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Original Research Article

N-Methyl-N-Nitroso-Urea Induced-DNA Damage Response in the Breast Tissues of Female Albino Rats Treated with Ethanol Extract of *Solanum erianthum* D. Don

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ABSTRACT

Solanum erianthum has been traditionally recognized for its anti-inflammatory and anticancer properties; however, the mechanism by which its ethanol leaf extract (ELESE) mitigates *N-methyl-N-nitroso-urea* (NMU)-induced breast tissue damage remains unclear. This study evaluated the tumor-suppressive and antioxidant potential of ELESE on NMU-induced mammary gland tumors in female albino rats. A total of thirty (30) adult female albino rats were sectioned into six groups which comprises: control, NMU-group, ELESE-50 mg/kg, ELESE-100 mg/kg, ELESE-200 mg/kg, ELESE-400 mg/kg. 50 mg/kg of the NMU was administered via the buccal cavity to groups except the control, while ELESE was concomitantly administered to each group with the use of a cannula for 30 days and the following parameters got evaluated: Glutathione (GSH) increased significantly ($p<0.01$) in ELESE-200mg/kg and ELESE-400 mg/kg compared to NMU group. Superoxide dismutase (SOD) significant ($p<0.01$) in ELESE-100, 200, 400 and 50 mg/kg respectively compared to the NMU-group. Catalase (CAT) level insignificant ($p<0.05$) in ELESE-200 mg/kg, 50 mg/kg, and 100 mg/kg in comparison with the NMU group, significant ($p<0.01$) in ELESE-400 mg/kg. Malondialdehyde (MDA) level reduced significantly ($p<0.01$) in ELESE-50 mg/kg and 400 mg/kg. Hemoglobin (Hgb) levels in ELESE-groups reduced significantly ($p<0.01$) in 400mg/kg compared to NMU-group. Hematocrit (HCT) level in the ELESE 50 mg/kg group surge significantly ($p<0.1$) compared to the NMU-group. γH2AX molecular marker was used to study DNA damage. The tail DNA percentage reduced in ELESE-400 mg/kg and 50 mg/kg compared to the NMU-group, demonstrating a protective effect against genotoxicity and indicating antioxidant effect. ELESE may ameliorate nitroso-methyl- urea (NMU)induced breast tissue damage

Keywords: Nitroso-Methyl-Urea (NMU), DNA Damage, Breast tissues, Antioxidant, *Solanum erianthum*, Antioxidant.

Introduction

Solanum erianthum (*S. erianthum*) belongs to the Solanaceae family, popularly known in the Southwestern part of Nigeria as 'ewuro-Igbo or Gbogbo nise' (meaning "cure-all" due to its traditional medicinal use), while in the South-Eastern part of Nigeria, it is sometimes called 'Ukwu ojii' (depending on locality), but not popular in Northern Nigeria. It is a shrub or small tree often called Potato true. [1] It is a fast-expanding annual tree that may grow to altitudes of between 4 and 10 meters, with a stem diameter of up to 20 cm, and can thrive at heights from sea level. [2]

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The genus *Solanum* belongs to the nightshade family (Solanaceae) and comprises 2500 or more species used as traditional food and in medical care. [3] *S. erianthum* is an excellent source of pure antioxidants and is beneficial for halting the progression of various cellular oxidative stress phases[4]. A boiled solution of *Solanum species* leaves is purportedly utilized in West Africa for the treatment of malaria, leprosy, venereal illnesses and promoting liver function. It also acts as a stimulant and purgative.[5] In Ghana, the leaf extracts of *S. erianthum* are considered appropriate to make a hot drink, and purposefully cultivated as both food and medicine.[6] Nitrosomethylurea (NMU) is a pale yellow, direct DNA alkylating carcinogen belonging to the nitrosamine class of chemicals. [7] It causes adenine-thymine (AT) to guanine-cytosine (GC) transition mutations by moving the amino group from adenine to cytosine, and transition mutations via the methylation of nucleobases in nucleic acids, which is how it exerts its toxicity.[8] Its direct carcinogenic, mutagenic, and teratogenic potentials have been demonstrated.[9] In experimental animals, NMU has been demonstrated to cause DNA damage that results in a variety of cancers, such as colorectal, gastric, esophageal, breast, and retinal degeneration. [10]

Comet Assay (solitary cell Electrophoresis Assay) is an adaptable and responsive technique for assessing DNA damage and single-cell DNA repair ability.[11] The utilization of the comet assay has grown

significantly for over a decade due to its sensitivity, simplicity and the minimum count of cells needed for reliable findings.[12] Traditionally, comet experiments employ cell suspensions steeped in agarose on a microscope slide that are fragmented by exposure to high-salinity and detergent solutions.[12] A supercoiled DNA nucleoid is left behind when membranes and other soluble cell components are removed during lysis. The fragments of DNA will move to the positive electrode (anode) under electrophoretic conditions, creating a characteristic "comet tail." Overall, the number of fragmented strands is equivalent to the quantity of DNA in the head and the tail.[13]

Comet assay has been harnessed for the evaluation of genotoxicity of metallic elements, DNA fragmentation due to the impact of radiation from natural or environmental source, phytotoxicity of small-scale materials, DNA damage induced by organic pollutants such as azo dyes, alkylating agents, aromatic hydrocarbons with benzene ring, pesticide and herbicides. [12] This assay was used in recent time to detail on the importance of genomic stability and homology recombination during the doubling of DNA. Moreover, comet test is considered essential in delineating the macro nucleotide (DNA) damaging effect caused by chemicals including mitomycin, ethyl methane sulfonate, methyl methane sulfonate, and/ or hydrogen peroxide nitrosamine (NMU)

