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RESEARCH ARTICLE **Bhartiya Krishi Anusandhan Patrika**

Cytogenetic Infractions of Latex Extract of the Floristic Dumbcane (*Dieffenbachia amoena*) on Mitotic Chromosomes of Onion (*Allium cepa*)

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ABSTRACT

Background: *Diffenbachia amoena* commonly called dumb cane is a houseplant found in homes, offices, banks and landscape premises as ornamental plants. This family of plants has shown high levels of acute and chronic toxicities with high cytogenic, mutagenic, carcinogenic, genotoxic potentials. Several studies had implicated some phytochemicals contained in the whitish latex sap including saponins, glycosides, tannins and oxalates to be responsible for the toxicity of this houseplant to plant cells and proteins.

Methods: Cytological investigation protocol was used to determine cytogenetic infractions such as chromosome stickiness, lagging chromosomes, bridged chromosomes, deletions and chromosomal aberrations. The phytochemicals contained in the latex were determined using high performance liquid chromatography while computational biology approach was used to determine the latex phytochemicals interactions with the onion plant proteins using SIB stitch of expasy.org.

Result: Cytogenetic studies reveals that Dumb cane causes significant effect and infractions in the cytogenetics of onion cells ranging from chromatid-type breakage-fusion-bridge, chromosome stickiness, lagging chromosomes, bridged chromosomes, deletions and chromosomal aberrations. The latex sap from the stem contains oxalates, saponins, glycosides, tannins, alkaloids to varying degree which impairs photosynthetic and biochemical processes in the plant system. Phytochemicals-proteins interactions revealed that oxalates impairs and inhibits the formation and functionality of alanine glycoxylatetransferase (GRHPR), Chromobox homolog 5 chromosome (CBX5), alpha ketaglutarate dehydrogenase (AGXT) and glyoxylate reductase (OGDH) genes. Hence, there is need for enlightenment of the public on the dangers and toxicity of this houseplant in rural community households, urban cities, offices, recreational parks, business centers where the use of this deadly plant as ornamental is still very predominant.

Key words: Chromosomal aberrations, Cytotoxicity, Dumbcane, Genotoxicity, Leaf extract, Onions.

INTRODUCTION

Plants are known to have ornamental, nutritional, industrial and medicinal properties which enable them to be utilized in the decoration of residence, generation of staple food, raw plants in industries (Pankaj and Kumari, 2013) and in the prevention and treatment of diseases and ailment such as flu, typhoid, malaria, hypertension and diabetes mellitus *etc*. and this knowledge has been passed down from one generation to another using various forms of documentation (Tulachan *et al.,* 2014). Naturally occurring compounds of plants and animals origin exist in different forms as saponins, flavonoids, resins, terpenoids, alkaloids, glycosides, tannins (Mecina and Montenoti, 2016), amongst others and have been used primarily as sources of medicinal therapy from time immemorial (Olorunfemi *et al.,* 2011). Some of these naturally occurring compounds in plants are toxic at low or high concentrations to plants and animals and some of theseplants have also been used in indoor ornamental decorations in most exotic homes, offices, banks, school premises and relaxation centers by people grossly ignorant of their grave dangers (Shebab, 2000, Sofowora, 2008).

The name dumb cane is derived from a temporary speechlessness after chewing a piece of the stem. Juices or sap from the stem contains oxalates and other substances which irritate the mucousal membranes and cause rapid swelling and inflammation of the tongue and throat. Ingestion

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burning sensations to the mouth and throat, swelling of the mouth and throat, hoarse voice, nausea, vomiting and blindness if sap is in contact with the eyes (Sheela and Thorat, 2017). This family of plants has shown high levels of acute and chronic toxicities with high cytogenic, mutagenic, carcinogenic, genotoxic potentials.

