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Anti-proliferative potential of Aqueous Leaf Extract of *Ocimum gratissimum* on L-ARG+MSG induced Prostate Cancer in Male Albino Rats targeting ANAX7 geneMinari, J.B.^{1*} and Egwu, F.L.¹*Department of Cell Biology and Genetics University of Lagos, Lagos State, Nigeria¹**Author for Correspondence*: jminari@unilag.edu.ng***Abstract**

Prostate cancer is a heterogeneous disease with varied morphological appearances, molecular features, behaviour, and response to therapy. The aim of this study was to evaluate the anti-proliferative potential of aqueous extract of *Ocimum gratissimum* leaves on L-ARG+MSG induced prostate cancer in male albino rats. Aqueous leaf extract of *Ocimum gratissimum*, twenty-five (25) male albino rats, L-Arginine and Monosodium glutamate given at 90:30mg/kg/bwt were used in this work. Phytochemical screening of aqueous leaf extracts of *Ocimum gratissimum* reveals the presence of Saponnins (13.87 ± 0.19) as highest while Phenol (8.50 ± 0.06) was the least. Gel electrophoresis showed that DNA bands obtained suggested a possible L-ARG+MSG-induced damage which was significantly prevented owing to the effect of the aqueous leaf extract of *Ocimum gratissimum* in dose dependent manner. The histological examination showed layer of gland with large lumen, many stroma and few epithelial nuclei and layer of small large lumen. The Prostate specific antigen (PSA), was elevated in the group induced with the L-Arginine and Monosodium glutamate but were progressively reduced in those treated with increasing concentrations of the extract. This study has shown that the leaf extract could significantly reduce the proliferation of cell against L-ARG+MSG- induced prostate cancer in male albino rats.

Keywords: Prostate Cancer, L-ARG, MSG, *Ocimum gratissimum*, PSA, ANAX7 gene

Introduction

Cancer is a group of genetic and cellular disease usually caused by the fundamental defects in the regulation of cell division and characterised by cell proliferation (Cairns *et al.*, 2011). Among the chief cause of cancer is the disruption of normal cell behaviour by mutations (Chen *et al.*, 2011). DNA damage arises by the spontaneous cleavage of chemical bonds in DNA, by reaction with genotoxic chemicals in the environment (Ananya, 2012). DNA damage can also arise as a result of the reaction with certain chemical by-products of normal cellular metabolism (Kim *et al.*, 2011b). Prostate cancer is the second most frequent cancer to occur in men worldwide after lung cancer (Ferlay *et al.*, 2008). According to Jemal *et al.* (2011), Prostate cancer is one of the most common malignancies and third leading cause of cancer deaths in developed countries. Prostate tumours development and progression involve a complex series of events, including dysregulation of cellular differentiation, excessive proliferation and resistance to apoptosis (Cairns *et al.*, 2011). Annexin 7 is a candidate tumour suppressor gene (TSG) implicated in prostate cancer and function in a number of cellular processes involving calcium signalling (Olufemi and Olayiwola, 1999). It is the most evolutionary conserved member of the annexin gene family and located on human chromosome 10 in the q21 region (Woo and Kim, 2013). ANX7 a biomarker gene in prostate cancer progression and is down regulated during prostate malignancy.

The main treatment for prostate cancer is radical prostatectomy in which the tumour is surgically

removed. Treatment option such as surgery and radiation therapy are effective against early stages of prostate cancer, patients with hormone-independent advanced stage prostate cancers typically face a poor prognosis with limited treatment options (Damber and Aus, 2008). Anti-androgen therapy is a treatment option which initially reduces prostate cancer cell proliferation, but castration-resistant prostate cancer will develop eventually (Sharifi *et al.*, 2010). The therapeutic options for castration-resistant prostate cancer are limited and treatment responses to currently existing therapies are often unsatisfactory and cytotoxic, causing severe toxicity and eventual chemoresistance (Berthold *et al.*, 2008). Therefore, there is a need to search for alternative therapeutic options to target advanced and metastatic prostate cancer cells.

Cancer chemoprevention with natural phytochemical compounds is an emerging strategy to prevent, impede or cure cancer. The use of plants such as *Ocimum gratissimum* whether herbs, shrubs or trees on parts or whole in the treatment and management of diseases and disorders date back to prehistoric days (Akindele and Busavo, 2011). The overall aim of this research was to determine the anti-cancer properties of aqueous leaf extract of *Ocimum gratissimum* on prostate cancer induced albino rat.

Materials and methods

Plant material: A sample of fresh leaves of *Ocimum gratissimum* were collected in a Garden within Yaba area, Lagos Nigeria in the month of December 2015. The plant was identified and authenticated by a taxonomist (Herbarium Unit of Botany Department), UNILAG, Lagos where a voucher specimen number (LUH 6335) was deposited.

Chemical used: L-Arginine, Monosodium glutamate was purchased from Mosco Chemical Store Bariga, Lagos, Nigeria, the primer and DNA extraction kits were purchased from Jena Bioscience, Germany. All other chemicals were obtained commercially and are of analytical grade.

Methodology

Preparation of plant extract and extraction:

The aqueous extracts of the plant were obtained using the Hot Extraction Method as described by Yadav and Agarwala (2011).

