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Research Article

CYTOTOXIC EFFECTS OF FERMENTED AFRICAN LOCUST BEAN SEEDS ON A BREAST CANCER CELL

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ABSTRACT

Objective: There is a growing scientific evidence of the health-enhancing benefits of fermented food especially in cancer treatment and prevention. Fermented African locust beans (FALB) are a condiment with many medicinal activities and consume in many West African countries. Breast cancer is the most common cancer among women globally. This study investigated the cytotoxicity of FALB extracts on breast cancer (MCF-7) cells overtime and at different concentrations.

Methods: The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to quantify the extent of cytotoxicity of the extracts.

Results: The aqueous extract of FALB had an half maximal inhibitory concentration (IC_{50}) of 1.51 and 0.98 mg/mL on exposure for 24 and 48 h, respectively, against MCF-7 cells. Comparatively, the IC_{50} obtained for the same extract against normal human fibroblasts was 1.90 and 1.37 mg/mL, respectively.

Conclusion: The results obtained here suggest some measure of selective cytotoxicity by the aqueous extract against transformed as compared with normal cells. These findings present an important lead to the usefulness of this condiment in cancer treatment. Further studies are recommended.

Key words: Fermented African locust bean seeds, Breast cancer cell, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Cytotoxicity, *iru*.

INTRODUCTION

The technology of fermentation has long been exploited for the main purpose of extending the shelf life of foods. However, some fermented products are now reported to contain enhanced bioactive components and exhibit various bioactivities including anticancer effects [1-3]. Natural fermentation of *Parkia biglobosa* (Jacq.) (African locust bean) seeds yields a popular, nutritious condiment that is used for sauce and soup seasoning in many West African countries. The fermented African locust bean (FALB) seeds (Fig. 1) are locally called "*iru*" and "*dawadawa*" in the southern and northern parts of Nigeria, respectively. It has been extensively studied with many bioactivities reported. The potential usefulness of African locust beans in the prevention, treatment, and management of various diseases (using various models) such as diabetes mellitus [4], hypertension [5], antioxidant, and hypolipidemic effect [6] has been reported.

Breast cancer is a major public health menace. It is the most common cancer among women globally and is the second leading cause of death [7,8]. Worse still, the psychological disturbances associated with diagnosis and management of breast cancer are enormous, resulting in anxiety, trauma, depression, and negative body image [9,10]. However, there has been recent attention on the role of dietary factors as a contributor to cancer growth and spread. Dietary factors are also known to play an important role in both the development and prevention of breast cancer [11]. Foods high in fiber such as fruits, uncooked vegetables, and non-sugary diet have been documented to reduce the risk of cancer occurrence. In addition, many fermented foods such as kefir [12], kimchi [13], and most recently sauerkraut, a fermented vegetable of Germany [14], have been reported to have cancer preventive properties. In light of the aforementioned, this study investigated the possible cytotoxic effect of both aqueous and methanolic extracts from FALB seeds extract on human breast cancer and normal fibroblasts cells, as a fundamental step to elucidate the potential anticancer property of the condiment.

METHODS

FALB seeds and its solvents extraction

Indigenously prepared [15] FALB seeds were obtained from a commercial producer and smoothened to paste in the laboratory using a mortar and pestle. Aqueous extract was obtained by soaking the paste in distilled water for 48 h. The supernatant was collected and the residue filtered through a mesh cloth to collect all the extracts. The extract was freeze dried to obtain a dried marc. For the methanolic extract, a known weight of the original paste was extracted with methanol (1:5 w/v) in the Soxhlet apparatus for 18 h. The methanol solution was subsequently concentrated in a rotary evaporator (Buchi RII, Switzerland) at 40°C.

Cell culture and growth

The human breast epithelial adenocarcinoma cell lines (MCF-7) and normal human fibroblast (KMST-6) were obtained from the Biotechnology Department, University of the Western Cape, South Africa. The cells were cultured in complete Dulbecco's Modified Eagle's Medium (DMEM Life Technologies, USA) supplemented with 10% fetal bovine serum (FBS Life Technologies, USA) and 1% pen-strep cocktail (100 U/mL penicillin and 100 µg/mL streptomycin, Lonza, USA) in a humidified incubator – Labotec, South Africa (5 % CO_2 in air at 37°C)

Assessment of cytotoxicity of aqueous and methanolic extracts from FALB seeds on MCF-7 and KMST-6 cells

KMST-6 and MCF-7 cells were seeded at 6×10^4/mL and 1×10^5/mL, respectively, in a volume of 100 µL (depending on their respective proliferation rate) into 96-well plates and incubated for 24 h under standard conditions. The cells were thereafter treated with various concentrations (0.5–2.5 mg/mL) of aqueous and methanolic extracts from FALB for 24 and 48 h. Cells in control wells were not treated with any extract. Cell viability after treatment was assessed using the

Fig. 1: Fermented African locust bean seeds

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma-Aldrich St. Louis, USA) assay. Here, 10 µL of 5 mg/mL MTT (dissolved in phosphate-buffered saline) was added to each well of a 96-well plate containing treated and untreated cells and then incubated at 37°C for 4 h. The formazan crystals were thereafter solubilized by adding 100 µL dimethyl sulfoxide and absorbance was read at 570 nm using the POLARStar Omega plate reader (BMG Labtech, Germany). Experiments were performed in triplicates and the half maximal cytotoxic concentration (IC_{50}) was estimated from the linear regression curve.