In recent years, research has shown that DNA damage response regulates the cell cycle and signal transduction, and that its malfunction can result in genomic instability and tumour growth; hence, it has emerged as one of the new targets for breast cancer treatment.[14] Breast tissue malignancy is the fourth leading cause of cancer related mortality and the second most common tumor idiocy among women globally.[15]

The pathophysiology of breast cancer involves DNA damage response (DDR) pathways, with harmful heritable mutations in homologous recombination (HR)-linked genes like BRCA1/2 accounting for around 10% of cases.[16] Furthermore, in recent years, medications that target the DNA damage response pathway as well as associated treatments have been developed.[17] The death rate from breast cancer has decreased owing to the recent developments in the introduction of new, powerful anti-cancer treatments combined with early detection techniques.[8] However, breast cancer is still a problem, and the present anticancer therapy techniques need to be improved further.[18] Therefore, this research is aimed to investigate the preventive anti-inflammatory and anti-cancer effects of the ethanolic extract of *Solanum erianthum* in NMU-induced DNA damage in the breast tissues of female albino rats.

Materials and Methods

Plant collection and identification

The leaves of the plant were obtained on 13th November, 2021 in Alimosho, Lagos State, Nigeria's Ayobo-Ipaja neighbourhood (N 6° 36' 47.052", E 3° 15' 57.2652"). Dr. G. Nodza of the Herbarium Unit, Botany Department, University of Lagos, Akoka, Yaba, Lagos, performed identification and authentication of the plant. The specimen's voucher number, LUH 8925, was entered into the database for specimen identification.

Preparation of ethanol leaf extract of S. erianthum (ELESE)

With an impact mill, the dried leaves of *S. erianthum* were ground into a fine, smooth powder. A modified version[19] protocol was used for this. A double-layered Mosklim sieve cloth was used to filter the 179 g of powdered leaves after they had been steeped in 2L of 70 percent ethanol for 72 hours. The filtrate was dried using a UNISCOPE laboratory model SM-9023 at 40ºC for 5 days.

Animal Preparation

Thirty adult female albino rats weighing 130 g –135 g and 5-7 weeks old were collected from the College of Medicine, University of Lagos, Nigeria. The rats were divided into six (6) groups of five (5) each and allowed to acclimatize for seven days. The experimental rats contained in a plastic enclosure were fed with pelleted food and water ad libitum in an animal house under a consistent temperature of 27 to 30℃ and had 12-hour cycles of light and darkness.

Preparation of the Chemical

Specifically, 2 g of NMU was dissolved in 25 mL of phosphate-buffered or citrate-buffered saline (pH 4.0) prior to administration (NMU) was freshly prepared each time of use following a slightly modified protocol[7]. Intraperitoneal injections were administered once weekly, and given at a dose of 50 mg/kg for four (4) weeks [7]. A modified regimen was used to induce the first malignancy, in which a 50 mg/kg dose of the NMU was given after 7 days of acclimation. The second injection was given after another 14 days, once until the four weeks of weekly treatment were complete. These rats were weighed weekly and checked for invasive growth and formation of breast lesions.

The percentage of yield using this formula:

$$\%Yield = \frac{weight\ of\ extract(119g)}{weight\ of\ plant\ material\ (179g)} \times 100$$

Experimental Animal

The thirty (30) female rats were placed in six (6) groups at random, comprising of 5 rats per group and given the following treatments for 30 days: ELESE was administered orally once daily through the use of a canula for each group except the NMU-group and the control-group, which were given only food and water. The other groups received the following doses of ELESE: 50 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg on daily basis, in addition to the weekly administration of NMU.

All experimental procedures involving animals were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and were approved by Ethical Review Committee of College of Medicine, University of Lagos under the approval number CMUL/HREC/06/18/801.

After the 30 days administration of treatment, the animal's blood volume of 0.5 ml from each group was taken for testing through orbital veins to evaluate the haematological variables, then the rats were euthanized by inducing them with fumes of anaesthetic (chloroform) to cause weakness and rapid death before harvesting the liver and breast tissues for biochemical assays. The breast tissue was evaluated for antioxidants and DNA fragmentation using the comet assay.

Hematological Assays

The haematological assays were conducted using a Mindray haematological analyzer. Whole blood cell counts were performed using 0.5 ml of the samples of blood in the EDTA-treated tubes. Haemoglobin were measured in grams per liter (g/l)[20]

Tissue homogenization

The liver samples were taken, cleaned in a 1.15% potassium chloride solution that was extremely cold, blotted, and weighed.[21] They were then blended with a Teflon homogenizer and 0.1M phosphate buffer (pH 7.2). The resultant homogenate was centrifuged, after which it was taken out of the centrifuge, and the resultant liquid was taken out and kept at -20°C in a refrigerator for analysis. The centrifuge had been running for fifteen minutes at a speed of 2500 revolutions per minute.

Catalase analysis

The activity of catalase in µ/ml was obtained by a dichromate-acetic acid method. In this assay, dichromate undergoes reduction in acetic acid to form chromic acetate; due to heating in the presence of hydrogen peroxide, perchromic acid is transiently generated, allowing absorbance to be measured at 620nm. 0.5 ml of the sample was mixed to a reaction solution that has 5 ml of 0.2 M, 0.4 M distilled water, and 0.01M buffer (pH 7.0). The addition of 2ml of a dichromatic acid solution (potassium dichromate (5%) and glacial acetic acid; 1:3 v/v) and subjecting the mixture to high heating effect at a temperature of 60°C for a period of 10 minutes serve to stop the process and readings of the absorbance at 620 nm.[22]

Superoxide Dismutase (SOD) activity assessment

Superoxide dismutase assessment was performed using the approach according to [23]. The total reaction volume of 3 ml contained 2.95 ml of