Induction: All the experimental Rats used for this work were purchased from the Department of Zoology University of Lagos. The experimental animals were given calculated doses of L-Arginine and Monosodium glutamate in ratio 3:1 at (90:30 mg/g/bwt) concentrations intragastrically by gavage method as described by Alfredo *et al.* (2004). The animals were bred in our laboratory under ideal conditions of temperature, humidity, and light, and they were fed with appropriate ration in pellets and filtered water.

Experimental design: Animals (25 male albino Rats) with average weight of 150- 250g were classified into groups, of 5 animals each. The groups were as follows:

Group PC (Positive control) Control experiment feed with water and food

Group NC (Negative control) L-ARG and MSG Induced rats only

Group TA -L-ARG and MSG Induced rats treated with *O. gratissimum* extract (300 mg/g b wt)

Group TB -L-ARG and MSG Induced rats treated with *O. gratissimum* extract (400 mg/g b wt)

Group TC -L-ARG and MSG Induced rats treated with *O. gratissimum* extract (500 mg/g b wt)

Plant extract administration and treatment:

After induction with L-ARG and MSG, group TA, TB and TC were administered and treated with daily doses of (300, 400 and 500) mg/kg body-weight of the aqueous plant extract *Ocimum gratissimum* leaves by gavage method for a period of 15 consecutive days respectively.

Experimental site: The work was carried out in the animal house (Botanical Garden), University of Lagos, 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 experiment with whole animals.

Phytochemical screening of the plant extract

Preliminary phytochemical screening (Qualitative and Quantitative analysis) of the aqueous leaf extract of *Ocimum gratissimum* was carried out by methods of analysis described by Horborne (1973) and Sofowara (1993).

Histological Determination

For microscopic evaluation, liver and prostate tissues were fixed in a fixative (10% formal saline) and embedded in paraffin, sectioned at 4 to 5 μm and subsequently stained with haematoxylin/eosin. Sections were studied under light microscope at 40 and 100 magnifications. Slides of all the treated groups were studied and photographed. All specimens were compared histologically with a reference (Ito, 1986)

Statistical analysis

Statistical analysis of data was assessed by One-way Analysis of Variance (ANOVA) using the GraphPad Prism software package 5.0 for windows from Graphpad Software Inc. Newman-Keuls Multiple Comparison Test was used to determine the group which showed the most significant reduction in weight as shown in the appendix. The results were expressed as the mean \pm SEM for the 5 rats in each group. Values of $p < 0.05$ were considered statistically significant. The DNA migration distances and fragment sizes were determined using Excel.

Prostate specific antigen

The PSA Enzyme Immunoassay is a solid phase two-site immunoassay. The principle of this test is based on the formation of the coated antibody-antigen-antibody-enzyme complex.

DNA Extraction

The DNA extraction protocol used for this study was a modified procedure (Nishiguchi *et al.*, 2002).

Primer

The primer used for this work was bought from Jena Bioscience (Germany). The sequences are: Forward primer (ANXA7F) - 5' CAC CTGGGCTGTGACGCTGCT3' and Reverse primer (ANXA7R) - 5' CCCTCCTACTGGCCCAATAGCC 3'.

DNA Agarose Gel Electrophoresis (AGE)

Agarose (1.5g) was weighed into 100ml TBE buffer to give 1.5 percent agarose. It was then autoclaved for 3minutes. After the solution had cooled, 20 μl of SYBR safe dye was added and then poured into the electrophoretic chamber after which it was allowed to solidify. DNA ladder was loaded in the first well to monitor the sizes of the fragments. The analysis was done at 100V for 1hr 30minutes.

PCR Amplification of Annexin VII (ANXA7) gene

PCR was carried out in 0.40M Tris Hcl, 0.1M Ammonium sulphate, 1.5Mm Mgcl₂, 1Mm dNTPs of each 200 μM dATP, dCTP, dTTP and dGTP, Blue, yellow dyes and DNA polymerase. Amplification was performed using Peltier Thermal Cycle PTC-100 from MJ Research in a total reaction volume of 50 μl containing 10 μl master mix, 34.6 μl dd-water, 0.2 μl each of forward and reverse primer and 5 μl of DNA. Amplification was done with an initial denaturation temperature of 94 $^{\circ}\text{C}$ for 3min followed by 30 cycles: denaturation at 94 $^{\circ}\text{C}$ for 1min, annealing at 61 $^{\circ}\text{C}$ for 1min, extension at 72 $^{\circ}\text{C}$ for 4min and 1 cycle of final extension at 72 $^{\circ}\text{C}$ for 15min. PCR product was resolved by agarose gel electrophoresis and purified with QIAquick Gel Extraction Kit (Jena Bioscience, Germany) according to manufacturer's protocol.

Results

The result obtained for the qualitative phytochemical screening of the aqueous leaf extract of *Ocimum gratissimum* is shown in Table 1. The phytochemical screening revealed that alkaloids, reducing sugar, flavonoids, saponins, tannins, and phenol were present while isoprenoids, anthraquinones, cardiac glycosides and Steroids was absent. The quantitative phytochemical analysis of selected phytochemicals present in the aqueous extract of *Ocimum gratissimum* is shown in Table 2. Alkaloids were found to be present in the highest amount (11.43 \pm 0.09) while phenol was found to be in the lowest amount (8.50 \pm 0.06). Serum prostate specific antigen (PSA) activities are shown in Table 3. The NC (negative control) induced with L-ARG+MSG PSA activities were found to be present in the highest value (1.7 \pm 0.4099), the PC (positive control) has PSA

