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Amelioration of nickel chloride-induced breast cancer in rats treated with allicin and *Allium sativum* extracts

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ABSTRACT: To analyse PRDX5 gene from induced breast cancer rats treated with *Allium Sativum* extracts, ninety female albino rats with average weight of 186.4g were classified into 6 groups namely NC, PC, NCAI, NCAII, NCAIII and NCGI, of 5 animals each. The experimental groups received different concentrations of both Allicin extract and $NiCl_2$. The NC. which is Negative Control, received 20 mg/kg/bwt of $NiCl_2$ only. While the PC, the Positive Control receive distilled water only. The other groups received different concentration of Allicin (50mg. 100mg and 200mg) and 20mg/kg/bwt of $NiCl_2$. Animals treated with allicin extract has significant increase in body weight at the beginning of weeks 3 to 5. There was fluctuation in weight of group that receive the treatment NCAIII and NCGI. No significant differences were observed between NC and $NiCl_2$ treated rats of NCGI, NCGII and NCGIII as the weight of the animals increased across the weeks. There was amplification in the *PRDX5* gene located in breast tissue of rats. Rats treated with allicin and garlic extract shows a distinct band at 100 base pair. However, rats in the groups (NC, NCAI and NCAII) ID not have visible bands at 100bp due to the DNA damage caused by $NiCl_2$. Rat treated with NCG II and III have a clear band. Histological assay revealed the presence of seromucous gland proliferation and atrophy with surrounding stromal fibrosis, ductal hyperplasia, ductal hyperplasia and intraductal proliferation with serous acinal hyperplasia and mucinous glands. The present study showed that allicin and garlic extract from *A. sativum* could be used as a preventive measure against $NiCl_2$-induced breast cell proliferation in the breast tissues of female albino rat.

Keywords: *PRDX5,* allicin, breast cancer, gene'

Introduction

Breast cancer is the commonest female malignancy globally (Barros et al., 2004; Forbes, 1997). It is the most common cause of cancer death among women in 140 of 184 countries worldwide and the most frequently diagnosed cancer among women which now represent one in four of all cancers in women (Globacan, 2018). In Nigeria breast cancer has overtaken cervical cancer as the leading female malignancy (Solanke and Adebamowo, 1996). It is also the commonest cancer among women in South Africa (Hoffman *et al.*, 2000). In Nigeria, as in most developing countries, late presentation with unfavourable prognosis is common (Gukas *et al.,* 2005). The African patients is likely to present with a more aggressive tumour than

her Western counterpart and is likely to die from the disease (Gakwaya *et al.,* 2008). It is characterized by high mortality, younger age distribution; more advanced stage distribution and increased frequency of high-grade tumors, as found in African–American women (Jedy-Agba *et al.*, 2012).

Breast cancer is a public health problem as it is the second commonest cancer with increasing incidence (1 in 8 women aged 45-55) in the world. It is the second most common cause of death after lung cancer in the West (Jemal *et al.,* 2009). It is important to note that although over 90% of breast disease is benign, breast cancer is easily diagnosed as the suspicious lump is mostly discovered by the patients who calls the attention of the physician. Most breast cancers are associated with fibrous tissue proliferation (scirrhous) and consequently the tissue surrounding the growth contract clinically and presents as dimpling of the skin and in-drawing of the nipple (Brinton *et al.,* 2018). Local spread occurs in 40% of breast cancer patient at presentation and 75% of lymphatic drainage is to the ipsilateral axilla. Following the definitive diagnosis of breast cancer using 'triple' assessment (clinical, pathological and radiological) the patient should have appropriate staging tests i.e. a metastatic work-up prior to decision on management (Ravdin *et al.,* 2007). The hypothesis underlying the screening for malignant disease is that the detection and treatment of cancers at an asymptomatic stage enables the cure of lesions which would be incurable if left until patient present with symptoms (Ravdin *et al.,* 2007).

Although the majority of breast cancer cases are considered sporadic, about 10% may harbor predisposing germline mutations (Cancer Genome Atlas, 2012). These mutations differ in their penetrance and associated breast cancer risk (Ghoussaini *et al.,* 2013). About 25% of hereditary breast cancer is associated with mutations in highly penetrant genes including *BRCA1*, *BRCA2*, *TP53*, *PTEN*, *STK11* and *CDK1* (Shiovitz and Korde, 2015). These mutations are associated with a lifetime risk of developing breast cancer that exceeds 50% (Ghoussaini *et al.,* 2013). *PRDX5* is a gene that has been implicated in cancer and increased levels have been reported in aggressive Hodgkin's lymphomas, in malignant mesothelioma, in breast carcinoma, in ovarian carcinoma and in thyroid cancer (Gerard *et al.,* 2005). Reduced levels of *PRDX5* expression have been described only in adrenocortical carcinoma (Kinnula *et al.*, 2002).

It is the unique atypical 2-Cys *PRDX* in mammals, widely expressed in tissues at different levels, with a large subcellular distribution including the mitochondria, the peroxisomes, the cytosol and the nucleus (Seo *et al.,* 2000). PRDX5 is a thioredoxin peroxidase that acts mainly by reducing alkyl hydroperoxides and peroxynitrite via cytosolic or mitochondrial thioredoxins. PRDX5 is a cytoprotective antioxidant enzyme rather than a redox sensor, able to act against endogenous or exogenous peroxide attacks. Several treatment options such as surgery, chemotherapy, radiation therapy are used for treatment of breast. However, a lot of short comings have been associated with breast cancer treatment options. In order to proffer a possible better and cheaper means of preventing breast cancer among women, this study was carried to evaluate the chemopreventive and antiproliferative properties of ethanolic extract of allicin from *Allium sativum* on nickel chloride ($NiCl_2$) induced cell proliferation as a result of mutation in PRDX5 gene of nickel chloride induced breast cancer tissues of female albino rats.

Garlic (*Allium sativum*) is a widely distributed plant used throughout the world not only as a spice and a food, but also as a folk-medicine. Previous studies show that many *Allium* plants other than *A. Sativum* and *A. cepaare* are of great importance due to their uses as flavoring agents, antioxidants, fragrance and therapeutics (Stajner *et al.,* 2006). The antioxidant activity of *Allium* species is due to a variety of sulphur-containing compounds and their precursors, but it is also related to other bioactive compounds such as polyphenols, dietary fiber and microelements (Nencini *et al.,* 2007). Other studies show that allicin (the main active compound in garllic) prevents the development of the atherosclerotic process, reduces serum cholesterol, normalizes lipoprotein balance, decreases blood pressure and has antithrombotic and anti-inflammatory activities (Sela *et al.,* 2004; Miron *et al.,* 2006).