0.05 M sodium carbonate buffer (pH 10.2), 0.02 ml of liver sample, and 0.03 ml of epinephrine (2nM) in 0.005 N HCL. The standard cuvette has 2.95ml of buffer, 0.03 ml epinephrine, also containing 0.02 ml of distilled water. The rise in absorbance at 480 nm was used to gauge SOD's ability to stop adrenaline from oxidizing on its own. At one-minute intervals for three minutes, the absorbance at 480 nm was recorded.[2]

Reduced Glutathione (GSH) Determination
The technique published by [23] was used to determine the amount of reduced glutathione levels in the tissues. 1.0 ml of liver tissue was prematurely degraded with 0.1 ml of 10% trichloroacetic acid and spun at 650 x g for a period of 5 min to get liquid. Additionally, 0.5 ml of the precipitate was treated with 3.0 ml of phosphate buffer and 0.5 ml of Ellman's reagent, which contained 19.8 mg of 5,5-dithiobis-(2 nitrobenzoic acid) (DTNB) dispersed in 100 ml of 0.15 M sodium nitrate (pH 8.0), and absorbance was read at 412 nm.

Malondialdehyde (MDA) assessment
By monitoring the emergence of reactive chemical molecules, the degradation of lipids was quantified. Using the technique described, malondialdehyde (MDA), a sign of lipid peroxidation that causes damage, was discovered by [24]. Two milliliters of the supernatant of the homogenate of the liver sample were combined with a 1:1:1 ratio of TCA-TBA-HCL reagent (thiobarbituric acid 0.37 percentage, 0.24 N HCL, and 15 percent Tricarboxylic acid, thiobarbituric acid, and hydrochloric acid were combined for the reaction, and the mixture was heated at 100℃ for 15 minutes before cooling. The materials were spun at 3000 rev per min for 10 minutes, and flocculent elements were removed. The absorbance reading was taking at a wavelength of 532 nm over the blank solution after the supernatant was removed. The molar extinction coefficient for the malondialdehyde was determined.

Preparation of Alkaline Single-Cell Gel Electrophoresis
The method published by [25] was used, with a slight modification. Three replicates of adult female albino rats' breast tissues were chosen at random from each group of healthy control animals, NMU-group (50 mg/kg), ELESE-50 mg/kg, ELESE-100 mg/kg, ELESE-200 mg/kg, and ELESE-400 mg/kg were harvested and stored in sample bottles for 12 hours before being taken to the laboratory. Each breast tissue was put in a 1ml cold Hank's Balanced Salt Solution (HBSS) solution that contained 20 mM EDTA and 10 percent dimethyl sulfoxide (DMSO). The breast tissues were divided into tiny fragments, allowed to settle, removed, and processed by combining 5-10µl with 75µl low melting point agarose (LMPA). Each slide has an optimal call number of 10ul cells, samples were in three biological and technical replicates, making a total of 54 slides of ELESE-50, 100, 200, 400 mg/kg, NMU-group and normal control group under the electrophoresis under pH >13 alkaline conditions.

Determination of DNA damage
10µl of cells were placed per slide after slides were taken from a solution of lysis buffer and placed in opposite layer on a gel vessel, tilting as close to one another as feasible, for two hours at 4℃. The electrophoresis buffer storage tanks were replenished to the liquid level as needed with a fresh pH>13 electrophoresis buffer, completely covering the slides. For 20 minutes, the hydroxide buffer was used with the film slides for DNA to unwind and express alkaline-labile distortion. The electric source was put on at 24 volts and set to 300 milliampere of current for 30 minutes while the light was reduced.
After the power was switched off for 30 minutes, the 54 slides were carefully taken out of the buffer and put on a tray with drainage holes. After coating the slides for 5 minutes with a neutralization buffer, the procedure was carried out twice. The slides were coated with Giemsa Stain, left to dry for five minutes, and subsequently immersed in cooled distilled water to get rid of any remaining particles. Slides that had been drained were maintained in cold 100% ethanol for 20 minutes to dehydrate them, dried, and then baked at 50℃ for 30 minutes before being prepared for grading.

Determination of microscopic imaging cells
A digital microscope camera was used to take pictures of the comets while the slides were magnified by x40 under a microscope[26]. The software Image J's invert colour feature was employed to flip the colours of the images (the open comet software only detects bright objects in a dark background, which is usually the reverse of what light microscopy produces). The open-source OPEN COMET plug-in for the image-editing program IMAGE J was then used to examine the comets in the photographs. Briefly, the software was used to open all of the photographs for a specific group. After selecting an output folder, the run button was selected to automatically find and score the comet in the images. An effort was made to guarantee that at least 100 cells were analyzed per sample.

Statistical Analysis
Statistical Products and Service Solutions (SPSS) edition 17.0 was utilized to analyze the generated date using one-way assessment of variance (ANOVA), and the findings were given as mean SED. The LSD (Least Square Difference) post hoc test was used to compare means, and a significant result was defined as ($p<0.01$) and the distinction among groups was carried out with turkey post hoc test.

Results and Discussion

The average weight of rats in the NMU-category reduced, but the variation is of no statistical significance ($p>0.05$) after the first two weeks; however, there was a little weight gain during the last week of the experiment, while the average weight of rats in the Normal control was relatively stable, as shown in Table 1. The rats in the ELESE-50 mg/kg group experienced reduced weight, but the difference was not statistically significant ($p>0.05$) in weight during the second and last week, while the rats in the ELESE-100 mg/kg and ELESE-200 mg/kg groups experienced weight loss until the fourth week, but the variation is not statistically significant ($p>0.05$). However, the weights of the rats in the ELESE-400 mg/kg group increased, but the difference was not statistically significant ($p>0.05$) until the fourth week. NMU toxicity's adverse effects and disease symptoms were seen. The initial weight before drying was 179 g; the weight after drying (119 g) was taken to calculate the percentage of yield.

