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Comparative evaluation of genotoxic suppressant effect of *Paederia foetida* (L) and *Terminalia arjuna* (Roxb.) on chromatic aberration induced in *Allium Cepa* model by capsaicin

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Abstract
Capsaicin is an active component of chili produced by genus Capsicum sp. In spite of the various beneficial effects of capsaicin, it has also been reported with toxic effects in terms of dermatological injury, ophthalmic injury, gastro-intestinal problems and systemic toxicity. On other hand, there are many plant metabolites reported with toxicity suppression activity. So, the present study was designed to evaluate genotoxicity suppressing property of Paederia foetida (PF) and Terminalia arjuna (TA) induced by pure capsaicin and methanolic extract of Capsicum chinense (MCC) in Allium cepa model. Five treatments (Normal, Capsaicin, MCC, PFMCC, TAMCC) were formulated to study the effect. Mitotic index was observed and calculated by using Foldscope. The result showed mitotic index in capsaicin as 14.29 ± 2.22 on day one followed by negligible number of dividing cells. The mitotic index was recorded to be 15.89 ± 2.54 in day one and 18.91 ± 4.13 in day three for TAMCC. In case of PFMCC, 17.91 ± 2.35 in day one and 23.54 ± 2.35 in day three. Thus, it could be concluded that PFMCC showed significant increase in normal dividing cells as compared to TAMCC on third day which surpass toxicity of capsacin. Further studies on PF would help to get an efficient herbal formulation for suppression of genotoxicity of various toxic compounds.

Keywords: genotoxicity, *Allium cepa*, capsaicin, *Capsicum chinense*, *Terminalia arjuna*, *Paederaria foetida*

Introduction

Capsaicin, the active component of chili which provides hotness in chili. Despite having various beneficial effects, it has also been reported with mutagenic character. It may increase cell viability and proliferation of prostate cancer inducing cells (*Malagarie-Cazenave et. al. 2009*) [10]. Studies has shown its association with respiratory failure and acute respiratory inflammation with damage to epithelial cell in *in-vivo* condition (Reilly et. al. 2003) [13]. Chilies are most highly consumed spice among all in India. In North-eastern part of India, Naga chili is also consumed as one variety of chili. In 2007, it was recorded as the "World Hottest chili pepper" by Guinness World Record with a rating of 1 million Scoville Heat Units. Consumption of only chilies in long run might cause adverse health issues due to its accumulation in body. But our food preparation is such that this toxic effect of capsaicin might get diluted with other plant metabolite that we consume.

Northeast India has always been known as the biodiversity hub with numerous plant species. There are many plant species of high therapeutic value which are unexplored till date. Medicinal plants can be highly beneficial in reducing toxicity since they are loaded with phytoconstituents. Accordingly, the present study was conducted to examine the capability of *Terminalia arjuna* (TA) and *Paederia foetida* (PF) in reducing the toxicity induced by capsaicin in *Allium cepa* bulbs. *TA* is a miracle herb used since ancient times to cure heart problems. The active components this herb which are been reported are phenolic compounds terminic acid and arjunolic acid, phenolic acids-ellagic acid and gallic acid, glycosides-arjunetin and arjunosides IV, flavones, tannins, oligomeric proanthocyanidins, lactones, B-sitosterol and casuarinin (*Gupta et. al., 2018*) [6]. Studies have shown methanolic extract of TA has intense antimicrobial, antioxidant and anticytotoxic activity (*Rahman and Sultana 2012*). PF is commonly used curry leave of Northeast India and in Ayurveda, is considered as alterative, antiarthritic, antispasmodic, diaphoretic, expectorant and stomachic. It is also used in asthma, bowel complaints, diarrhoea, diabetes, seminal weakness etc. Its dried fruit extract has the capacity to reduce toothache. Also, it has been reported to be used against gout, vesical calculi, diarrhoea, dysentery, piles, inflammation of the liver and emetic (*Blatter et. al., 1981; Indian Materia Medica 2002*) [3, 8]. Taking into consideration of the medicinal property of the plants, the present study was conducted to observe their activity against capsaicin toxicity as TA is pronounced medicine of Ayurveda and PF is medicinal plus used as vegetable in Northeast Indian household. Genotoxic cells were observed under Foldscope, a paper microscope inspired by origami. It has magnification up to 2000X and 2micron resolution. It is an ultra-affordable microscope with optical quality similar to that of conventional research microscopes (*source:www. Foldscope.com*).

