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# Effect of ethanolic garlic extract and fatty acid on histology and DNA related damage of nickel chloride induced carcinoma in albino rats

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**Abstract**
Bioactive component from medicinal plants continues to be the centre of research in treatment of ailments. In this study, we evaluated the inhibitory effect of ethanolic Garlic extract and its HPLC fatty acid elute on the histology and DNA of nickel chloride induced female albino rats (*Rattus norvegicus*). The result showed disparities in weight across groups throughout the experimental period, with the group treated with fatty acid having the highest weight. Tumour was observed in the negative control group. Result from PCR gene amplification (ULBP3, STX17, PRDX5, Il2 and CTLA4) showed distinct bands, which suggest the DNA was not damage. The histology showed variation in aggressiveness of nickel chloride pathogenesis in relation to treatment. This study help confirm the chemo-preventive properties of Garlic and suggest that, at least in part it fatty acid constitute fat deposition in tissues, could be detrimental to it ameliorative efficacy.

**Keywords:** *Allium sativum*, cancer, medicinal plants, HPLC, nickel chloride

## 1. Introduction

Nickel and it compounds are employed as manufacturing chemicals in the production of metal alloys, batteries, electroplating utensil/jewelry, surgical staples, coins and other industrial processes [1, 2]. Exposure to the nickel compounds have been implicated in several *in vitro*, animal and human studies as carcinogen and toxic agents, while it has also been described as a source of industrial, environmental and occupational health hazard [3, 4, 5]. However, the carcinogenic potency of nickel compounds in research studies tend to be inversely related to the solubility of the compounds [6]. Previous researches on Nickel and its compounds have been concerned with its role in cancer of the respiratory tract through inhalation [7, 8]. Nickel chloride is a water-soluble salt, which could present a dangerous influence on human health through percutaneous adsorption and ingestion via food and drink [9, 10, 11]. Other reports have also suggested that Nickel chloride could causes damage to DNA and cells via the indirect production of Reactive Oxygen Species [12, 5], which could be a mechanism for the induction of cancer. Hence, it becomes imperative to investigate the latent damage of Nickel chloride on tissue and genetic integrity through experimentation and profound possible solution to treatment.

Ongoing research throughout the world to seek out alternative effective means of cancer treatment, as conventional treatments are often associated with high risk dosage of drugs, toxicity, severe side effects and higher cost of treatment [13, 14]. As part of sustainable means of treating diseases, the uses of medicinal plant holds therapeutic potentials and have shown to play key role in treating a number of diseases including cancer [15, 16]. Garlic has been widely acclaimed to be an effective anti-proliferation agent against different form of carcinoma [17]. It most active compound is Allicin, an organosulfur compound from the catalytic action of Allinase on Alliin when cloves Garlic is crushed [18]. The viability of the bioactive component of these medicinal plants with high effectiveness and lower side effects is much desirable. A comparative study on it phytochemicals and fatty acid content can help to elucidate it health-promoting activity in inhibiting cancer initiation. Therefore, the aim of this study was to investigate the chemo-preventive effects of ethanolic Garlic extracts and its HPLC fatty acid elute on tumorigenesis in Albino rats exposed to Nickel Chloride.

## 2. Materials and Methods

### 2.1 Materials

Fresh Garlic bulbs were purchased from the Bariga Market, Lagos State, Nigeria. The cloves was identified and authenticated as *Allium sativum* by a taxonomist at the Herbarium Unit, Department of Botany, University of Lagos. Ninety female albino rats (Wistar) were obtained from the animal house, University of Lagos Teaching Hospital, ldi Araba, Lagos. All chemicals and reagents used throughout these research were commercially obtained and of analytical grade. Primers and DNA extraction kits were ordered from InqabaBiotec West Africa Ltd (IBWA).

### 2.2 Preparation of ethanol Garlic extract

The Garlic cloves were separated and dried at room temperature away from sunlight for fourteen (14) days. The outer dry clove sheaths were peeled off to induce Allicin (diallyl thiosulfinate) production, and the dried garlic cloves were further crushed. The ethanol Extraction was done according to the extraction technologies for medicinal and aromatic plants outlined by Sukhdev *et al*., 2008 [19]. Two (2) kg was macerated in a stoppered glass container containing ethanol for 48 hours with frequent agitation. The mixture was filtered using a 200 mm mesh followed by vacuum filtration through a Whatman filter paper. The filtrate was concentrated using a rotary evaporator at 45°C into a paste and further under dry air.