White blood cell (WBC) levels in the experimental rats in the NMU-group increased with a slight statistically significant ($p>0.05$) by 0.093 % compared to the normal control, as shown in Table 2. In the ELESE-50 mg/kg group, the amount of white blood increased by 37.59 and 43.44 % compared to the experimental rats in the NMU group and the normal control groups, respectively in similar patterns ($p>0.05$). It was also higher ($p>0.05$) in the ELESE-100 mg/kg group with 23 % and ELESE-200 mg/kg with 15.11 % WBS also increased ($p>0.05$). by 31.12 and 24.00 %. An increase ($p>0.05$) of 21.12 and 13.13 % was observed in the ELESE-400 mg/kg.
Red blood cell (RBC) level decreased ($p>0.05$) by 1 % in the NMU-group when compared to the control group. A similar decrease ($p>0.05$) also occurred in the ELESE-50 mg/kg group, which was by 0.6 and 1.6 % in comparison to the control and NMU groups, respectively. In the ELESE-100 mg/kg, there was an increase of 1.5 in the control to 2.5 % in the NMU-group. RBC level in ELESES-200 mg/kg was 4.49 % higher ($p>0.05$) in comparison with the group serving as control, while 5.4 % in the NMU-group. Nevertheless, in the ELESE-400 mg/kg group, there was a decrease ($p>0.05$) by 5.39 % and 4.4 % respectively, as shown in Table 2.
Haemoglobin (Hgb) level results were suppressed in the NMU-group compared to the control, 2.2 % lower ($p<0.05$) in the ELESE-50 mg/kg in relation to the group representing the control, and 1 % higher ($p>0.05$) in the NMU-group. In the ELESE-100 mg/kg group, it was 1 % lower and 2.2 % higher, but there existed no statistically significant distinction ($p>0.05$) in the same order. The ELESE-200 mg/kg group was higher ($p>0.05$) by 0.18 % compared to the control and by 6.3 % compared to the NMU-group. In the ELESE-400 mg/kg group, the level of Hgb was 5.9% lower ($p>0.05$) compared to the control and 2.7 % in comparison to the NMU-group, respectively, as shown in figure (Figure 1a.)
There were notable variations ($F=54.50$, $p<0.01$) in GSH content across the groups as shown in Figure1. The NMU-group was

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15.75 ± 1.91 umol/g, 39 % lower when compared with the normal control (25.87 ± 2.81 umol/g). ELESE-50 and 100 mg/kg groups had GSH content of 5.36 ± 1.99 umol/g, and 9.45 ±0.85 umol/g, respectively, but ELESE-200 and 400 mg/kg categories presented GSH levels of 18.06 ± 3.00 umol/g and 19.11 ± 3.16 umol/g, respectively, 12.7 % and 26.1% higher than the NMU treated group.
The activities of antioxidant parameters significantly (F=11.62, p<0.01) decreased with the amount of superoxide dismutase (SOD). In the NMU-set, SOD was 7.17 ± 1.68 u/g, 65 % lower compared to normal control (20.96 ± 4.0 u/g). However, the NMU-induced decrease in SOD activity was reversed significantly by ELESE. At 50mg/kg, SOD activity was 13.36 ± 1.99 u/g (46 % higher than the NMU group); At ELESE-100 mg/kg, 14.50 ± 2.51 u/g (50.5 % higher in comparison

Table 1: Effect of ethanolic leaf extract of *S. erianthum* (ELESE) treatments on average body weights

Treatment	Week One (g)	Week Two (g)	Week Three (g)	Week Four (g)
NMU-group	118.80±9.91	121.70±4.35	80.90±56.66	86.80±60.70
Normal Control	133.40±4.67	130.40±5.17	137.20±7.22	135.40±7.71
ELESE-50 mg/kg	105.20±5.83	96.50±35.52	108.70±6.53	88.30 ±46.97
ELESE-100 mg/kg	127.90±5.80	124.20±9.89	119.30±57.95	109.30±57.94
ELESE-200 mg/kg	121.80±7.80	120.90±11.21	110.40±39.65	108.80±39.06
ELESE-400 mg/kg	121.70±4.35	121.20±3.85	129.00±3.20	128.10±3.28

Variables are displayed as the standard deviation of the SEM (n=6). In all of the groups, there is no statistically significant difference (p>0.05).

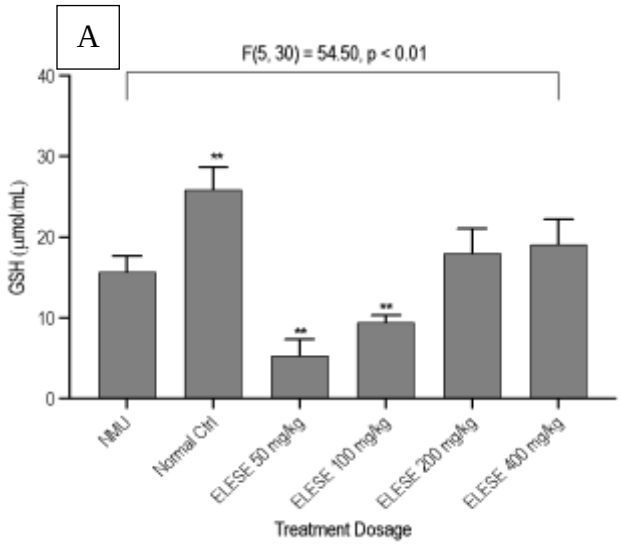
Key:
NMU= 50mg only
ELESE-50 mg/kg = ELESE $_{50\ mg/kg}$ + NMU $_{50\ mg/kg}$
ELESE-100 mg/kg = ELESE $_{100\ mg/kg}$ + NMU $_{50\ mg/kg}$
ELESE-200 mg/kg = ELESE $_{200\ mg/kg}$ + NMU $_{50\ mg/kg}$
ELESE-400 mg/kg = ELESE $_{400\ mg/kg}$ + NMU $_{50\ mg/kg}$