activities of 1.1 ± 0.3564 . The PSA activities of TA (induced and treated with 300mg of extract), TB (induced and treated with 400mg of extract) and TC (induced and treated with 500mg of extract) are 1.6 ± 0.2588 , 0.9 ± 0.1924 and 1.2 ± 0.2074 respectively. Table 4 shows the effect of different concentrations of aqueous extract of *Ocimum gratissimum* on absolute Increase and variation in body weight of male albino rats administered with L-ARG+MSG. There was a significant reduction ($p < 0.05$) in the weight of rats that received 300mg/g b wt dose of the extract when compared with those that took neither of the extract nor L-ARG and MSG (positive control) within days 3, 6, 12 and 15. Animals that received both L-ARG+MSG and treated with 500mg of extracts showed a significant increase in weight ($p > 0.05$) within days 3, 6, 9, 12 and 15. However, there was decrease in weight, sickling signs and weakness observed the first 12 days after L-ARG and MSG administration in the animals of the negative control. The effects of aqueous leaf extract of *Ocimum gratissimum* on the survival rate of rat induced with prostate cancer using L-ARG+MSG is also shown in Figure 1. There was no death recorded in the first two days. Death was recorded in the NC (Negative control) group in day 5, 7, 11, 13 and 15 of L-ARG+MSG administration. The highest number of deaths was recorded between day 3 to 9. However, there was equal death in NC, TA and TC. Histological section of the prostate tissue of experimental rats from the positive control (PC) is shown in Plate 1. This revealed the presence of Layer of Gland with large lumen, many stroma and few epithelial nuclei (HE-100x) magnification. Plate 2 shows the histological section of the prostate tissue of L-ARG+MSG induced-prostate cancer in rats from negative control (NC). This revealed the presence of Layer of small, large lumen, few

stroma and many epithelial nuclei. The histological section of the prostate tissue of L-ARG+MSG induced-prostate cancer in rats treated with 300mg/g b wt of aqueous leaf extract of *Ocimum gratissimum* is shown in Plate 3. This revealed the presence of Layer of gland with small, large lumen, few stroma and few epithelial nuclei at (HE-100x) magnification. Plate 4 shows the histological section of the prostate of L-ARG+MSG induced-prostate cancer in rats treated with 500mg/g b wt of leaf extract of *Ocimum gratissimum*. This revealed the presence of Large Glands units with many Stroma, lumen and few epithelia nuclei. Plate 5 shows the histological section of the liver tissue of L-ARG+MSG exposure in rats from TA. This reveals the presence of Layer of vascular sinusoids hepatocytes at (HE-100x) magnification. The histological section of the liver tissue of L-ARG+MSG exposure in rats from PC is shown in Plate 6. This reveals Layer of vascular sinusoids hepatocytes at (HE-100x) magnification. Plate 7 shows the histological section of the liver of L-ARG+MSG exposure in rats from TB. This revealed Layer of vascular sinusoids hepatocytes. The histological section of the liver tissue of L-ARG+MSG exposure in rats from NC is shown in Plate 8. This reveals Layer of liver with tumour cells and moderate steatosis at (HE-100x) magnification. Plate 9 shows the histological section of the liver tissue of L-ARG+MSG exposure in rats from TC. This revealed the presence of Layer of vascular sinusoids hepatocytes at (HE-100x) magnification. Plate 9 shows the effect of aqueous leaf extract of *Ocimum gratissimum* on L-ARG+MSG-induced prostate cancer using DNA gel electrophoresis of ANXA7 gene. This revealed different band patterns in PC, NC, TA, TB and TC.

Table 1: Qualitative Analysis of Aqueous Extract of *Ocimum gratissimum* Leaves.

| S. No. | Phytochemical Components | Aqueous Extract of <i>O. gratissimum</i> Leaves |
|--------|--------------------------|---|
| 1. | Alkaloids | |
| 2. | Reducing sugar | + |
| 3. | Isoprenoid | - |
| 4. | Anthraquinones | - |
| 5. | Flavonoids | + |
| 6. | Saponins | + |
| 7. | Tannins | + |
| 8. | Cardiac glycosides | - |
| 9. | Phenol | + |
| 10. | Steroids | - |

Key: (+) Presence of Phytochemical(s)
 (-) Absence of Phytochemical(s)

Table 2: Quantitative Analysis of Aqueous Extract of *Ocimum gratissimum* Leaves

| S/No | Phytochemical Components | % Composition |
|------|--------------------------|---------------|
| 1. | Phenol | 8.50 ±0.06 |
| 2. | Saponins | 13.87 ±0.19 |
| 3. | Flavonoid | 9.20 ±0.06 |
| 4. | Alkaloids | 11.43 ±0.09 |
| 5. | Tannin | 10.50 ± 0.06 |

Mean values (%) of phytochemical screening of O. gratissimum leaves showing Mean±SE

