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Original Article

Cell viability and genotoxic potential of *Kigelia africana* fruit: implications for traditional medicine safety

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ABSTRACT

Traditional medicine plays a crucial role in healthcare and disease management in African communities; however, the quality and toxicity of these remedies remain largely unknown, particularly concerning the chronic conditions that are managed by traditional medicines. Despite the widespread use of *Kigelia africana* as a traditional medicine for chronic conditions, its genotoxic and mutagenic effects remain unclear. This study aimed to address this knowledge gap by examining the genotoxic and mutagenic properties of aqueous and ethyl acetate crude extracts of *Kigelia africana* fruits obtained from the Kazungula District of Zambia. The study used quantitative fluorescence microscopy to determine mutagenicity and genotoxicity and compared the results with the Ames results obtained in this study. The study found that the aqueous extract had no effect on cell viability, while the ethyl acetate extract caused a dose-dependent reduction in cell viability. The effects of the fruit extracts on His+ revertants were comparable to those of the negative control, showing no mutagenic effects. The study also found that the average nuclear area of Vero cells increased significantly with the highest concentration of the ethyl acetate fruit extract, but there was no significant effect on the multi+dual/mononucleated Vero cell ratio following a 48-hour exposure. This study demonstrated for the first time that *K. africana* fruit extracts are not cytotoxic and do not have mutagenic potential and that the extracts do not exhibit genotoxic effects at therapeutic doses.

KEYWORDS: traditional medicine, *Kigelia africana,* mutagenic effect, genotoxic effect, cell viability, His+ revertants.

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INTRODUCTION

There is a significant incidence of herbal medicine use among African patients. According to a recent

study conducted by Hikaambo *et al.* (2022), a significant proportion (approximately 92%) of individuals diagnosed with type 2 diabetes in Zambia utilize herbal therapies. This observation was made within the context of the country's main referral hospital [1]. According to a study conducted by Logiel *et al.* (2021), a significant proportion (68%) of seniors in Uganda opt for traditional medicine as the primary form of treatment for various ailments [2]. Notwithstanding the documented prevalence of traditional medicine use for disease management and prevention among African patients, there is inadequate research investigating the quality, dose, and toxicity profiles of these traditional medicine practices [3, 4].

Kigelia africana Lam [Benth.] is a plant that has been endorsed by traditional African medical practitioners for its therapeutic potential in addressing a range of health issues, including chronic conditions like diabetes [5-7]. Several studies have established the effectiveness of *Kigelia* extract [8-10]. However, research on the safety profile of fruits is limited [11]. Abdul-Hafeez *et al.* (2018) investigated the mutagenic potential of *Kigelia* stem and leaf extracts [12]. However, caution should be exercised when extrapolating these findings to fruit extracts because of the potential variations in phytochemistry between the fruit and bark/stem [13, 14]. Furthermore, disparities in the geographical provenance of the plant may result in discrepancies in the phytochemical constitution of the fruit and, consequently, its toxicity profile [15, 16].

To ensure the provision of high-quality traditional medicines, it is imperative to prioritize both their efficacy and safety. The scope of drug safety extends beyond acute and chronic hazardous exposure, encompassing cytotoxicity and genotoxicity. The significance of this matter arises from the fact that the administration of mutagenic medications frequently results in alterations at physiological, biochemical, and genetic levels in humans [17]. Hence, it is of utmost importance to investigate the genotoxicity of pharmaceutical compounds, including traditional medications, employed in the management of chronic ailments. Further, investigations are warranted to examine the genotoxic effects of antidiabetic medications in individuals diagnosed with diabetes because of the potential of these treatments to enhance pre-existing mutations. This necessitates a comprehensive understanding of their effects on the human cells [17-19]. As indicated earlier, the number of cytotoxic and genotoxic studies conducted on traditional medicines administered to individuals with chronic illnesses remains limited despite their persistent and widespread use [3].

Water is frequently employed as a solvent in traditional practices for the extraction of phytoconstituents [20]. Traditionally, aqueous extracts derived from *K. africana* have been employed for the treatment of many medical problems [7]. Nevertheless, it is imperative to acknowledge that the use of various solvents throughout the extraction process can potentially influence the cytotoxic properties of plants [3]. It is crucial to guarantee that the phytoconstituents included in plant extracts do not compromise the viability of cells, regardless of the solvents employed for extraction. This is because a decrease in cell viability indicates that the plant extracts possess cytotoxic properties [21]. According to MacGregor and Jurd [1978], some flavonoids possess genotoxic and mutagenic properties [22]. According to a study conducted by Muyenga *et al.* in 2015, it has been documented that *Kigelia* crude extracts contain flavonoids [10]. Hence, this study for the first time assessed the cytotoxic, genotoxic and mutagenic properties of aqueous as well as ethyl acetate extracts of the fruits of *Kigelia africana*, obtained from plant species growing in the Kazungula District of Zambia, located in Southern Africa.

MATERIALS AND METHODS

Plant material

The fruit was obtained from the Kazungula district in the Southern Province of Zambia during November. Subsequently, it underwent authentication procedures at the Department of Biological Sciences of the School of Natural Sciences at the University of Zambia.

Plant sample preparation

The freshly harvested fruit underwent cutting, crushing, and subsequent air drying within

a designated shed for approximately five days. Subsequently, the desiccated fruit was pulverized using a mortar and pestle followed by filtration to obtain a uniform powder. This powder was subsequently preserved in a temperature range of 8-10 °C.