The plant is native to tropical America and the West Indies, especially Costa-Rica and Colombia but presently it can be found in many tropical and subtropical climates, including African, Nigeria, Cross River State and Calabar. Reports of its toxicity has not been popularized even as at the time of this report and thus the need for the creating awareness through research study and publishing results of the findings to prevent further damages and deaths arising from the continual use of these plant species as houseplant or ornamental in homes, offices and premises in the area (Joshi *et al.,* 2013). Phytochemical studies conducted on *Dieffenbachia* plant species have implicated some of the phytochemicals such as alkaloids, saponin, glycocides and oxalates to be responsible for the acute and fatal toxicities associated with the whitish liquid sap of the plant species (Blessing *et al.,* 2009).

Dieffenbachia amoena toxicity and mutagenic profile and studies of Cytotoxic and Genotoxic potential have been conducted in order to assess the risk of ingesting these plants for food or medicinal purpose (Abu and Duru, 2006).

Among assays concerning cytotoxic potential stands out *Allium cepa*, because it can be used to determine both cytotoxic and genotoxic effects of many kinds of chemical and those of vegetables origin (Dutta, 2002).

Thus this paper seeks to create the desired awareness and enlightenment on the dangers of this houseplant ornamental plant *Dieffenbachia* species with a view to saving lives and humanity.

MATERIALS AND METHODS

Experimental site and period of study

The research experiment on the cytogenetic infractions of latex extract of the floristic dumbcane (*Dieffenbachia amoena*) on mitotic chromosomes of onion (*Allium cepa*) was carried out in the Department of Genetics and Biotechnology, Faculty of Biological sciences, University of Calabar, Calabar Nigeria. The research was carried out from March 2021 to September, 2022.

Preparation of plant extract of *Dieffenbachia amoena*

Freshly harvested leaves of *D. amoena* (Fig 1) were chopped into smaller pieces and macerated with the hand to get the whitish latex sap which was used immediately for the pre-treatment of onion root tips. Also 0.02 g 8-hydroxyquiniline in 100 ml of also prepared and used as control for pre-treatment of onion root tips.

Cytological treatment of onion root tips with latex sap and 8-hydroxyquinolene

Allium cepa (onion bulb) (Fig 2) bought from watt market in Calabar was germinated in a conical flask containing water. When the young roots attained about 1-2 cm in length, onion bulbs were transferred into petri-dishes containing undiluted 100% concentration of whitish latex sap of *Dieffenbachia amoena* and allowed to grow for 24 hours. 8-Hydroxyquinoline was used as control. The young root-tips were then harvested at the termination of the designated time period and subjected to cytological analysis.

In fixation, the root-tips were removed from each concentration of the dump cane latex sap and control, washed in water, dried and fixed using prepared alcoholic fixatives the Carnoy's solution of glacial acetic acid and ethanol in the ratio 3:1(v/v) respectively and stored overnight in a refrigerator at 4°-6°C. After fixation, the root-tips were hydrolyzed in IN HCl (1 ml of HCl: 99 ml of water) and put in a water bath at 60°C acid temperature for 6-8 mins.

Microscope slides were prepared by placing the root tips on the slide and with a surgical knife, the root-tip were excised leaving the milky part on the slide while discarding the other part. Using a scalpel, the milky root-tip was macerated. Maceration is done to further loosen the cell structure. The macerated root-tips were stained using 1-2 drops of aceto-orcein stain and then a cover slip was placed over the prepared slide, mounted and observed for cytological infractions with the aid of AMSCOPE 1000 MA digital camera attached to the microscope.

Data analysis

Data from the microscope analysis was taken on dividing and non- dividing cells on three slides in each treatment combination. The types and number of abnormalities were scored and recorded for each slide. Collected data was analyzed using simple descriptive statistics. At the end of the experiment, the following parameters such as, number of chromosomes, sticky, lagging, bridged, total aberrant chromosomesand mitotic index (ratio between the number of mitotic cells and the total number of cells scored and expressed in percentage) and the cytotoxic effect of the dump cane latex sap on onion root tip cells were scored at different mitotic stages using 100-200 cells count each.

