



## Chemo-preventive Activity of Ethanolic Extracts of *Newbouldia laevis* and *Olax subscorpioidea* Leaves on Methylnitrosourea–induced Stroma Fibrosis in Breast Tissues of Female Albino Rats

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**ABSTRACT:** Breast cancer represents the most common neoplastic disease in females, accounting for up to one third of new diagnoses of women's cancer in certain regions of the world. The chemo-preventive activity of ethanolic extracts of *Newbouldia laevis* and *Olax subscorpioidea* leaves on methylnitrosourea (MNU)–induced stroma fibrosis in female albino rats was evaluated. Quantitative and qualitative phytochemical screening was carried out to identify and determine the quantity of bioactive compounds. Haematoxylin and Eosin staining procedure was used to assess breast tissues of experimental animals. Cancer antigen (CA) markers 15-3, 27-29 and carcinoembryonic antigen (CEA) in the blood of experimental animals were evaluated using an automated procedure. Quantitative phytochemical screening showed that *Olax subscorpioidea* and *Newbouldia laevis* contained alkaloid in the highest amount. Histopathological assay revealed the presence of stromal fibrosis in the breast tissues of 8 out of 10 rats administered with MNU. Levels of CA 15-3, CA 27-29, CEA were significantly ( $p < 0.05$ ) elevated in MNU administered group in comparison to the negative control. Treatment with *Olax subscorpioidea* leave extract significantly ( $p < 0.05$ ) ameliorated CA 15-3, CA 27-29 and CEA levels. However, treatment with *Newbouldia laevis* leave extract significantly ( $p < 0.05$ ) ameliorated CA 15-3 and CEA levels only. Findings from this study showed that ethanolic extracts of *Olax subscorpioidea* and *Newbouldia laevis* leaves have chemo-preventive activity in MNU-induced stroma fibrosis in the breast tissue of female albino rats. However more studies on the discovery and validation of bioactive components of the plants are needed.

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Cancer is a group of diseases that cause cells in the body to change and grow out of control (Klug *et al.*, 2018). Breast cancer represents the most common neoplastic disease in females, accounting for up to one third of new diagnoses of women's cancer in certain regions of the world (Ntekim *et al.*, 2022). Breast cancer has been identified as one of the main cause cancer-related deaths among women during some last decades (Ntekim *et al.*, 2022). Recent advances in the introduction of novel potent anti-cancer therapeutics in association with early detection methods led to a

decrease in the mortality rate of breast cancer (Joshi, 2019). However, the scenario of breast cancer is yet going on and further improvements in the current anti-cancer therapeutic approaches are needed (Hadisaputri *et al.*, 2021). Plants and plant-based medicaments have been used as the basis of many of the modern pharmaceuticals that is being used today in order to treat various ailments (Gajalakshmi *et al.*, 2012). Plants have been the source of well known anticancer (Garcia-Oliveira, *et al.*, 2021). Despite the discovery of many drugs of natural origin, the search for new

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anticancer agents is still necessary, to increase the range available and to find less toxic and more effective drugs. *Newbouldia laevis*, (Seem. or Boundary Tree) is a medium sized angiosperm and tropical plant belonging to the family of *Bignoniaceae* growing up to a height of about 7-20 m tall depending on the region where it was found, and the stem grows vertically with few branches. It is widely used traditionally for the treatment of malaria, prostate cancer, wounds and eye problems (Nwauzoma and Dappa, 2013). Its anti-oxidant and *in vitro* cytotoxicity has also been reported (Anaduaka *et al.*, 2014; Gbadamosi and Erinoso, 2016). *Olox subscorpioidea*, on the other hand is a shrub, belonging to the family of *Olacaceae*, up to 10m high bole to 60 cm girth with long thin, drooping branches, of deciduous forest (Ahmad *et al.*, 2021). It has been documented as part of traditional recipe for the treatment of various diseases in Africa. It possesses anti-oxidant, anti-inflammatory and anti-proliferative, anti-asthma, anti-diabetes, anti-cancer, anti-rheumatism and anti-typhoid property (Kazeem *et al.*, 2015; Poopla *et al.*, 2021). Methylnitrosourea (MNU), belonging to a group of compounds classified as nitrosamines is a pale-yellow sand-like alkylating solid compound (Sajjadi and Bathaie, 2016). It exerts its toxicity by transferring its methyl group to nucleobases in nucleic acids, causing AT:GC transition mutations (Minari and Ugochukwu 2018). It has been proven to exert direct carcinogenic, mutagenic and teratogenic potential (Sajjadi and Bathaie, 2016). (Tsubura *et al.*, 2011). MNU has been shown to induce various cancers in experimental animals including retinal degeneration, esophageal, breast cancer, photoreceptor degeneration, gastric and colorectal malignancies (Xiong *et al.*, 2016). This present study was therefore aimed at evaluating the anti-inflammatory and chemo-preventive activities of ethanolic extracts of *Newbouldia laevis* and *Olox subscorpioidea* leaves on methylnitrosourea (MNU)-induced carcinogenesis and inflammation in female albino rats.