Two different extracts were prepared, using distilled water and ethyl acetate. In both extracts, 1 g of powdered fruit material was macerated in 10 mL of solvent (either distilled water or ethyl acetate) for 48 h with uninterrupted stirring using a mini magnetic stirrer at a temperature of 28 °C. The crude extracts were filtered using Whatman® No. 1 filter paper and further sterilized using Millipore membrane filters. The dried crude extracts and fractions were reconstituted in dimethyl sulfoxide (DMSO) to obtain a final concentration of 100 mg/mL. To ensure complete solubility, the samples were sonicated and stored at 4 °C until use. The samples were then subjected to the Ames test, cytotoxicity assay on *C3A hepatocytes*, and genotoxicity assay on *Vero cells*.

The Ames test was performed at a concentration of 100 µL/plate, which is equivalent to 10 mg/ plate of dry plant matter. On the other hand, the cytotoxicity and micronucleus tests utilized a quantitative fluorescence microscopy assay with serial dilutions resulting in concentrations of 31.25, 62.5, 125, 250, and 500 µg/mL.

Bacterial samples for Ames test

The Ames test kit used in this study was procured from Sigma and consisted of commercially available strains of *Salmonella typhimurium* (*S. typhimurium*) TA97, TA98, and TA100. C3A hepatocytes generated from a human hepatoma were acquired from the American Type Culture Collection.

Cell cultures for cyto- and genotoxicity

Selected cell lines were maintained in 10 cm treated polystyrene culture dishes in the relevant culture medium supplemented with 10% FBS and 1% (v/v) penicillin/streptomycin and incubated in a humidified incubator at 37 °C with 5% CO_2. Cultures were assessed daily for contamination using an Axiovert 40C inverted microscope (Carl Zeiss, Germany) and sub-cultured when they reached approximately 80% confluence. Furthermore, the culture medium was replaced every third to fourth day to stimulate and maintain optimal growth conditions.

The C3A hepatocarcinoma and Vero African monkey kidney cell lines were purchased from the American Type Culture Collection and Cellonex (South Africa), respectively.

Preliminary toxicity evaluation for Ames test

Prior to conducting the mutagenicity experiment, a preliminary toxicity test was performed on the extracts using the *Salmonella typhimurium* strain TA98. In this cytotoxicity assay, a mixture of *S. typhimurium* cells and 100 µL of plant extract was inoculated onto nutrient agar plates and incubated for growth. It was concluded that extract samples with a viability of over 50 percent are non-toxic compared to the negative control consisting solely of the solvent. The percentage of viable cells was determined by assessing the colony forming units per plate (CFU/plate) of the control group, which consisted of *S. typhimurium* TA98 cells without any extracts. The CFU/plate count in the control group was considered 100 percent.

Ames test assay

The Ames assay was conducted following the experimental procedure outlined by Maron and Ames (1982), which is endorsed by the OECD 471 Guidelines and Annex V to Directive 67/548/EEC [23]. Some slight modifications and adaptations were made to the protocol to assess the ability of *K. africana* to induce genetic mutations in *Salmonella typhimurium* (*S. typhimurium*) strains TA97, TA98, and TA100. The analyses utilized the TA97 and TA98 strains as the foundation for assessing frameshift mutations, whereas the TA100 strain was used to evaluate the likelihood of base pair exchange mutations.

The experiments were performed using preincubation methodology. To serve as a negative control for all samples, a 10 percent solution of dimethyl sulfoxide (DMSO) was used. The positive control for *S. typhimurium* TA97 and TA98 strains consisted of 4-nitroquinoline 1-oxide (4-NQO) at a concentration of 5 g per plate. Sodium azide (SA) was used as a positive control for the TA100 strain at a concentration of 10 mg per plate.

A dose-finding experiment was performed for each test strain. A series of diluted DMSO concentrations, ranging from 0 to 100 ml/L were evaluated for each sample. For the experiments, a volume of 0.5 mL of a sodium-phosphate buffer solution with a concentration of 0.1 mol/L and a pH of 7.4 was introduced. The resulting mixture was incubated on a shaker at 37 °C. Following a preincubation period of 30 minutes, a volume of 2 mL of top agar solution, which included L-histidine and D-biotin at a concentration of 0.05 mol/L, was introduced into the mixtures containing strains of *S. typhimurium*. Subsequently, each of these mixtures was evenly distributed on a plate supplemented with glucose agar. Following the solidification of the top agar layer, the plates were incubated for 72 h. Quantification of revertant colonies was conducted for each tester strain of *S. typhimurium* and the treatment cohort. A favorable outcome was determined when there was an increase in the average number of revertant colonies within each treatment group.

C3A hepatocyte cytotoxicity assay

C3A hepatocytes, originating from human hepatoma, were cultured in 10 cm culture dishes using a complete medium consisting of minimum essential medium (MEM) supplemented with 1% non-essential amino acids, 10% fetal bovine serum (FBS), and penicillin/streptomycin. The cells were maintained at 37 °C in a humidified environment containing 5% carbon dioxide (CO_2).

The cells were seeded into 96 well plates at a density of 5000 cells per well in 100 aliquots. Subsequently, the plates were incubated overnight to allow the cells to adhere.

Dilutions of each extract were performed in complete medium at double the required final concentrations. A 100 µL aliquot of each dilution was added to 100 µL of the cells adhered to the 96 well plate, resulting in final concentrations of 15.6, 31.25, 62.5, 125, and 250 µg/mL. A final concentration of 30 µM melphalan was used as the positive control. The cells were then incubated for 48 h at 37 °C in a humidified environment containing 5% CO_2.