This study was therefore carried out to evaluate the anti-cancer potential of different concentrations of allicin on *PRDX5* gene from the breast tissues of the nickel chloride induced rats.

Materials and Methods

Allicin was extracted from Garlic at the College of Medicine, Lagos University Teaching Hospital, Idi- Araba using ethanol. PRDX5 gene sequence from *Rattus norvegicus* (Norway rat) was gotten from NCBI (Gene bank) and primers were designed based on the sequence, primer3 plus was used to validate the best out of all the listed primers. Primer and DNA extracting kit were purchased from INQABA Biotec, South Africa, and DNA extraction buffer was prepared in University of Lagos Central Research Laboratory. All other reagents used were of analytical grade and prepared in all glass and with distilled water.

Animal and Experimental designs

The 90 adult albino rats used for the study were obtained from the College of Medicine, Lagos University Teaching Hospital, Idi-Araba, Lagos Nigeria at six weeks of age and left to acclimatise for two weeks. The animals were housed in standard clean rat cages at 25^{o}C, fed with standard pellet and tap water *ad labium*. They were maintained under uniform conditions of natural photoperiod (12hours light/dark cycle), and relative humidity (61-95%). Experiment was carried out in the Animal House of the Department of Cell Biology and Genetics, University of Lagos, Lagos Nigeria accordance with the rules in Nigeria governing the use of Laboratory Animals as acceptable.

At the commencement of the experiment, 90 female albino rats with average weight of 186.4g were classified into 6 groups, of 5 animals each. The experimental groups received different concentrations of both Allicin extract and $NiCl_2$:

Group NC – (Negative Control): Rat treated with 20 mg/kg/bwt of $NiCl_2$ only.

Group PC – (Positive Control): Rat treated with distilled water only.

Group NCAI – Rat treated with 20 mg/kg/bwt of $NiCl_2$ + 50 mg/kg/bwt of allicin extract daily
Group NCAII – Rat treated with 20 mg/kg/bwt of $NiCl_2$ + 100 mg/kg/bwt of allicin extract daily
Group NCAIII– Rat treated with 20 mg/kg/bwt of $NiCl_2$ + 200 mg/kg/bwt of allicin extract daily
Group NCGI – Rat treated with 20 mg/kg/bwt of $NiCl_2$ + 50 mg/kg/bwt of garlic extract daily
Group NCGII – Rat treated with 20 mg/kg/bwt of $NiCl_2$ + 100 mg/kg/bwt of garlic extract daily
Group NCGIII – Rat treated with 20 mg/kg/bwt of $NiCl_2$ + 200 mg/kg/bwt of garlic extract daily.

Chemicals used

Nickel chloride ($NiCl_2$) was purchased commercially from Lagos Island market, and all other chemicals and drugs used were obtained commercially and of analytical grade.

Preparation of Plant Extract and Extraction

Allium sativum, known as Garlic was cleaned and peeled in the Biochemistry Laboratory of the College of Medicine, Lagos University Teaching Hospital. It was cut to pieces and crushed in 70% ethanol for 30 minutes. The outer skin of the garlic cloves was peeled and crushed in a garlic press. The pressed garlic was then collected in a beaker and mixed thoroughly. 700-900mg of the pressed mash was weighed and transferred to a 50ml centrifuge tube. Using a volumetric pipette, 25ml of cold water was delivered to the sample and immediately capped and shaken vigorously for 30 seconds. Heat transfer was avoided from hands by holding the tube cap while shaking. An additional 25ml of cold water was added and shaken for 30 more seconds to dilute and mix the solution. Each sample is filtered through 0.45µm glass filter into HPLC vial and capped for injection (Li *et al.,* 2011).

Tumor Induction

Mammary gland tumors were induced by a freshly prepared single dose of 20 mg/kg/bwt of $NiCl_2$ given through intraperitoneal injection by gavage method as described by Bordes and Papillion, 1983. All the 90 female albino rats, with an average weight of 186.4g (152–212g).

Plant Extract Administration and Treatment

Rats were palpated weekly to check for tumor appearance. After induction with $NiCl_2$, group NCAI, NCAII, NCAIII, NCGI, NCGII and NCGIII were administered and treated with daily weekly doses of (50, 100 and 200) mg/kg/ body-weight of the plant extract of *Allium sativum* and allicin for the period of 6 weeks respectively. Treatment of animals lasted for six (6) weeks. The experimental and control animals were carefully checked daily and their weight taken weekly. Each rat had 6 pairs of mammary glands that were checked by inspection, touching and palpitation (Barros *et al.,* 2004). Rats were sacrificed at the end of the sixth week by cervical dislocation. The breast tissues from each animal were sliced off using a surgical blade and fixed in formalin saline for histology using hematoxylin and eosin staining. Blood of animals were collected into a sample bottle for DNA extraction using agarose gel electrophoresis. The work was carried out in the animal house of the University of Lagos, Lagos, Nigeria in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiment with consent from the University of Lagos Ethics Committee guidelines for experiments with whole animals (World Medical Association, 2008).

Primer

The primer sequence was designed from National Center for Biotechnology Information (NCBI) and validated using primer 3 plus. The sequence of the forward and reverse primer is 5'-TCTTTGGGAATCGTCGGCTA-3' and 5'-TGGAGGAGATGGGAGAGTCA-3' respectively.

Histological Determination

Histological evaluation was performed on a mammary gland tissues and were fixed in a fixative (10% formaline) and embedded in paraffin wax. The sections were cut at 4–5 um in thickness, stained with hematoxylin and eosin. Sections were viewed under the light microscope at 40 and 100 magnifications. Slides of all the treated groups were studied and photographed (Hould, 1984).

Immunohistochemistry Detemination

Immunohistochemistry staining was performed according to the following protocol. Sections from paraffin- embedded tissue microarrays were cut to 4um, deparaffinized in xylene and rehydrated through graded alcohols.

DNA Extraction protocol

DNA was extracted from blood of rat, using the extracting kit method. 200µl of blood sample was mixed and 200µl of Biofluid with 20µl of proteinase K in a microcentrifuge tube and incubated at 55^0C for 10 minutes. 1 volume of genomic binding buffer was added to the digested sample and mixed thoroughly. The mixture was transferred into a zymo spin collecting tube and centrifuge at 12,000rpm for 1 minute. 400µl DNA Pre-Wash buffer was to added to the mixture in a new collection tube and re-centrifuge for another 1 minute before the collection tube was discarded. 700µl g-DNA wash buffer was added and centrifuge at 12,000 rpm for 1 minute before empty the collection tube. 200µl g-DNA wash buffer was later

added and centrifuge at 12,000 rpm for 1 minute. DNA was eluted by transferreing to a clean micro centrifuge tube and 50µl DNA elution buffer was added and incubated at 55^0C for 5 minutes, and then centrifuge for 1 minute.