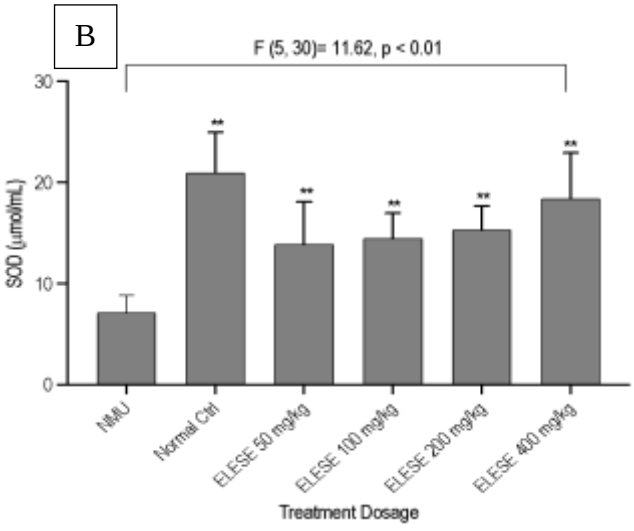
Table 2: Effect of ethanol extracts of *S. erianthium* (ELESE) on some Haematology Parameters

Group	WBS (x10^9/L)	RBC (x10^{12}/L)	HCT (%)	Hgb (glc)
NMU-group	7.47±1.31	8.62±0.2648.00±0.69	15.73±0.31	
Normal Control	6.77±1.91	8.71±0.5749.90±5.11	16.27±1.10	
ELESE-50 mg/kg	11.97±1.35	8.56±0.4451.50±1.18	15.90±0.10	
ELESE-100 mg/kg	8.80±3.21	8.85±0.5149.53±0.92	16.10±0.35	
ELESE-200 mg/kg	9.83±1.11	9.12±0.3949.93±1.971	16.33±0.67	
ELESE-400 mg/kg	8.60±3.36	8.24±0.2147.27±2.36	15.30±0.66	

Variables are displayed as the standard deviation of the SEM. (n=6). In all of the groups, there is no statistically significant difference (p>0.05)

Key:
NMU= 50mg only
ELESE-50 mg/kg = ELESE $_{50\ mg/kg}$ + NMU $_{50\ mg/kg}$
ELESE-100 mg/kg = ELESE $_{100\ mg/kg}$ + NMU $_{50\ mg/kg}$
ELESE-200 mg/kg = ELESE $_{200\ mg/kg}$ + NMU $_{50\ mg/kg}$
ELESE-400 mg/kg = ELESE $_{400\ mg/kg}$ + NMU $_{50\ mg/kg}$





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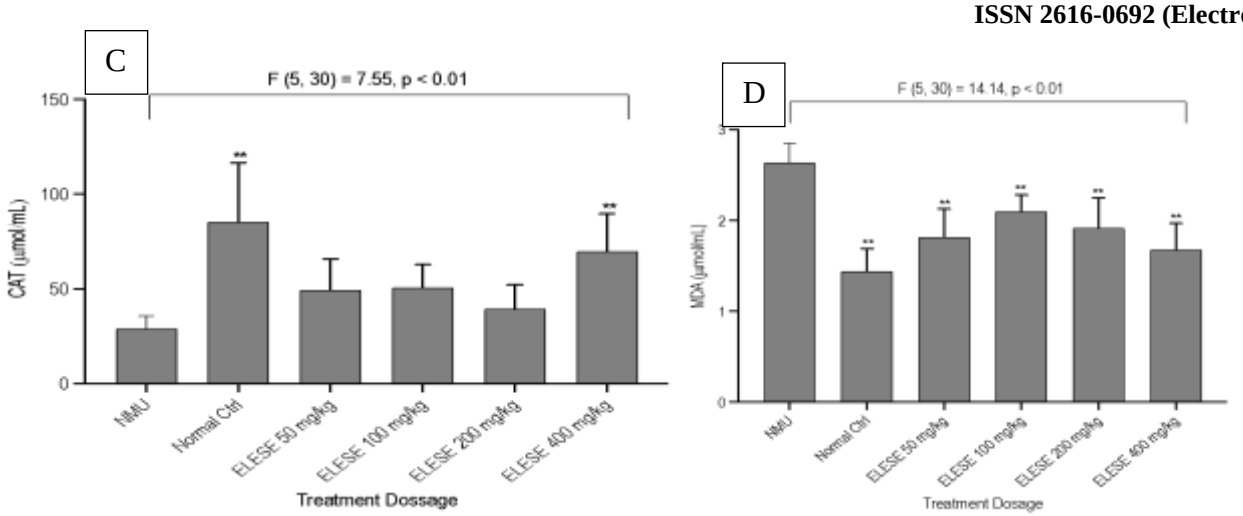

Figure 1: (**A**) Impact of ELESE on oxidative stress indicator GSH in the breast caused by NMU in rats. **(B)** Impact of ELESE on breast-specific SOD oxidative stress indicators generated by NMU. **(C)** Effect of Methyl-Nitrosourea on the oxidative stress parameters in experimental rats treated with ethanolic extracts of *S. erianthium* (ELESE), **(D)** Effect of ELESE on NMU-induced oxidative stress markers MDA in the breast. Results are presented as the mean SEM (n=6). In comparison to the NMU and the treated groups, *P<0.05; **P<0.01; ***P<0.001. One-way ANOVA statistical level of significance analysis, followed by the Tukey post hoc multiple comparison test