Chemical-proteins interactions of phytochemicals showing genotoxicity in biological systems

Several research and reports carried out on *Dieffenbachia* species has implicated the following phytochemicals and antioxidant as responsible for the toxicity.

1. Oxalates
2. Glycosides
3. Saponins
4. Tannins

The European molecular chemical-proteins interaction network analysis (STITCH) online interactive program was used to identify each of the phytochemicals in Dieffenbachia sap and the proteins they interact with in the plant biochemical systems to identify possible toxic effects and damages on biological systems.

RESULTS AND DISCUSSION

Cytotoxicity of *Dieffenbachia amoena* latex sap on onion mitotic chromosomes

Analysis of altered chromosomes showed they were derived from a chromatid-type breakage-fusion-bridge (BFB) cycle due to the impact of latex sap on chromosomes and DNA.

Fig 1: Household floristic dumb cane (*Dieffenbachia amoena*) plant used as ornamental plants.

Fig 2: Growing onion bulb with root tips.

Investigations on mitotic cell divisions in onions as influenced by *D. amoena* latex sap treatment reveals the following chromosomal abnormalities; Stickiness, Multipolar chromosomes with spindles, Multipolar chromosomes without spindles, Fragments and bridges (Fig 3a), Lagging chromosomes (Fig 3b), unequal chromosome distribution, star shape arrangement of the chromosomes, Increased cell size, failure in cell plate formation, stoppage of spindle apparatus with abnormalities like, stickiness, fragments, bridges, lagging or dysfunction, unequal distribution. Normal chromosomes from control are shown in Fig 4.

Results shows chromosome number in Onion = 8 = n; Diploid number = 2n = 16; Haploid number = n = 8; 2n = 2× = 16 (Table 1).

Sticky chromosomes

The chromosomal infractions and effect of latex sap of *D. amoena* plays a significant role in reducing cell division processes and reveals a serious impact on growth of the onions bulbs in the field (Fig 5). Cytogenetic infractions is seen in chromosomes that remains sticky and refuse to unwind leading to aberrations due to stickiness thus impacting great economic loss to farmers and causing food insecurity.

Bridged chromosomes

The effect of latex sap of *D. amoena* Interferes with mitotic phases of cell division and hinders onion growth through bridge segregating error and fusion with sister chromatids (Fig 6). It also directly reduced growth of onion bulbs due to bridge chromosomes breaking at anaphase resulting in the poor growth and yield status of onion plants.

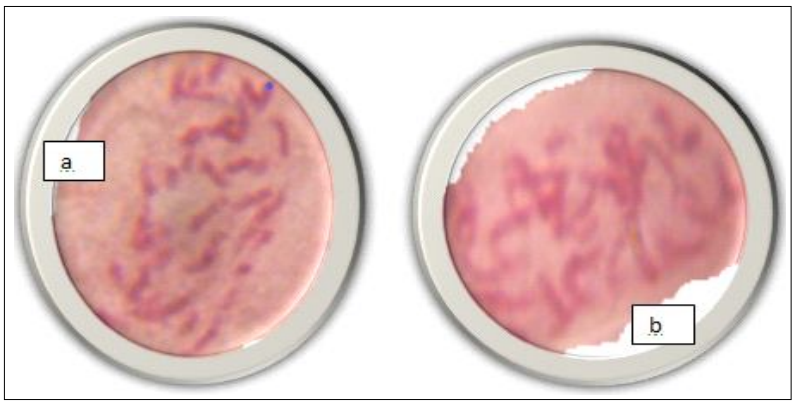

Fig 3: Mitotic chromosomes of onion (*Alium cepa*) pretreated with 8-hydrooxyquinilene (a) and dunbcane latex (b).