Spectrophotometric Analysis

The spectrophotometric analyses of the extracted DNA were carried out to determine the quality, concentration and purity of the DNA. This was done by initial blanking of the spectrophotometer with 55µl of TE buffer, after which 5µl of the DNA samples was added to it. The absorbance value was obtained at O.D 230nm, 260nm, 280nm and 320nm wavelength, concentration of DNA and the absorbance ratio at 260 and 280 nm (A260/280) were also obtained to determine the purity of the DNA.

Electrophoretic analysis

The amplification product was separated on a 2% agarose gel and electrophoresis was carried out at 80V for 2 hours. After electrophoresis, DNA bands were visualized by ethidium bromide staining. 100bp ladder was used as DNA molecular weight marker.

Statistical Analysis

Data are shown as means ± SEM. Statistical significance of the results obtained for various comparisons was estimated by applying one way of variance (ANOVA) followed by students t-test and the level of significance was set at $p<0.05$.

Results

Figures 1 and 2 show samples against the wavelength at 260:280nm absorbance values and concentrations in ng/ul of the DNA in the samples respectively. It was observed that all groups have a high absorbance peak at 260nm except the positive control that has 1.78. However, group NCAI, NCAIII and NCGI have their absorbance greater than the negative control indicating high purity and quality DNA when compare to others. Variation in DNA in concentrations were also observed for different experimental animals groups with group NCGI having the highest concentration and positive control with the least concentration.

Plate 1 shows Nickel chloride ($NiCl_2$) induced-breast cancer in an experimental rat. Tumour was seen on the breast region of some of the rat across all groups that received the nickel chloride. This occurred 3 weeks after the administration of the carcinogen.

Histological section of the breast tissue of experimental rats from the positive control (PC) is shown in Plate 2a and 2b respectively. This revealed the presence of normocellular serous glands, fibrosis, muscle and stroma at (HE-100x and 40x) magnification.

Plate 3a shows the histological section of the breast tissue of $NiCl_2$ induced-breast cancer in rats from negative control (NC). This revealed the presence of ductal dialation and intraductal proliferation under x40 magnification while seromucous proliferation and ductal carcinoma in-situ were identified in 3b under x100 magnification as a result of animals not been treated.

Plate 4a reveals the histological section of the breast tissue of $NiCl_2$-induced cell proliferation of breast cancer rat that were treated with 50 mg/kg/bwt of allicin extract (NCAI). The presence of $NiCl_2$- ductal atrophy, periductal fibrosis, atrophy of glands with proliferation and seromucous gland were identified under x100 magnification in Plate 4a while ductal hyperplasia was identified in plate 4b under x100 magnification

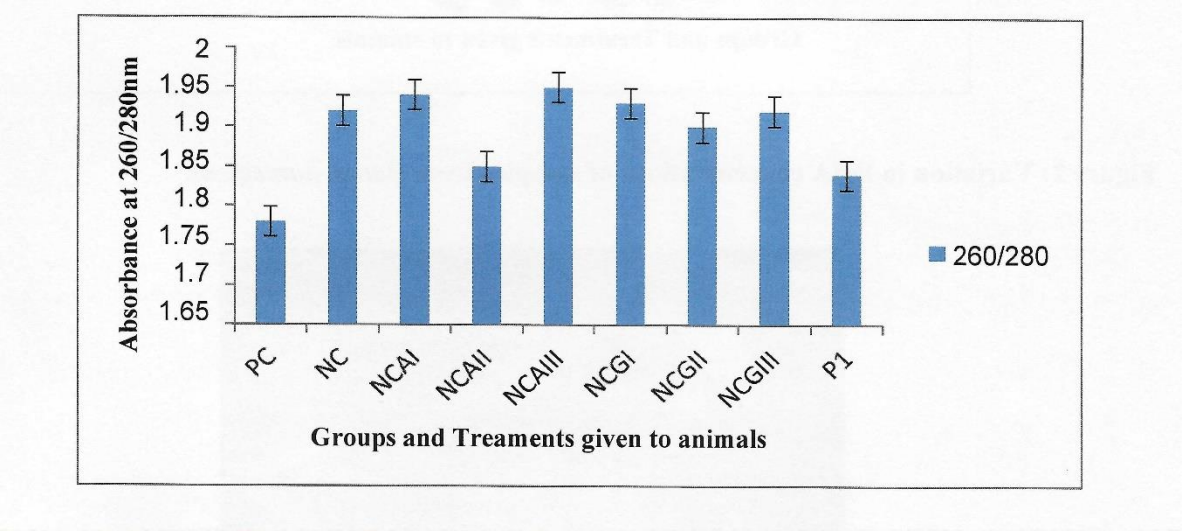

Figure 1: Absorbance peak of DNA samples extracted from Rattus norvegicus at wavelength 260nm:280nm (DNA Purity)