Cell viability was calculated as described below:

$$\%\ \text{cell viability} = \left(\frac{\text{Average absorbances of treated cells}}{\text{Average absorbances of control}}\right) \times 100\%$$

Statistics

All data were analyzed using the GraphPad Prism software version 5. Data are expressed as mean with standard error of mean. One-way analysis of variance followed by Dunnett's multiple comparison *post hoc* test was used to determine statistical significance ($p<0.05$).

RESULTS AND DISCUSSION

Results

Cytotoxicity investigations are one of the most important, fundamental validation steps to ascertain the potential toxicity of a test substance including plant extracts or biologically active compounds of diverse origin [16,17]. Such studies are particularly needful in evaluation and development of anticancer agents using various bioassays on a number of different cell lines.

Cytotoxicity of FALB seeds extract to MCF-7

Aqueous extract from fermented locust bean seeds significantly reduced the viability of MCF-7 cells in a dose-dependent manner on 24 h treatment (Fig. 2). The number of viable cells at each treatment concentration was significantly reduced ($p<0.05$) compared to that of the control group (untreated). The only exception to this is between the control and 0.5 mg/mL extract-treated MCF-7 cells. On extension of treatment time to 48 h, aqueous FALB extract was significantly cytotoxic to MCF-7 cells at the varying concentrations examined. Cytotoxic activities of aqueous extract of FALB were time dependent, the IC_{50} values were 1.51 and 0.98 mg/mL for 24 and 48 h, respectively, implying increased potency with increased incubation time.

Again methanolic extract from FALB seeds induced both time- and concentration-dependent cytotoxicity on MCF-7 cells. The number of viable MCF-7 cells was statistically significant ($p<0.05$) between the untreated and all the treated groups, meaning methanolic extract from

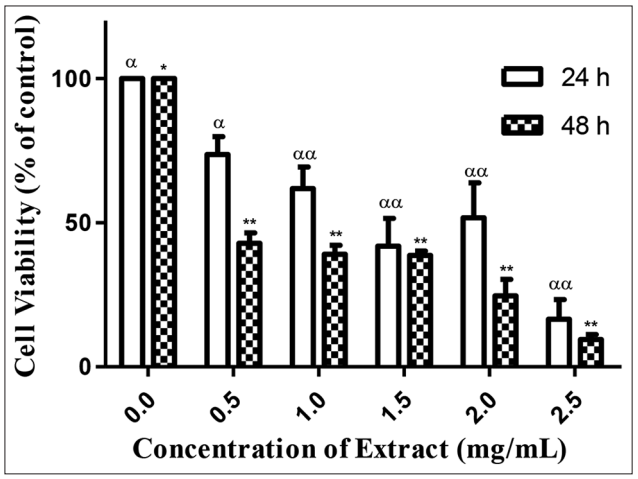

Fig. 2: Time- and concentration-dependent cytotoxic activities of aqueous extract of fermented African locust bean seeds on MCF-7 cells. †MCF-7 cells were treated with increasing concentrations of extract for 24 h; cell cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay. ‡- α and αα imply not statistically different and statistically ($p<0.05$) different from control (α), respectively, at 24 h investigation while ** implies a statistically significant difference ($p<0.05$) from control * at 48 h. §Bars represent mean±standard error of the mean of three independent experiments