The medium was removed from the cells by aspiration, and 100 µL of 5 µg/mL Hoechst 33342 in phosphate-buffered saline was added to each well. The plates were incubated for 30 min prior to the addition of 10 µL of 100 µg/mL PI solution to each well. Image acquisition was performed immediately utilizing an Image Xpress Micro XLS widefield automated fluorescence microscope (Molecular Devices). This microscope was equipped with DAPI and Texas Red filters as well as a 10x Plan Fluor objective. Nine images were obtained per well, capturing approximately 70% of the entire well surface area. The Multiwavelength Cell Scoring module of the Meta Xpress software was utilized for image analysis. The number of viable cells (nuclei solely stained with Hoechst 33342) and non-viable cells (nuclei stained with both Hoechst 33342 and PI) were recorded and imported into Excel for subsequent analysis.

Micronucleus assay to determine genotoxicity

Vero cells were used as an experimental model to assess the genotoxicity of *Kigelia* fruit extracts. The cells were seeded into 96-well plates at a density of 3000 cells per well in 100 µL aliquots. The plates were incubated overnight to allow the cells to adhere. Subsequently, the cells were treated with 15.625, 31.25, 62.5, 125, 250, and 500 µg/mL of the extracts for 48 h under controlled conditions of 37 °C and 5% CO_2. Griseofulvin was employed as the positive control, with concentrations ranging from 0.94 µM to 30 µM. Following the incubation period, cells were fixed in a 4% formaldehyde solution for 15 min. A working solution of Hoechst 33342 was prepared in phosphate-buffered saline (PBS), resulting in a final concentration of 5 µg/mL. The fixative was removed by aspiration before introducing 100 µL of Hoechst 33342 working solution. The cells were stained for 15-30 min. Subsequently, images were acquired using the DAPI filter on the Image Xpress Micro XLS Widefield Microscope manufactured by Molecular Devices. The acquired images were analyzed using the Micronucleus Application Module of MetaXpress software. The collected data were exported to Microsoft Excel, for analysis and processing.

Ethical approval

The study was approved by the Research Ethics Committee of Mulungushi University School of

Medicine [SMHS-MU3-2020-12], the National Health Research Authority of Zambia, and the Research Ethics Committee of the University of Namibia (HG-/162/2021).

RESULTS

S. typhimurium TA98 cell viability after exposure to both fruit powder extract samples

In the present study, a preliminary assessment was conducted to evaluate the cytotoxicity of *Kigelia africana* fruit extracts against the strain TA98. The impact of *Kigelia africana* fruit extracts on the viability of *S. typhimurium* tester strains, which were employed in mutagenicity assays, was investigated. The findings from the analysis of the two fruit extract samples indicated no observable toxic effects of significance. The results are presented in Table 1.

Mutagenic potential of *Kigelia africana* extracts on *S. typhimurium* strains TA100, TA97 and TA98

The assessment of mutagenicity of the extracts of *Kigelia africana* fruit powder revealed that there was no statistically significant difference in the number of His+ revertant colonies caused by both the aqueous and ethyl acetate extracts of *Kigelia africana* for *S. typhimurium* strains TA97, TA98, and TA100 (p=0.7). Nevertheless, the frequencies of His+ revertants, triggered by the positive control mutagens, exhibited a substantial elevation in the spontaneous mutation rate across all three strains of *S. typhimurium*. This resulted in a statistically significant difference between the experimental and positive control groups (p=0.004). Please refer to Table 2 for further details.

C3A hepatocyte cell viability after exposure to fruit powder extracts

The viability of cells exposed to *Kigelia africana* aqueous extract ranged between 102.1 and 103.9% compared to the viability of untreated cells which was set at 100%. These differences were statistically insignificant ($p > 0.05$). Nevertheless, when cells were exposed to the ethyl acetate extract, there was a noticeable decrease in cell viability, which was directly proportional to the increase in dosage. A statistically significant difference in cell viability was observed between the aqueous and ethyl acetate crude extracts at 250 µg/mL ($p = 0.019$) as well as between the aqueous extract and melphalan ($p < 0.0001$) (see Figure 1 below).

Table 1. *S. typhimurium* TA98 cell viability after exposure to both crude fruit extract samples.

Sample	Number of CFU/plate [mean +/- SD]	Viability %
Ethyl acetate crude extract	25.60 ± 3.50	68.30
Aqueous crude extract	23.80 ± 3.06	69.45

Table 2. Mutagenic potential of *Kigelia africana* crude extracts on *S. typhimurium* strains TA100, TA97 and TA98.