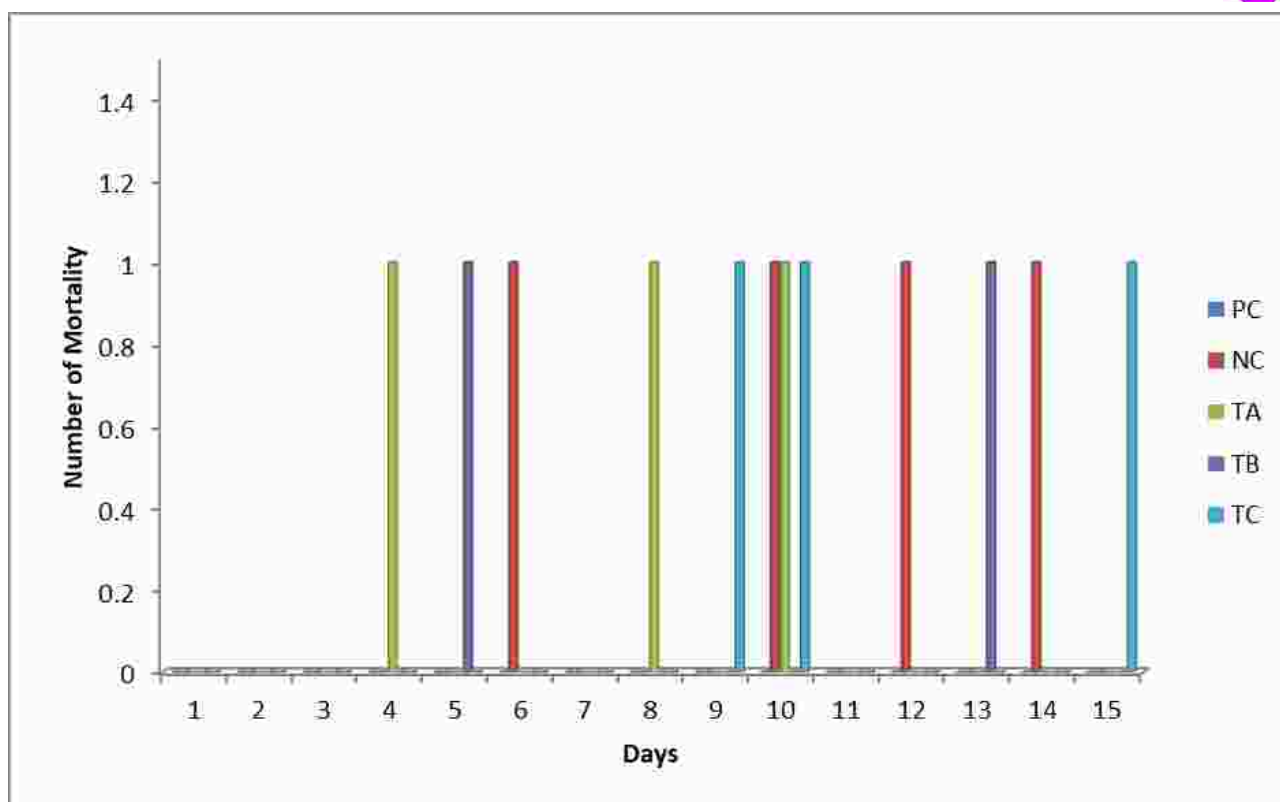


Figure 1: The mortality rate of L-ARG+MSG –induced and treated in different animal groups
Key

NC (Negative control) –Induced rats only

PC (Positive control)– Control experiment

TA– Induced rats treated with *O. gratissimum* extract (300 mg/g b wt)

TB– Induced rats treated with *O. gratissimum* extract (400 mg/g b wt)

TC–Induced rats treated with *O. gratissimum* extract (500 mg/g b wt)

Table 3: Prostate Specific Antigen (PSA) activities of L-ARG+MSG-induced and treated in different animal groups.

| S. No. | Treatment Groups | PSA Value (ng/dl) |
|--------|------------------|-------------------|
| 1. | PC | 1.1±0.3564** |
| 2. | NC | 1.7±0.4099* |
| 3. | TA | 1.6±0.2588 |
| 4. | TB | 0.9±0.1924** |
| 5. | TC | 1.2±0.2074** |

*Significantly different from PC **Significantly different from NC

Key: NC (Negative control) –Induced rats only

PC (Positive control) – Control experiment

TA– Induced rats treated with *O. gratissimum* extract (300 mg/g b wt)

TB– Induced rats treated with *O. gratissimum* extract (400 mg/g b wt)

TC–Induced rats treated with *O. gratissimum* extract (500 mg/g b wt)

Table 4: Body weights of L-ARG+MSG induced and treated in different groups

| | PC | NC | TA | TB | TC |
|-----------------|-------------|-------------|-------------|-------------|-------------|
| BASELINE | 250.20±2.36 | 250.20±3.01 | 252.60±6.23 | 249.80±2.91 | 258.40±3.45 |
| DAY 3 | 210.25±4.11 | 245.40±4.88 | 251.00±5.02 | 245.00±2.87 | 268.40±4.91 |
| DAY 6 | 211.33±2.09 | 238.20±2.81 | 250.40±4.46 | 241.32±2.55 | 260.75±4.03 |
| DAY 9 | 196.50±4.71 | 225.80±3.33 | 243.50±3.27 | 247.00±2.83 | 259.33±3.77 |
| DAY 12 | 205.50±3.81 | 208.80±3.69 | 188.67±3.11 | 250.50±3.01 | 259.33±2.63 |
| DAY 15 | 206.50±3.60 | 209.80±4.01 | 189.69±2.04 | 251.50±3.22 | 260.44±2.96 |

Key : NC (Negative control) – Induced rats only

PC (Positive control) – Control experiment

TA– Induced rats treated with *O. gratissimum* extract (300 mg/g b wt)

TB– Induced rats treated with *O. gratissimum* extract (400 mg/g b wt)

TC– Induced rats treated with *O. gratissimum* extract (500 mg/g b wt)



