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EVALUATION OF THE PHYTOCHEMICAL COMPOSITIONS AND GENOTOXIC POTENTIALS OF SOME ANTI-HEMORRHOID HERBAL PREPARATIONS SOLD IN NIGERIA

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ABSTRACT

The phytochemical analysis and cytotoxicity induced by extracts of packaged herbal preparations used for the treatment of hemorrhoids on root meristems of *Allium cepa* L. (onion) was conducted. Quantitative phytochemical analyses were carried out on the eighteen packaged herbal samples obtained from the six geopolitical zones in Nigeria. The developed roots of *Allium cepa* were transferred to control and infusions of the herbal products for 48 h and harvested between 7:30am and 8:30am West African Time for cytological studies. Results of the phytochemical analysis showed that there were significant ($P<0.05$) differences in the phytoconstituents (tannins, saponins, flavonoid, alkaloids, phenols and glycosides) of the samples. This suggests that the contents of these samples vary from product to product, therefore their potency and effects on cell cycle may also vary. In this study, chromosome aberrations such as C-mitosis, fragmentation, spindle disturbance and vagrant chromosomes were observed in all except three of the collected samples. Also, negative Relative Division Rate (RDR) and lower Mitotic Index (MI) compared to controls were recorded in sixteen samples which suggest that most of these products were genotoxic. The findings in this study suggests that anti-hemorrhoid herbal preparations should be taken with caution because of their toxic effect.

Key words: Phytochemical, Cytotoxic, Hemorrhoids, Aberrations, Anti-mitotic. Depth, Geothermal Energy.

INTRODUCTION

Traditional herbal preparations have been used for thousands of years in developing and developed countries for the treatment and /or management of a variety of diseases and conditions (Singh, 2015). This is due to their natural origin and minimal side effects. According to Biozid *et al.* (2020), a good number of people living in Africa use traditional medicine, especially herbal medicine for their basic health care needs. The high dependence on herbal medicine by people in developing countries rests on its easy accessibility, steady availability and affordability (Azeez and Isiugo-Abanihe, 2019). This according to Shu *et al.* (2019) led to the production of natural preparations marketed in cities and villages with assertions that it can effectively cure infectious ailments. Despite the long existence and continuous use of herbal preparations, Elujoba *et al.* (2005) reported that many nations have not given herbal remedies the needed attention.

Gami (2011) reported that humans are prone to hemorrhoids (pile) because the erect posture of man puts a lot of pressure on the veins in the anal region. According to Azeez and Isiugo-Abanihe (2019), hemorrhoid gets worse when there is a rupture in the blood vessels around the anus leading to painful conditions. Soladoye *et al.* (2010) reported that about one-quarter of the African populace have had hemorrhoids at age 50 while 50% to 85% of the World population have been reported to be affected by hemorrhoids at some points in their life. In spite of the fact that hemorrhoids and its associated discomfort have affected man for thousands of years, its natural cause is still unclear which leads to slender treatment options (Douglas, 2001). According to him, herbal treatments and nutritional therapy are safe and effective therapy for prevention and treatments of hemorrhoids. These herbal preparations for treatment of pile are packed in plastic bottles and hawked around in towns and cities in the form of 'agbo jedi' across Nigeria (Azeez and Isiugo-Abanihe, 2019).

Alege and Anthony (2020) and Ayo-Lawal *et al.* (2020) reported that among members of the

genus *Allium*, common onion (*Allium cepa*) has proven to be the most frequently used plant bioassay for the assessment of cytotoxicity and genotoxicity of toxic substance. Akinpelu *et al.* (2018) and Wijeyaratne and Wadasinghe (2019) reported that the consistent/constant use of *Allium cepa* for genotoxic assessment of substances rest on the rapid growth of its roots and fewer number of chromosomes in the cells. According to Firbas and Amon (2014) and Fiskesjo (1985), International Program on Plant Bioassay (IPPB) accepted *Allium cepa* root tip cells bioassay as a valid tool for assessing hazardous substances in the environment.