Sample	Number of His + /plate		
	TA97	TA98	TA100
Negative control	22.00 ± 6.00	26.00 ± 5.00	145.82 ± 22.50
Positive control	680.10 ± 44.00	720.00 ± 32.00	1300.90 ± 112.20
Ethyl acetate crude extract	21.20 ± 0.30	25.40 ± 0.50	150.70 ± 10.50
Aqueous crude extract	20.08 ± 0.60	24.70 ± 0.78	145.80 ± 11.30

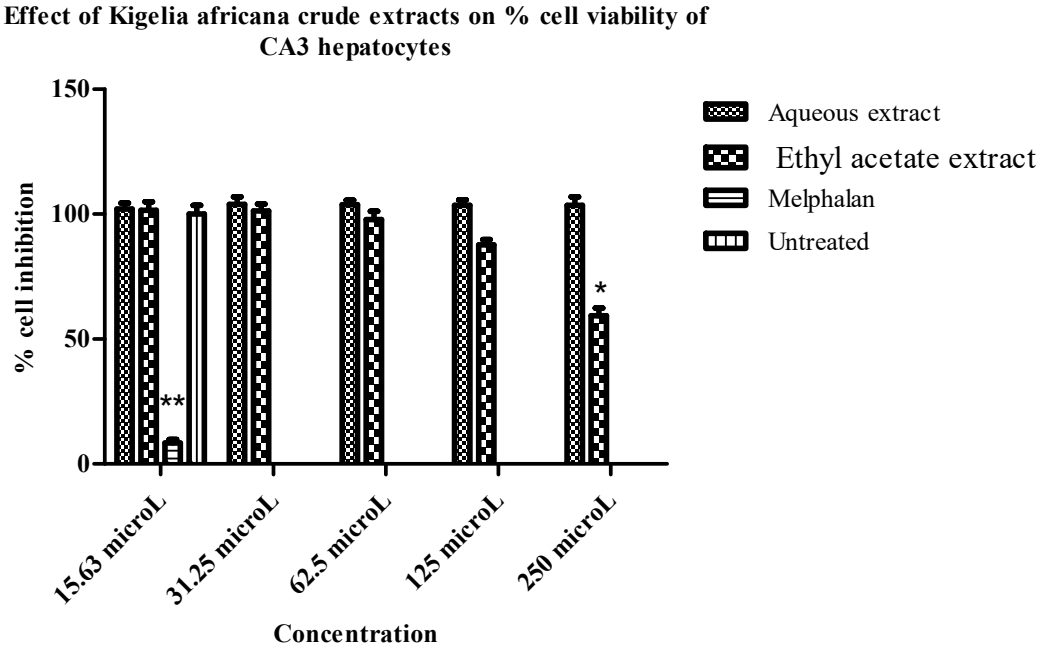

Figure 1. Effect of *Kigelia* crude extracts on % cell viability of C3A hepatocytes. *p<0.05; **p<0.001 compared to control.

However, even at the highest dose of 250 µg/mL, there was still a cell viability of 59%.

Genotoxic evaluation using Vero cells

The sample of ethyl acetate (EtOAc) demonstrated a cytotoxic impact on Vero cells at doses of 250 and 500 µg/mL, as depicted in Figure 2 below. Similar observations were found for the elevated concentrations of the positive control (7.5, 15, and 30 µM), while the aqueous extract did not affect the total cell number.

The aqueous extract exhibited a marginal although statistically significant elevation in the percentage of micronucleated cells at the three most concentrated levels (Figure 3). The highest concentration resulted in a significant rise in the percentage, from 2.68% in the control group to 3.8%. The observation did not translate into a substantial rise in multinucleation (refer Figure 3) or nuclear size (refer Figure 4). The positive control, known to be an aneugen, exhibited a considerably more pronounced rise in the percentage of micronucleated cells, accompanied by substantial and statistically significant increases in both ploidy and nuclear size.

The extract of ethyl acetate (EtOAc) did not result in a notable rise in the proportion of micro- or multinucleated cells, as depicted in Figures 3 and 4. However, a statistically significant elevation in the average nuclear area was observed, as illustrated in Figure 5 below. The observed alteration is likely attributable to the cytotoxic properties of the extract at elevated concentrations.

Note: The error bars in the figures represent the standard deviation of quadruplicate measurements obtained from a single experimental trial. Statistical significance was assessed using the two-tailed Student t-test, with significance levels denoted as **p<0.05 and ***p<0.001 when compared to the control group.

DISCUSSION

A preliminary cytotoxicity assessment of *Kigelia africana* fruit extracts on the *S. typhimurium* strain TA98 indicated that neither of the fruit extract samples exhibited any noticeable toxic effects that could be considered substantial or indicative of possible harm. Given that the survival rate of *S. typhimurium* TA98 cells exposed to both samples exceeded sixty percent,