Plate 1: Histological Section of the liver L-ARG+MSG-induced in rats from PC (He100x) (Layer of gland with large lumen, many stroma and few epithelial nuclei)

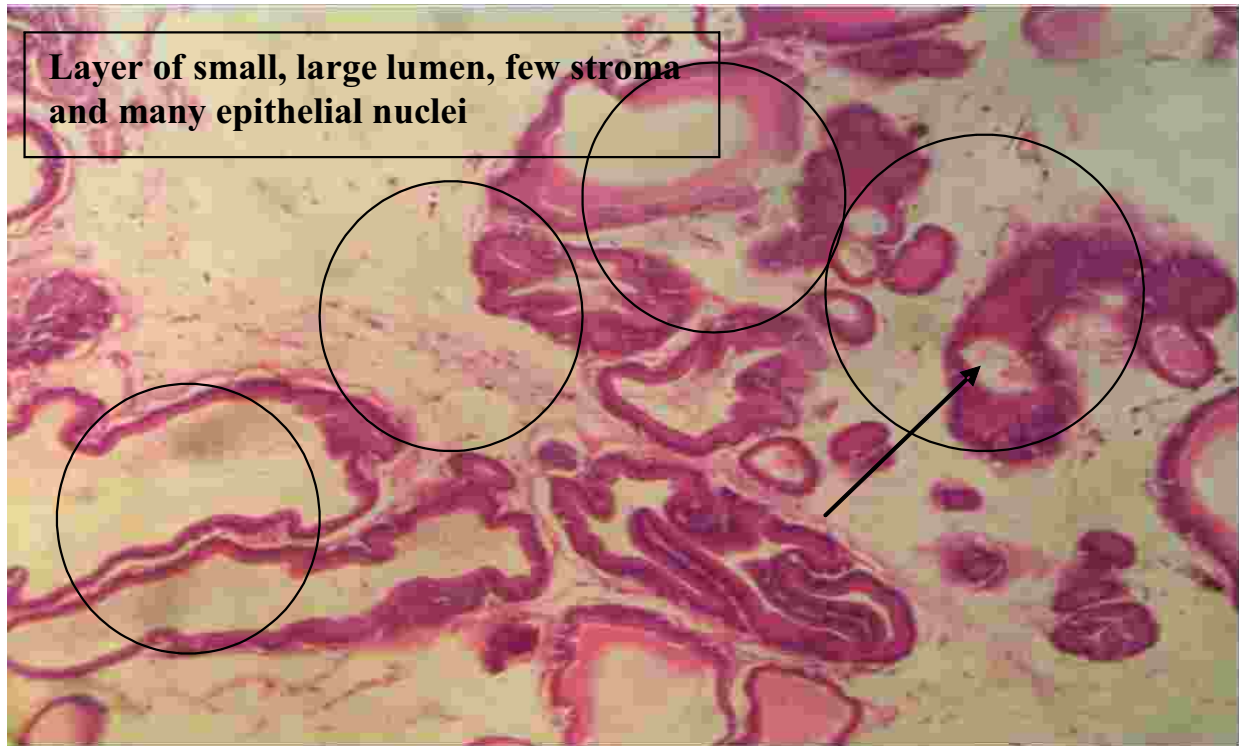


Plate 2: Histological Section of the liver L-ARG+MSG-induced in rats from NC HE-100x (Layer of small, large lumen, few stroma and many epithelial nuclei)