Materials and Methods

***Chemicals*:** All chemicals and reagent of analytical grade (*HiMedia, Fisher scientific*) were used. Capsaicin standard was purchase from *HiMedia*.

***Collection and Processing of sample*:** Around 350 gm of *Capsicum chinense* (CC) was collected from Kolongpar and Khanapara market of Guwahati and dried under sunlight (*obtained 120 gm after dried*). Barks of *T. arjuna* (TA) and leaves of *P. foetida* (PF) were collected from Nagaon district of Assam. The cold extraction method was done by using 70% methanol and samples were stored in 4°C.

***Phytochemical Screening*:** Crude methanolic extract of *TA* and *PF* was subjected to phytochemical analysis to detect the presence of tannins, flavonoids, saponins, terpenoids, alkaloids, phenolic compounds, steroids (Sofowora, 1993 [14]; Edeoga *et al*. 2005 [4]; Harborne, 2005) [7].

***Total Flavonoid Content*:** Aluminium chloride method was used where, Rutin was used as standard (*Asma and Amzad 2015*). 0.1 ml of plant extract (*stock conc. 1mg/ml*) was added to 0.3 ml distilled water followed by 0.03 ml NaNO2 (5%) and incubated for 5 min at 25°C. Later 0.03 ml $AlCl_3$ (10%) was added and after 5 min, the reaction mixture was treated with 0.2 ml (1mM) NaOH. Finally, the reaction mixture was diluted to 1 ml with water and absorbance was measured at 510 nm. All tests were performed in triplicates. The flavonoid content was calculated from a Rutin standard curve and expressed as mg/g Rutin equivalent (RE) of dry extract.

***Total Phenol Content*:** *Folin-Ciocalteu* method was used for determination (*Uddin et. al., 2015*). Gallic acid was used as standard. 0.5 ml of the plant extract (100 µg/ml) was mixed with 2 ml of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 765 nm. The total phenolic content was determined against standard curve of Gallic acid. The content of total phenolic compounds expressed as mg/g gallic acid equivalent (GAE) of dry extract.

***Fourier Transformation Infrared Spectroscopy (FT-IR) Analysis*:** Dried powder of the plant extracts was used for FT-IR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each extract was loaded in FT-IR spectroscope with a Scan range from 400 to 4000 cm-1 with a resolution of 4 cm^{-1}.

***Genotoxicity of Capsaicin*:** *Allium cepa* bulbs were taken as a test model for observing the capsaicin toxicity (Grant). The *A.cepa* root tips were allowed to grow in distilled water and after a growth of about 2cm they treated with different doses of capsaicin standard, methanolic extract of dry Naga chili, methanolic extract of TA and PF.

Table 1: Different treatments and its concentration

Test Group	Treatment	Dose given
1	Distilled water	200µl
2	Capsaicin standard 100µg/ml	200µl
3	Methanolic extract of *Capsicum chinense* (MCC) 1mg/ml	200µl
4	Methanolic extract of *Terminalia arjuna* (MTA) and *Capsicum chinense* (MCC) 1mg/ml	200µl
5	Methanolic extract of *Paederia foetida* (MPH) and *Capsicum chinense* (MCC) 1mg/ml	200µl

Root tips were cut and fixed in the fixative (ethanol: acetic acid in 3:1 ratio) for 15 minutes and hydrolyzed in 0.1N HCL for 30 minutes.

The tips were then washed with distilled water and put on a clean microscope slide. The tips were stained with Aceto-orcein stain for 2 minutes. Slides were observed under foldscope to view cellular changes (*Foldscope DBT India, Nokia 7 plus India)* and mitotic index was calculated. Each day changes were observed and recorded. Cellular changes were observed under *Foldscope* (a paper microscope with 140X magnification and 2micron resolution) coupled with Nokia 7 plus mobile phone.

Results

Phytochemical screening showed presence of potent phytochemicals such as alkaloids, tannins, flavonoids, phytosterols, saponin and phenols. Alkaloids, tannins, flavonoids and saponin was found to be more in TA extract. Both the extracts, TA and PF showed phytosterols and phenols to be present equally. Total flavonoid content of TA extract showed higher amount of flavonoid as compared to PF with 125±0.3mg/g RE and 110±0.2mg/g RE, respectively. Also, total phenolic content was found to be high in PF with 140±0.35mg/g GAE as compared to TA 130±0.4mg/g GAE. IR spectra showed presence of O-H stretching peak at 3383.89 cm^{-1} and C=C stretching at 1611.60 cm^{-1} for *TA* whereas, O-H stretching at 3404.37 cm^{-1} and C-H stretching at 2920.30 cm^{-1} was observed in *PF*.

