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Tetracarpidium conophorum (AFRICAN WALNUT) HUTCH. & DALZIEL: ANTIMITOTIC ACTIVITIES OF WATER EXTRACTS USING THE *Allium cepa* ASSAY.

SEINO Richard Akwanjoh[1], **ENDUM** Lucas Akacha[1], **NGYANYIYI** Abdoul[2]

1. *Laboratory of Genetic Toxicology & Orthopterology (LAGTO), Department of Zoology, Faculty of Science, The University of Bamenda, P.O. Box 39 Bambili- Bamenda, Cameroon.*

2. *Applied Biology and Ecology Research Unit (URBEA), Department of Animal Biology, Faculty of Science, University of Dschang. P.O. Box 57 Dschang, West Region, Cameroon.*

ABSTRACT

Purpose: In Cameroon, the African walnut *Tetracarpidium conophorum* is used in the traditional treatment of prostate cancer. The antimitotic activity of this nut is yet to be investigated. The purpose of this study is to evaluate the antimitotic activities of this African walnut *Tetracarpidium conophorum* since cancer is a disease of mitosis.

Methods: Five aqueous extracts of boiled nuts were used to investigate the antimitotic properties of *Tetracarpidium conophorum* by the *Allium cepa* assay. Percentage sprouting and growth of roots of treated *A. cepa* were measured and compared to the control which was water. Mitotic index, mitotic inhibition index and chromosomal abnormalities were determined on treatment of *A. cepa* bulbs with various aqueous concentrations of *Tetracarpidium conophorum.*

Statistical analysis: The Student's t test and the SPSS 15.0 statistical package was use for the analysis.

Results: Aqueous extracts of *Tetracarpidium conophorum* inhibited root sprouting and growth in *A. cepa*. Inhibition was proportional to concentration of aqueous extract and was greater than 50% at 30% concentration of the extract. Chromosomal abnormalities recorded included bridges, laggards, ghost cells, chromosome fragments and Anaphase vagrants.

Conclusion: Our findings show cytotoxic and genotoxic effects of these concentrations of GA and indicate that the difference among control and treatment groups were statistically significant. Therefore, *Tetracarpidium conophorum* possesses antimitotic properties and hence is a potential anticancer agent that require further studies and development.

Key words: *Tetracarpidium conophorum*, Mitotic Index, Mitotic Inhibition, Chromosomal abnormalities, *Allium cepa.*

INTRODUCTION

The African walnut, *Tetracarpidium conophorum* (Müll. Arg.) Hutch. et Dalz, is a climbing shrub that is native to tropical Africa, and can be found from Sierra Leone to the Democratic Republic of Congo. In eastern Nigeria it is known as ukpa (ibo), in western

Nigeria it is called awusa or asala (Yoruba), and in Northern Nigeria it is commonly referred to as gawudi bairi (Hausa) (Chijoke, et al. 2015; Kanu et al. 2015; Ojobo et al. 2015). In Cameroon it is called kaso or ngak, among other local names (Burkill 1985) and hawkers commonly refer to the nuts as cashew nuts. The plant belongs to the genus Juglans and the family Juglandaceae. It is an edible economic plant that is common to the Cameroonian forest zone and is now cultivated in the western and central regions in cocoa and coffee farms. This species is cultivated mainly for the nuts that are cooked and eaten as a snack (Adebona, 1988; Oke (1995) as well as a tradimedicine against prostate cancer. The leaves and nuts of *T. conophorum* have been variously used in traditional medicine for the treatment of male sterility, constipation, toothache, eczema, pruritus, psoriasis, common cold, and prostate cancer (Oke, 1995; Odugbemi & Akinsulire, 2008; Bamindele et al. 2015; Ayodeji et al. 2018; Uhunmwangho et al. 2022).

The phytochemical analysis of *T. conophorum* nut has been reported to include saponins, flavonoids, phenols, tannins and alkaloids and ascorbic acid (Udedi et al, 2013, 2014; Akomolafe et al. 2015, 2017; Olusola et al, 2021). These phytochemicals and nutrients confirm the role the nuts of *T. conophorum* play in nutrition and health (Nwaoguikpe et al. 2012; Udedi et al. 2013, 2014). Studies have also shown that hydrogen cyanide and steroid content of raw seed nuts was higher than for cooked seed nuts (Apeh et al. 2014).