Banu and Catherine (2015) reported that natural plant phytochemicals unlike pharmaceuticals cure diseases without causing harm to human beings and are therefore considered "man-friendly medicines". Contrary to this assertion, Bent (2008) opined that the widespread belief that herbal preparations are harmless and without risk is a misconception. According to De-smet (2002), several side effects have been reported following continuous usage of herbal products. The inherent toxic nature and the side effects of medicinal herbs have been attributed to adulterations (Elujoba *et al.*, 2005). Lack of standardization (Onyegbule *et al.*, 2017) and insufficient studies on safety of herbal preparations (Muhammad and Awaisu, 2008) have been identified as the major problems militating against development of herbal medicine in Nigeria. Douglas (2001) reported that botanical treatments of hemorrhoids have been poorly researched. Cletus *et al.* (2017) evaluated the effectiveness of selected plants for the treatment of hemorrhoids in Bali, North-East, Nigeria. Soladoye *et al.* (2010) carried out similar study in South – Western, Nigeria while Azeez and Isuigo-Abanihe (2019) restricted their survey of anti-hemorrhoids herbal preparations to Oyo State, Nigeria. Okpuzor and Oloyede (2009) reported that tri-herbal drug for the treatment of hemorrhoids in Nigeria did not cause death of rats but was associated with sluggishness, general weakness and bloody eyes in these experimental animals. Alimba *et al.* (2016) carried out cytogenotoxic and haematotoxic screening of herbal pills used for the treatment of hemorrhoids in Nigeria. They therefore recommended studies on the toxicological assessments of anti-hemorrhoids herbal preparation sold in Nigeria. Against this background, the study was carried out to determine the phytochemical components and genoxicity induced by some herbal preparations for the treatment of hemorrhoids sold across the six geo-political zones in Nigeria.

MATERIALS AND METHODS

Collection of herbal preparations

Eighteen herbal preparations sold in plastic bottles for the treatment of hemorrhoids (piles) were purchased from eighteen different towns and states across the six geopolitical zones in Nigeria. The herbal samples were purchased directly from vendors and markets at these locations. The locations of sample collected are shown in Table 1.

Table 1: Towns, States and Geo-political Zones of the Collected Herbal Samples for Treatment of Hemorrhoids

Sample codes	Towns	States	Geo-political zones
A	Anyigba	Kogi	North Central
B	Jos	Plateau	North Central
C	Ilorin	Kwara	North Central
D	Sokoto	Sokoto	North West
E	Kiyawa	Jigawa	North West
F	Kano	Kano	North West
G	Jalingo	Taraba	North East
H	Yola	Adamawa	North East
I	Damaturu	Yobe	North East
J	Ado-Ekiti	Ekiti	South West
K	Ibadan	Oyo	South West
L	Lagos	Lagos	South West
M	Port Harcourt	Rivers	South South
N	Calabar	Cross Rivers	South South
O	Yenagoa	Bayelsa	South South
P	Aba	Abia	South East
Q	Abakaliki	Ebonyi	South East
R	Owerri	Imo	South East

a. Phytochemical analysis

A 350 mL volume of distilled water was poured into 10 g of each ground herbal sample in an airtight bottle to prepare the infusion. This was covered and left for 48 h before subjecting the filtrates to phytochemical analysis. Quantitative analysis of the six phytochemical components (i.e., Tanins, Saponins, Flavonoids, Alkaloids, Phenols and Glycosides) was done in triplicates according to the methods outlined by Evans (2002) and Banu and Catherine (2015).