Table 1: Chromosomal aberration in onion root tips as influenced by latex sap of *Diffenbachia amoena.*

Parameter	Method	Dumbcane latex sap pre-treated onion root tips	8-hydroxyquinoline pretreated root tips (Control)
Chromosome number (2n)	Cytological analysis	16	16
No. of Sticky chromosomes (%)	Cytological analysis	87.43	2.12
Bridged chromosomes (%)	Cytological analysis	90.21	1.42
Lagging chromosomes (%)	Cytological analysis	88.86	3.98
Total aberrant chromosomes	Cytological analysis	266.50	7.52

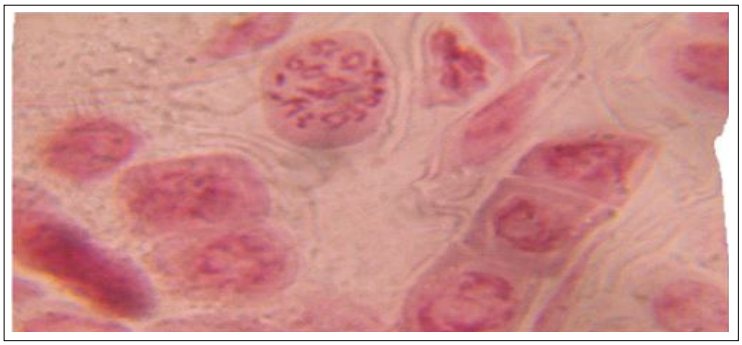

Fig 4: Normal metaphase chromosomes on onion in control experiment.

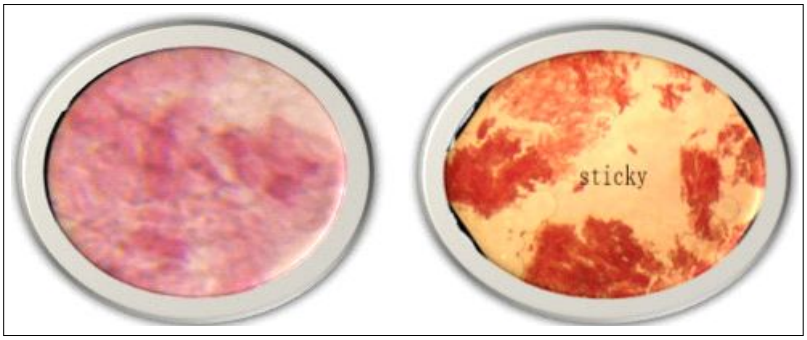

Fig 5: Unequal distribution and sticky chromosomes of *Allium cepa* as influenced by dumbcane latex sap.

Fig 6: Bridge chromosomes of *Allium cepa* as influenced by dumbcane latex sap.

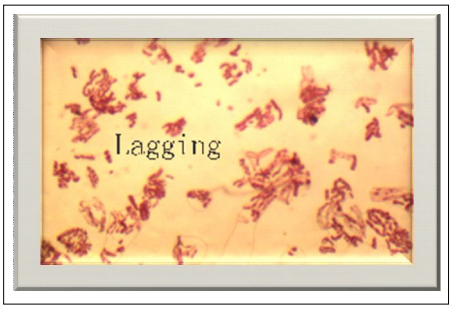

Fig 7: Lagging and dysfunctional chromosomes of *Allium cepa* as influenced by dumbcane latex sap.

Lagging chromosomes

The chromosomal infractions of latex sap of *D. amoena* directly impair segregating chromosomes at mitotic stages of cell division in the onion plants. Lagging chromosomes refuse to overlap during crossing over and cause chromosomal infractions and abnormalities which directly affect growth and reproduction of the onion plants (Fig 7).

Total aberrant chromosomes

This reveals the total number of aberrant and abnormal cells in the onion plants (Table 1). Aberrant chromosomes are induced by deletions, additions, duplications and inversions occasioned the presence of sticky, bridged or lagged chromosomes.These are responsible and highly implicated in chromosomal mutations in the onion exposed to the highly toxic latex sap of *D. amoena*.

Genotoxicity of latex sap of *Dieffenbachia amoena* on onion root tips

Oxalates in Dieffenbachia and protein interactions in onion plants

Oxalates (Fig 8 and 9) are naturally occurring molecules found in abundance in dieffenbachia species and many other plant species especially edible plants and leads to photosynthetic inefficiency in plants.