The seeds of *T. conophorum* have also been shown to possess good glycemic control of diabetes mellitus by protecting the liver against oxidative damage that is induced by hyperglycemia (Bamindele et al. 2015). Oil from the seed of *T. conophorum* have also been reported to contains bioactive components that may oppose prostate carcinogenesis induced by MCA (Uhunmwangho et al. 2022). Carvalho et al. (2010) reported that the methanol extract showed concentration-dependent growth inhibition towards human kidney and colon cancer cells.

Since cancer is mitosis, screening for the antimitotic properties of aqueous extracts of *T. conophorum* nuts, could be of immense importance in the confirmation of the presence of some bioactive agent in the control of prostate cancer.

MATERIALS AND METHODS

Collection of plant material

Boiled cashew nuts (Fig. 1) were purchased in the local Bamenda market located in the North West Region of Cameroon.

Preparation of aqueous extract

Pre-cooked nuts *T. conophorum* were obtained from the Bamenda city main market. The shells cracked to obtain the seeds (Fig. 2a) which were cut into small pieces and air dried before grinding into a coarse powder. Three (300) grams of the powdered seeds were stirred in 1 litre of distilled water and allowed to stand for 24 hours. The mixture was then filtered through a No 4 coffee filter paper and the extract stored in a refrigerator at 4^{o}C and used in subsequent experiments as a stock solution. Five concentrations (v/v) of the extract were prepared from the stock (5, 10, 20, 30 and 40%) to study the mitotic and genotoxic effects. Distilled water was used as the control.

Cytotoxic evaluation

Average sized onion bulbs (2 - 3 cm diameter) were obtained for the Bamenda main market. Before use, the loose and dry outer scales of onion bulbs were removed. Also, the dry roots at the bottom plate of the onion bulbs were carefully scraped off without destroying the root primordia.

Five Onion bulbs were respectively placed with the root primordia immersed in the various extracts (0, 5, 10, 20, 30 and 40%) of *T. conophorum* and incubated in the open laboratory at 25^{0}C. After 72 hours, the number of roots sprouted on each onion bulb were recorded and the lengths of the roots measured using a transparent ruler. Five replications of this experiment were made.

The percentage inhibition of root sprouting was calculated using the formula:

$$\textit{Root sprouting inhibition (\%)} = \frac{\textit{Mean No of roots in control} - \textit{Mean No of roots in treated group}}{\textit{Mean No of roots in control}} X$$

The percentage inhibition in root growth was calculated using the formula:

$$\textit{Root growth inhibition (\%)} = \frac{\textit{Mean root lenth in control} - \textit{Mean root length in treated group}}{\textit{Mean root length in control}} X\ 1$$

Cytogenetic evaluation

To study the genotoxic effect of the extracts of *T. conophorum*, root tips from each treatment were fixed in Conoy's solution (1:3 acetic - alcohol) for 24 hours after which they were transferred to 70% ethyl alcohol and stored in a refrigerator at 4^{o}C until used. Chromosome smears were prepared from the fixed root tips using the methods of Shama & Shama (1983). The chromosome smears were temporally preserved by sealing the edges of the slides with colourless nail polish. Five slides were prepared for each treatment and control. Two hundred cells were examined per slide to determine mitotic index (MI) and chromosome abnormalities (CA). The slides were examined with the X40 objective lens of the Fisher binocular microscope. Mitotic indices were calculated by

dividing the number of cells in mitotic division by the total number of cells examined per concentration of the extract. The mitotic inhibition was calculated using the following formular:

Mitotic inhibition = (MI in control – MI in treated group) x 100

MI in control

Chromosome abnormalities were determined by comparison with the proposals of Celik & Aslanturk (2010), Olorunfemi et al. (2013) and Owolarafe et al. (2020).

Statistical analysis

Differences in number of roots sprouted and length of roots for the different treatments were compared using the Students' t-test. The SPSS 15.0 statistical package was used for the analysis.

RESULTS

Cytotoxic assay

The present investigation showed that all the tested concentrations of the aqueous extract of *T. conophorum* inhibited root sprouting and root growth (length) as compared to the control. A progressive reduction in the number of roots (Fig. 1) was observed as the concentration of the water extract of TC increased. The number of roots sprouted was drastically reduced on treatment with 40% extract. Further examination also revealed normal root morphology and colour in all the treatments.

Fig. 1: Roots sprouted in the different treatments of *A. cepa* bulbs with *T. conophorum* nuts.