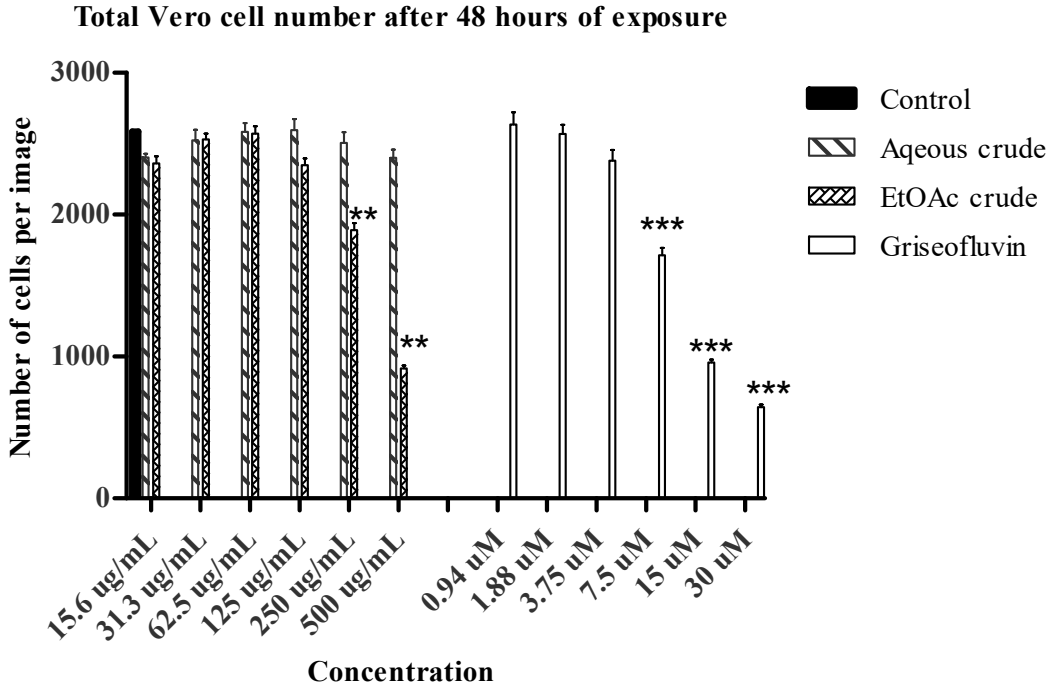

Figure 2. Cytotoxic effect of *Kigelia* fruit extracts at different doses on the number of Vero cells following a 48-hour exposure. **p<0.005 and ***p<0.001 when compared to the control group.

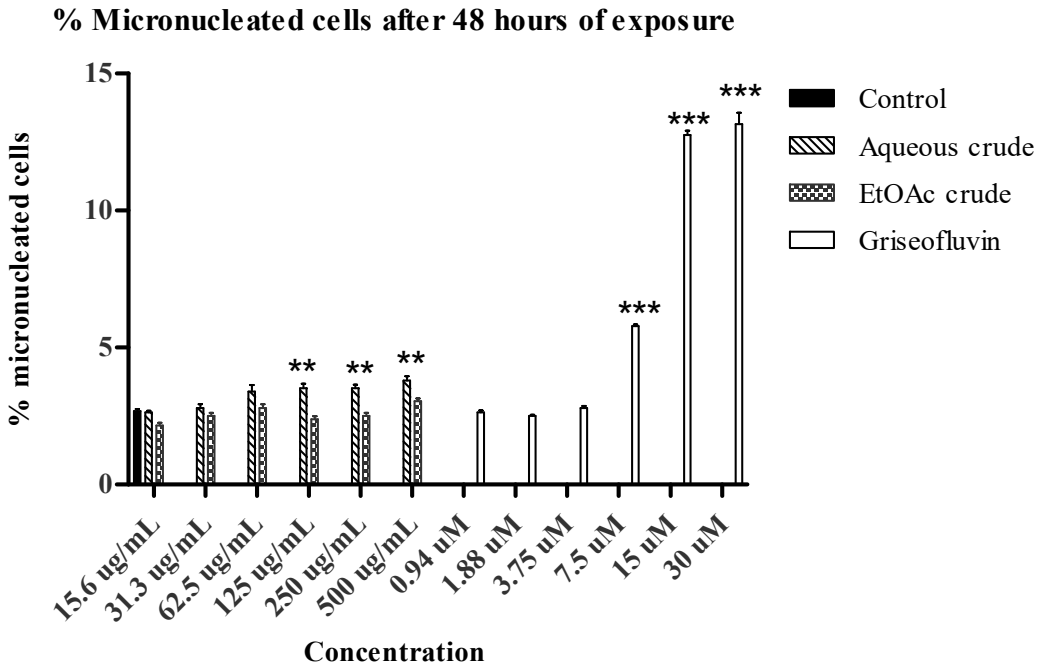

Figure 3. Percentage of micro-nucleated Vero cells following a 48-hour exposure to two *Kigelia africana* fruit extracts and griseofulvin as positive control. **p<0.05 and ***p<0.001 when compared to the control group.

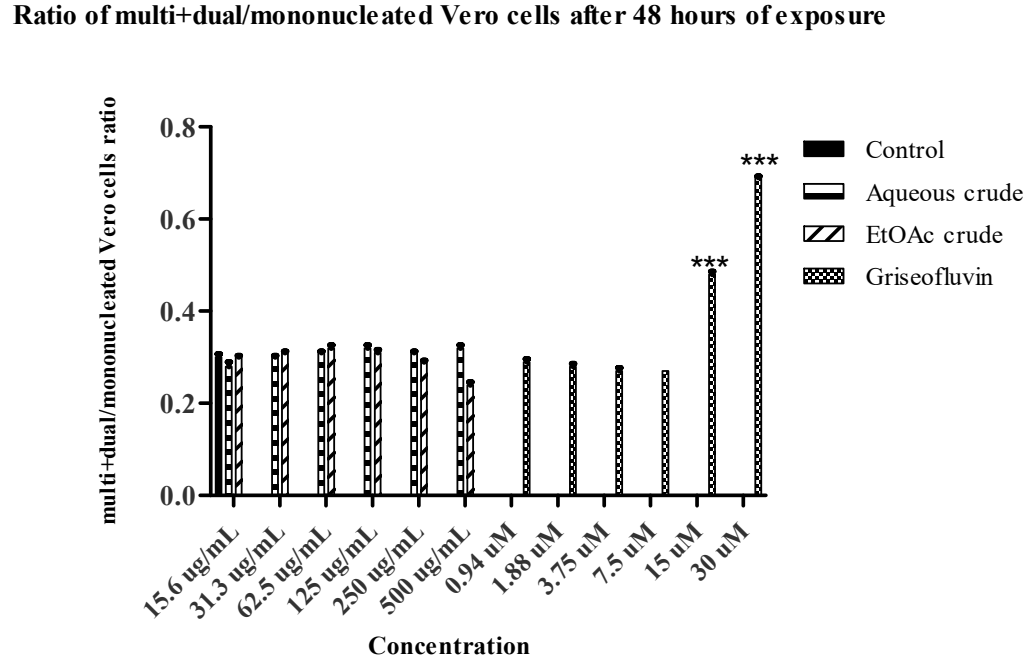

Figure 4. Effect of *Kigelia* fruit extracts at different concentrations on multi+dual/ mononucleated Vero cell ratio following a 48-hour exposure. ***p<0.001 when compared to the control group.

