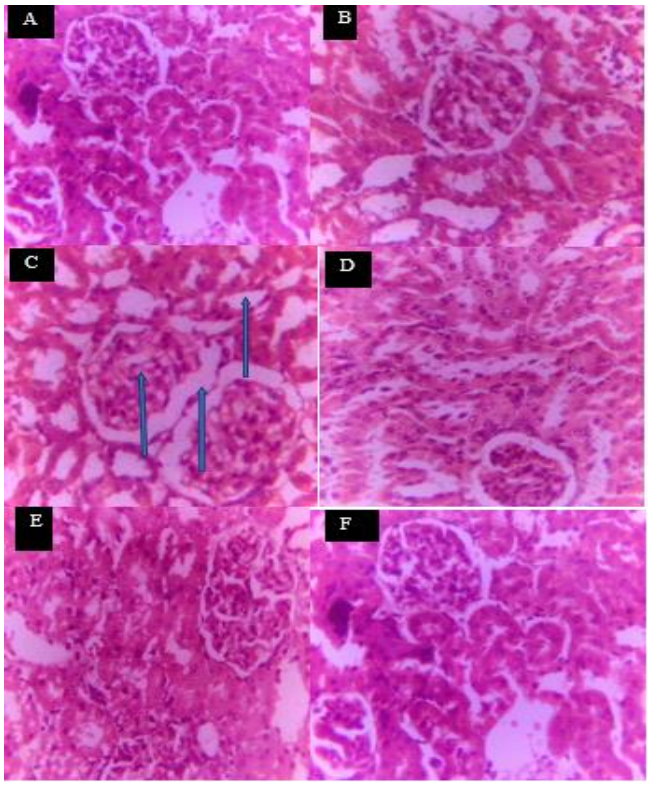

Plate III: Photomicrograph of a Section of Kidney following 28 Days Oral Administration of Methanol Aerial Parts Extract of *Indigofera stenophylla* on DMBA Induced Breast Tumor in Wistar Rats (H and E, 250 X magnifications)

Key:
A (Normal control), B (Negative control), C (150 mg/kg MAEIS treated group), D (300 mg/kg MAEIS treated group), E (600 mg/kg MAEIS treated group), F (Positive control), Yellow colour (glomerulus), blue colour (Bowman capsule) and green colour (tubules).

significant disruption to the hemopoietic system.[46,47] The MAEIS has no significant effect on LYM, GRAN, MID, RBC and HGB concentration which is in agreement with that reported in the literature.[43] This study showed that MAEIS has minimal effect on hematological profile and selective antitumor activity in breast tumor. MAEIS has remarkable benefits on the hematological parameters with an attribute that further confirmed its anti-inflammatory and antioxidant activity.

The evaluation of breast tissue based on pathological diagnostic criteria for human breast tissue is characterized by several features; the terminal duct's enlargement with the capacity to spread into the nearby adipose tissue, irregular curvature of the ductal lumen, occasional shedding of the cells accompanied by phagocytic histiocytes, clustering of the proliferative ducal epithelial cells, formation of secondary ductal lumen (papillary) and morphological diversity of the cells in terms of size.[48] The evaluation of breast tissue encompasses the presence of proliferating acni in the mammary glands, hyperplasia of epithelial cells in layered formations, necrotic cells and polymornuclear cells. Additionally, it includes dense formation of fibrotic cells, reduced number of adipose cells, which are being replaced by fibroblasts and apoptotic cells as indicators.[49] The attenuation of proliferative activity exhibited by MAEIS further confirmed the antitumor activity of MAEIS. This study corroborates with that reported in the literature.[48] The absence of histological changes in the liver and kidney is a crucial indicator of lack of hepatotoxicity and kidney toxicity.[50,42] This implies absence of any structural and morphological changes in the liver and kidney.[50,42] This showed that MAEIS has minimal or no detrimental effect on both the liver and the kidney.**Conclusion**

The methanol aerial parts extract of *Indigofera stenophylla* (MAEIS) contains secondary metabolite with chemo-preventive potential against DMBA-induced breast tumor in rats.

Conflict of Interests

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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