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Research Article

Cytotoxic Effects of (5) Medicinal Plants on Mitosis in *Allium cepa* Root Tips

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Abstract: The study was conducted to investigate the effects that plant extracts from 5 medicinal plants may have on mitosis in *Allium cepa*. Root of *A. cepa* were immersed in alcoholic extracts at the concentrations of 0, 25, 50, 75 and 100 mg/mL, respectively for each of the following plants: *Gnetum africanum* Welw., *Lasianther aafricana* P. Beauv, *Ocimum gratissimum* Linn., *Telfairia occidentalis* Hook F. and *Vernonia amygdalina* Del. Leafy vegetable which are commonly used in herbal medicine. Results obtained show that the various concentrations of the extracts from test plants had toxic effects on the cells, which caused significant reduction ($p<0.05$) in the mitotic index when compared with the control. Other effects were prophase inhibition, the delay of mitosis and nuclear lesion. The cytotoxic effect makes a case for a precaution in the use of the leafy extracts in herbal medicine practice.

Keywords: Alcohol, *Allium cepa*, cytotoxic effect, mitosis

INTRODUCTION

Medicinal plants are a common, cheap and renewable source of pharmacological active substances and because of this it is extremely important that genotoxicity tests are applied to the active ingredients of these preparations in order to assess their mutagenic potential (Apostolides *et al*., 1996).

The plants *Lasianthera africana* P. Beanv. (*Icacinancea*), *Telfairia occidentalis* Hook. F. (*Cucurbitacea*), *Gnetum africanum* Welw. (*Gnetaceae*). *Vernonia amygdalina* Del.; and *Ocimum gratissimum* Linn. *Lasianthera africana* are natives of the mostly tropical rain forest zone and are widely cultivated in home gardens and used as leafy vegetables (Dutta and Mukerji, 1952). The stem and leaves of *Lasianthera africana* have been reported to be rich in chemical compounds which prevents internal bleeding as well as provide other beneficial effects to the body (Ebana *et al*., 1996). The leaf extract of *T. occidentis* is drunk to treat anaemia (Etukudo, 2003). The leaves of *Gnetum africanum* are eaten raw by the Igbo's community as vegetable salad when sliced and mixed with palm oil and salt (Etukudo, 2003). The leaves of *V. amgdalina* are widely used for treating fevers as a quinine substitute. The leaves extract is also used for rheumatism. *Ocimum gratissimum* leaves extracts are used in the treatment of headache, diarrhea, worms and kidney function (Seung-Joo-Lee *et al*., 2004). This study seeks to throw some light on the possible cytotoxic effects of the alcoholic extracts of these vegetable and medicinal plants which are so commonly used in Nigeria communities.

MATERIALS AND METHODS

Collection of plant material: *Lasianthera africana* P. beavu., *Telfairia occidentalis* Hook F., *Gnetum africanum* Welw., *Vernonia amygdalina* Del. and *Occimum gratissimum* Linn were obtained from Botany Research Garden in University of Uyo. The plants were identified at the Herbarium unit of the Department of Botany and Ecological Studies, University of Uyo, where the voucher specimens have been deposited. The leaves were harvested and taken to the pharmacognosy Laboratory for the extraction. The leaves were air dried and pulverized using mortar and pistle in the laboratory as described by Mukhtar and Tukur (1999). Thereafter, alcoholic extracts of the plants were prepared using the method of Fatope *et al*. (1993). The extracts were serially diluted to the concentrations of 0, 25, 50 and 100 mg/mL, respectively.

Treatment of onion root tips: Young sprouting roots at the base of the onion bulbs were immersed in the different concentrations of the plant extracts for 12 h. Thereafter, the onion bulbs were returned to distill water for 12 h to observed if there was recovery from any possible damage. The roots were fixed and stained using the aceto-orcein and mounted on slides. The slides were examined using high power objectives of the microscope (40x objectives). One hundred cells were examined on each slide and three slides were prepared for each concentration. Mitotic Index (M1) was recorded and cells with abnormalities were examined. The statistical evaluation was performed using one way analysis of variance and where this was

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significant, the Least Significant Difference (LSD) analyses was used to separate the means.

RESULTS

Table 1 shows the mean mitotic indices of the control and the various alcoholic extract concentrations of the medicinal plants after 12 h treatments.