AGXT (Fig 10) is alanine-glyoxylate aminotransferase protein that process essential amino acids production in the onion plant cells and their further metabolism. These enzymes are disrupted by oxalates as antioxidants and interrupt the processing and production of essential amino acids in the plant system.

CBX5 (Fig 11) is the chromobox homologue 5 which is a component of heterochromatin that recognizes and binds

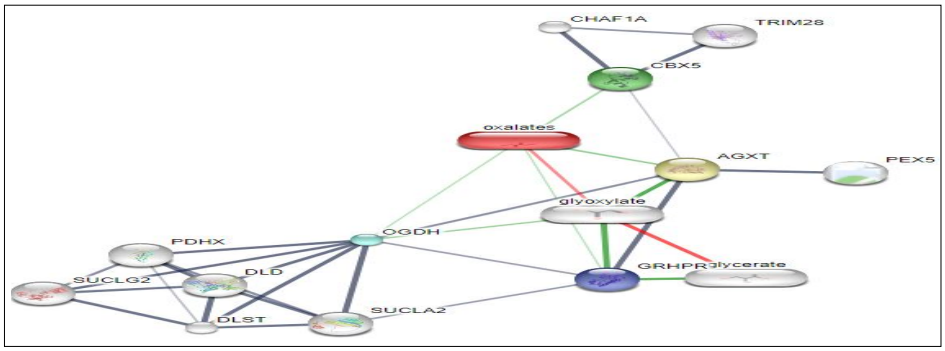

Fig 8: Oxalate-proteins interactions in biological systems.

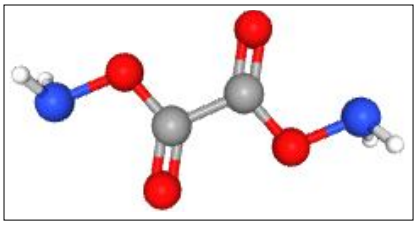

Fig 9: Oxalate chemical structure.

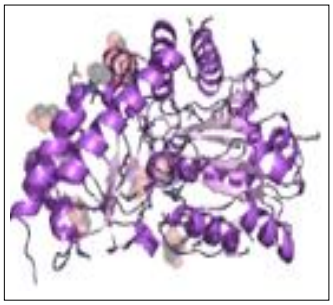

Fig 10: AGXT.

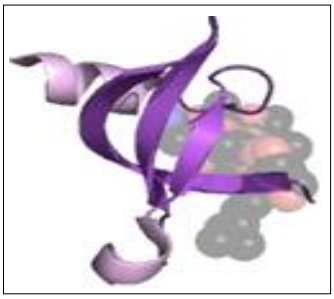

Fig 11: CBX5.

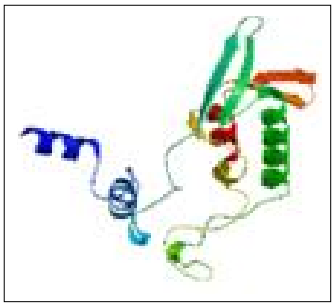

Fig 12: OGDH.

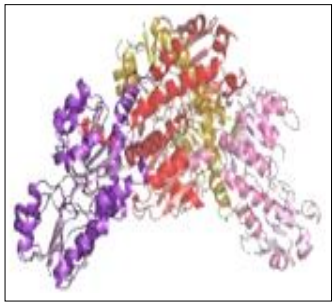

Fig 13: GRHPR.

histone H3 proteins tail which has been methylated at Lysine 9 (H3K9me), leading to epigenetic repression of the plant chromosomes. Oxalates from *Diffenbachia amoena* latex interacts with the CBX5 and lamin-B receptor, disrupts and inhibits the possible complex association of the heterochromatin and the inner membrane which together take part in formation of functional kinetochore and by further interacting with MIS12 complex proteins.This causes inversion of spindles during crossing over and thus impedes cell division processes in plants.