Key:
NMU= NMU 50mg only
Normal Ctrl = Normal Control
ELESE-50 mg/kg = ELESE $_{50\ mg/kg}$ + NMU $_{50\ mg/kg}$
ELESE-100 mg/kg = ELESE $_{100\ mg/kg}$ + NMU $_{50\ mg/kg}$
ELESE-200 mg/kg = ELESE $_{200\ mg/kg}$ + NMU $_{50\ mg/kg}$
ELESE-400 mg/kg = ELESE $_{400\ mg/kg}$ + NMU $_{50\ mg/kg}$

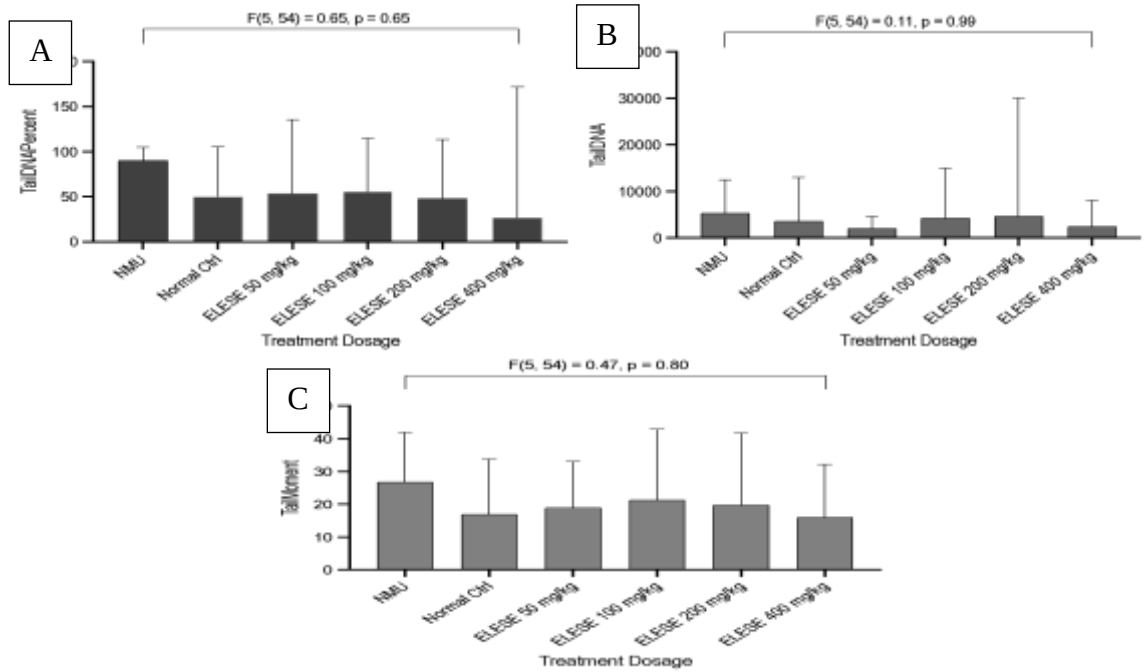

Figure 2: (A) Tail DNA associated with ethanolic leaf extract of *S.erianthum* (ELESE) dosage treatment. There was no statistically significant difference (F=0.11, p=0.99) in the tail DNA damage parameters. **(B)**Tail DNA percentage associated with ethanolic leaf extract of *S. erianthum* (ELESE) dosage treatment. None of the differences were statistically significant. (F= 0.65, p= 0.65) in the tail DNA percentage across the groups. **(C)** Olive moment (OTM) associated with ethanolic leaf extract of *S. erianthum* (ELESE) dosage treatment. The olive tail moment (OTM) did not differ between the groups in a way that was statistically significant (F= 0.47, P= 0.8).

Key:
NMU= NMU 50mg only
Normal Ctrl = Normal Control
ELESE-50 mg/kg = ELESE $_{50\ mg/kg}$ + NMU $_{50\ mg/kg}$
ELESE-100 mg/kg = ELESE $_{100\ mg/kg}$ + NMU $_{50\ mg/kg}$
ELESE-200 mg/kg = ELESE $_{200\ mg/kg}$ + NMU $_{50\ mg/kg}$

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$\text{ELESE-400 mg/kg} = \text{ELESE}_{400\ \text{mg/kg}} + \text{NMU}_{50\ \text{mg/kg}}$

with the NMU-group); ELESE-200 mg/kg (15.36 ± 2.33 u/g), 53.3 % higher than the NMU-group and ELESE-400 mg/kg (18.47 ± 4.7 u/g), 61 % higher than the NMU-group (Figure 1b).

The level of catalase activities differed significantly ($F=7.55$, $p<0.01$) from one group to another. There was a 66 % decrease in CAT activity in the vehicle-NMU treated group (29.00 ± 6.61 u/g) compared to the normal control with 85.35 ± 31.28 u/g. ELESE-50 mg/kg treatment increased CAT activity (49.49 ± 16.32) by 42 % compared with the vehicle-NMU group. ELESE-100 mg/kg (50.68±12.35), ELISE-200 mg/kg (39.44 ± 12.8) treatments increased CAT activity by 53 % compared with the NMU group, while in the ELESE-400 mg/kg group (69.80 ± 19.97) by 58.4 % compared to the NMU group (Figure 1c).