## MATERIALS AND METHODS

**Chemical compound/Carcinogen:** Methylnitrosourea (MNU) was supplied by Hangzhou Sage Chemical Company Ltd, Hangzhou, China. It was prepared by dissolving in phosphate/citrate-buffered saline at pH 4.2 (1 part buffer to 14 parts saline) shortly before administration.

**Leave collection and preparation:** Leaves of *Olox subscorpioidea* and *Newbouldia laevis* were collected from Otta, Ogun state, Nigeria. The leaves were identified and confirmed taxonomically in the Herbarium Unit of the department of Botany,

University of Lagos, Lagos. Voucher specimens with the number LUH 9785 and LUH 9786 for *Newbouldia laevis* and *Olox subscorpioidea* respectively were deposited in the University herbarium for future reference. The leaves of *Olox subscorpioidea* and *Newbouldia laevis* were air-dried at room temperature and pulverized into a uniform material using an electric blender machine. Plant extraction (1000 g of pulverized material) was done with 80% ethanol at 70°C by continuous percolation using Soxhlet extractor for 24 hours. The resulting extract was concentrated at 40°C in a rotary evaporator to yield a dark green mass of weight 90 g (9.93%). The obtained crude extract was packed aseptically in airtight plastic containers and stored at 4°C until required. The percentage yield of the extract was calculated using the formula:

$$\%Yield = \frac{\text{weight of extract}}{\text{weight of plant material}} \times 100$$

**Experimental Animals and Study Design:** Ninety female albino rats, 30 days of age were used in this study. The rats were kept in cages with 10 rats each and were maintained under conditions of an average of 12 hours of light and 12 hours of darkness. Experiment was carried out in the animal house of the Department of Cell Biology and Genetics, University of Lagos, Lagos, Nigeria in accordance with the rules in Nigeria governing the use of laboratory animals as acceptable internationally with ethical approval from College of Medicine, University of Lagos Health Research Ethics Committee (HREC). Animals were given a high-fat diet and water *ad libitum*. All group of rats received 100mg/kg/wt of MNU except those in group B and E which received distilled water and extracts respectively. Varying dose of the extracts were administered as illustrated below.

### Dosage

Group A: Rats that received 100mg/kg/wt of MNU  
 Group B: Rats that received distilled water (Control)  
 Group C: Rats that received 100mg/kg/wt of extract + 100mg/kg/wt of MNU  
 Group D: Rats that received 200mg/kg/wt of extract + 100mg/kg/wt of MNU  
 Group E: Rats that received 400mg/kg/wt of extract + 100mg/kg/wt of MNU  
 Group F: Rats that received 400mg/kg/wt of extract only

**Induction of cancer in animals:** Cancer was induced using a modified protocol of Sajjadi and Bathaie, 2016. Intraperitoneal route of administration was used for MNU induction. The experimental groups received 100 mg/kg/body weight of MNU once per week for the

first four weeks. However, treatment of animals with ethanolic extract of *Olox subscorpioidea* and *Newbouldia laevis* leaves daily lasted for six weeks. The experimental and control animals were carefully checked daily, and weight taken weekly. At the end of the experiment, collection of blood sample was done by orbital venous plexus bleeding in plain bottles kept in slanting position to induce separation of serum from whole blood and centrifuged (5000 rpm for 5 minutes) in plain sterile bottles for tumour markers evaluation. The rats were sacrificed by cervical dislocation, their organs were harvested and fixed in formalin for histopathological assay.

**Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay:** The free radical scavenging activity of the extract, based on the scavenging of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated according to the procedure described by (Cuendet *et al.*, 1997; Burits and Bucar, 2000). An aliquot of 0.5 mL of extract in ethanol (95%) at different concentrations (25, 50, 75, 100µg/mL) was mixed with 2.0 ml of reagent solution (0.004 g of DPPH in 100 ml methanol). The control contained only DPPH solution in place of the sample while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 minutes the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm. The scavenging effect was calculated using the expression:

$$\text{Inhibition} = \frac{[A_0 - A_1]}{A_0} \times 100 \quad 1$$

Where  $A_0$  is the absorption of the blank sample and  $A_1$  is the absorption of the extract.