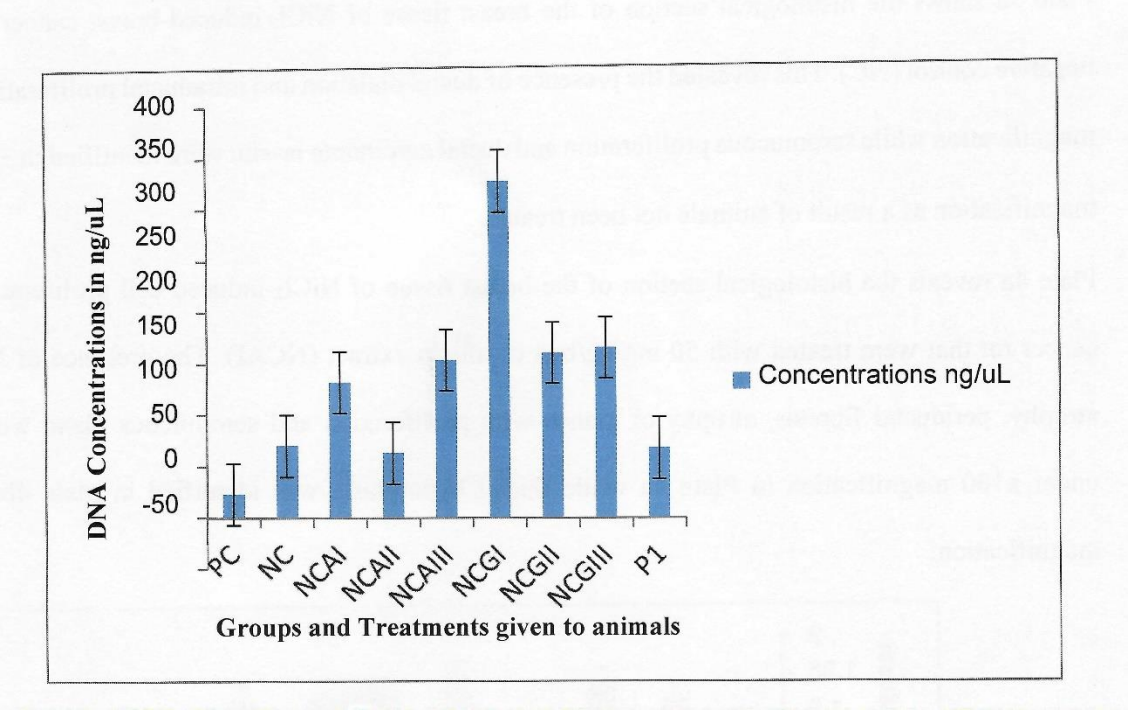

Figure 2: Variation in DNA concentrations of samples from *Rattus norvegicus.*

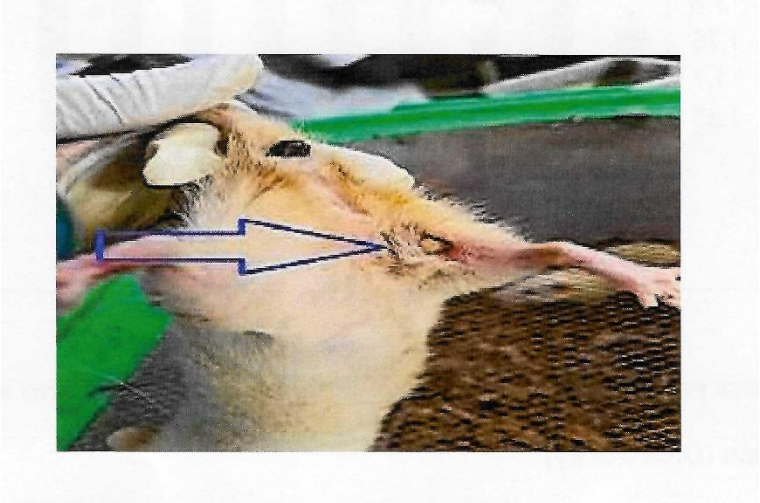

Plate 1: $NiCl_2$ induced-breast cancer at the breast region of rats treated with NiCl2 only. Arrows indicate the location of $NiCl_2$ induced tumour (NC)

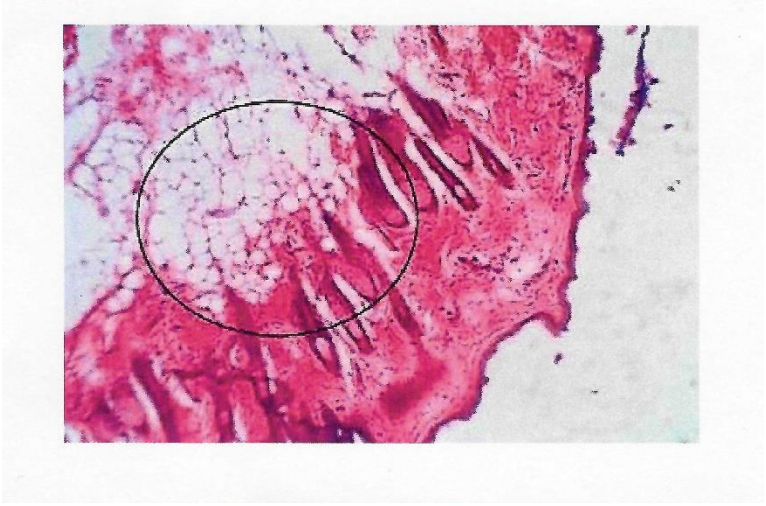

Plate 2a: Histological section of the breast tissue of NiCl2 induced breast cancer in rats from PC (HE-100X) (Normocellular serous glands and fibrosis). PC (HE-100X)

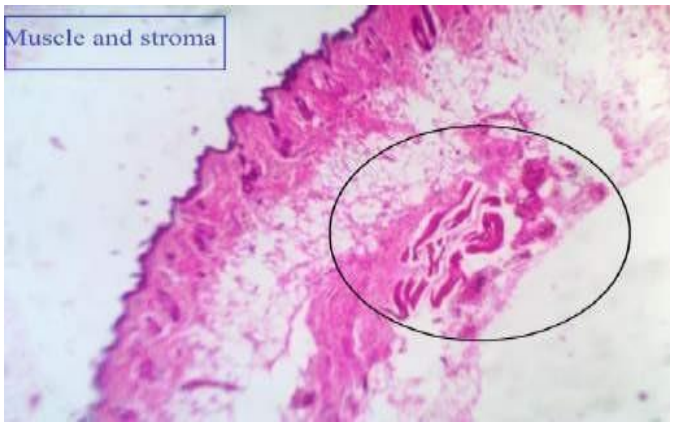

Plate 2b: Histological section of the breast tissue of NiCl2 induced breast cancer in rats from PC (HE-40X) (muscle and stroma). PC (HE-40X)

Plate 3a: Histological section of the breast tissue of NiCl2 induced breast cancer in rats from NC (HE-X40) (Ductal dilation and intraductal proliferation).
NC (HE-40X)