The malondialdehyde (MDA) level of every group revealed a noticeable difference. ($F=14.14$, $p<0.01$). However, in the NMU-group (2.64± 0.21 umol/g X 10^3) MDA was significantly increased by 45.4 % compared to the normal control (1.82 ±0.31 umol/g X 10^3). In the ELESE 50 mg/kg group, MDA levels were dramatically lowered by 31.4 % when compared with the NMU-vehicle control treated (Figure 1d).

No statistically significant difference was found. ($F=0.11$, $p=0.99$) in the tail DNA damage parameters. The result in Figure 5 revealed that the NMU-group showed the highest range of 5356.90 ± 7101.31 μm, 33.15 % tail DNA damage above the mean value of the normal control which was at 3580.90 ± 9381.41 μm, 33.15 % fewer than the NMU-group. The 100 mg/kg ELESE dosage was with 4262.14±10699.27μm, 20.43 % reduction in damage, ELESE-200 mg/kg dose value of 4688.06 ± 25342.31 μm was at 1.26 %. On the other hand, there was a drastic reduction in the ELESE-400 mg/kg group with the range value of 2413.24 ± 8553.79 μm (54.95 %) and 20526 ± 7101.31 μm in ELESE-50 mg/kg group with 61.6 % in tail DNA damage reduction (Figure 2a). The NMU group had the highest tail DNA damage value; however, among the other groups, 50 mg/kg and 400 mg/kg doses had the lowest tail DNA damage value.

None of the differences were statistically significant. ($F= 0.65$, $p= 0.65$) in the tail DNA percentage across the groups. The NMU-group had the highest percentage tail DNA mean value of 90.13 ± 14.39 μm at 44.39 %. The normal control animal group was at the mean value of 50.12 ± 55.20 μm with the same percentage less. There was an increase in the ELESE-50 mg/kg group at 53.51±82.90μm, with 40.63 % in tail DNA percentage reduction, while it was 38.18% in ELESE-100 mg/kg with 55.71±58.80 μm mean value compared to the NMU group respectively, as shown in Figure 6. At 48.02 ± 65.42 μm there was a decline in the ELESE-200 mg/kg group and a 46.72 % decrease in tail DNA. The ELESE-400 mg/kg group had a mean value of 26.62 ± 133.83 μm, 70.46 % decrease in damage compared to the NMU group (Figure 2b). Tail DNA percentage across the treatment groups at different doses was 50 % and 48 % in 50 mg/kg and 200 mg/kg ELESE doses, with normal control animals showing no significant difference ($p>0.01$).

No statistically significant variation was found. ($F= 0.47$, $P= 0.80$) in the Olive tail moment (OTM) across the groups. This is a measure of DNA damage that combines Tail length – how far the DNA has migrated during electrophoresis. The NMU-group was at 15.75 ± 10.62 μm, 40.44 % higher in contrast to the usual control group and other groups. The olive tail moment population declined at different doses from ELESE-100 mg/kg mean value of 12.07 ± 13.90 μm, 23.33 % OTM while in ELESES-200 mg/kg (10.3 ± 12.65 μm) was 34.41 %. Reduction was higher by 39.49 % at the mean value of 9.53 ± 12.59 μm in the ELESE-50 mg/kg group and 69.71 % at 4.77 ± 29.16 μm mean value for the ELESE-400 mg/kg group (Figure 2c).

The decrease in weight experienced by the NMU group may be attributed to the toxic effect of methyl-nitrosourea. This is in concordance with a similar study of some experimental rats which showed an increase in weight due to the toxicity of NMU.[7] However, it could be inferred that the extract works better at higher doses (400 mg/kg) owing to the increased weight over time. Overweight is a common and ongoing problem among many breast cancer survivors. The effect of body weight increase in diseased patients with breast cancer is connected with adverse health challenges.[27] Weight gain has been reported in 50-96 percent of women undergoing breast cancer treatment, with many continuing to gain weight, particularly some who manage their body mass index while receiving treatment, report growing additional kilograms in the months and years following finishing their course of treatment.[26] According to a similar cross-country clinical trial, 18.6 % of women lost weight, 14.1 % gained weight, and 67.3 % of them maintained their weight.[27]

The lower number of white blood cells (WBC) of animals found in the NMU-group in comparison to the ones in 50 mg/kg and 200 mg/kg of ELESE-dosage groups agrees with a similar research work in which it was discovered that the total white blood cell and platelet counts were greatly reduced. [28,29]

S. erianthum could be said to have played a major role in reversing the decrease in WBC as evidenced by the increase in WBC level shown by ELESE-100 mg/kg.

Even when treated with different doses, the red blood cell (RBC) and hematocrit (HCT) mean values for the NMU-group and ELESE-groups in this study did not significantly differ. This shows that the level of erythrocytes and hematocrit in the other groups is similar to that in the healthy control group. A complete red blood cell count, or hematocrit count, can be used to check for diseases like leukaemia, dehydration, malnutrition, and anaemia. RBC increases before and after treatment, while dropping during and after it, similarly with haemoglobin levels reported prior to and following therapy in individuals with breast malignancy in certain previous research. This shows the extract was able to reverse the anaemic effect of NMU in exposed rats. Premenopausal obese women's WBC counts were found to have no connection to breast cancer.[30] A similar study found that among breast cancer patients, hematocrit rises both during and after therapy in comparison to haematological parameters before and after treatment. When compared to the NMU-group, the ELESE-groups' haemoglobin (Hgb) levels are lower. Thus, there was no apparent distinction between the groups and the control groups. This is in support of a similar study, which found that haemoglobin values were considerably lower following the start of cancer treatment [31].