Determination of saponin

The spectrophotometric method was used for saponin analysis. A 10 mL volume of the infusions from each sample was measured into 250 mL beaker and 100 mL of Butyl alcohol (Butanol) was added. The VDY agitator was used to obtain homogeneity by shaking the resulting compound for 5 h. Subsequently, 200 mL of 40% saturated magnesium carbonate ($MgCO_3$) was poured into the filter mixture. The resulting mixture was filtered through Whatman filter paper number 2 (pore size 8 μm) until a clear solution was obtained. A 2 mL volume of 5% Iron (III) Chloride ($FeCl_3$) solution was added to the mixture and filled up with distilled water to 50 mL mark. Thereafter, the set up was left to settle for 30 min. As done for the samples, 2 mL of 5% $FeCl_3$ solution was likewise added to standard solutions prepared from saponin stock. After colour development, Agilent Spectrophotometer was set at wavelength of 380 nm to read the absorbance of the standard saponin and samples.

Determination of tannins

A total volume of 10 mL of infusion from each sample was measured into 10 mL of distilled water. The setup was allowed to settle at room temperature for 30 min before intermittent agitation for a period of 30 min. Into separate 50 ml volumetric flasks, 2.5 mL of this mixture was added followed by 2.5 mL of standard tannic acid solution and 1 mL of Folin-Denis' reagent was added. Finally, 2.5 mL of saturated sodium carbonate (Na_2CO_3) solution was added. The solution was incubated for 90 min at room temperature after the content was diluted to 50 mL mark. The absorbance was read off the spectrophotometer at a wavelength of 250 nm.

Determination of phenolic

A 10 mL volume of each of the infusion from herbal samples was poured into different test tubes. This was followed by addition of 10 mL of 50% aqueous ethanol and left for 2 h at room

temperature. Thereafter, the mixture was filtered into 50 mL volumetric flask using Whatman filter paper (number 2) then 2.5 mL of Folin-Denis' reagent was added to each filtrate and allowed to stand for 30 min after which 50mL of saturated sodium carbonate (Na_2CO_3) was added to the mixture and left for 20 min. The absorbance of each of herbal sample was taken at 760 nm.

Determination of flavonoids

The flavonoid contents of the samples were determined using Aluminum Chloride colorimetric method by mixing 10 mL of the infusions with 0.1 mL of Aluminum Chloride, 1.5 mL of methanol, 2.8 mL of distilled water and 0.1 mL of Potassium acetate. After 30 min, the UV visible spectrophotometer was used to measure the absorbance of the reacting mixture at 413 nm.

Determination of alkaloids

From each herbal infusion, 50 mL of 10% acetic acid solution was taken and mixed properly with ethanol. The content was filtered after 4 h. One quarter ($^1/_4$) of the initial volume of the filtrate was evaporated followed by the addition of three drops of concentrated ammonium hydroxide (NH_4OH) to bring out the alkaloids. A 1% NH_4OH solution was used to wash the precipitate as it was filtered with Whatman number 2 filter paper. The precipitate was dried for 30 min at 60 ^oC before been reweighed. By weight difference, the quantity of alkaloid was measured and shown in percentage of the difference in weight using this formula:

$$\%\ \text{Alkaloids} = \frac{W2 - W1}{W} X\ 100$$

W = Weight of Sample
W_1 = Weight of empty filter paper
W_2 = Weight of filter paper + precipitate

Determination of glycosides

The herbal infusion from each sample was treated with 1 mL of $FeCl_3$ reagent (mixture of 1 mL of 5% $FeCl_3$ solution and 99 mL of glacial acetic acid). Concentrated H_2SO_4 was added in drops to the resulting solution while its absorbance was measured at 350 nm.

b. Cytotoxicity study

Small onion (*Allium cepa*) bulbs ranging from 12 cm to 15 cm in diameter and weighing between 3.00 g to 3.50 g were considered for this study. The loose outer scales were removed and the root primordials (in the reduced stems) were allowed to contact with distilled water in beakers until roots developed. Ten onions were grown per sample while five onion bulbs with satisfactory root developments were selected for further study. The developed roots were thereafter transferred into labeled beakers containing 100% concentration of the infusions from each of the eighteen herbal preparations while distilled water served as the control for the study. This was done following the methods outlined by Udebuani *et al.* (2016). After 48 h of growing the roots in the infusions and control, 1 cm of the root tips were cut into specimen bottles between 7:30am and 8:30am West African Time (WAP). Each treatment was replicated five times and arranged in Completely Randomized Design (CRD). The study proceeded in series of stages according to the method outlined by Akinleye (2007).