**Reducing power assay:** Various concentrations of the extracts (20 to 100 µg/mL) in 1.0 mL of deionized water were mixed with phosphate buffer (2.5 mL) and potassium ferricyanide (2.5 mL). The mixture was incubated at 50° C for 20 min. Aliquots of trichloroacetic acid (2.5 mL) were added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes the upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared ferric chloride solution (0.5 mL). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1 to 16 µg/mL) was used as standard. Percentage increase in reducing power was calculated as stated below:

$$\% \text{ IRP} = \left[ \frac{A_1}{A_0} \right] - 1 \times 100 \quad 2$$

Where IRP = increase in reducing power;  $A_1$  is absorbance of test solution;  $A_0$  is absorbance of blank. The antioxidant activity of aqueous extract of root bark of *Olox subscorpioidea* and *Newbouldia laevis* expressed as  $IC_{50}$  and compared with standard.

**Cancer antigen measurement:** An automated procedure involving serum measurement of markers by ELISA (ES300/Elecsys2010; Roche Diagnostics) was used to evaluate cancer antigen (CA) 15-3, cancer antigen (CA) 29-27 and carcinoembryonic antigen (CEA).

**Statistical analysis:** Statistical analysis was carried out using Statistical Product and Service Solutions (SPSS) version 26. Analysis of variance (ANOVA) was used to analyse differences in the weight, and cancer specific markers between the data obtained in the control group of rats and the experimental group of rats. All comparisons with  $P \leq 0.05$  and  $P \leq 0.01$  were considered as significant and highly significant respectively.

## RESULT AND DISCUSSION

The qualitative phytochemical screening of ethanolic extract of *Olox subscorpioidea* leaves and *Newbouldia laevis* leaves as shown in Table 1 revealed the presence of alkaloid, terpenes, flavonoid, phenol, saponin in both extracts of *Olox subscorpioidea* leaves and *Newbouldia laevis* leaves. Phytochemicals such as flavonoid, saponins and alkaloid compound isolated from medicinal plants has been shown to possess anticancer and anti-inflammatory activities (Gutierrez, 2012).

**Table 1:** Qualitative phytochemical screening of ethanolic extract of *Olox subscorpioidea* leaves and *Newbouldia laevis* leaves

Phytochemicals	<i>Olox subscorpioidea</i>	<i>Newbouldia laevis</i>
Alkaloid	+	+
Flavonoid	+	+
Phenol	+	+
Phytic acid	-	+
Saponin	+	+
Tannins	+	-
Terpenes	+	+
Cardioactive glycoside	+	+
Anthraquinones	+	+
Reducing sugar	+	+

Key: + = Present, - = Absent

Figure 1 and 2 shows the quantity of phytochemicals present in ethanolic extract of *Olox subscorpioidea* leaves and *Newbouldia laevis* leaves. Alkaloid which is notably present in the highest amount in both extracts has also been reported to act as a potent antineoplastic property which inhibits the uncontrolled growth of cancer cells (Jadid *et al.*, 2017). A growing body of antioxidant isolated from plant has been

identified to date to support health and wellness, as the disbalance of cellular redox homeostasis, which is the reactive oxygen species (ROS) and the antioxidant system, contributes to the pathogenesis of almost all of diseases (Manea *et al.*, 2012). DPPH assay is relatively rapid and efficient method to evaluate free radical scavenging activity. DPPH can accept an electron or hydrogen radical to form a stable diamagnetic molecule. Changes in color, from purple to yellow indicates a decrease in absorbance of DPPH radical. Principally, the discoloration of DPPH is stoichiometrically associated with the number of electrons acquired which resulted in the existence of a

free radical scavenger (Ngonda, 2013). Figure 3 demonstrates that *Olox subscorpioidea* leave extract has more capacity to donate proton which resulted in the ability to act as free radical scavengers compared to the control (ascorbic acid). This indicates that the *Olox subscorpioidea* leave extract may act as primary antioxidants. This was further authenticated by the IC<sub>50</sub> result (Table 2) which showed that both the control (ascorbic acid) and *Olox subscorpioidea* are intermediate antioxidant while *Newbouldia laevis* is a weak antioxidant as reported by Nurul *et al.*, 2017.