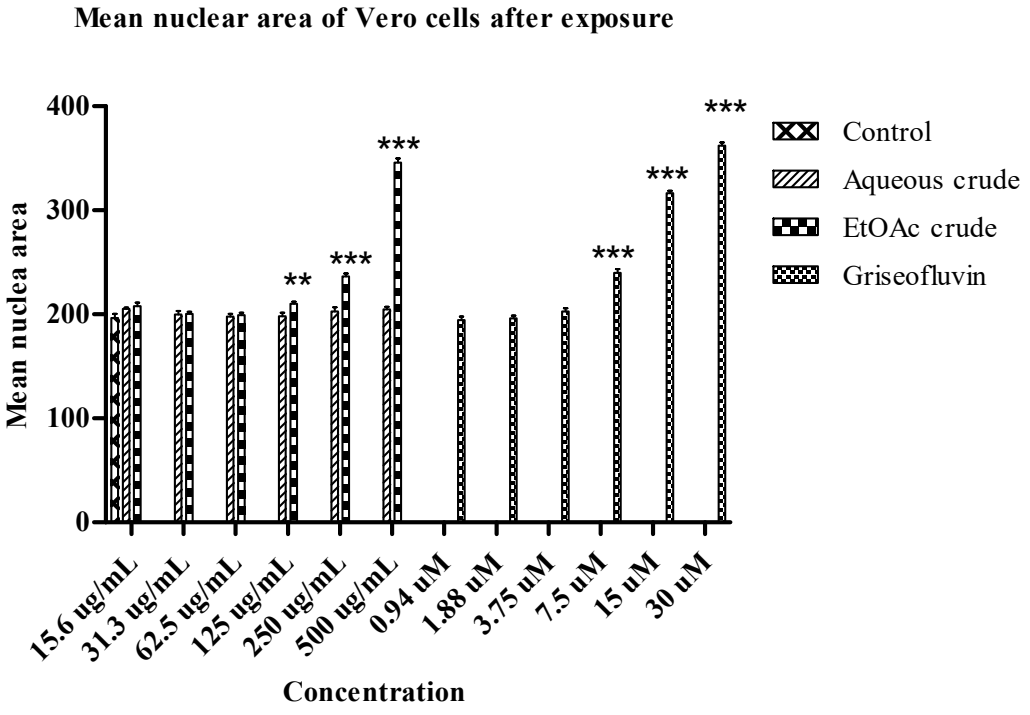

Figure 5. Effect of *Kigelia* fruit extracts at different concentrations on mean nucleus area of Vero cells following a 48-hour exposure. **p<0.05 and ***p<0.001 when compared to the control group.

it was plausible to assess the mutagenic potential of the fruit extracts in *S. typhimurium* strains TA97 and TA100 without encountering the possibility of false-negative or inconsistent outcomes (as indicated in Table 1). The *Salmonella* strains TA98 and TA100 were selected due to their reported 93 per cent sensitivity in identifying plant extracts that had mutagenic potential [24]. Using the Ames test the study demonstrated the effect of the extract both on the frame-shifting mutagenicity with TA97 and TA98 strains, as well as base-pair substitution mutagenicity with TA98 [23, 25]. We concluded that the extracts do not possess mutagenic or clastogenic properties in bacteria or cultured mammalian cells, regardless of the presence or absence of S9 activation. There was no significant difference seen in the counts of revertants induced by ethyl acetate and aqueous extracts compared to the negative control. Thus, in this study, we have made a pioneering contribution towards the mutagenicity profile of *Kigelia africana* extracts by demonstrating that both of these extracts do not induce base pair replacement or frameshift gene alterations in the bacterial cells utilized within the scope of this study. The results we have presented bear resemblance to findings published previously by Abdul-Hafeez and colleagues as well as Eldeen and colleagues [12, 26]. Notably, these earlier investigations centered on the stem and bark extracts of *Kigelia africana* originating from Egypt, as well as leaf extracts from the same plant grown in Sudan. It is noteworthy that, in contrast, our study is the first to specifically explore the mutagenic profile of fruit extracts of *Kigelia africana*, which are frequently employed by patients in Zambia. In contrast to the studies conducted by Eldeen and Van Staden (2007), who employed a single strain of *Salmonella* (TA98), and Abdul-Hafeez *et al.*, who utilized both TA98 and TA100, the present investigation incorporated three strains of *Salmonella* (TA97, TA98, and TA100) [12, 26]. In summary, based on the findings obtained from the Ames test, it can be inferred that neither frame-shifting nor point mutations played a significant role. This conclusion is drawn from the absence of any noteworthy disparity between the outcomes observed with the fruit extracts and the negative control.