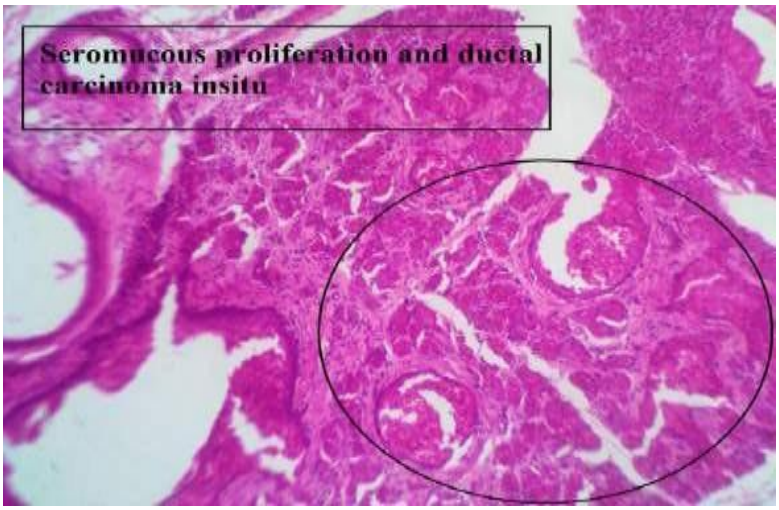

Plate 3b: Histological section of the breast tissue of $NiCl_2$ induced breast cancer in rats from NC (HE-X100) (Seromucous proliferation and ductal carcinoma *in-situ*).
NC (HE-100X)

Plate 4a: Histological section of the breast tissue of NiCl2 induced breast cancer in rats from NCAI (HE-100X) (Ductal atrophy, periductal fibrosis, seromucous gland proliferation and atrophy).
NCAI: Rat treated with 20 mg/kg/bwt of NiCl2 + 50 mg/kg/bwt of allicin extract daily.

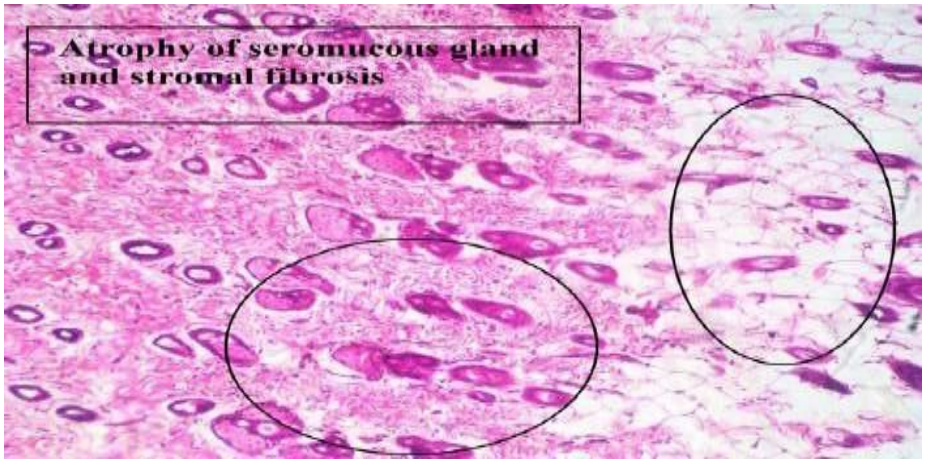

Plate 4b: Histological section of the breast tissue of $NiCl_2$ induced breast cancer in rats from NCAI (HE-100X) (Atrophy of seromucous gland with surrounding stromal fibrosis)
NCAI: Rat treated with 20 mg/kg/bwt of $NiCl_2$ + 50 mg/kg/bwt of allicin extract daily.

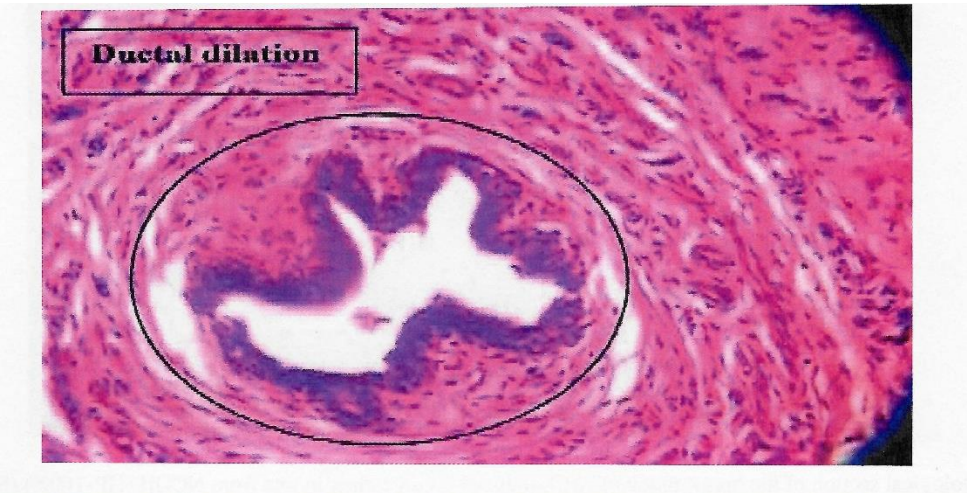

Plate 5a: Histological section of the breast tissue of $NiCl_2$ induced breast cancer in rats from NCAIII (HE-100X) (Periductal fibrosis and ductal epithelium hyperplasia)
NCAIII: Rat treated with 20 mg/kg/bwt of $NiCl_2$ + 200 mg/kg/bwt of allicin extract daily.

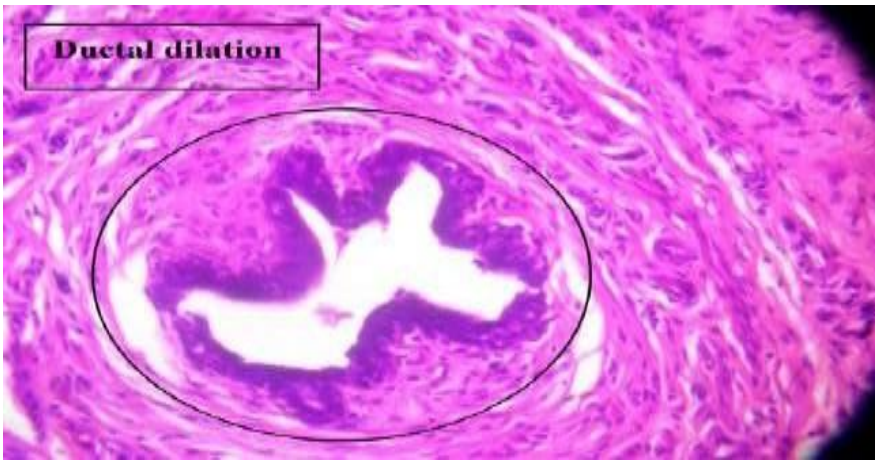

Plate 5b: Histological section of the breast tissue of $NiCl_2$ induced breast cancer in rats from NCAIII (HE-400X) (Ductal dialation)
NCAIII: Rat treated with 20 mg/kg/bwt of $NiCl_2$ + 200 mg/kg/bwt of allicin extractdaily.