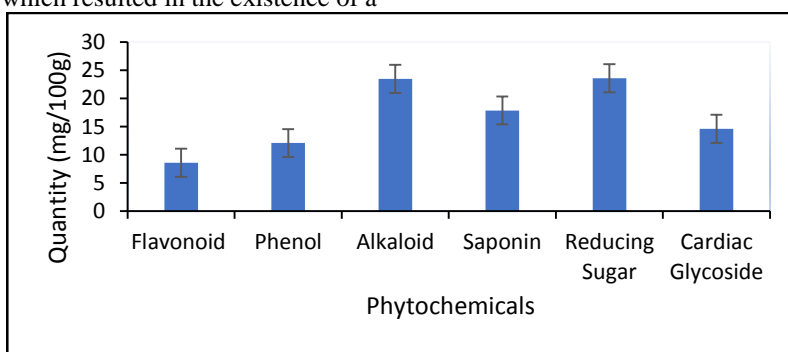


Fig 1: Quantitative phytochemical screening of crude extract of *Olox subscorpioidea* leaves

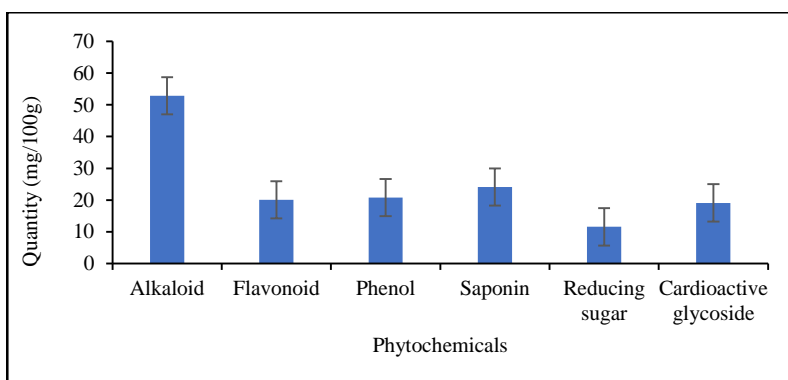
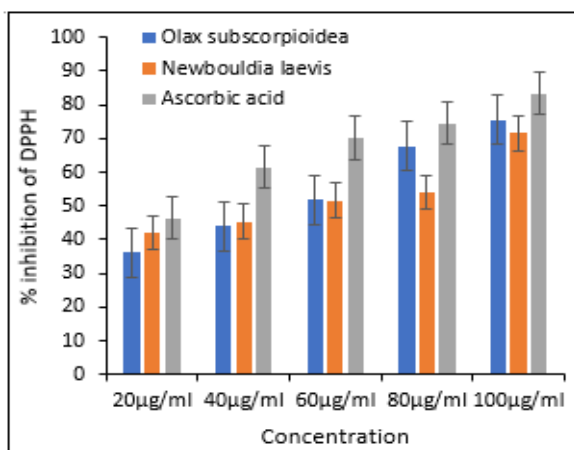


Fig 2: Quantitative analyses of selected phytochemicals present in crude extract of *Newbouldia laevis* leaves

This is in concordance with earlier reports of antioxidant activity of *Newbouldia laevis* and *Olox subscorpioidea* (Habu and Ibeh, 2015; Konan *et al.*, 2015; Tsado *et al.*, 2016; Seun *et al.*, 2018; Salemcity *et al.*, 2020; Ahmad *et al.*, 2021). Plant phenols and flavonoid have been documented to synergize and act as reducing agents, hydrogen donors, singlet and triplet oxygen quenchers or decomposing peroxides agents (Kouassi *et al.*, 2013). The phenol and flavonoid content of both crude extracts may explain the reason for their antioxidant activity (Figure 1 and 2). A growing body of evidence indicated that some plants' saponins have strong antioxidant activities *in vitro*, noting that they may be the novel potential antioxidant candidates which may rely on their free

radical scavenging abilities (Tapondjou *et al.*, 2011; Bi *et al.*, 2012; Chen *et al.*, 2014). Saponin being the second most abundant phytochemical after alkaloid for both extracts could be suggested to have contributed to the antioxidant potential of both extract (Figure 1 and 2). In Figure 4, the presence of antioxidants in the crude extract of *Newbouldia laevis* and *Olox subscorpioidea* resulted in the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> by donation of an electron. It was noted that there was increasing reducing power as concentration increased (Figure 4). The crude extract of *Olox subscorpioidea* which showed greater reducing power than the control and *Newbouldia laevis* may be suggested to have exhibited such property owing to the synergistic effect of the sugars and phenol present in *Olox*

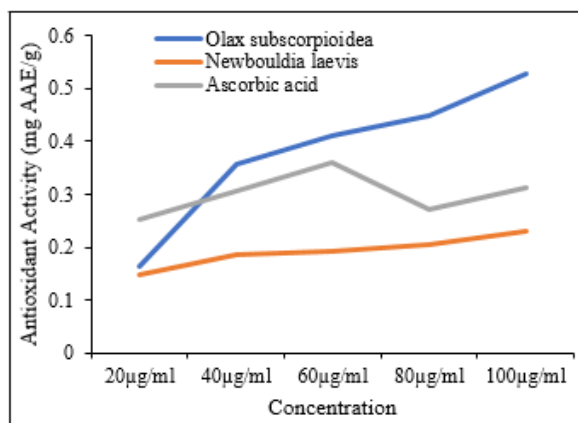
*subscorpioidea* in higher amount compared to *Newbouldia laevis* (Figure 1 and 2). This is in alignment with an earlier hypothesis suggesting that the synergistic interaction of sugars (or sugar-like compounds) and phenolic compounds forms part of an integrated redox system, quenching reactive oxygen specie (ROS) and contributing to stress tolerance.