The findings of this investigation indicate that the ethyl acetate extract of *Kigelia* fruit exhibited a decline in cell viability of the C3A hepatocytes that was proportional to the dosage administered. Notably, the highest concentration of 250 µ/L resulted in a cell viability of 59%. Conversely, the aqueous extract continually showed a substantial level of cell viability throughout the experiment. Nevertheless, the observation of a cell viability over 50% solely at the highest dose (IC50 > 250 µg/mL) still indicates that the phytochemicals extracted by ethyl acetate solvent, along with their metabolites, pose minimal danger of inducing hepatoxicity. The utilisation of herbal medications and nutritional supplements has been linked to hepatotoxicity [27, 28]. Consequently, from the results of this study, it is improbable that the aqueous and ethyl acetate extracts would induce hepatotoxicity in individuals. Moreover, implementing a dose adjustment to the ethyl acetate extract during treatment would also result in a decrease in potential hepatotoxicity.

The genotoxicity of the *Kigelia* fruit extracts was assessed using the MTT assay, and the results were compared with the Ames results obtained in this work. The MTT test conducted on Vero cells is a dependable method that can provide valuable insights into the potential clinical implications of the plant extracts investigated in the study [3, 29]. According to Rampa *et al.* (2022), if the detected cytotoxicity testing result exceeds 55%, it would be appropriate to proceed with a genotoxicity test [30]. However, in this case, even at the highest dose the ethyl acetate extract recorded a 59% cell viability. The ethyl acetate extract had a cytotoxic impact on Vero cells, similar to the observed effect on C3A hepatocytes at high concentrations. Similar observations were found for the elevated concentrations of the positive control (7.5, 15, and 30 µM). However, the observed hepatoxicity can be avoided in the case where lower concentrations are used. Further, studies have reported efficacy of the fruit extracts in the management of diabetes, even at average doses, in animals [10].

The results indicate that the aqueous extract exhibited a minimal but statistically significant rise in the proportion of micro-nucleated cells at the three highest doses, as depicted in Figure 3.

Nevertheless, there was no substantial rise in multinucleation (Figure 4) or enlargement of the nucleus (Figure 5) observed in conjunction with this. The observation of an augmented nuclear size may indicate the possibility of genotoxicity associated with the extracts at very high doses. However, the positive control, known to be an aneugen, exhibited a considerably more notable rise in the percentage of micro-nucleated cells, which was accompanied by substantial and statistically significant increases in ploidy and nuclear size. Thus, based on the findings of this investigation we may conclude that the extract does not exhibit an aneugenic impact on the cells. In contrast, the EtOAc fruit extract did not exhibit a significant effect on the percentage of micro- or multinucleated cells, as depicted in Figures 2 and 3. However, a notable increase in the mean nuclear area was observed, as illustrated in Figure 5. The observed alteration can be attributed to the cytotoxic properties of the extract at elevated concentrations. Similar studies conducted on antidiabetic drugs like Sitagliptin have demonstrated genotoxicity at high doses of about 500 µg/mL [31]. However, the genotoxicity test in human subjects only showed genotoxicity at the highest doses of treatment which was about 1000 µg/mL [32]. Thus, there is a need to conduct genotoxicity studies in patients who have been exposed to the aqueous fruit extracts for a long period of time.

CONCLUSION

The importance of ensuring the safety of traditional medicine, such as *Kigelia africana*, for the management of chronic conditions cannot be overstated. Using quantitative fluorescence microscopy and the Ames test, this study has shown for the first time that the aqueous and ethyl acetate extracts of *Kigelia africana* fruit do not reduce cell viability. Additionally, the study demonstrated that these fruit extracts do not cause mutagenicity and are not genotoxic when used within therapeutic dosage ranges. However, it is recommended to conduct further genotoxicity studies in patients who use these fruit extracts for prolonged periods to manage chronic conditions like diabetes.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest to declare.

REFERENCES

1. Hikaambo, C. N., Namutambo, Y., Kampamba, M., Mufwambi, W., Kabuka, R., Chulu, M., Nanyangwe, N., Banda, M., Chimombe, T., Muungo, L. T. and Mudenda, S. 2022, J. Biomed. Res. Environ. Sci., 3(1), 74-81. https://doi.org/10.37871/jbres1402
2. Logiel, A., Jørs, E., Akugizibwe, P. and Ahnfeldt-Mollerup, P. 2021, Afr. Health Sci., 21(3), doi:10.4314/ahs.v21i3.52.
3. Anywar, G., Kakudidi, E., Byamukama, R., Mukonzo, J., Schubert, A., Oryem-Origa, H. and Jassoy, C. 2021, Front. Pharmacol., 12. https://www.frontiersin.org/articles/10.3389/fphar.2021.615147
4. Matsabisa, M. G., Tripathy, S., Dassarma, B., Chabalala, H. P. and Mukherjee, P. K. 2022, Evidence-Based Validation of Herbal Medicine [Second Edition]. Elsevier; p. 561-586. https://doi.org/10.1016/B978-0-323-85542-6.00027-5
5. Bello, I., Shehu, M. W., Musa, M., Zaini Asmawi, M. and Mahmud, R. 2016, J. Ethnopharmacol., 189, 253-276. doi: 10.1016/j.jep.2016.05.049.
6. Kasali Félicien Mushagalusa, Kadima Justin Ntokamunda, Peter Emanuel, L., Mtewa Andrew, G., Ajayi Clement Olusoji, Tusiimire Jonans, Tolo Casim Umba, Ogwang Patrick Engeu, Weisheit Anke and Agaba Amon Ganafa. 2021, Front. Pharmacol., 12. doi:10.3389/fphar.2021.757090.
7. Muyenga, T. A., Ezaela, C. E., Mushabati, F., Bamitale, SD-K. and Kibuule, D. 2021, J. Prev. Rehabil. Med., 3(2), https://journals.unza.zm/index.php/medicine/article/view/537.