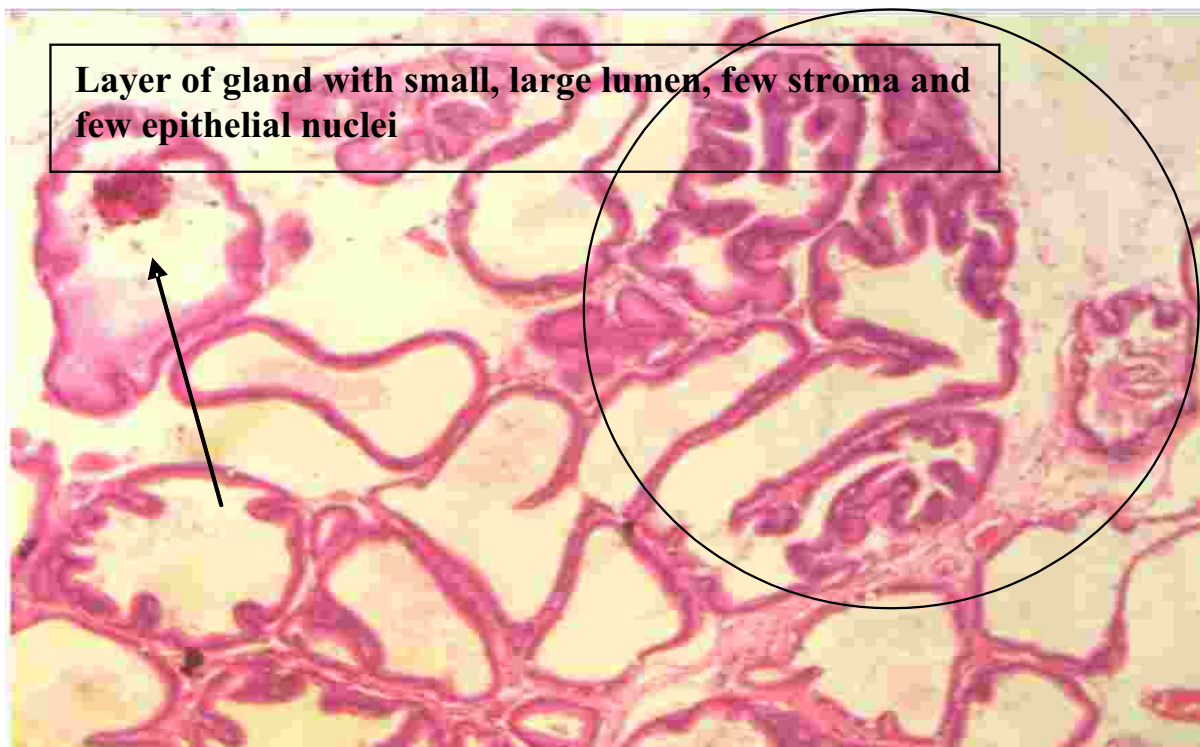


Plate 3: Histological Section of the prostate tissue of L-ARG+MSG-induced in rats from TA HE-100x (Layer of gland with small, large lumen, few stroma and few epithelial nuclei)

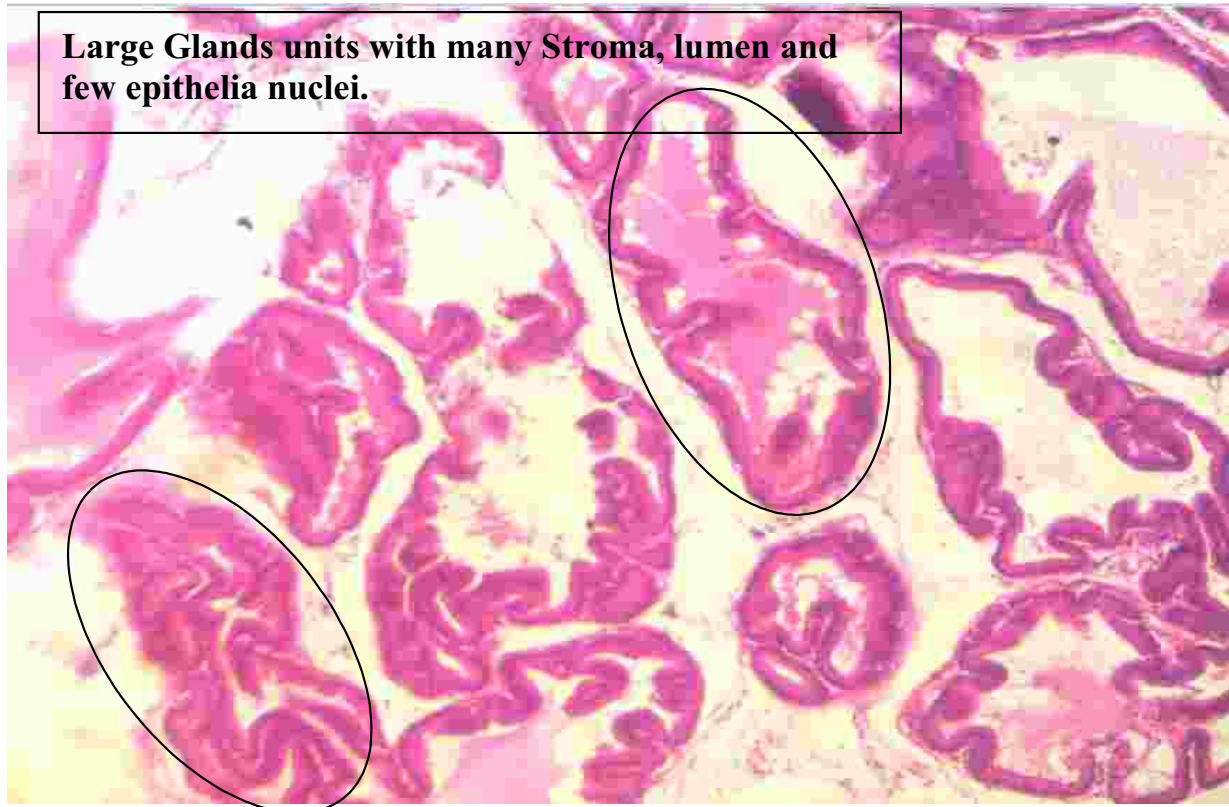


Plate: 4 Histological Section of the Prostate Tissue of Induced-Prostate Cancer in Rats from TC (HE-100x) (Large Glands units with many Stroma, lumen and few epithelia nuclei)