**Fig 3:** Free radical scavenging potential of crude extract of *Olax subscorpioidea* and *Newbouldia laevis* leaves

**Table 2:** IC<sub>50</sub> of crude extract of *Olax subscorpioidea* and *Newbouldia laevis*

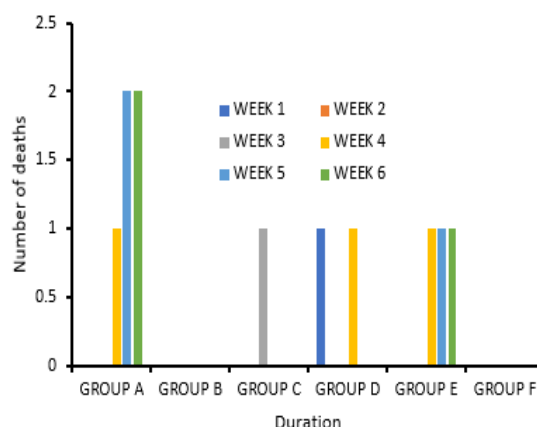
Crude extract	IC <sub>50</sub> (µg/ml)
<i>Olax subscorpioidea</i>	80.52
<i>Newbouldia laevis</i>	123
Ascorbic acid	54.2



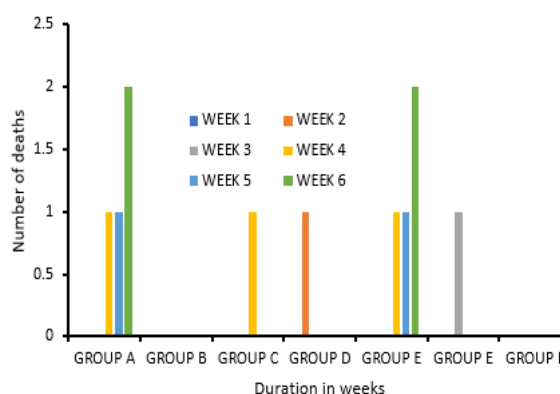
**Fig 4:** Reducing Power of crude extract of *Olax subscorpioidea* and *Newbouldia laevis*

The difference between the average weight of experimental rats in Group D and E in table 3 and Group C in Table 4 were statistically significant ( $p < 0.05$  and  $p < 0.01$  respectively). The average weight of experimental rats used in this study increased with time however, the least increase in weight was seen in Group A (Table 3 and 4). This

might be because of the toxic effect of the MNU administered. The highest number of deaths occurred in Group A for both treatments (Figures 5 and 6), however, group E in Figure 6 also showed high death rate. It could be explained that the toxic effect of the MNU administered to the rats without treatment had resulted to the high death rate recorded, while the high death rate also recorded in group E (Figure 6) might be the resultant effect of high dose of *Newbouldia laevis* leave extract being ineffective at a concentration of 400mg/kg/wt. No dead was recorded in groups B and F (Figure 5 and 6). N-Nitroso-N-methylurea (NMU) is a DNA alkylating agent whose major toxic effects result from severe damage to hematopoietic, lymphoid, and other tissues that have rapid rates of cell turnover (Sajjadi and Bathaie, 2016). Its carcinogenesis in animal species has been tested including mice, rats, Syrian golden, Chinese, and European hamsters, guinea pigs, rabbits, gerbils, pigs, dogs, and monkeys (Tsubura *et al.*, 2011). It induces benign and malignant tumors following its administration by different routes, including ingestion (Xiong *et al.*, 2016).



**Fig 5:** Effects of MNU on the survival rate of rats treated with *Olax subscorpioidea* leaf extract



**Fig 6:** Effects of MNU on the survival rate of rats treated with *Newbouldia laevis* leaf extract

**Table 3:** Effect of MNU on the average weight of rats treated with *Olox subscorpioidea* leave extract

Week	Group A(g)	Group B(g)	Group C(g)	Group D(g)	Group E(g)	Group F(g)
Wk 1	44.15±1.66	32.86±0.66	29.43±0.43	32.14±0.70*	35.43±1.07*	45.71±2.51**
Wk 2	45.11±1.97	55.45±0.59	38.14±1.18	37.71±1.36*	46.86±3.03*	48.50±3.94**
Wk 3	50.87±2.27	53.30±0.69	73.14±1.67	65.71±2.99*	71.43±3.92 *	71.17±9.03**
Wk 4	52.88±3.21	76.27±0.93	83.71±1.57	86.33±3.87*	82.71±3.35*	96.20±3.38**
Wk 5	55.06±6.91	92.62±1.49	97.14±3.06	106±7.22*	101.83±4.7*	96.00±3.70**
Wk 6	55.35±6.44	95.60±1.87	97.3±3.06	106±3.10*	102.23±3.7*	97.00±2.10**