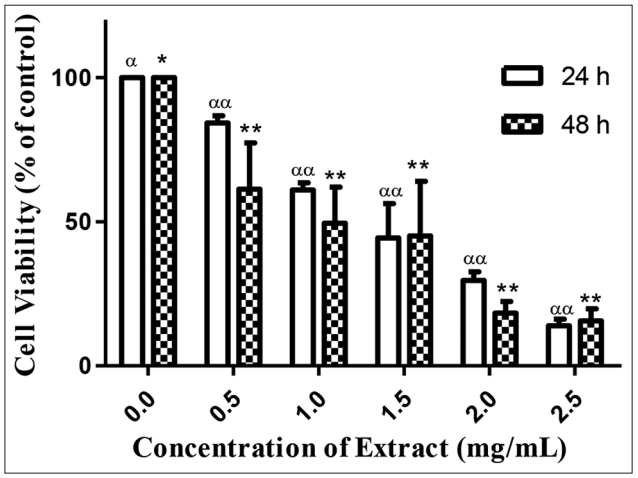

Fig. 3: Time- and concentration-dependent cytotoxic activities of methanolic extract of fermented African locust bean seeds MCF-7 cells. †MCF-7 cells were treated with increasing concentrations of extract for 24 h; cell cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay. ‡: αα implies statistical ($p<0.05$) significant difference from control (0.0) at 24 h investigation while ** means same to the control (*) at 48 h treatment. § – bars represent mean±standard error of the mean of three independent experiments

FALB seeds induced statistically significant reduction in the number of viable cells in MCF-7 cells (Fig. 3). Again, activity increased with incubation time as evidenced in the estimated IC_{50}. The latter were 1.91 and 1.28 mg/mL for 24 and 48 h treatment times, respectively.

Cytotoxicity of FALB seeds extract to KMST-6 cells

Aqueous extract from FALB seeds was again significantly cytotoxic to KMST-6 cells at all the concentrations investigated at 24 h (Fig. 4). The IC50 value was, however, higher on KMST-6 cells compared to MCF-7, estimated to be 1.90 mg/mL. This value was higher compared with what was required in the MCF-7 cells (1.51 mg/mL). Again at 48 h, there was

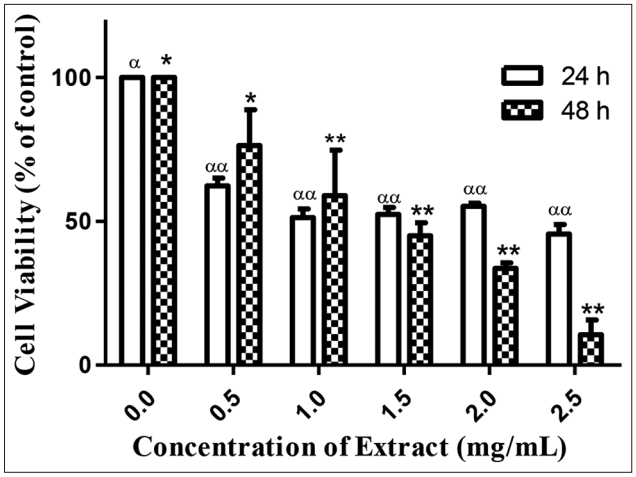

Fig. 4: Time- and concentration-dependent cytotoxic activities of aqueous extract of fermented African locust bean seeds on KMST-6 cells. †KMST-6 cells were treated with increasing concentrations of aqueous extract from fermented African locust bean seeds for 24 and 48 h; cell cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay. ‡The treatment of KMST-6 cells with aqueous extract of fermented African locust beans for 24 h elicited a statistically significant ($p<0.05$) difference between all the treated groups (αα) and the control (α). ** implies a statistically significant difference at 48 h to the control (*), on the other hand, * shows none. §Bars represent mean±standard error of the mean of three independent experiments

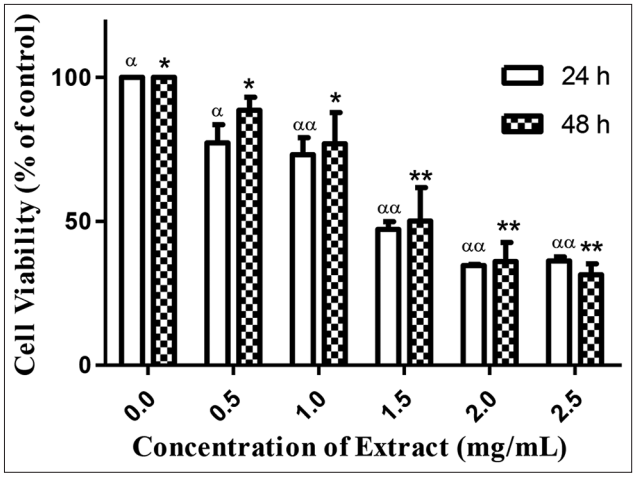

Fig. 5: Time- and concentration-dependent cytotoxic activities of the methanolic extract of fermented African locust bean seeds KMST-6 cells. †KMST-6 cells were treated with increasing concentrations of extract for 24 h; cell cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay. ‡Bars with the symbol (α) are not statistically ($p<0.05$) different from control (0.0) at 24 h while bars with αα are different. Bars with the symbol * are not significantly ($p<0.05$) different from control (0.0) while bars with ** are statistically different at 48 h investigation. §Bars represent mean±standard error of the mean of three independent experiments

no significant difference ($p<0.05$) between the number of viable cells in the untreated and cells treated with 0.5 mg/mL extract. However, at higher concentrations of extract, there were significant differences. IC_{50} at 48 h was 1.37 mg/mL.