### 2.3 Phytochemical screening of the plant extract

Preliminary Qualitative phytochemical screening of the ethanol extract of garlic was carried out by methods of analysis described by Handa *et al*., 2008 and Trease and Evans, 1983, at the Biochemical Laboratory, University of Lagos Teaching Hospital. The Garlic ethanol Extract was tested for presence of Saponin, Tannin, Flavonoid, Glycoside, Steroid, Terpenoids, Antraquinone, Alkaloid, Fehling and Molisch [20, 21].

### 2.4 Fatty acid analysis using High Performance Liquid Chromatography (HPLC)

The HPLC analysis of ethanolic extract was carried out performed using a CLARUS 500GC HPLC system at the Rolab Laboratory, University of Ibadan. The separation was performed on a reverse- phase column C18 (100 mm × 3 mm, 3μm) at ambient temperature. The mobile phase consists of acetonitrile and purified deionized (20:80) water and the separations were performed by using isocratic mode. The flow rate was 1.0 ml/min. The samples detection was done at 220 nm by digital multimeter (DMM). All chromatographic data were recorded by a computer loaded with a data acquisition program.

### 2.5 Experimental animals

*Rattus norvegicus* rats were used as experimental animals in this study. The rats were housed in rat cages at room temperature (18-25 $^{0}$C) in the Cell Biology and Genetics Animal Laboratory, University of Lagos. They were feed with rat pellets and liberally provided tap water ad libitum. They were aclimitizated to the housing for a period of two (2) weeks before experimental procedures and treatment was administered. Experimental animals were treated in accordance with the national and institutional guidelines for the protection of the animals' welfare during experiments as acceptable internationally according to World Medical Association Helsinki Declaration, 2013.

#### 2.5.1 Experimental design

The experimental animals were distributed into eight groups (n=5). The rats were administered with a single intramammary injection dose of 20mg kg-1 Nickel Chloride, excluding the positive control group (Group PC). All rats were inspected daily for physical condition. The first exposures of the fractionated treatments were given at 3 days after inducement. Treatments were given administered daily orally. Group TA, TB and TC were administered 50, 100 and 200 mg kg-1 HPLC fatty acid elute respectively, while Group GA, GB and GC were administered 50, 100 ad 200 mg kg-1ethanolic garlic extract. Group NC was Negative Control.

#### 2.5.2 Blood Collection

Twenty-Four hours after the last treatment administration, the experimental rats were sacrificed by cervical dislocation and the blood collected by bleeding through the jugular vein in EDTA containing sample bottles.

#### 2.5.3 Tissue Collection and Processing

Breast tissues was dissected out from anesthetized rats at sacrifice, and stored appropriately labeled sample bottles containing 10% formalin buffer. The Histological sample was embedded in paraffin wax, sectioned at 4-5 um and subsequently stained with hematoxylin and eosin (H&E). The H&E stained sections of the tumours was studied under the light microscope at 4, 10 and 40 magnifications.

### 2.6 DNA Extraction, Polymerase Chain Reaction (PCR) and Electrophoretic analysis

The DNA extraction and PCR was carried at the Central Research Laboratory, University of Lagos. Quick-DNA MiniprepPlus Extracting Kit used was purchased from INQABA Biotech, South Africa, the protocol was based on spin column using the manufacturer's manual guide. The blood samples were pipetted into an Eppendorf tubes. The DNA kit manufacturer's protocol was followed to obtained high purity genomic DNA from the samples. The quality of DNA extracted was checked by UV-Spectrophotometric Analysis (EppendorfBioPhotometer Plus) and Gel electrophoresis. The gene sequence for this study was obtained on the GenBank section of NCBI website, the primer design was based on sequence in database and primer sequence obtained from literature. Primer3 plus was used to validate the listed primers for optimized selection. The primers were synthesized and order from INQABA Biotech, South Africa. The PCR was run at annealing temperature of 55oC. The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80 V for 1 h 30 min. The DNA bands were visualized using ethidium bromide stain.