Analyses of variance of the mitotic indices indicated significant differences between the various concentrations of the extracts in *Gentum africanum*, *Lasianthera africanan*, *Ocimum gratissimum*, *Telfairia occidentalis* and *Vernonia amygdalina*. Least Significant Difference (LSD) shows that mitotic index in root tips treated with the various concentrations of the alcoholic extracts of all the medicinal plants except *V. amygdalina* was significantly depressed ($p<0.05$) at all concentrations when compared with the control. However, there was no significant difference in the mitotic index at the concentrations of 75 and 100 mg/mL in the root tips treated with *T. occidentalis* extract.

Table 2 shows the mean mitotic indices in the control and in the various concentrations of the alcoholic extracts of the medicinal plants after 12 h recovery in distilled water.

The mean mitotic indices in the control and the various concentrations of the alcoholic extracts of the medicinal plants after 12 h recovery in distilled water are shown in Table 2. Analyses of variance of the mitotic indices indicated significant difference between the various extract concentrations. The analyses indicated that in *G. africanum*, *L. africana* and *O. gratissimum* the mitotic index was significantly reduced ($p<0.05$) at all concentrations when compared with the control. There was no apparent tend in the reduction of mitotic index but the data showed that this was most severe in the recovery treatment of 25 mg/mL *G. africanum* and 75 mg/mL *L. africana*.

Figure 1A shows that the mean numbers of cells in prophase stage was more strongly reduced in *Gnetum africanum*, *Lasianthera africana*; and *Ocimum gratissimum* than in *Telfaira occidentalis* and *Vernonia amygdalina* at all the concentrations. There was also a noticeable increase in the mean number of prophase cells in *Telfairia occidentalis* as the concentration of the aqueous extract increased.

From the graph in Fig. 1B it is evident that the mean number of cells in the metaphase stage was strongly increased in *Telfairia occidentalis* and *Vernonia amygdalina* above that of the control at 25 mg/mL concentration. This number dropped below that of the control at 50 mg/mL and increased when the roots were exposed to 75 mg/mL concentration of the aqueous extracts. However only the number of cells in *T. occidentalis* exceeded that of the control. At 100 mg/mL, the number of cells in the *T. occidentalis* treatment exceeded that of the control by 50% while that of *Vernonia amygdalina* dropped to about half of the control. *Gnetum africanum*, *Lasianthera africana* and *Ocimum gratissimum* showed reduction of the metaphase stage at all the concentrations.

The graph in Fig. 2C shows that the mean number of cells in anaphase was reduced in *Gnetum africanum*, *Ocimum gratissimum* and *Vernonia amygdalina* with the increased in concentration of the aqueous solutions while in *Telfairia occidentalis*, there was a noticeable increased with increased in the concentrations when compared with the control. *Lasianthera africana* had the most severe depressive effect at 50 mg/mL treatment.

The graph in Fig. 1A cells shows that the mean number of cells in prophase was more strongly reduced in *Lasianthera africana* and *Ocimum gratissimum* than in *Gnetum africanum*, *Telfairia occidentalis* and *Vemonia amygdalina* after 12 h recovery period. There was also a noticeable increase in the mean number of prophase cells in *Vernonia amygdalina* at 75 and 100 mg/mL concentrations.

Table 1: Concentrations of alcoholic extract of the medicinal plants after 12 h treatment

Mitotic index					
Concentration mg/mL	*G. africanum*	*L. africana*	*O. gratissimum*	*T. occidentalis*	*V. amygdalina*
0	18.36	18.36	18.36	18.36	18.36
25	9.21*	10.51*	12.64*	13.71*	14.79
50	10.51*	10.59*	12.25*	12.54*	10.15
75	10.66*	11.64*	11.62*	16.48	10.66
100	9.58*	10.11*	11.19*	17.48	9.58
LSD ($p<0.05$)	3.80	2.34	2.91	2.96	-

*: Significantly different from the control ($p<0.05$)

Table 2: Concentrations of the alcoholic extracts of the medicinal plants after 12 h recovery

Mitotic index					
Concentration mg/mL	*G. africanum*	*L. africana*	*O. gratissimum*	*T. occidentalis*	*V. amygdalina*
0	18.36	18.36	18.36	18.360	18.36
25	9.48*	10.98*	12.59*	13.540*	14.56
50	10.55*	11.56*	11.08*	12.254*	11.07*
75	11.88*	9.12*	12.04*	16.330	14.06*
100	11.14*	10.38*	11.30*	16.850	11.82*
LSD ($p<0.05$)	4.53	2.27	2.53	4.120	4.04

*: Significantly different from the control ($p<0.05$)