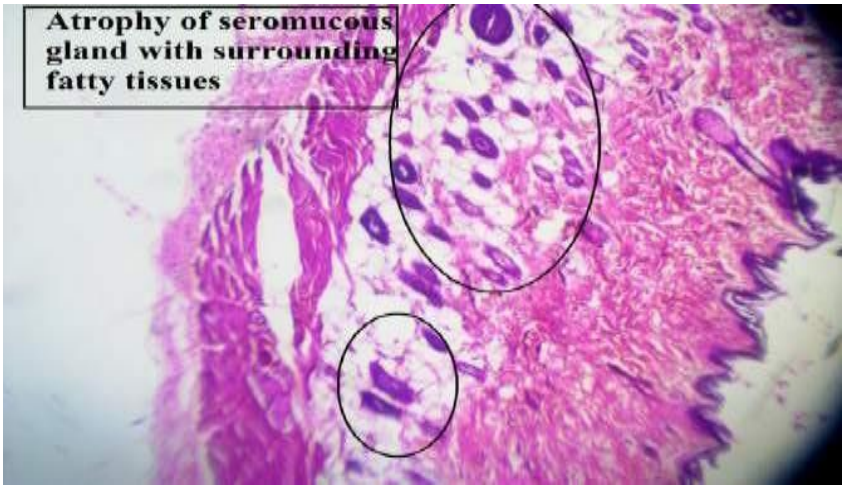

Plate 6: Histological section of the breast tissue of $NiCl_2$ induced breast cancer in rats from NCGI (HE-100X) (Periductal fibrosis, atrophy of seromucous gland and ductal dilation)
NCGI: Rat treated with 20 mg/kg/bwt of $NiCl_2$ + 50 mg/kg/bwt of garlic extract daily.

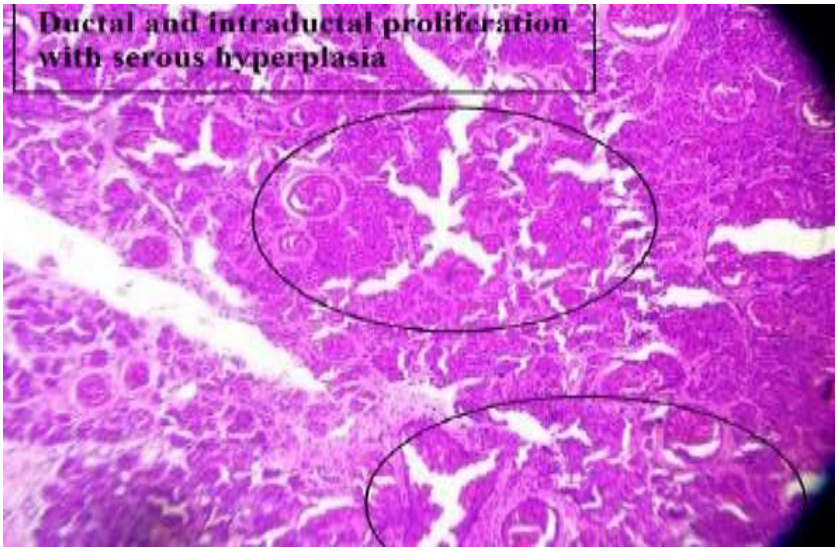

Plate 7: Histological section of the breast tissue of $NiCl_2$ induced breast cancer in rats from NCGII (HE-100X) (Ductal proliferation, serous hyperplasia and intraductal proliferation)
NCGII: Rat treated with 20 mg/kg/bwt of $NiCl_2$ + 100 mg/kg/bwt of garlic extract daily.

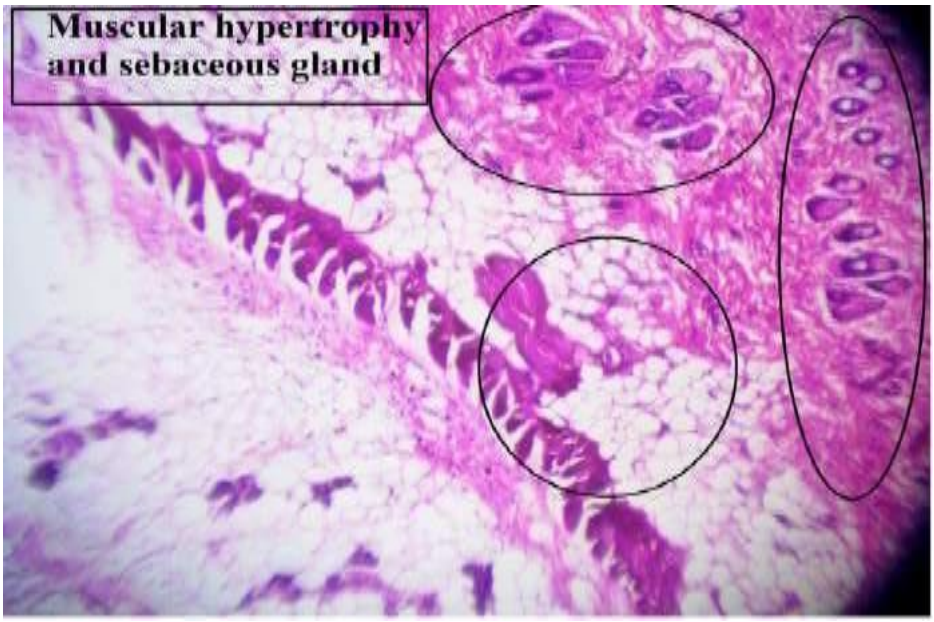

Plate 8: Histological section of the breast tissue of $NiCl_2$ induced breast cancer in rats from NCGIII (HE-100X) (muscular hypertrophy and sebaceous glands)
NCGIII: Rat treated with 20 mg/kg/bwt of $NiCl_2$ + 200 mg/kg/bwt of garlic extract daily.

Plate 5a shows histological section of the breast tissue of $NiCl_2$ induced-breast cancer in rats treated with 200 mg/kg/bwt of allicin extract from *A. sativum* (NCAIII) . The histological section reveals the presence of periductal fibrosis and ductal epithelium hyperplasia at an objectives of x10 and plate 5b reveals the histological section that show ductal dialation at x40 objective lens.

Plate 6 shows histological section of the breast tissue of $NiCl_2$ induced-breast cancer in rats treated with 50 mg/kg/bwt of garlic extract (NCGI). The histological section reveals the presence of periductal fibrosis, atrophy of seromucous gland and ductal dilation at a magnification of 100X.