The changes in percentages of *A. cepa* roots that were treated with different concentrations of TC for 72 h is given in Table 1. The data in Table 1 revealed that number of roots sprouted and the length of roots in 5% TC extract was not significantly different ($p>0.05$) to the control.

Table 1.: The effects of treatment with different concentrations of *T. conophorum* (TC) for 72 h on *A. cepa* roots. *Means followed by different letters are significantly different at P □ 0.05.*

Concentration	Mean No of roots sprouted	% inhibition of root sprouting	Mean length of roots	% inhibition of root growth in length
Control	72.8 ± 2.3^{a}	0.0^{a}	5.38 ± 3.5^{a}	0.0^{a}
5%	69.9 ± 1.8^{a}	3.98^{b}	4.34 ± 1.0^{a}	19.33^{b}
10%	51.2 ± 2.8^{b}	29.67^{c}	1.64 ± 2.0^{b}	69.52^{c}
20%	36.0 ± 3.2^{c}	50.55^{d}	0.98 ± 2.1^{c}	81.78^{d}
30%	17.2 ± 3.6^{d}	76.37^{e}	0.96 ± 2.2^{c}	82.16^{d}
40%	13.8 ± 2.7^{d}	77.84^{e}	0.88 ± 4.1^{c}	83.64^{d}

Groups that have no letters in common in column differ significantly from the control group (Distilled water), **$p<0.01$, $\alpha= 0.05$.**

However, the mean number of roots sprouted and the lengths of the roots decreased with increase in concentration of TC seed extracts. Inhibition of root sprouting and root growth was greater with increasing concentration of the TC seed extract. The decreases in root lengths in all TC concentrations were statistically significant compared to the control which was water with the exception of 5% TC. The data in Table 1 also revealed that inhibition percentage of *A. cepa* root sprouting and root growth increased with concentration of TC seed extract. However, after 20%TC nut extract concentration, though inhibition of root sprouting and root growth was very high the increases were not significant. An inhibition in growth of root length was indicative of high level of toxicity of the TC extracts.

Cytogenotoxicity

Generally, Mitotic Index (MI) decreased when the *T. conophorum* (TC) concentration increased (Table 2). At the highest (40%) TC concentration, MI was reduced from 10.60 to 4.53% when compared to the control group. After the treatment of *A. cepa* bulbs with the five concentrations of TC for 72 h, MI of the control group was 10.60 %, whereas it decreased to 8.36% at 5% TC, to 6.66 % at 10% TC, to 5.82% at 20% TC, to 5.09% at 30% TC and to 4.53 % at 40% TC (Table 2). Thus, the lowest MI was observed in the treatment with 40% TC. MI inhibition was not very high for all the TC treatments but increased proportionately with concentration of TC nut extract. MI inhibitors are known to blocks cell growth by stopping mitosis (cell division). It therefore follows from our results that TC nut extract could be a good source of an antimitotic agent.

Table 3.: The effects of different concentrations of *T. conophorum* (TC) for 72 h on mitotic index in A. cepa root tips. *Means followed by different letters are significantly different at P □ 0.05.*

% TC concentration	No of dividing cells	% Mitotic Index (MI)	% Mitotic Inhibition
Control	2120	10.60 ± 0.00^{a}	00.00^{a}
5	1672	8.36 ± 0.12^{b}	21.13^{b}
10	1332	6.66 ± 0.12^{c}	37.17^{c}
20	1164	5.82 ± 0.13^{d}	45.09^{d}
30	1018	5.09 ± 0.13^{e}	51.98e
40	906	4.53 ± 0.22^{f}	57.26^{f}

Groups that have no letters in common in column differ significantly from the control group (Distilled water), **p<0.01, α= 0.05.**

Chromosome aberrations were monitored for treatments with all the concentrations of *T. conophorum*. An increase in the amounts of abnormal cells was positively correlated with the increase in *T. conophorum* concentration. The recorded abnormalities were, stickies, nuclear vacuoles, laggards, vagrant chromosomes and chromosome bridges (Fig. 2). As a result of the obtained findings, different concentrations of *T. conophorum*, especially treatments with concentrations above 40%, might be potential threats to the genetic material of the *A. cepa* root tips.