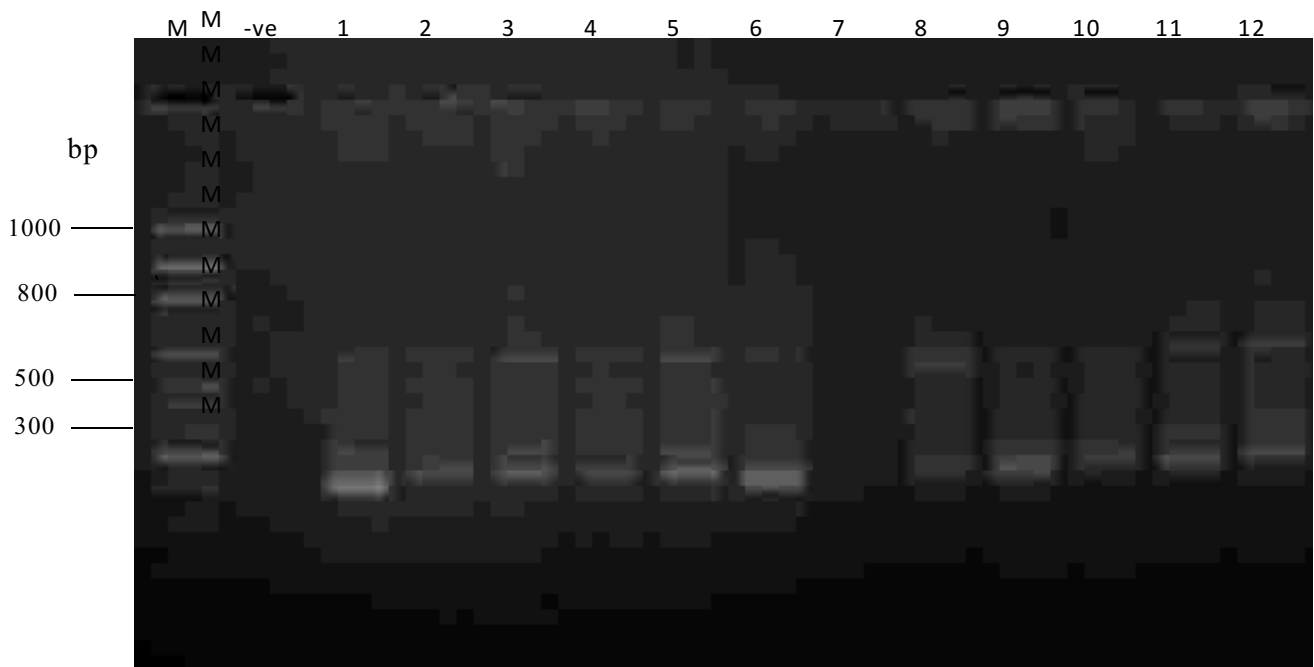


Plate 5: Effect of aqueous leaf extract of *Ocimum gratissimum* on L-ARG+MSG-induced prostate cancer on different band patterns of DNA gel electrophoresis of *ANXA7* gene.

Key: Lane M represents the marker.

Lane –ve represent the negative lane without DNA.

NC (Negative control) –Induced rats only, bands represented in lane 5 and 6

PC (Positive control) – Control experiment, bands represented in lane 1 and 2

TA– Induced rats treated with *O. gratissimum* extract (300 mg/g b wt), bands represented in lane 3 and 4

TB– Induced rats treated with *O. gratissimum* extract (400 mg/g b wt), bands represented in lane 7 and 8.

TC –Induced rats treated with *O. gratissimum* extract (500 mg/g b wt, bands represented in lane 9, 10, 11, 12.

Discussion

The chemopreventive potential of naturally occurring bioactive compounds have been demonstrated with trials in prostate, breast and colon cancer (Steward and Brown, 2013). Compounds that inhibit cancer initiation are traditionally termed 'blocking agents'. Anticancer potentials of many medicinal plants at sub pharmaceutical doses could synergize to delay or disrupt the development of aggressive disease (Lu *et al.*, 2004). Medicinal plants possess biological properties such as antiapoptosis, antiaging, anticarcinogen, anti-inflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han, 2007). Due to this development, the anti-proliferative potential of aqueous leaf extract of *Ocimum gratissimum* on L-ARG+MSG-induced prostate Cancer in male Albino Rats' was evaluated. L-Arginine and Monosodium glutamate are important components of diets and drugs. Phytochemical analysis of the result presented in this study confirm the presence of constituents such as alkaloids, reducing sugar, flavonoids, saponins, tannins and phenols (Table 1) which are known to exhibit remarkable medicinal as well as physiological activities with specific mode of action. This is in an agreement with Adewole (2014) on the proximate and phytochemical constituents of *Ocimum gratissimum*. However, the presences of alkaloid in this study contradict the report of Afolabi *et al.* (2007) who reported the absence of alkaloid in his work. This could be due to the fact that, the location of the plant might be a significant factor in determining the presence or absence of essential phytochemicals. Environmental factor such as interactions with micro and macro elements as well as the biosynthetic mechanism is another possible factor. However, the presence of saponins and flavonoids in this work agrees

with the report of Effriam *et al.* (2000), who reported their presence to exhibit antioxidant activity and as effective scavengers of free radicals. The quantitative phytochemical screening of selected phytochemicals present in the aqueous extract of *Ocimum gratissimum* is shown in (Table 2). Alkaloid was found to be in highest amount (11.43 ± 0.09) while phenol was found to be in lowest amount (8.50 ± 0.06). The relative high amount of alkaloids (an antioxidants/free radical scavenger) could possibly be responsible for the chemopreventive property of the aqueous leaf extract *Ocimum gratissimum* against L-ARG+MSG induced-prostate cancer. Flavonoids are important group of polyphenols widely distributed among the plant flora. Structurally, they are made of more than one benzene ring in its structure (a range of C15 aromatic compounds) and various reports support their use as antioxidants or free radical scavengers (Kar, 2007). Phenol was quantitatively determined to be (8.50 ± 0.06). Bishayee *et al.* (2013) reported the anti-proliferative effects of phenol in various cell lines. The presence of phenol exerts its anti-proliferative potential during cancer progression in the prostate tissues. Polyphenols particularly are among the diverse phytochemicals that have the potential in the inhibition of carcinogenesis (Gross, 2004). The significant reduction in the body weight of experimental rats that took 300mg/ml and 400mg/ml of extract could be a possible indication of toxicity of the aqueous extract of *Ocimum gratissimum*. (Table 4). The extract may have been metabolised to a toxic end product which could have interfered with the gastric function and decreased found consumption efficiency (Chokshi, 2007). Interesting, the significant increase in the body weight of rats that received 500mg/ml observed in Table 4, could be due to the effectiveness of the aqueous leaf extract of *O. gratissimum* in activating the immune system to overpower the