For the methanolic extract, investigation at 24 h revealed that there was no significant difference in the number of viable cells between the untreated and 0.5 mg/mL treated cells. Beyond this value, the extract was significantly cytotoxic to the cells. The estimated IC_{50} concentration at 24 h was 1.67 mg/mL. With increase in incubation time to 48 h, the extract became less potent contrary to what was observed in MCF-7 cells. This was evidenced in IC_{50} increase to 1.71 mg/mL. Furthermore, concentrations higher than 1.0 mg/mL were required to induce statistically significant cytotoxicity to KMST-6 cells (Fig. 5).

DISCUSSION

Various *in vitro* cell-based assays have been designed and used to rapidly determine the cytotoxic activity of several compounds. This is important in that it can provide the knowledge of the relative efficacy of such agents before an empirical *in vivo* investigation [18]. One of such assays is the MTT assay. The MTT assay [19] is a high-throughput cell-based sensitive, quantitative, and reliable colorimetric assay that measures viability, proliferation, and in turn cytotoxicity in cells. The assay is based on the capacity of mitochondrial dehydrogenase enzymes in viable/living cells to convert the yellow water-soluble substrate MTT into a water-insoluble, dark blue/purple formazan product. The latter precipitates in the cellular cytosol can be dissolved after cell lysis and measured, whereas cells being dead following a toxic damage, cannot transform MTT. The quantity of the product formed is directly proportional to the number of viable cell and inversely proportional to the degree of cytotoxicity. The reaction is mediated by dehydrogenases enzymes associated with the endoplasmic reticulum and the mitochondria [20].

In this study, time- and concentration-dependent cytotoxicity of aqueous and methanolic extracts of FALBs on MCF-7 and KMST-6 cells was investigated using MTT assay. The aqueous extract, the readily edible state of the condiment induced statistically significant cytotoxicity to both MCF-7 and KMST-6 cells. However, the IC_{50} in both cells give clues to the extent of cytotoxicity. Higher values were obtained in both treatment time frames on the KMST-6 cells compared with the MCF-7 cells. This is indicative that the extract was more cytotoxic to the latter compared with the former. It is also interesting to know that aqueous extract activity increased with time.

In contrast to the aqueous extract, the methanolic extract exhibited an even more discriminatory pattern of activities between cancer and non-cancer cells. Cytotoxic activity of methanolic extract of FALB was both time and concentration dependent on MCF-7 cells. Statistically significant activities were obtained at all the concentrations investigated on MCF-7 unlike in KMST-6 cells. Concentrations above 1.0 mg/mL were required to induce significant cytotoxicity on KMST-6 cells at 24 and 48 h, respectively. Again cytotoxic activity of methanolic FALB on KMST-6 cells did not increase with time unlike in MCF-7 cells. In all, KMST-6 cells showed relative resistance to the cytotoxic effect of methanolic FALB. Put together, these results show that FALB extracts exhibited higher cytotoxic effect on the MCF-7 cell line, whereas low cytotoxicity on the KMST-6 cells. Kuete *et al*. (2018) [21] previously reported that catechin derivatives extracted from *P. biglobosa* were selectively active against leukemia cell lines. In addition, cytotoxic effects of other spice and fermented products similar to locust bean seeds have been reported. For instance, the cytotoxicity of saffron (dried stigmas of *Crocus sativus* L.) to HepG2 and HeLa cell lines has been reported [22]. Similar to FALB seeds, saffron is commonly used as a spice, food colorant, and medicinal plant. Fermented soybean food – Doenjang, was also reported to have antimutagenic and cytotoxic effect on cancer cells [23]. The report further suggests that the anticancer activity might have been acquired through the fermentation process.

Functional microorganisms are reportedly involved in the natural fermentation of foods and beverages. These microorganisms are capable of transforming the chemical constituents of raw materials from animal/plant origin during food fermentation, thus improving bioavailability of nutrients as well as sensory, nutritional, and health-promoting properties of such food [24,25]. The results of this study

add to the increasing body of scientific data pointing to availability of nutritive, functional compounds in many fermented foods. These compounds are known to potentially modulate specific target functions in the body when consumed, thereby contributing to the health and well-being of consumers [24].

CONCLUSION

Conclusively, to the best of our knowledge, this is the first attempt to investigate bioactivity of this condiment on breast cancer cells, this study found that aqueous and methanolic extracts of FALB seeds are significantly cytotoxic to breast cancer cells. This is a promising lead into further investigation, isolation, characterization, and development of FALB seeds into useful nutraceutical product(s) with cytotoxic potential on cancer cells.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

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