Values are means of 10 replicates ± S.E.M; Values carrying superscript (\*\*) were significance ( $p < 0.01$ ); Values carrying superscript (\*) were significant ( $p < 0.05$ )

**KEY: Group A:** Rats that received 100mg/kg/wt of MNU; **Group B:** Rats that received distilled water (Control) **Group C:** Rats that received 100mg/kg/wt of extract + 100mg/kg/wt of MNU; **Group D:** Rats that received 200mg/kg/wt of extract + 100mg/kg/wt of MNU; **Group E:** Rats that received 400mg/kg/wt of extract + 100mg/kg/wt of MNU; **Group F:** Rats that received 400mg/kg/wt of extract only

**Table 4:** Effect of MNU on the average weight of rats treated with *Newbouldia laevis* leave extract

Week	Group A(g)	Group B(g)	Group C(g)	Group D(g)	Group E(g)	Group F(g)
Wk 1	44.15±1.66	32.86±0.66	44.15±7.13***	32.86±1.31	71.46±0.94	51.43±2.19
Wk 2	45.11±1.97	55.45±0.59	45.12±6.79***	55.45±2.13	68.94±1.71	51.26±2.85
Wk 3	50.87±2.27	53.30±0.69	50.87±4.58***	53.30±6.88	72.86±2.21	56.33±4.41
Wk 4	52.88±3.21	76.27±0.93	52.88±6.48***	76.27±1.65	72.04±1.47	55.06±2.01
Wk 5	55.06±6.91	92.62±1.49	55.06±9.55***	92.62±2.99	94.03±2.45	65.30±4.05
Wk 6	55.35±6.44	95.60±1.87	55.35±6.45	95.60±8.05	95.33±3.28	61.93±4.05

Values are means of 10 replicates ± S.E.M; Values carrying superscript (\*\*) were significance ( $p < 0.01$ ); Values carrying superscript (\*) were significant ( $p < 0.05$ )

**KEY: Group A:** Rats that received 100mg/kg/wt of MNU; **Group B:** Rats that received distilled water (Control); **Group C:** Rats that received 100mg/kg/wt of extract + 100mg/kg/wt of MNU; **Group D:** Rats that received 200mg/kg/wt of extract + 100mg/kg/wt of MNU; **Group E:** Rats that received 400mg/kg/wt of extract + 100mg/kg/wt of MNU; **Group F:** Rats that received 400mg/kg/wt of extract only

The levels of CA 15-3, CA 27.29, CEA were significantly ( $p < 0.05$ ) elevated in NMU administered group in comparison to negative control (Table 5 and 6). Treatment with *Olox subscorpioidea* leave extract significantly ( $p < 0.05$ ) ameliorated CA 15-3, CA 27.29 and CEA levels.

This is in concordance with Nassan *et al.*, 2018 in which administration of *T. officinale* extract for 4 consecutive weeks decreased the elevated CA15-3 levels detected in DMBA administered rats while carcinogenic group showed highly significant levels.

However, treatment with *Newbouldia laevis* leave extract significantly ( $p < 0.05$ ) ameliorated CA 15-3 and CEA levels while there was no significant difference in the levels of CA 27.29.

CA 15-3 was highly significant ( $p < 0.001$ ) in both treatments (*Olox subscorpioidea* and *Newbouldia laevis* leave extract). Breast cancer

specific antigen markers such as cancer antigen 15-3, (CA 15-3), cancer antigen 27-29 (CA 27.29), carcinoembryonic antigen (CEA) are frequently applied for screening and monitoring of many cancers and possible factor that may regulate it.

CA15-3, is a high molecular weight glycoprotein (300-450 kDa), and mucin belonging to a large family of glycoproteins encoded by the MUC 1 gene, that are heterogeneously expressed on the apical surface of epithelial ducts and acinic breast cells which is secreted in milk normally.

However, at cancerous state, CA 15-3 drains into the blood perfusion because of disrupted breast morphology (Fakhari *et al.*, 2019).

Cancer antigen 27-29 (CA 27-29) is used to predict early recurrence of disease in women with treated carcinoma of the breast with reference range less than 38U/mL.