Fixation

Fixation of the root tips was done using a mixture of glacial acetic acid and absolute ethanol (1:3, v/v). The harvested root tips were immediately transferred into the fixative in labeled vials. The vials were thereafter kept in the refrigerator for 24 h. The fixative helps to keep the cells in their natural condition.

Hydrolysis

After 24 h, the root tips were taken out of fixatives and washed thoroughly in distilled water. Hydrolysis meant to soften the root tips were achieved by treating the fixed root tips with 1N HCl in water bath at temperature of 60^oC according to the methods of Wijeyarantne and Wadasinghe (2019).

Squashing and staining

The hydrolyzed root tips were washed with distilled water and each tip was placed on a clean glass slide. The meristematic tips were cut with blade and the remaining part discarded. The meristems were treated with few drops of aceto-carmine stain before covering with cover slips. Squashing was done using the wider surface of a

cylindrical search pin until a cloudy liquid was seen. Filter paper was used to remove extra stain by carefully exerting pressure. Limpid fingernail varnish was used to seal the margins of the cover slip to prevent the preparation from drying up.

For each treatment, five slides were prepared for each sample while ten microscopic views were considered per sample to give a total of fifty microscopic views per sample. The following counts were recorded from each microscopic view at 1000X magnification: total number of dividing cells, number of cells at interphase, number of cells at prophase, number of cells at metaphase, number of cells at anaphase, number of cells at telophase, and number of the different aberrant cells observed. This was done according to the methods described by Ping *et al.* (2012) with slight modifications. Altogether, a total of 1,500 cells were considered per sample. The percentage of cells with chromosomes irregularities, Mitotic Index (MI) and Relative Division Rate (RDR) for cells exposed to herbal infusions in relation to the control were determined by formulae outlined by Malode *et al.* (2012) as given below:

Percentage of Aberrant Cells (PAC) was calculated using the formula:

$$(PAC) = \frac{Total\ number\ of\ abnormal\ cells}{Total\ number of\ cells\ examined}\ X\ 100$$

$$Mitotic\ Index\ (MI) = \frac{Total\ number\ of\ dividing\ cell}{Total\ number of\ cells\ examined}\ X\ 100$$

The numbers of prophase, metaphase, anaphase and telophase were summed to represent the total number of dividing cells. The calculation of Relative Division Rate (RDR) was carried out as follow:

$$(RDR) = \frac{\%\ of\ dividing\ cells\ in\ treated\ root\ tips - \%\ of\ dividing\ cells\ in\ control\ root\ tips}{100 - \%\ of\ dividing\ cells\ in\ control\ root\ tips}\ X\ 100$$

Chromosome observation

The 4X, 10X, 40X objectives of the Digital model of the light microscope was employed to observe the slides. Photomicrographs of the normal mitotic stages and aberrant cells were taken at 400X magnifications.

Data Analyses

Data generated in triplicates on phytochemical components were analyzed using Analysis of Variance (ANOVA) and separation of significant means was done using the Least Significant Difference (LSD). P values < 0.05 were considered significant for all comparisons.

RESULTS

It was observed that all the six phytochemical components analyzed (tannins, saponins, flavonoid, alkaloids, phenols and glycosides) in Table 2 showed statistically significant differences (P<0.05) among the samples.

Sample M had significantly high tannin (13.54 mg/g), saponin (4.88 mg/g), flavonoids (12.04 mg/g) and phenol (449.23 mg/g) content while sample C had significantly high alkaloids and glycosides values of 2.32 mg/g and 0.96 mg/g respectively. Significantly low tannins, saponins, flavonoids and glycosides values of 4.56 mg/g, 1.23 mg/g, 2.86 mg/g and 0.16 mg/g respectively were recorded for sample H while sample G had significantly low alkaloids (1.16 mg/g) and phenols (99.63 mg/g) contents.