Genotoxicity Suppressant Study: Each day cellular changes were observed in different treatments and mitotic index was calculated as shown in table 2.

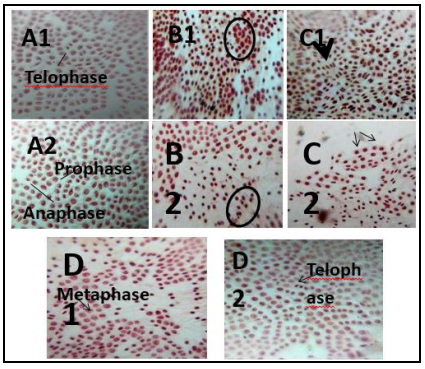

Fig 1: Normal mitotic stages (A1, A2); Damaged cells (circled) after treatment with capsaicin std. (B1); similar damaged cells treated with chili extract (B2); Reduced damage after treatment with P. foetida (C1, C2) and cells treated with T. arjuna (D1, D2). *Images taken under Foldscope (140X magnification) coupled with Nokia 7 plus mobile.

Table 2: Different treatment and its mitotic index

Treatments	Mitotic Index		
	Day 1	Day 2	Day 3
Distilled water	16±1.13	21±2.15	27±3.11
Capsaicin standard	9±0.11	4±3.36	2±4.11
Methanolic extract of *C. chinense* (MCC)	14.29±2.22	14.11±1.37	13.98±2.11
Methanolic extract of *T. arjuna* (MTA) and *C. chinense* (MCC): TAMCC	15.89±2.54	16.76±1.33	18.91±4.13
Methanolic extract of *P. foetida* (MPF) and *C. chinense* (MCC): PFMCC	17.91±2.35	20.34±2.11	23.54±2.35

Normal division of cells with mitotic stages such as telophase, prophase and metaphase were observed in distilled water treated tips. Cellular changes such as abnormal growth of cells, sticky nucleus, abnormal division pattern of cells and enlarged nucleus were observed in capsaicin standard and MCC. Mitotic division of cells stopped in repeated dose treatment 2 and 3. Whereas treatments MTA and MPF showed normal dividing stages on repeated doses. Treatment MTA and MPF were able to suppress the toxic effect of MCC. Mitotic index in MCC was recorded to be 14.29 ± 2.22 in day one followed by negligible number of dividing cells in day two and three. The mitotic index of the other treatments was recorded to be 15.89 ± 2.54 in day one to 18.91 ± 4.13 in day three (TAMCC) and 17.91 ± 2.35 in day one to 23.54 ± 2.35 in day three (PFMCC). Thus, it could be concluded that PFMCC is a better suppressant of genotoxicity induced by MCC as compared to TAMCC.

Discussion

The present study reveals the toxicity of capsaicin in gene level was seen in the *A. cepa* tips in *in vitro*. Severe cellular damages were recorded in the consecutive days of the study in case of pure capsaicin. On contrast, extracts of *TA* and *PF* were able to suppress the toxic effects of MCC upon treatment with herbal extracts. MPF was found to be a better genotoxicity suppressant than MTA with mitotic index of *23.54±2.35* on third day of the study. Significant increase in the mitotic index of onion tips marks the anti-toxicity of the extract. Methanolic extracts of *TA* and *PF* were found to contain potent phytochemicals such as phenols, flavonoids, tannins, phytosterols which may attribute towards their ability to reduce toxicity. The total phenolic content of *PF* was higher than TA which might be the reason for better activity. Studies are reported for *PF* showing anticytotoxic, thrombolytic and anti-diabetic activities (*Ahmed et. al., 2014*). IR spectral analysis shows the presence of various functional groups such a phenol, methyl, carboxylic, polysaccharides and amide. These groups may be present due to various photochemical content of these extracts which aids towards their anticytotoxic effects. Therefore, MPF can be further studied for their anticytotoxic activity in animal models for better findings.

Conclusion

From the present study, it could be concluded that MPF and MTA both are capable of minimising the toxic effects of capsaicin in *A. cepa* root tips. MPF and MTA contains various phytochemicals of which the phenolic and flavonoid content were determined. The phenolic content of MPF was higher whereas, total flavonoid content of MTA was found to be higher. Further studies on these herbal plants can be useful for formulation of pharmacologically active products capable of reducing the toxic effects of not only capsaicin but other toxic components of plant, animal or microbial origin. Molecular studies on their structure can be done to identify the core component responsible for their anticytotoxic property.

Conflict of Interest

None

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