Plate 7 shows histological section of the breast tissue of $NiCl_2$ induced-breast cancer in rats treated with

One hundred mg/kg/bwt of garlic extract (NCGII). The histological section reveals the presence of ductal proliferation, serous hyperplasia and intraductal proliferation at 100X magnification.

Plate 8 shows histological section of the breast tissue of $NiCl_2$ induced-breast cancer in rats treated with 200 mg/kg/bwt of garlic extract (NCGIII). The histological section reveals the presence of muscular hypertrophy and sebaceous glands at 100X magnification.

The effect of different concentrations of allicin and garlic extract on the body of weight female albino rats during the study is shown in Table 1. There was a gradual increase in the weight of rats evaluated during the study across the treatments evaluated. However, there was fluctuation in weight of group that received the treatment (NCAIII and NCGI). There is significant difference ($p< 0.05$) in treatment between weeks 3 to 5. However, there is no significant difference between week 1 and week 2. The allicin extract (NCAI, NCAII and NCAIII) administered to rats are not significantly different from weeks 3 to 5, However NCAI and NCAII are not significantly different ($p>0.05$) from the positive control but NCAIII is significantly different ($p< 0.05$) from PC, and not significantly different from the NC. The garlic extract (NCGI, NCGII and NCGIII) are not significantly different at different concentrations that was administered to rats and this effect was noticed form weeks 3 to 5. There is significant different in weight between the garlic extract and the positive control.

It was observed that there was significant different between allicin and garlic extract. However, rats that received 200mg/kg/bwt of allicin extract has no significant difference when compared to the rat given garlic extract.

Plate 9 shows the DNA fragmentation images for the control groups (NC and PC) and the treatment

groups (NCAI, NCAII, NCAIII, NCGI, NCGII and NCGIII) obtained by single cell gel electrophoresis amplicons of *PRDX5* gene from the DNAs obtained from the breast tissues of the rats.

Table 1: Effects of allicin and garlic extract from *A. sativum* on the weight trend of experimental animals over a period of 5 weeks

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5
PC	190.50±0.50	193.17±3.25	201.40±1.64^{b}	202.60±2.42^{b}	205.13±1.03^{b}
NC	190.13±1.03	189.33±4.73	190.67±0.58^{a}	190.17±1.04^{a}	190.33±1.53^{a}
NCAI	191.33±2.08	186.33±8.08	200.00±1.00^{b}	203.00±2.65^{b}	207.33±1.15^{b}
NCAII	193.40±2.43	193.93±6.90	201.30±2.86^{b}	204.93±4.50^{b}	212.00±2.00^{b}
NCAIII	185.53±9.30	188.33±5.77	194.67±1.53^{ab}	193.33±3.06^{a}	195.33±2.08^{a}
NCGI	189.60±3.27	192.53±1.36	190.17±1.04^{a}	189.33±0.58^{a}	184.33±1.15^{a}
NCGII	189.87±0.81	190.40±5.03	195.23±2.04^{ab}	193.63±1.48^{ab}	199.00±1.00^{ab}
NCGIII	188.50±4.95	191.33±0.58	191.10±1.01^{a}	191.67±0.58^{a}	192.87±0.81^{a}
F	0.918	0.779	26.45	20.33	131.2
P=0.05	0.518	0.614	<0.001	<0.001	<0.001

Column values (Mean±SEM) followed with similar alphabets are not significantly different (DMRT, P>0.05).

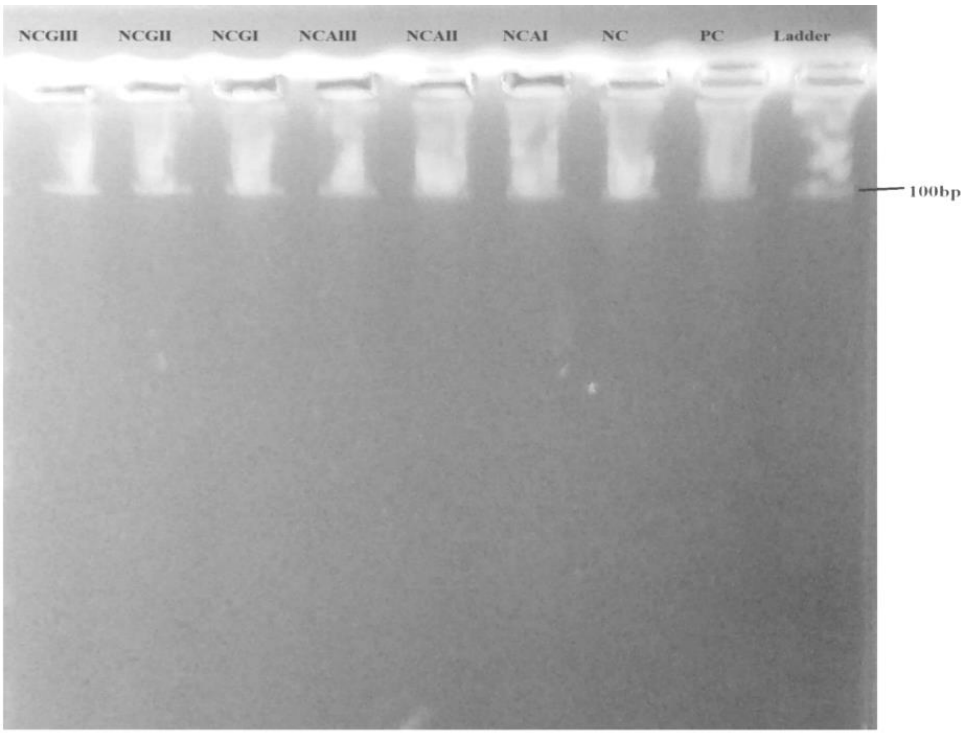

Plate 9: Electrophoresis bands of PCR amplicons of PRDX5 gene of DNAs obtained from the breast tissues of NiCl2 induced-breast cancer in rats

Discussion

Breast cancer is a major cause of death among women both in developed and less developed countries and despite improvement in modifiable associated risk factors. This explains why there is still ongoing study to identify the association between other risk factors that may possibly responsible for the growing burden of breast cancer globally (Abudu *et al*., 2007). In Nigerian, Breast cancer (BC) is the most common type of cancer among women (Akinyemiju *et al*., 2015), with an overall age standardized rate (ASR) of 52.2 per 100,000 as reported by (Albrektsen *et al*., 2010). Previous studies affirmed that breast cancer among Nigerian women differs across the states of the federation. For instance, in northern part of Nigeria breast cancer is the second most common diagnosed in women while western part has breast cancer as the most diagnosed cancer among their women (Anyanwu *et al*., 2011).