All the mitotic stages observed showed statistically significant differences (P<0.05) with respect to the different herbal preparations studied (Table 3 and Plate 1 A-I). These stages are number of cells at interphase stage, number of cells at metaphase stage, number of cells at anaphase stage, number of cells at telophase stage. There was remarkable decrease in the number of cells at prophase stage when treated with herbal preparations. The four chromosome aberrations recorded in this study are C-mitosis, fragmentation, spindle disturbance and vagrant chromosomes. None of the herbal samples produced all the four aberrations. Sample O produced three out of the four aberrations observed in this study while fragmentation is the only aberration induced by sample C. It was also observed that 66.7%, 5.6%, 27.8% and 27.8% of the studied herbal remedies for treating hemorrhoids produced C-mitosis, chromosome

fragmentation, spindle disturbance and variant chromosomes respectively.

From Table 4, the highest percentage of aberrant cells (2.22%) was recorded in sample O while samples G, K, and P did not produce any aberration. The lowest significant Mitotic Index (MI) was recorded for sample E with 19.48% while sample G had highest statistical significant Mitotic Index value of 33.49%. Only samples C (+0.40%) and G (+3.33%) representing 11.11% of the studied herbal samples studied had positive Relative Division Rate while the remaining sixteen samples (representing 88.89% of the studied herbal products) had negative Relative Division Rate with the highest inhibitory effect recorded in sample J (-16.04).

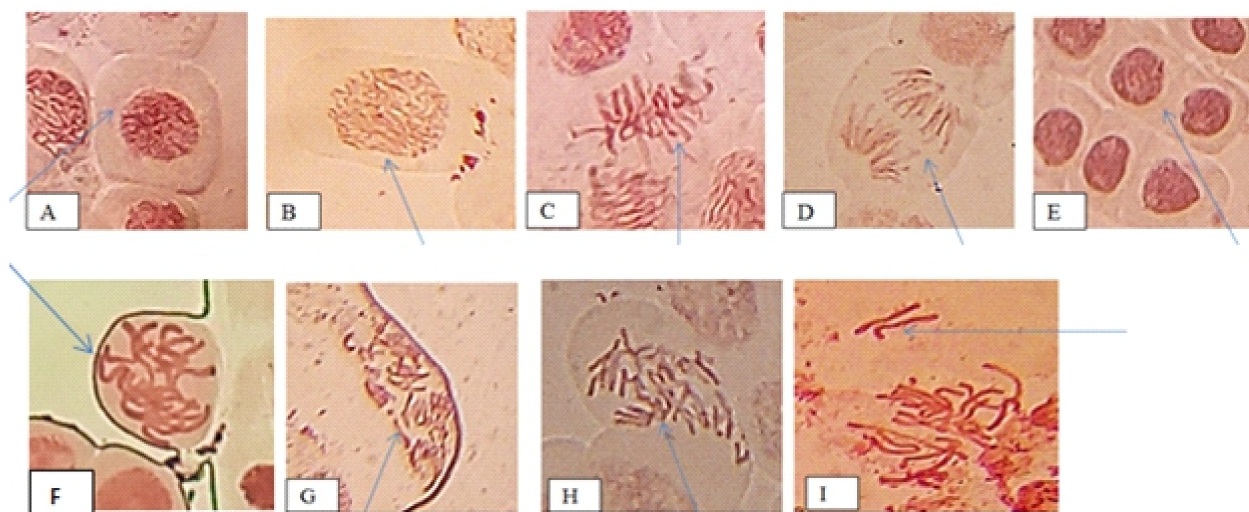

Figure 1A-I: *Allium cepa* root tip cells showing normal stages of mitotic division and aberrant cells from root tips treated with herbal preparations for the treatment of hemorrhoids (A) Normal interaphase (B) Normal prophase (C) Normal metaphase (D) Normal anaphase (E) Normal telophase, (F) C-mitosis (G) Chromosome Fragmentation (H) Spindle disturbance (I) Vagrant chromosomes (Magnification ×400).