This investigation demonstrated that the extract had a beneficial impact on the level of GSH in the ELESE-groups via the increase seen following treatment. The abundance of electrophiles, glutathione (GSH), is more actively conjugated, helping to prevent oxidative damage to cells.[32,33]

Superoxide dismutase (SOD) activity was decreased ($P<0.01$) in the NMU-group compared to the ELESE groups in this study. In the groups that received ELESE-50 mg/kg and ELESE-400 mg/kg. SOD levels steadily increased ($P<0.01$). SOD is a very important enzyme that captures free radical attack as the first line of defence towards oxidative damage. [33,35] It was increased ($P<0.01$) in the control group in comparison to extract-treated groups, which may have lessened the toxicants' effects. This indicates the possibility that the extract has intrinsic factors to normalize SOD level after induction with carcinogens as a result of high levels of polyphenols and alkaloids.[34] By comparing the NMU-group to the control group, a substantial decline in the concentrations of enzymatic antioxidants, such as SOD, was seen.

The catalase (CAT) activity in ELESE-50 mg/kg dose significantly lowered ($P<0.01$) the compared to control. CAT increased ($P<0.01$) in the ELESE-400 mg/kg treatment group compared to the ELESE-200 mg/kg and ELESE-100 mg/kg group respectively. The normal control had the highest ($P<0.01$) level of CAT, while the lowest was observed in the NMU-group. This finding is supported in a similar work on *Operculina turpethum*, which revealed a decrease in the catalase level of cancer-bearing animals after treatment with ELESE when compared to the normal control group, which suggests that the extract shows antioxidant activity. [36,37]

Malondialdehyde (MDA) is a useful indicator of environmental stress-induced oxidative damage to lipids brought on by the degradation of unsaturated fatty acids.[34,37]. All ELESE-groups had low MDA according to the analysis; however, compared to the control group, there was no significant difference; nevertheless, it was far greater

(P<0.01) in the NMU-group. Malondialdehyde (MDA) levels rose in the NMU group, probably due to lipid peroxidation, which is one of the factors that can cause gene changes that result in breast cancer. Treatment with ELESE lowered the level of cell oxidative stress lipid peroxidation (MDA) due to alkaloids, steroidal saponin and glycoalkaloids present in the extract, which are the primary biochemical markers of the plant, and because these biologically active compounds, anticancer components, steroidal alkaloids and glycoalkaloids play a significant part in both conventional and modern medical practices.[37,41, 42]

The study hypothesizes that the daily fluctuations in lipid peroxidation's phase shift may add to the development of cancer. [37] Research on MDA level was conducted using a comparison approach with 30 cancerous breast individuals and 30 healthy controls. The outcomes of that research revealed that the mean MDA level among breast cancer patients was significantly greater than the control group (3.040.36 nmol/l), with a p-value of 0.001 displaying significant distinction in MDA amount between breast tumour individuals and the controls [38,39]. Regarding the result from this study, it's clear that ELESE reduced MDA levels in all the treatment groups. *S. erianthum* leaf is a very good source of antioxidants among several plants. In the tail DNA damage analysis, groups given 50 mg/kg and 400 mg/kg of ELESE presented a reduction in damage compared to the NMU group, and also showed the lowest mean value in tail-DNA damage and olive moment parameter, compared to others. It was also noted that these particular ELESE treatment groups were the best to reduce tail DNA damage level and tail DNA percentage, indicating the effect of the ELESE-groups. This corroborates reports that the extract of the leaf of Solanum *erianthum* has membrane-stabilizing properties that have been explored in the evaluation of its anti-inflammatory properties in vitro.[39] When compared to a related species: *Solanum torvum*, *Solanum erianthum* contains the highest levels of alkaloids, which have the potential to serve as an anti-cancer agent and stabilize membranes. The tail DNA percentage damage was significantly the lowest at 400 mg/kg dose with 25 percent. This suggests that the best dose in reducing the effect of tail-DNA damage and lower damage in the olive moment length in breast cancer is ELESE-400 mg/kg. Olive tail Moment (OTM) across the ELESE-groups at different doses and in the normal control animals showed no significant difference. The NMU group's Olive moment was higher than other treatment groups. The Olive tail moment refers to the tail moment computed.[40] OTM's ability to detect differences in DNA distribution is thought to make this metric helpful in defining heterogeneity within a cell population. This study's observation of DNA damage may be related to the other results. However, no research has demonstrated the utility of ELESE in controlling tail DNA damage in breast cancer when NMU, utilized in this study, is the initiating carcinogen to induce oxidative stress, trigger programmed cell death, and mimic damage DNA in human mammary gland tumor. According to the results of this study, ELESE may be a viable option as an anti-cancer drug, perhaps independently in breast cancer or in conjunction with oral squamous cell carcinoma in many studies.

Conclusion

The outcomes of this research showed that S. *erianthum* leaf has anti-cancer properties as shown by its ability to reduce the effect of carcinogenesis. It is also a good source of antioxidants, as seen in its potency to reduce the amount of oxidative stress markers like malondialdehyde, and surge in the level of superoxide dismutase and catalase; both protective defenses thus preventing cell damage. *Solanum erianthum* leaf also reversed body weight loss due to breast cancer in female albino rats. The ethanol leaf extract of *S. erianthum* at 200 mg/kg and 400 mg/kg doses would be good doses in maintaining the stable weight of the rats during breast cancer treatment. RBC levels increased, thus useful in anemic conditions, and white blood cell (WBC) levels were sustained in NMU-induced malignancy among rats in category treated with ethanol leaf extract of *S. erianthum* (ELESE) at various doses. DNA damage was drastically reduced to a very appreciable value after treatment with 400 mg/kg of *S. eriathum* leaf extract. The plant showed therapeutic potential for the management of breast cancer when administered correctly. Breast cancer treatment, management, and prevention could thus benefit from the use of *Solanum erianthum leaf.*

Conflict of Interest

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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