However, several studies have assessed the risk factors associated with breast cancer and many of them have been identified as established factors. Increasing age has been identified by many studies as risk factors (Ebughe *et al*., 2013). In addition, the use of oral contraceptive, lifestyle factors such as alcohol consumption and high fat diet have been identified by many studies as risk factors for breast cancer (Brown *et al*., 2010). Recently, some studies have shown that histological types of breast cancer is associated with reproductive factors, age at first birth and other hormone related risk factors (Galukande *et al*.,2013).

The electrophoregram in this study showed that DNA extracted from *R. norvegicus* showed both slow and fast movements respectively. The spectrophtometric results shown in Figures 6 and 7 with the absorbance ratio of DNA samples ranging from 1.78 to 1.95 indicate a good quality DNA which is in support with the work of Oboh *et al* (2009), and that every other contaminant like RNA, polysaccharides have been removed. Spectrophotometric readings showed that animal that received distilled water only (PC) has the least concentration and absorbance ratio at 260nm which could be as a result of normal growth without the influence of the carcinogen, group NCAI, NCAIII and NCGI have the highest concentration of (132, 154 and 357ng/µl) respectively, has the highest purity value around 1.94, 1.95 and 1.93 respectively which is good for molecular studies.

The visible evidence of nickel chloride administration ($NiCl_2$) induced breast cancer was seen around the breast region of rat treated with $NiCl_2$ only (Plate 1) and histological section on it was shown in (Plate 4). Tumour was noticed 3 weeks after the carcinogen was administered. According to the European Chemical Agency (2018), they showed that different nickel compounds can be used to induce carcinogenicity through intraperitoneal and injection administration, and this process result in oxidative damage, generation of reactive oxygen species (ROS) or inhibition of repair and inhibit DNA repair enzyme (Chen *et al.,*2010).

The histopathological classification of breast disease is subjective and, despite an attempt to provide clear guidelines, the inter-observer variability is known to be high according to Sloane *et al.*, (1994). The presence of $NiCl_2$-induced ductal atrophy, periductal fibrosis, seromucous gland proliferation with ductal hyperplasia, ductal epithelium hyperplasia and ductal dilation in the histological sections of breast tissues of $NiCl_2$-induced breast cancer of rats that were treated with allicin extract at 50 and 200mg/kg/bwt concentration (Plates 4a, 4b, 5a and 5b) respectively suggest that $NiCl_2$-induced rat mammary carcinomas have been shown to arise from the ductal elements of the mammary gland and significant variability in tissues induced with the carcinogen and neoplastic transformation with an indication of proliferation. This corresponds with the studies that were reported by Uriel *et al., (*1983). However, these changes were found to vary from the group that receive $NiCl_2$ only (Plate 3a and 3b) where the tumour is more pronounced and not treated. Animals treated with allicin extract has significant increase in body weight at the beginning of weeks 3 to 5 which is contrary to the work of Lee, (1967) that rats given allicin extract inhibit the increase in body weight, and that the influence on weight cannot be attributed to toxic effects. These variations are likely to occur due to the preventive effect of different concentrations of allicin extract administered to the rats.

The presence of $NiCl_2$-induced sebaceous gland hyperplasia, ductal dilation with periductal fibrosis, ductal dilataion with intraductal proliferation, acinial hyperplasia and muscular hypertrophy in the histological sections of the breast tissues of rats treated with garlic extract in (Plates 6, 7 and 8) suggests a

neoplastic transformation which is an indication of $NiCl_2$-induced cell proliferation and ductal carcinoma in-situ which correspond to the studies of Mehta *et al* (2001). However, these changes were found to vary in occurrence among the different groups used for the present study. These variations might be due to the preventive effect of the different concentrations of garlic extract (50, 100 and 200mg/kg/bwt) administered to rats. The presence of proliferating cells in the stroma as well as serous acinal hyperplasia (Plate 8) strongly suggests that the carcinogen acts on different cells in the breast tissue and, although the stroma itself does not undergo neoplastic transformation, it plays an important role in the carcinogenic process (Grossfeld *et al.*, 1998).

The reduction in weight of NCAIII that received 200mg/kg/bwt concentration (Table 1) of allicin extract indicate the possible toxicity of the extract. The extract may have indeed metabolized to a toxic end point product which thereby affect the weight of animals. Fluctuation in weight was observed in group that receive the treatment NCAIII and NCGI (Table 1) probably as a result of the action of the animal immune system. The immune system of rats might have acted against the carcinogen within those weeks. No significant differences were observed between NC and $NiCl_2$ treated rats of NCGI, NCGII and NCGIII as the weight of the animals increased across the weeks. This is an indication of the possible toxicity of garlic extract from *A. sativum* suggesting the possible interaction between phytochemical components due to increase in concentration given to animals.

The results as shown in Plate 9 indicate that there was amplification in the *PRDX5* gene located in breast tissue of rats. Rats treated with allicin and garlic extract shows a distinct band at 100 base pair. However, rats in the groups (NC, NCAI and NCAII) did not have visible bands at 100bp due to the DNA damage caused by $NiCl_2$. Rat treated with NCG II and III have a clear band which shows that the garlic extract at high concentration can repair the damage DNA from $NiCl_2$. Animals that received distilled water only (PC) has a faint band that shows that the DNA was present but probably due to high voltage and buffer. Rat induced with nickel chloride only (NC) did not show any visible band as a result of the damage been from the carcinogen.

Cancer is one of big problems in both developing and developed countries. Various synthetic drugs have been used to treat cancer but they have limitations due to their toxic effects on the normal health cells. Therefore, medicinal plant and extracts from these plants largely contributed to human being health and it contains various secondary metabolites which show their potential towards numerous disease treatments.

The present study showed that allicin and garlic extract from *A. sativm* could be used as a preventive measure against $NiCl_2$-induced breast cell proliferation in the breast tissues of female albino rat. Histological assay revealed the presence of seromucous gland proliferation and atrophy with surrounding stromal fibrosis, ductal hyperplasia, ductal hyperplasia and intraductal proliferation with serous acinal hyperplasia and mucinous glands which suggest the presence of cells undergoing initial proliferation stage preceding carcinogenesis. The knowledge obtained from this study serves as a resource base and can be scientifically exploited for future research in breast cancer chemoprevention. Nevertheless, further studies and more research need to be done to optimize the quality of extract, effective dose and its specificity on breast cancer susceptibility genes

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