Table 2: Phytochemical compositions of the eighteen herbal preparations studied

Herbal Samples	Tannins (mg/g)	Saponin (mg/g)	Flavonoids (mg/g)	Alkaloids (mg/g)	Phenols (mg/g)	Glycosides (mg/g)
A	8.17^{de}	2.07^{d}	9.91^{d}	1.25^{h}	144.02^{k}	0.57^{ef}
B	5.73^{i}	1.68^{e}	8.75^{e}	1.24^{h}	135.94^{l}	0.60^{e}
C	7.02^{g}	2.07^{d}	5.16^{f}	2.32^{a}	115.20^{m}	0.96^{a}
D	6.72^{h}	1.26^{f}	11.14^{c}	1.43f	235.83^{e}	0.55^{f}
E	6.66^{h}	1.27^{f}	11.54^{b}	1.44^{ef}	225.76^{f}	0.59^{ef}
F	5.64^{i}	1.34^{f}	9.83^{d}	1.45^{ef}	187.50^{i}	0.87^{b}
G	7.74^{f}	1.27^{f}	4.08^{g}	1.16^{i}	99.63^{q}	0.28^{hi}
H	4.56^{k}	1.23^{f}	2.86^{j}	2.26^{b}	168.09^{j}	0.16^{j}
I	6.98^{g}	1.25^{f}	3.94^{h}	1.18^{i}	102.43^{pq}	0.25^{i}
J	8.04^{e}	1.24^{f}	3.31^{i}	1.35^{g}	111.84^{no}	0.35^{g}
K	6.60^{h}	1.25^{f}	3.25^{i}	1.34^{g}	111.35^{o}	0.31^{gh}
L	4.95^{j}	2.14^{d}	3.25^{i}	1.23^{h}	104.84^{p}	0.32^{gh}
M	13.54^{a}	4.88^{a}	12.04^{a}	1.54^{c}	449.63^{a}	0.85^{b}
N	9.56^{c}	4.78^{ab}	11.50^{b}	1.47^{de}	366.88^{b}	0.87^{b}
O	11.43^{b}	4.71^{b}	11.18^{c}	1.48^{de}	294.00^{d}	0.85^{b}
P	8.42^{d}	2.93^{c}	11.08^{c}	1.50^{d}	206.91^{h}	0.94^{a}
Q	7.72^{f}	2.92^{c}	12.12^{a}	1.44^{ef}	318.28^{c}	0.78^{cd}
R	7.90^{ef}	2.95^{c}	11.44^{b}	1.38^{g}	217.76^{g}	0.69^{d}
LSD Values	0.29	0.17	0.50	0.04	9.55	0.04

❖ Means with the same alphabets in the same column are not significantly different at $P<0.05$

Table 3: Effects of the eighteen herbal preparations on cytological parameters of *Allium cepa* root tip