OGDH (Fig 12) is the oxoglutarate (also called alpha-ketoglutarate) dehydrogenase (lipoamide). This oxalate glutarate complex catalyzes the overall conversion of the oxalate glutamine complex to succinyl-COA and CO2, revealing multiple copies of three enzymatic components which interrupts with protein expression and metabolism in the plant cells.

GRHPR (Fig 13) is glyoxylatereductase which inhibits the processing and reduces hydroxypyruvate to D-glycerateglyoxylate and vice versa of the plant biochemical system.

Glycosides in Dieffenbachia species and their interactions in genes

GBA3 is Cytosolic beta-glycosidase protein gene (Fig 14). Glycosidase is an enzyme primarily involved in the internal absorption and metabolism of dietary flavonoid glycosides and is able to hydrolyze a broad variety of glycosides including phytoestrogens and cyanogens. It possesses beta-glycosylceramidase activity and involved in a nonlysosomal catabolic pathway of glycosylceramide (Fig 15). GBA3 reacts with linamarin (Fig 16) which is a cyanogenic glucoside found

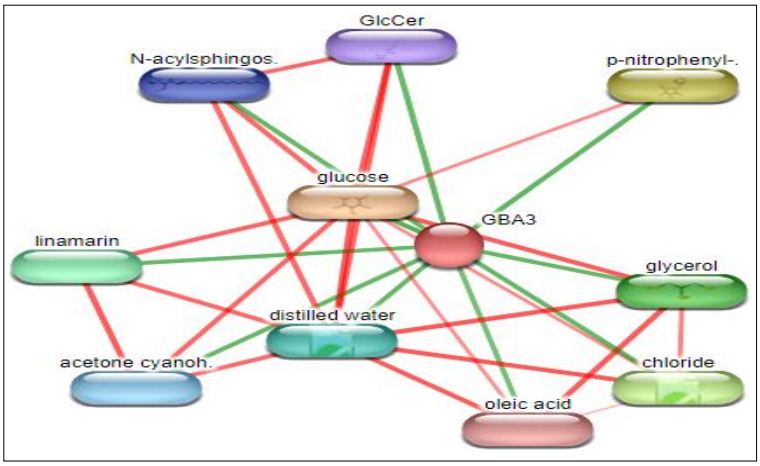

Fig 14: Glycoside-protein interactions in biological systems.

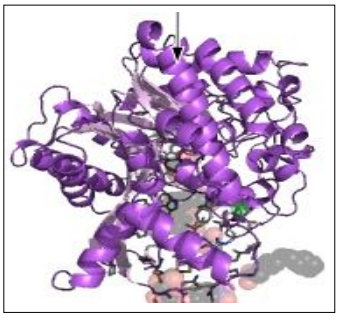

Fig 15: Protein structure of GBA3.

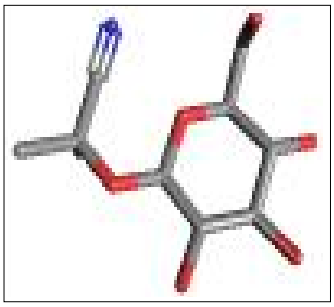

Fig 16: 3D Chemical structure of linamarin.

in the leaves and roots of Dieffenbachia, cassava, lima beans and flax. It is a glucoside of acetone cyanohydrin. Upon exposure to enzymes and internal flora in plant biochemical systems, linamarin and its methylated relative lotaustralin, decomposes to the toxic chemical hydrogen cyanide. However, the toxicity is believed to be induced by ingestion of acetone cyanohydrin, the breakdown product of linamarin.

Saponins in dieffenbachia species and their interactions in protein genes

CYP93E1 is beta-amyrin 24-hydroxylase, a heme-containing cytochrome P450 and involved in the biosynthesis of saponins whose chemical structure is shown in Fig 18. The availability of excess saponins in the plant tissue induces hyperactivity with the cytochrome protein gene thereby degrading it within a very short time which is harmful to the plant tissue. This protein hydroxylates specifically the C-24 methyl group of the triterpenes beta-amyrin and sophoradiol. Saponins specifically react and affect minor genes specifically of uncharacterized proteins in the plant cells as shown the network interaction diagram in Fig 17.