**ULBP3 primer**

F(5'-CGAGAGAACTTTGCACTATG-3'),
R(5'-GGCACTTTCACGTATAACTT -3')

*STX17 primer:*

F(5'-AGGATGTCCGAAGATGAGG-3'),
R(5'-CACTTCTTAACACTGTTCCA-3')

*PRDX5 primer:*

F(5'-TCTTTGGGAATCGTCGGCTA-3'),

R(5’-TGGAGGAGATGGGAGAGTCA-3’)
*Il-2 primer:*
F(5'-AGGATGTCCGAAGATGAGG-3'),
R(5'-CACTTCTTAACACTGTTCCA-3')
*CTLA4 primer:*
F(5'-GTAGTCTCCTTGCCGTAGCC-3'),
R(5'-AGTGGAATGGCTCTGCTTAACC-3')

**2.7 Statistical analysis**
Statistical analysis was carried out using GraphPad Prism version 8. All values expressed as mean ± standard deviations (SD). Significant differences were established by using a repeated measure Analysis of Variance (ANOVA) based on the general linear model (GLM) and Tukey post-hoc test of multiple comparism was used to determine between group differences ($p < 0.05$).

## 3. Result
The results of qualitative phytochemical screening analysis of ethanolic extract of Garlic revealed the presence of Saponin, Tannins, Flavonoids, Glycosides, Steriod, Terpenoid and Alkaloids. However, Antraquinone, Fehling and Molisch were absent (Table 1).

**Table 1:** Qualitative analysis of an ethanolic extract of Garlic (*Allium sativum*)

| Phytochemical components | Test Result |
|---|---|
| Saponin | + |
| Tannin | + |
| Flavonoid | + |
| Glycoside | + |
| Steroid | + |
| Terpenoids | + |
| Antraquinone | - |
| Alkaloid | + |
| Fehling | - |
| Molisch | - |

Key: (+) = Present; (−) = Absent

The HPLC fatty acid analysis of Garlic acid are shown in Table 2, linoleic acid had the highest concentration of 23.92% area followed by oleic acid (19.33%), lauric acid (17.15%), eicosenoic acid (10.43%), palmitic acid (10.18%), elaidic acid (7.58%) and myristic acid (6.14%). The total saturated fatty acid is 38.29% while the total unsaturated fatty acid is 61.71%.
Figure 1 shows the effect of ethanolic extract of Garlic (*Allium sativum*) and fatty acid extract on the weight trend of experimental animals over a period of five weeks. The experimental rats in the negative control group showed repressed growth throughout the treatment administration. Rats administered with the Garlic extract at 100 mg/kg and 200 mg/kg showed median growth rate, while the 50 mg/kg garlic extract treatment had a decline in weight from the second week of treatment through the experimental period. There was a progressive increase in the weights of rats that were administer fatty acid extract at 100 mg/kg, and the positive control group. The 50 mg/kg fatty extract showed a sharp decline by the second week followed by a significant increase in growth from the three week of treatment. The negative control induced with nickel chloride only had repress growth until the end of the experimentation. Physical examination of experimental animals following administration of Nickel Chloride showed presence of localized tumour at the fifth week of treatment (Figure 2).

**Table 2:** HPLC profile of fatty acid of ethanolic extract of Garlic (*Allium sativum*)

| Component | Fatty Acid (Common name) | Saturation | Time (min) | Area/Height (s) | Area (%) |
|---|---|---|---|---|---|
| C8:0 | Octanoic acid (Caprylic acid) | saturated fatty acids | 15.517 | 16.3182 | 2.41 |
| C10:0 | Decanoic acid (Capric acid) | saturated fatty acid | 21.253 | 15.5395 | 2.32 |
| C12:0 | Dodecanoic acid (Lauric acid) | saturated fatty acid | 25.852 | 14.3593 | 17.15 |
| C14:0 | Tetradecanoic acid (Myristic acid) | saturated fatty acid | 30.232 | 10.6007 | 6.14 |
| C16:0 | Hexadecanoic acid (Palmitic acid) | saturated fatty acid | 33.902 | 7.545 | 10.18 |
| C16:1 | hexadec-9-enoic acid (Palmitoleic acid) | unsaturated fatty acid | 34.679 | 5.6946 | 0.2 |
| C17:0 | Heptadecanoic acid (Margaric acid) | saturated fatty acid | 35.6 | 4.9283 | 0.09 |
| C18:1n9t | Elaidicacid | unsaturated fatty acid | 37.224 | 10.5129 | 7.58 |
| C18:1n9c | Oleic acid | unsaturated fatty acid | 37.713 | 8.5729 | 19.33 |
| C18:2n6c | Linoleic acid | unsaturated fatty acid | 38.789 | 9.7792 | 23.92 |
| C20:1 | Eicosenoicacids | unsaturated fatty acids | 40.252 | 11.6958 | 10.43 |
| C18:3n3 | α-Linolenic acid | unsaturated fatty acid | 40.962 | 7.6459 | 0.25 |