Samples	TNC	NOI	NOP	NOM	NOA	NOT	NOC	NFC	NSD	NVC
Control	185.00^{c}	127.90^{abcd}	33.50^{a}	10.90^{cdef}	7.60^{cd}	5.80^{cde}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{c}
A	179.90^{abc}	137.10^{fgh}	25.40^{cde}	9.90^{defg}	3.00^{fg}	2.40^{f}	0.50^{cd}	0.00^{b}	0.90^{a}	0.00^{c}
B	179.40^{abc}	135.60^{a}	18.00^{hi}	11.80^{cde}	2.90^{fg}	8.10^{bc}	0.80^{bcd}	0.00^{b}	0.00^{b}	0.00^{c}
C	177.50^{abc}	121.50^{hi}	26.40^{bcd}	16.70^{a}	8.60^{c}	3.30^{f}	0.00^{d}	0.90^{a}	0.00^{b}	0.00^{c}
D	179.50^{abc}	142.10^{hi}	19.00^{ghi}	8.10^{gh}	5.30^{defg}	3.70^{ef}	0.00^{d}	0.00^{b}	0.00^{b}	1.20^{ab}
E	178.40^{abc}	143.70^{i}	12.10^{i}	7.20^{h}	7.90^{cd}	6.30^{bcd}	1.20^{abc}	0.00^{b}	0.00^{b}	0.00^{c}
F	178.70^{abc}	134.90^{defg}	20.60^{efghi}	16.30^{ab}	2.60^{g}	2.70^{f}	1.40^{abc}	0.00^{b}	0.00^{b}	0.00^{c}
G	186.10^{c}	123.60^{ab}	30.20^{ab}	13.60^{abc}	15.30^{a}	3.30^{f}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{c}
H	176.80^{abc}	131.80^{cdef}	21.30^{efghi}	12.30^{cde}	5.50^{def}	4.10^{def}	0.00^{d}	0.00^{b}	0.00^{b}	1.60^{a}
I	174.80^{ab}	127.10^{abc}	16.70^{h}	13.00^{bcd}	7.70^{cd}	6.80^{bc}	1.90^{a}	0.00^{b}	0.00^{b}	1.60^{a}
J	181.10^{bc}	133.60^{cdefg}	23.90^{cdef}	8.90^{fgh}	5.40^{def}	8.30^{b}	1.00^{abc}	0.00^{b}	0.00^{b}	0.00^{c}
K	179.20^{abc}	128.90^{bcde}	28.40^{abc}	11.30^{cdef}	6.90^{de}	3.70^{ef}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{c}
L	170.30^{a}	128.70^{bcde}	19.90^{fghi}	14.20^{abc}	3.70^{efg}	2.30^{f}	0.60^{bcd}	0.00^{b}	0.80^{a}	0.00^{c}
M	182.50^{bc}	129.30^{bcde}	24.30^{cdef}	11.60^{cde}	3.10^{fg}	11.60^{a}	1.00^{abc}	0.00^{b}	0.00^{b}	0.90^{b}
N	178.70^{abc}	129.90^{bcde}	22.00de^{fgh}	12.20^{cde}	6.40^{cde}	7.20^{bc}	0.80^{bcd}	0.00^{b}	0.00^{b}	0.00^{c}
O	184.40^{c}	130.50^{bcdef}	18.80^{ghi}	13.50^{abc}	11.40^{b}	6.00^{cd}	1.40^{abc}	0.00^{b}	1.10^{a}	1.60^{a}
P	185.90^{c}	129.30^{bcde}	23.10d^{efg}	16.90^{a}	13.30^{ab}	3.10^{f}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{c}
Q	184.10^{bc}	135.20^{efg}	18.60^{ghi}	9.30^{fgh}	12.40^{b}	5.90^{cde}	1.50^{ab}	0.00^{b}	1.10^{a}	0.00^{c}
R	185.30^{c}	139.40^{ghi}	22.90^{defg}	13.90^{abc}	6.40^{cde}	3.10^{f}	1.00^{abc}	0.00^{b}	0.70^{a}	0.00^{c}
LSD Values	0.68	0.63	0.48	0.31	0.32	0.24	0.79	0.25	0.46	0.15

❖ Means with the same alphabets in the same column are not significantly different at P<0.05

KEY

TNC- Total number of dividing cells
NOI- Number of cells at interphase stage
NOP- Number of cells at prophase stage
NOM- Number of cells at metaphase stage
NOA- Number of cells at anaphase stage
NOT- Number of cells at telophase stage
NOC- Number of cells with C-mitosis
NFC- Number of fragmented chromosomes
NSD- Number of cells with spindle disturbance
NVC- Number of cells with variant chromosomes

Table 4: Effects of the eighteen herbal preparations on cell division indices of *Allium cepa* root meristems