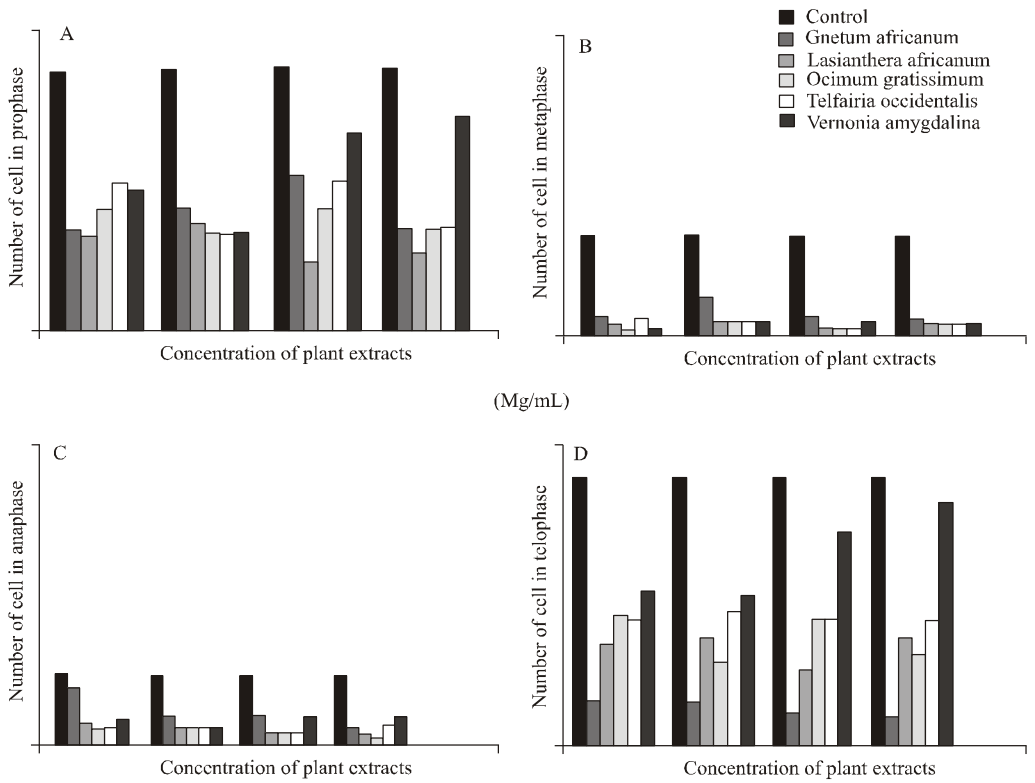

Fig. 1: Mean number of cells in telophase in the various concentrations after 12 h treatment and 12 h recovery in distilled water of *A. cepa* root tips, (A) prophase, (B) metaphase, (C) anaphase, (D) telophase