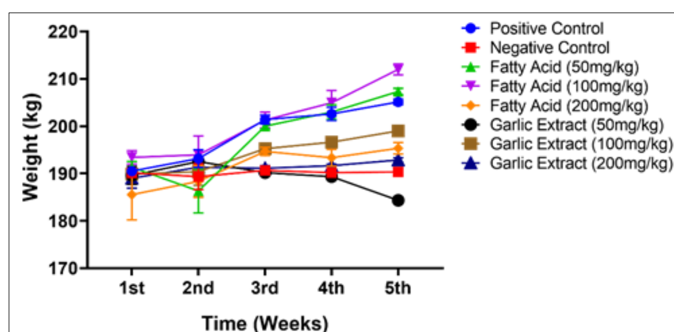


**Fig 1:** Effects of ethanolic extract of Garlic (*Allium sativum*) and fatty acid extract on Nickel Chloride induced albino rats.

The values are expressed as mean ± SEM of data in two experiments. ANOVA test was used for statistical analysis ($P < 0.05$)

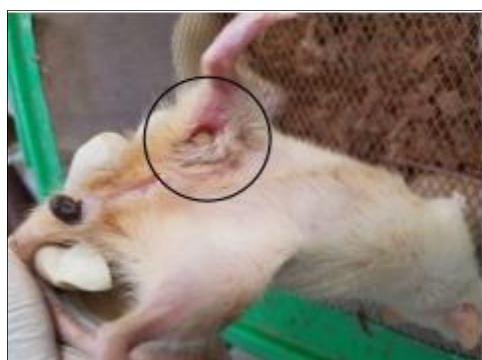

**Fig 2:** Peri-mammary tumor (0.5x0.5 cm) in the negative control group of experimental female albino rats

*Circle indicates the location of Nickel Chloride induced-tumor

Figure 3 shows the histological sections of breast tissues of the experimental albino rats. The negative control figure 3a shows Nickel Chloride induced lobular alveolar hyperplasia while figure 3e shows normocellular glands of the positive control. Figure 3b,c and d is the histological sections of rat breast treated with 50, 100 and 200 mg/kg ethanolic garlic extracts and figure 3f,g and h treated with 50, 100 and 200 mg/kg fatty acid extracts, with normal ducts lobular units in a background of adipose tissue.

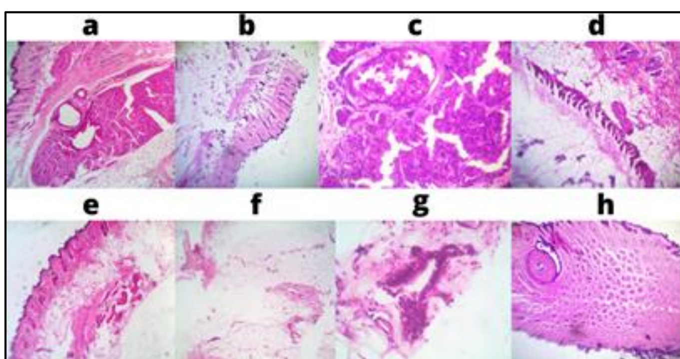


**Fig 3:** Effect of ethanolic garlic and fatty acid extract on breast tissue in Nickel Chloride induced albino rats. (a-h)

Figure shows a representative view from each group. (a) Negative Control (b)NiCl + 50 mg/kg fatty acid (c), NiCl + 100 mg/kg fatty acid (d) NiCl + 200 mg/kg fatty acid (e)Positive Control group (f)NiCl + 50 mg/kg ethanolic garlic extract (g)NiCl + 100 mg/kg ethanolic garlic extract and (h) NiCl + 200 mg/kg ethanolic garlic extract

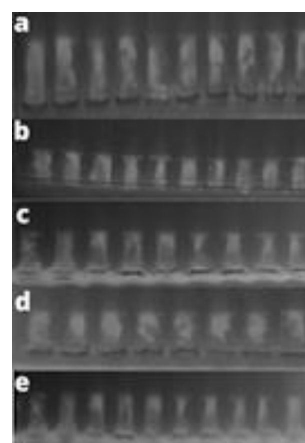


**Fig 4a, b, c, d and e:** are the electrophoretic band of the ULBP3, STX17, PRDX5, Il2 and CTLA4 genes respectively obtained from the blood of Nickel Chloride induced albino rats.