**Table 5:** Analysis of selected breast cancer specific antigen in rats treated with *Olox subscorpioidea* leave extract

Cancer Antigen	Group A (µl)	Group B (µl)	Group C (µl)	Group D (µl)	Group E (µl)
C.A 15.3	5.33 ± 0.52	1.70 ± 0.20*	1.81 ± 0.05*	1.00 ± 0.00*	2.47 ± 0.170*
C.A. 27.29	6.73 ± 2.00	3.73 ± 0.28*	2.00 ± 0.00*	2.17 ± 0.17*	1.67 ± 0.33*
CEA	0.90 ± 0.21	0.23 ± 0.13*	0.3 ± 0.06*	0.30 ± 0.60*	0.33 ± 0.03*

Values are means of 3 replicates ± S.E.M; Values carrying superscript (\*) were significant ( $p < 0.05$ )

**KEY: Group A:** Rats that received 100mg/kg/wt of MNU; **Group B:** Rats that received distilled water (Control); **Group C:** Rats that received 100mg/kg/wt of extract + 100mg/kg/wt of MNU; **Group D:** Rats that received 200mg/kg/wt of extract + 100mg/kg/wt of MNU; **Group E:** Rats that received 400mg/kg/wt of extract + 100mg/kg/wt of MNU

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**Table 6:** Analysis of selected breast cancer specific antigen in rats treated with *Newbouldia laevis* leave extract

Cancer Antigen	Group A (µl)	Group B (µl)	Group C (µl)	Group D (µl)	Group E (µl)
C.A 15.3	5.33 ± 0.52	1.70 ± 0.20***	1.88 ± 0.06***	2.01 ± 0.17***	2.13 ± 0.15***
C.A. 27.29	6.73 ± 2.00	3.73 ± 0.28	4.03 ± 0.28	4.20 ± 0.26	3.93 ± 0.43
CEA	0.90 ± 0.21	0.23 ± 0.13*	0.56 ± 0.04*	0.55 ± 0.07*	0.63 ± 0.03*

Values are means of 3 replicates ± S.E.M; Values carrying superscript (\*\*\*) were significance ( $p < 0.001$ ); Values carrying superscript (\*) were significant ( $p < 0.05$ )

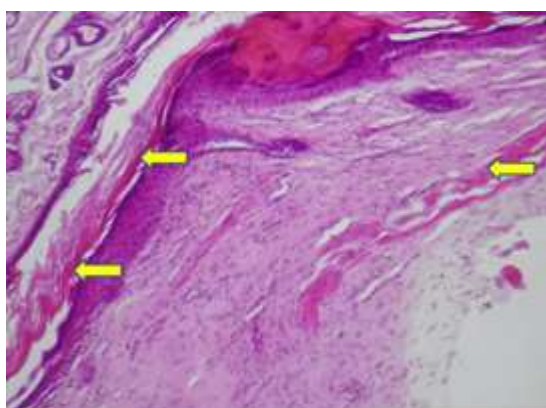
**KEY:** **Group A:** Rats that received 100mg/kg/wt of MNU; **Group B:** Rats that received distilled water (Control); **Group C:** Rats that received 100mg/kg/wt of extract + 100mg/kg/wt of MNU; **Group D:** Rats that received 200mg/kg/wt of extract + 100mg/kg/wt of MNU; **Group E:** Rats that received 400mg/kg/wt of extract + 100mg/kg/wt of MNU

*Histological sections of breast tissues of experimental rats:* Histopathological assay revealed the presence of stromal fibrosis in the breast tissues of 8 out of 10 rats administered with NMU (Plate 1). The neoplastic stage of breast cancer (stroma fibrosis) discovered in this study occurred in rats administered with NMU only without treatment.

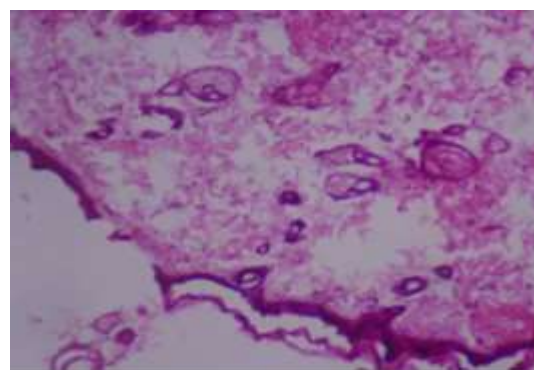
The absence stroma fibrosis (Plate 1B, Plates 2-6) in the other groups could be attributed to the chemo-preventive potential of the plant extracts. Stromal fibrosis is a histopathological finding categorized by dense collagenous breast mass with little glandular or vascular tissue (Kim *et al.*, 2015).