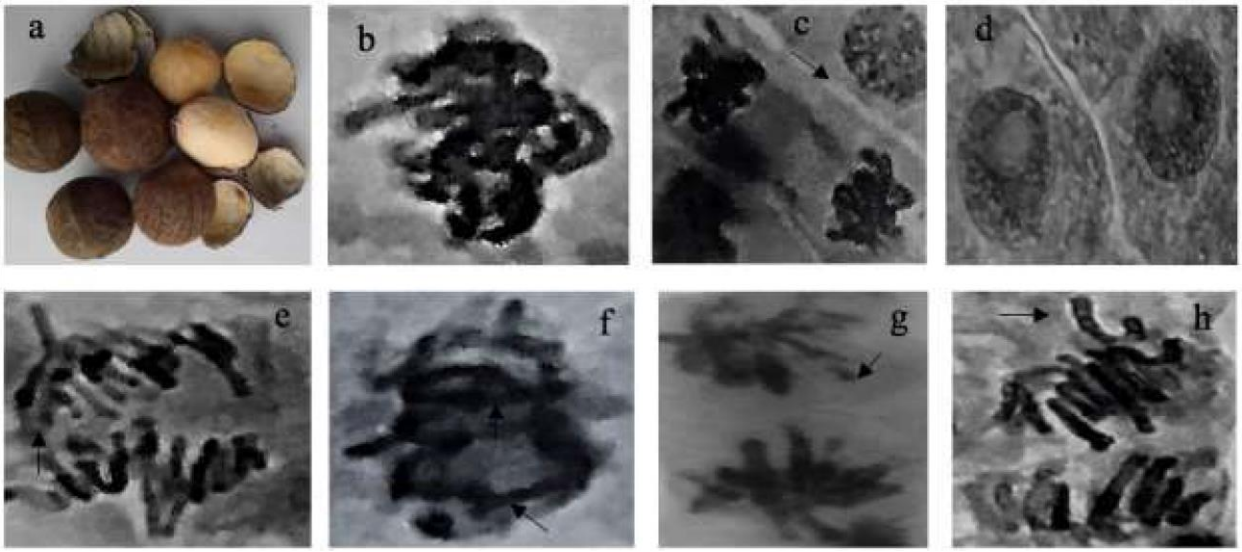

Fig. 2. Chromosome aberration recorded from *A. cepa* meristematic root tips treated with various concentration of aqueous extract of *T. conophorum* nuts: a) Boiled cashew nuts & seeds, b) Sticky chromosomes, c) nuclear vacuoles e) f) Vagrant chromosome g) Chromosome fragment, h) Anaphase laggard.

DISCUSSION

The cooked nuts of African Walnut, *Tetracarpidium conophorum*, are widely available and consumed in in Cameroon. These nuts have been shown to have an excellent profile for many ethnomedical uses (Ayeni et al, 2018). The present study has evaluated the antiproliferative and antimitotic effects of *T. conophorum* (TC) nut extract using the well-established *A. cepa* assay. The *Allium cepa* test is an important *in vivo test*, in which the onion roots come into direct contact with the extract thus enabling the prediction of possible damage to DNA. In this study this test has enabled us to assess inhibitory effects of TC seed extracts on root sprouting and growth. In our experiments, we found that the percentage of root sprouting and growth decreased with increasing TC concentrations. The inhibition in the sprouting and growth of *A. cepa* roots recorded in this study is an indication of a high level of toxicity of the TC extracts (Soliman and Ghoneam 2004).

A decrease in MI was recorded as the concentrations of TC increased. The changes in MI are indicators of cytotogenotoxic potential and antimitotic properties of TC (Nefic et al, 2013). The number of chromosomal abnormalities increased with increase in the concentration of TC nut extract. The presence of these chromosomal abnormalities in this study is further evidence of the genotoxic effects of the TC nut extract. Similar chromosomal abnormalities have been explained by changes in the movement of chromosomes around the equatorial plane of the cell. Further, mutagenicity leading to DNA damage often lead to chromosomal abnormalities (Soliman and Ghoneam, 2004; Abbas, 2017).

In this study chromosomal abnormalities were induced by high concentrations of TC. These abnormalities could be caused by several mechanisms, such as the mutagenic activity (deletion or insertion mutation), DNA damage (single or double-strand breaks), chromosomal aberration (Abbas et al. 2017).

CONCLUSION

The results obtained from this study indicated that TC is a potential antimitotic agent and hence confirms its traditional use in the treatment of prostate cancer. However, there is need for further in vitro and in vivo studies on mammals and especially humans.

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