toxicity level (Coler, 2011). The significant reduction in the weight of the negative group could be attributed to the genotoxic effect of L-ARG+MSG administered to the rats. This results in the overpowering of the immune system (Coler, 2011). The high mortality rates shown in figure 1, may be strongly linked to the combined toxicity of both the L-ARG+MSG and the extracts. This is in agreement with the report of Chokshi (2007) who reported that, phytochemicals extract of plants can be metabolised to a toxic end product (Chokshi, 2007). The adverse effect of arginine and monosodium glutamate has also been demonstrated in animal models (Abeer Waggas, 2009). The presence of large gland unit with large lumen, many stroma and few epithelial nuclei shown in the positive control revealed a normal prostate tissue (Gorelick, 2013). The presence of small, large lumen, few stroma and many epithelial nuclei from negative control, showed the presence of hyperplasia in the prostate tissue (Diamond *et al.*, 2004). The hyperlastic changed identified from NC could be due to the adverse effect of L-ARG+MSG (Egbonu *et al.*, 2010a). However, there were significant changes in the histological sections in groups from TA and TC. These variations might be due to the preventive effect of the different concentrations aqueous leaf extract of *O. gratissimum* administered to the rats from TA and TC. This suggest that, the antioxidant and free radical scavenging activities of various photochemical constituents detected in this study (table 2) acted significantly on the L-ARG+MSG- induced prostate cancer from TA and TC to repair the diseased prostate tissue. Table 3 showed the Prostate Specific Antigen (PSA) activities of L-ARG+MSG-induced and treated in different animal group from PC, NC, TA, TB and TC. The PSA activities of rats from NC, negative control is the highest (1.7 ± 0.4099). The elevation of PSA biomarker in detecting prostate cancer in this work agrees with report of Chen *et al.* (2011). They suggested an increase in PSA value activities is associated with prostate cancer. The PSA activities from TA, TB and TC reduced significantly when compared with the rats from NC. This suggest that, the antioxidant and free radical scavenging activities of various photochemical constituents detected in this

study (table 2) acted significantly on the L-ARG+MSG- induced prostate cancer from TA, TB and TC to lower the PSA activities. Plate 10 shows the effect of aqueous leaf extract of *Ocimum gratissimum* on L-ARG+MSG-induced prostate cancer on different band patterns of DNA gel electrophoresis of ANXA7 gene. The variation in the band patterns obtained from electrophoresis, especially in NC (Plate 5) may have arisen as a result of damage to the DNA or mutation which may have occurred as a result of the L-ARG+MSG administered. This is in agreement with previous reports (Klug, 2010; Mantoni *et al.*, 2006) which indicated that most cancer cells usually have mutations in genes regulating DNA damage responses or repair pathways and are often more susceptible to cell cycle arrest and death from treatment with genotoxic agents than normal cells. The similarities in the band patterns between lane 2 from PC and 3 from TA might be due to the prevention of excessive L-ARG+MSG-induced damage to DNA by aqueous leaf extract of *O. gratissimum*. The similarities in the band patterns between lane 2 from PC and lane 10 from TC as well as lane 12 from TB might be due to the prevention of excessive L-ARG+MSG-induced damage to DNA by aqueous leaf extract of *O. gratissimum*. These results agree with report of Espinosa *et al.* (2004) who reported that many chemotherapeutic agents such as plant-derived compounds are known to exert their effect by directly interacting with DNA or DNA-binding proteins. This interaction triggers DNA damage signalling pathways resulting in inhibition of proliferation or induction of apoptosis, depending on the extent of the damage (Kastan and Bartek, 2004). The difference in the band patterns of lane 1 and 2 from PC revealed the heterozygosity of the ANXA7 gene (Srivastava *et al.*, 2001b). The slight deviation of band patterns in lane 5 and 6 from NC, when compared to lane 1 and 2 from PC revealed lost in heterozygosity. This agrees with previous reports (Srivastava *et al.*, 2003; Zenklusen *et al.*, 1994). They reported that, prostate cancer progression is associated with loss of heterozygosity in ANXA7 gene. The homozygosity displayed in lane 3 and 4 from TA, revealed the inability of the aqueous leaf extract of *O. gratissimum* to restore the heterozygosity

of ANXA7 gene at 300mg concentrations. Lanes 9, 10, 11, and 12 from TC revealed heterozygosity in ANXA7 gene. This suggests the effectiveness of the aqueous leaf extracts at 500mg concentrations. This might be due to the prevention of excessive L-ARG+MSG-induced damage to DNA by aqueous leaf extract of *Ocimum gratissimum*.

Conclusion

The present study showed that the aqueous extract of *Ocimum gratissimum* leaves can be used as a preventive agent against L-ARG+MSG-induced prostate cancer in male albino rats. Further research is required to address some shortcomings including small sample size of this study to validate reliability and elucidate the underlying molecular mechanism of the present observations in animal models.

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