Stromal fibrosis is characterized by a histological finding of the proliferation of hypocellular fibrous stroma with destruction and atrophy of normal mammary ductal and acinar architecture (Shroff *et al.*, 2021).

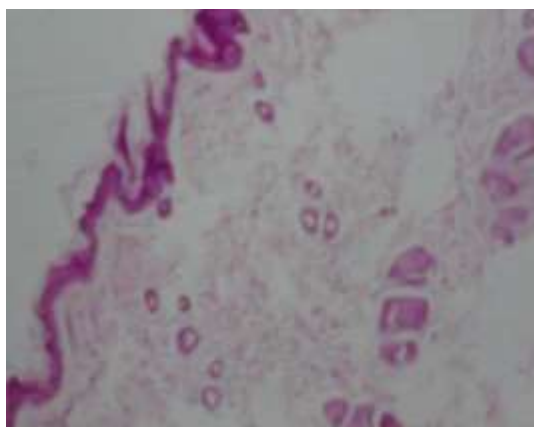
It is primarily characterized by proliferation of fibrous tissue that results in the obliteration of the mammary acini and ducts following hormonal stimulation from estrogen on fibroelastic tissue (Yilmaz *et al.*, 2018; Nassar *et al.*, 2019). It is has been documented as a process which occurs at the end stage of an inflammatory process or some type of breast involution and a leading cause of missed cases of breast cancer (Lee *et al.*, 2011; Nassar *et al.*, 2019)



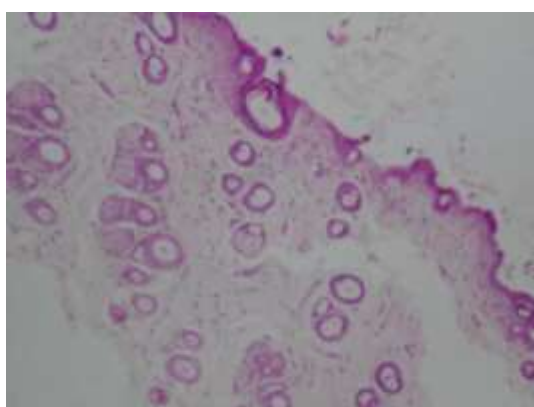
**Plate 1 (HE – 100X) (Stromal fibrosis):** PLATE 1: Rats that received 100mg/kg/wt of MNU



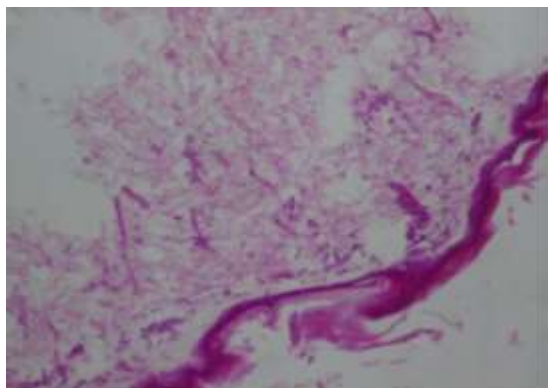
**Plate 2 (HE – 100X) (Normal study):** PLATE 2: Rats that received distilled water (Control)



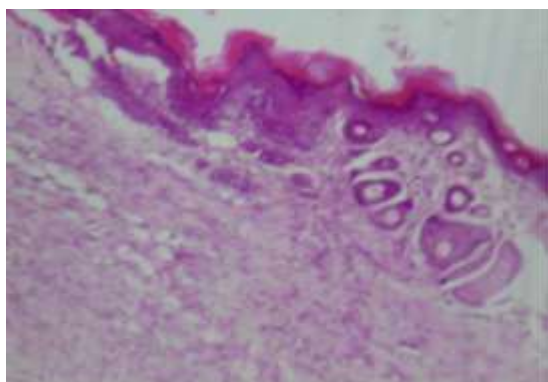
**Plate 3 (HE – 100X) (Normal study):** PLATE 3: Rats that received 100mg/kg/wt of extract + 100mg/kg/wt of MNU



**Plate 4 (HE – 100X) (Normal study):** PLATE 4: Rats that received 200mg/kg/wt of extract + 100mg/kg/wt of MNU



**Plate 5 (HE – 100X) (Normal study):** PLATE 5: Rats that received 400mg/kg/wt of extract + 100mg/kg/wt of MNU



**Plate 6 (HE – 100X) (Normal study) PLATE 6:** Rats that received 400mg/kg/wt of extract

Findings from this study has shown that ethanolic extract of *Olax subscorpioidea* and *Newbouldia laevis* leave has anti-proliferative and chemo-preventive activity in NMU-induced stroma fibrosis in the breast tissue of female albino rats, however more studies need to be carried in order to discover and validate active component responsible for action.

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