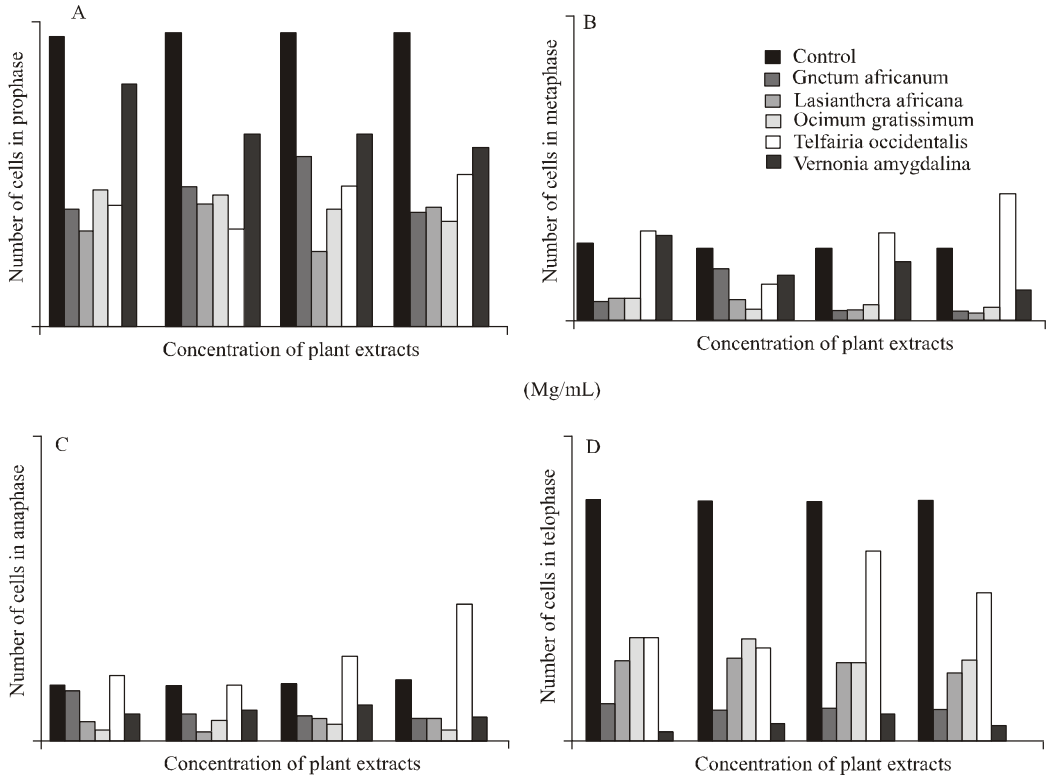

Fig. 2: Mean number of cells in telophase in the various concentrations after 12 h treatment in distilled water of *A. cepa* root tips, (A) prophase, (B) metaphase, (C) anaphase, (D) telophase

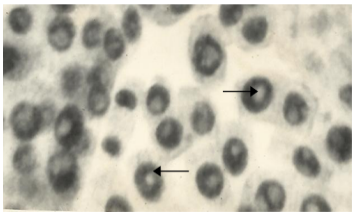

Fig. 3: Nuclear lesions (arrowed) in onions interphase cells treated with concentrations of 50 mg/mL of extract of *T. occidentalis* after 12 h treatment

The graph in Fig. 1B shows that mean number of cells in the metaphase stage was highly suppressed in *Gnetum africanum*, *Lasianthera africana*, *Ocimum gratissimum*, *Telfairia occidentalis* and *V. amygdalina.*

Figure 1C shows that the mean number of cells in anaphase was strongly reduced in *O. gratissimum* at the 100 mg/mL treatment with 12 h recovery compared to control, *V. amygdalina* was less suppressed at 75 and 100 mg/mL than *O. gratissimum*, *L. africana*, *G. africanum* and *T. occidentalis* (Fig. 3).

The graph in Fig. 1D shows that the mean number of cells in telophase was not so strongly reduced in *V. amygdalina* as in the other treatments at the 7 and the 100 mg/mL treatments with 12 h recovery.

The abnormality that was observed in the root tips cells of *Allium cepa* at all the concentrations of the aqueous extracts was the appearance of nuclear lesions which is characterized by appearance of clear areas in the nucleus. Also, no chromosomal aberrations were observed using the test plant extracts at all stages of mitosis, though nuclear lesions were observed. The extracts from the various test plants had toxic effects on cell division as it caused a general reduction in mitotic index when compared with that of the control.

DISCUSSION

The reduced mitotic indices were due to delay in cell division imposed by the test plant extracts. A similar result was obtained by Burim *et al*. (1999) and Dias and Takahashi (1994) on *Alphinia mutans* extract in *A. cepa* root tip cells. It is likely that the extracts affected the protein components of the chromatin, leading to their dissolution, hence the lesions.

The accumulation of prophase and telophase stages as observed in Fig. 1 and 2 may be due to lack of spindle fibres formation that would have introduced the cells to another stage, due to the interaction of the tested extracts. The results agree with the work of El-Ghamery *et al*. (2000) and Kumar and Rai (2007) on evaluation of cytological effects of Zn+ in relation of germination and root.

The prophase stage was found to be higher in all treated plants when compared with the control. Prophase stage in both cases had highest mean number of cells. This is followed by the telophase, anaphase and metaphase in that order. This differs from the findings of Moore (1976) where it was reported that herbicidal treatment of plants results to higher percentage in the order of prophase, telophase, metaphase and anaphase. Moore (1976) findings support preponderance of the prophase stage in the treated cells. Although cytotoxic effects was reversible with slight recovery in cell division after 12 h recovery in water. This agrees with the work of Itoyama *et al*. (1997) and Grisolia *et al*. (1995) which suggest that it is possible that a high concentration of any chemical will have an effect on the cell cycle; as has been shown for caffeine in *Drosophila prosaltan* and *Progostemun heyneanes* extracts in *A. cepa* root tip cells.

The greater number of cells at the interphase stage in the treated root tips suggest that the effects of the extracts was also to delay the initiation of mitosis thus the extracts had an inhibitory effect on mitosis. Similar results were reported by Kabarity and Malallah (1980) and Formina *et al*. (1989) with the root tips of *A. cepa*.

CONCLUSION

The extract of these five traditional medicinal plants used in this study have been shown to have cytotoxic and possibly genotoxic effects on *A. cepa* root tips. They decreased the mitotic index at all concentration and also induced nuclear lesion. In view of the cytotoxic data gathered in this study the use of the plants for herbal medicinal purposed should be with caution.

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