Figure 4: Electrophoretic bands of a: ULBP3 b:STX17 c:PRDX5 d:Il2 e:CTLA4 genes from the blood of Nickel Chloride induced albino rats

## 4. Discussion

Phytotherapy remain a commonly accessible means of treatment of diseases especially in low and middle income countries. While Garlic (*Allium sativum*) has gained reputation in the prevention and treatment of microbial infections, lowering blood pressure, diabetes, and cancer among other ailment [21, 22, 23]. Presence of phytochemicals such as Flavonoids, Glycosides, Alkaloids, Saponin, and Tannins which were reported in the qualitative phytochemical analysis of ethanolic extract of Garlic (*Allium sativum*) are in agreement with that of Ali and Ibrahim 2019 except for anthraquinones absent in our study [24]. These phytochemicals are known to exhibit remarkable medicinal effects, as reported in other medicinal plants [25]. Allicin (diallyl-dithiosulfinate) a sulfur-containing volatile component of garlic and an alkaloid have been generally regarded as the therapeutic agent in Garlic bioactivity [26, 27]. Allicin has a broad spectrum of cellular targets, particularly of interest is that report have shown it is effectiveness against microorganisms and cancer [28, 25].The chemotherapeutic effect of allicin against NiCl2-induced DNA damage may also be attributed to its ability to stimulate immune effector cells which includes T- cells [29].

The tumor outgrow seen in albino rats five weeks after injection with Nickel Chloride suggests the carcinogenic

effect of Nickel Chloride and it contribution to tumorigenesis initiation at the region. The Nickel Chloride induced albino rats treated with the fatty acid Extract show a general increase growth in weight, this suggest accumulation of the larger percentage of the fatty acid in the tissue of the animals. The influence of fatty acid on weight can neither be attributed to toxic effects. However, fluctuation in weight observed in some group that receive the treatment probably resulted from the adjusting action of the animal to stress. We suggest plant extracts should be defatted before use. At 100 mg/kg administration of garlic extract, there was steady growth of the rats. The lower 50 mg/kg garlic extract which resulted in a decline in weight and 200 mg/kg with repressed growth, implies that the amount of Garlic ingested need to be control to achieve optimum nutritive effect. However, readings from the growth studies allow is unclear as to the definite chemo-preventive effect of garlic extract against related pathological metabolism of nickel. Majority of the earlier reported bioactive component of Garlic are present in polar and mid polar medium [30, 31], this implies no nutritional or chemo-preventive effect were exhibited by the fatty acid increase in weight.

This study confirms the carcinogenic nature of Nickel chloride salt, observed from the negative control group. Only rats from this group had tumour growth on the mammary gland as seen in figure 2. Different nickel compounds have also been reported to induce carcinogenesis 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 [32]. The histomicrograph of the tissue obtained from the breast region shows variation in Nickel Chloride pathology. The positive control group indicates the feature of a normal breast tissue histological section without any neoplastic change. According to Sloane *et al*. 1994 the histopathological classification of breast tissue carcinoma is subjective, despite an attempt to provide clear guidelines the inter-observer variability is known to be high and there is a lack of uniformly agreed criteria [33, 34]. Neoplastic transformation is nevertheless accounted for as an indication of nickel compound-induced cell proliferation which is in accordance to the report given by Salnikow and Zhitkovich (2008) [35]. Treatment with Garlic extract at 100 mg/kg improved the histological changes of breast cancer-induced by reduced proliferative indexes of cancer-induced Nickel Chloride with moderate effect on growth. Previous studies indicated that Garlic extract (Allicin) inhibits the growth of tumor in mice. Garlic extract in fighting malignant cancer cells has been reported to increases caspase-3 activity in the human cancer cell lines, such as in hepatic, colon, prostate, and breast cancer [37]. The PCR product bands observed in the agarose gel implies that the genomic deoxyribonucleic acids (DNAs) isolated from experimental animals in the groups amplified (Figure 4). The speculated DNA damage might however not be in accordance to the literature research report given by Guo *et al*. 2019 that NiCl2 and Ni compounds induces DNA damage associated with carcinogenesis [36]. Therefore, the exact mechanisms of DNA damage caused by NiCl2 and Ni compounds are still unclear. However, the knowledge obtained from this study serves as a resource base and can be scientifically exploited for future research in Nickel chloride tumorigenesis and prevention.

## 5. Conclusions

In conclusion, Natural products found in medicinal plants have great promise for the treatment of cancer. Garlic extracts have chemo-preventive properties in the impediment of tissue cancer initiation. However, it fatty acid constitute fat deposition in tissues, which could be detrimental to it ameliorative efficacy. Further studies need to be done to optimize the quality of extract, effective dose and its specificity on especially in cancer susceptibility genes.

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