Tannins is 1, 3, 6-Trigalloyl glucose is an gallotannin whose chemical strucrure is shown Fig 20 can be found in the latex extract of *Diffenbachia amoena* but in relatively small amount whose effect is grossly overwhelm by other phytochemicals present in large amount. Tannins specifically affect minor genes specifically of uncharacterized proteins in the plant cells as shown in the network interaction diagram in Fig 19.

Araceae, which are the family of the floristic dumb cane, contain crystals of calcium oxalate, which are often known according to Koneman *et al.* (1997), to cause intense irritation experienced when handling or consuming the raw plant tissue of many genera in the family. This is contradicted by the fact that irritation generally is not produced by properly processed plants, because the crystals still remain even after heating (Johnson, 1995). Other compounds must therefore be involved which cause this reaction. Whether irritation is caused by enzymes or crystals, many genera of Araceae are included in the lists of poisonous plants (Groombridge, 1992). The poisonous compounds in *D. amoena* might likely be present in the non-polar fraction of the plant which is highly toxic to cell and DNA. However, the ingestion of the polar fraction may not be harmful to plants because it is non-toxic (Bors and Saran, 1991).

The present study result agrees with the findings and reports from many studies. Some of these reports includes those of Jiri *et al.* (2005), who studied the mitotic effect of leaf extract of *Ipomea carnea* on *Allium Cepa* and observed that the leaf extracts affected the mitotic frequency and caused some anomalies of root tip cells of onion, such as spindle inhibition, disturbed prophase and metaphase, lagging, sticky, bridges at anaphase and telophase and ring chromosomes in metaphases and telophase.

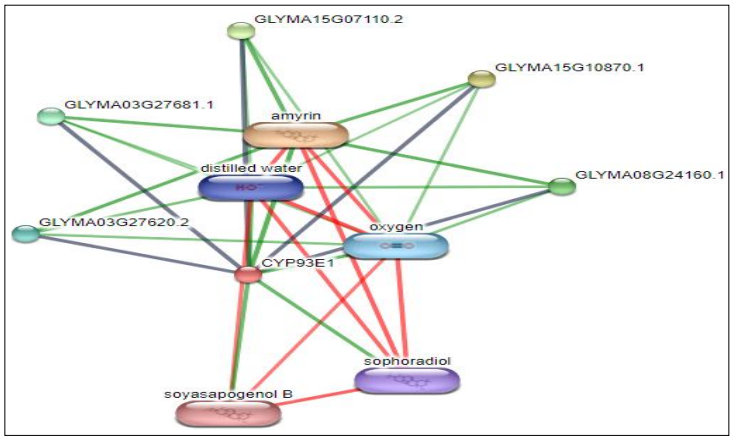

Fig 17: Chemical structure of saponins phytochemical compound.

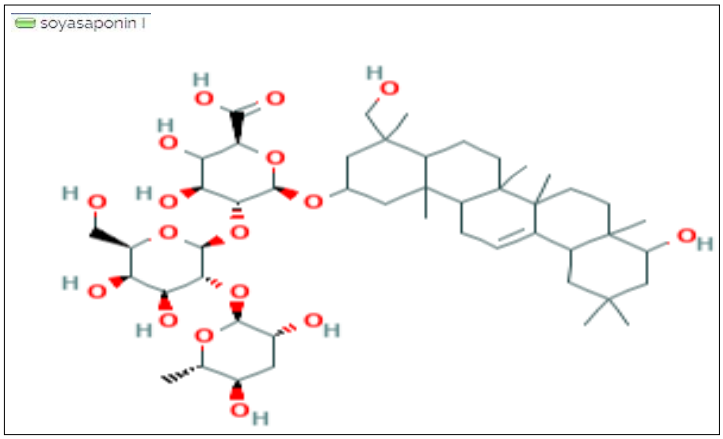

Fig 18: Saponin-protein gene interactions in plant biochemical systems.