8. Byrdie. 2021, Cosmetics. Byrdie. https://www.byrdie.com/kigelia-africana-5186027.
9. Fagbohun, O., Vincent, O., Adekola, M. and Msagati, T. A. M. 2020, Comp. Clin. Pathol., 29. doi:10.1007/s00580-020-03179-9.
10. Muyenga, T., Prashar, L., Bwalya, A. G. and Muungo, L. T. M. 2015, J. Med. Sci. Tech., 4(3), 201-206. https://library.adhl.africa/handle/123456789/11725
11. Farah, H., Elhussein, A., Khalid, H. and Osman, H. 2018, Adv. Res., 12, 1-9. doi:10.9734/AIR/2017/38539.
12. Abdul-Hafeez, E. Y., Karamova, N. S., Ilinskaya, O. N. 2018, Ecol. Genet., 16(3), 55-61. doi:10.17816/ecogen16355-61.
13. Joshi, R. K. 2023, Nat. Prod. Res., 1-5. doi:10.1080/14786419.2023.2202397.
14. Akintola, O. O., Aderounmu, A. F., Abiola, I. O., Abodurin, K. E., Adeniran, T., Agboola, F. and Olokeogun, O. S. 2020, J. Bioresour. Manage., 7(3), 131-142. doi:10.35691/JBM.0202.0146.
15. Hadju, V., Dassir, M., Putranto, A. and Sadapotto, A. 2021, Gaceta Sanitaria. 35, S396-S399. doi:10.1016/j.gaceta.2021.10.060.
16. Pacheco-Hernández, Y., Villa-Ruano, N., Lozoya-Gloria, E., Barrales-Cortés, C. A., Jiménez-Montejo, F. E. and Cruz-López, M. del C. 2021, Plants, 10(2), 325. doi:10.3390/plants10020325.
17. Goyal, K., Goel, H., Baranwal, P., Dixit, A., Khan, F., Jha, N. K., Kesari, K. K., Pandey, P., Pandey, A., Benjamin, M., Maurya, A., Yadav, V., Sinh, R. S., Tanwar, P., Upadhyay, T. K. and Mittan, S. 2022, Environ. Sci. Pollut. Res. Int., 29(41), 61993-62013. doi:10.1007/s11356-021-15442-9.
18. Eremina, N. V., Zhanataev, A. K., Lisitsyn, A. A. and Durnev, A. D. 2021, Ecol. Genet., 19(3), 219-240. doi:10.17816/ecogen70691.
19. Habib, S. L. and Rojna, M. 2013, Int. Sch. Res. Notices, e583786. doi:10.1155/2013/583786.
20. Abubakar, A. R. and Haque, M. 2020, J. Pharm. Bioallied Sci., 12(1), 1-10. doi: 10.4103/jpbs.JPBS_175_19.
21. Horváth, S. 1980, Toxicology, 16(1), 59-66. doi:10.1016/0300-483x[80]90110-9.
22. MacGregor, J. T. and Jurd, L. 1978, Mutat. Res., 54(3), 297-309. doi:10.1016/0165-1161[78]90020-1.
23. Maron, D. M. and Ames, B. N. 1982, Mutat. Res., doi:10.1016/0165-1161[82]90035-1.
24. Fabiana Gomes da Silva Dantas, Pamella Fukuda de Castilho, Adriana Araújo de Almeida-Apolonio, Renata Pires de Araújo and Kelly Mari Pires de Oliveira. 2020, Mutat. Res., 786, 108338. doi:10.1016/j.mrrev.2020.108338.
25. Bugda, H., Guven Ezer, B. and Rencuzogullari, E. 2022, Drug Chem. Toxicol., 46(5), 955-961. doi:10.1080/01480545.2022.2113096.
26. Eldeen, I. M. S. and van Staden, J. 2007, S. Afr. J. Bot., 73(3), 435-440. doi: 10.1016/j.sajb.2007.03.009.
27. Andrade, R. J. 2019, Clin. Liver Dis., 14(2), 49-50. doi:10.1002/cld.834.
28. Gómez-Lechón, M. J., Castell, J. V. and Donato, M. T. 2010, Methods Mol. Biol., 640, 389-415. doi:10.1007/978-1-60761-688-7_21.
29. Winikoff, S. E., Zeh, H. J., DeMarco, R. and Lotze, M. T. 2004, Measuring Immunity, Elsevier p. 343-349. doi:10.1016/B978-012455900-4/50291-9.
30. Rampa, K. M., van De Venter, M., Koekemoer, T. C., Swanepoel, B., Venables, L., Hattingh, A. C., Viljoen, A. M. and Kamatou, G. P. 2022, J. Ethnopharmacol., 282, 114596. doi:10.1016/j.jep.2021.114596.
31. Majeed Khan, H., Nawaz, H., Aerooj, H. and Sharif, A. 2021, Pak. J. Pharm. Sci., 34(5 Suppl.), 1939-1944. PMID: 34836863.
32. Yuzbasioglu, D., Enguzel-Alperen, C. and Unal, F. 2018, Food Chem. Toxicol., 112, 235-241. doi: 10.1016/j.fct.2018.01.003.