Herbal Samples	Mitotic Index (%)	Percentage of Aberrant Cells (%)	Relative Division Rate (%)
Control	30.69^{ghi}	0.00	0.00
A	23.45^{bc}	0.78	-11.40
B	23.26^{bc}	0.45	-11.71
C	31.47^{hi}	0.51	+0.40
D	20.79^{ab}	0.67	-15.21
E	19.48^{a}	0.68	-17.15
F	24.59^{bcd}	0.78	-9.95
G	33.49^{i}	0.00	+3.33
H	25.38^{cde}	0.91	-8.58
I	27.28^{cdefg}	2.00	-5.75
J	26.07^{cde}	0.55	-16.04
K	28.02^{defgh}	0.00	-4.61
L	24.37^{bcd}	0.82	-9.99
M	28.77^{efgh}	1.04	-3.59
N	27.06^{cdefg}	0.45	-5.88
O	29.19^{efgh}	2.22	-3.00
P	30.26^{fghi}	0.00	-1.45
Q	26.50^{cdef}	1.41	-6.88
R	25.35^{cde}	0.92	-8.55
LSD Values	0.37	-	-

❖ Means with the same alphabets in the same column are not significantly different at P<0.05

DISCUSSION

Allium cepa test according to Ayo-Lawal *et al.* (2020) is a generally accepted assay for the detection of cytotoxic and genotoxic agents in substances including medicinal plants. From this study, the eighteen herbal preparations studied contained tannins, saponins, flavonoid, alkaloids, phenols and glycosides in varying quantity. This suggests that the contents of the packed herbal samples vary from product to product, therefore their potency and effects on cell cycle may also vary. Akinpelu *et al.* (2018) attributed the bioactivities of medicinal plants to presence of secondary metabolites such as phenolics, terpenoids, cardiac glycosides, and flavonoids.

The presence of C-mitosis, chromosome fragmentation, spindle disturbance and variant chromosomes in sixteen of the eighteen anti-hemorrhoid herbal preparations studied suggests that these samples are genotoxic and contains substances that cause alterations in the genetic integrity of chromosomes at various stages of the cell cycle. The fact that all the four chromosome alterations reported in this study arose as a result of poor establishment of spindle fibre during cell division suggests that the infusions operate by distorting the process of spindle fibre formation. Mohammed and Najem (2020) stated that saponins exert anti-microtubules effect and polymerization of tubulin which impairs spindle filaments function. In line with this, Ayo-Lawal *et al.* (2020) opined that the induction of chromosome abnormality in dividing cells has significant implication on the integrity of genetic systems especially the DNA.

This study revealed that meristems grown in infusions from herbal samples G, K and P did not produce any chromosome aberration which indicates that the three herbal preparations were produced from plant materials that are not genotoxic.

The lower Mitotic Index (MI) in relation to control and negative Relative Division Rate (RDR) recorded for sixteen out of the eighteen herbal preparations for the treatment of hemorrhoids studied indicate that the samples exhibit inhibitory effects on cell division by interfering with replication processes of cellular DNA thereby inhibiting proliferation of cells during mitotic cell division. According to Saboo *et al.* (2014), anti-mitotic agents contain substances that block cell multiplication during cell division. The decrease in mitotic index according to Badr *et al.* (2020) and Jayasree *et al.* (2014) may result from block in G1 stage of cell cycle leading to suppression of DNA synthesis. Anti-mitotic agents obstruct mitosis and cause cell death through apoptosis by interacting with microtubules and tubulins in the cell (Govindappa *et al.*, 2015). This finding therefore reveals that most of the anti-hemorrhoid herbal products considered in this study are genotoxic and should be taken with caution.

CONCLUSION

This study revealed that some of the anti-hemorrhoid herbal preparations considered in this study are cytotoxic and genotoxic to the chromosomes which eventually impaired the integrity of genetic materials during cell division in *Allium cepa* meristems. In this study, genotoxic activities of most of the herbal preparations evaluated have been reported which indicates that the preparations should be taken with caution because of the possible toxic effects.

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