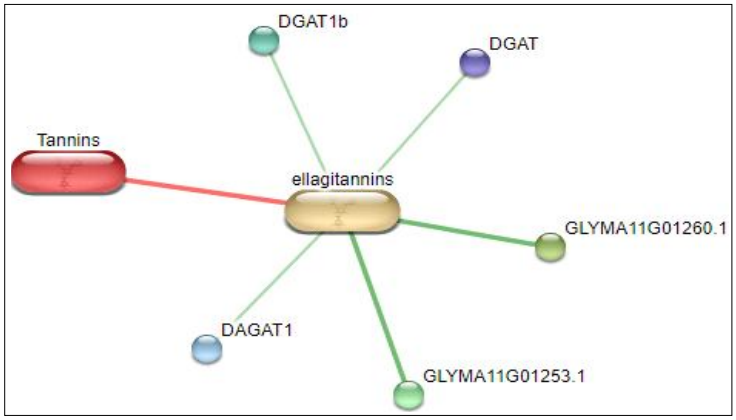

Fig 19: Tannins-protein gene interactions in plant biochemical systems.

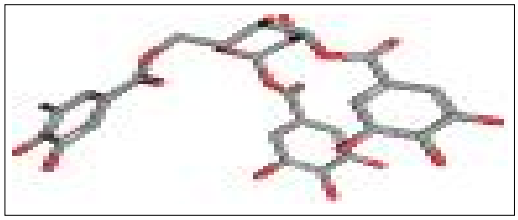

Fig 20: 3D chemical structure of Tannin.

In a similar reports, Jacob *et al.* (2013) studied the cytotoxic and genotoxic effect of cassava effluents using *Allium cepa* assay and reported rapid decrease in mitotic index with increasing effluents concentration and chromosomal aberration.

Also in collaboration with the present study, Fraonhne and Pfander (1984), had observes that a common effect of medicinal plant extracts containing oxytocin on root tip mitosis

of *Allium cepa* is an inhibition of spindle mechanism leading to the scattering of the chromosomes, stickiness of chromosomes, anaphase bridge and diverse kinds of abnormalities.

The findings of the present study conforms with the studies of Cragg *et al.* (1997), who treated onion root tips with different concentrations of tobacco smoke condensate and observed a number of abnormalities in somatic chromosomes. Elumalai *et al.* (2011), found the extract of *Teucrium pilosum* causes antimitotic effect. Aslanturk and Askincedik (2009), studied the cytotoxic and genotoxic effects of *Tridax procumbens* extracts on the mitotic root tips of *Allium Cepa* and they reported a reduction in mitotic index, suggesting that extract of *Tridax procumbens* have inhibitory effect on mitosis and mutagenic effect on cell division in root meristem of *Allium Cepa.*

CONCLUSION

Dumb cane causes significant effect in the cytotoxicity and chromosome infractions of onion cells ranging from chromatid-type breakage, fusion-bridge, chromosome stickiness, lagging chromosomes, bridged chromosomes, deletions and chromosomal aberrations. The study further revealed that a significant proportion of plant protein (genes) expressions are impaired by juices or sap from the stem which contains oxalates, saponins, glycosides, alkaloids and tannins which impairs photosynthetic activities and gene expressivity/functions.

The glycoside interacts with cytosolic beta glucosidase (GBA3) while saponins affect the transmitter proteins and the FOXB proteins in combination with alpha L rhamo, sephadex. All of these phytochemicals- proteins interactions are responsible for the observable associated symptoms and toxicity of *Diffenbachia amoena* plant latex.

Hence the urgent need for the enlightenment of the public on the cytotoxic and genotoxic effect of the houseplant especially in rural areas where this plant is still utilized as ornamental plant.

Conflict of interest: None.

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