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GENOTOXICITY ASSESSMENT OF VARIOUS EXTRACTS FROM *Ruta graveolens*

An important Medicinal and Aromatic Plant

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ABSTRACT

Ruta graveolens commonly named as rue, a folklore plant of family Rutaceae has been used in traditional medicine. Clastogenic effects of Ruta have already been proved in experiments done on mouse bone marrow cells. The present study deals with screening of crude plant extracts of Ruta graveolens for genotoxic response, Allium assay was performed for cytological analysis. Bridges were the most frequent kind of aberrations. The leaf ethanol extracts of Ruta graveolens was toxic compared to other extracts and provoked mitotic index of about 36±10.54 and had a statistically significant difference in relation to the positive control Ethyl methane sulfonate (EMS), which showed about 34.33 ± 3.89. Whereas root tips in negative control (tap water) showed a mitotic index at about 42.48 ± 4.99. Accordingly Ruta extracts should not be used for internal medicinal purposes until more thoroughly tested. This calls for a closer look at the genetic and toxicological effects in different test systems for human welfare.

Key words: *Ruta graveolens* , genotoxicity, *Allium cepa* , statistical analysis

Introduction:

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. Thus, the present investigation was carried out to evaluate the genotoxicity effect of *Ruta graveolens (Rue)* in experimental systems.

Members of the Rutaceae have been used in traditional medicine for various ailments including hysteria, gastrointestinal disorders, and menstrual problems. Ancient Egyptians and early Greeks used rue to improve eyesight. The juice of fresh rue has been used to relieve toothaches and ear aches. In Chinese medicine, rue is used to eliminate intestinal worms. It has been reported to possess antifungal (Oliva *et al.,* 2003), antibacterial (Ojala *et al.,* 2000), anti-inflammatory (Raghav *et al.,* 2005), antitumour (Preethi *et al.,* 2006) and cytotoxic activities (Ivanova *et al.,* 2005). Ruta has been reported to be useful for the treatment of multiple sclerosis (Bohuslavizki *et al.,* 1992) and also possesses hypotensive activity (Chiu and Fung, 1997). Rue oil has a flavor similar to the bitter oil in orange or lemon rinds which are used in cosmetics and foods. Fresh rue leaves are sometimes added to mixed salads, used in making pickles or put in cooked dishes for a bitter taste and flavour. In Italy, rue is used to flavor grappa, a type of brandy.

As Rue has been used extensively in traditional medicine, an attempt was made in this study to evaluate the genotoxic effect of the extracts from different parts of Ruta *graveolens in vitro* using *Allium cepa* assay by cytogenetic studies on chromosomal aberration.

Materials and Methods

Plant material

Saplings of *Ruta graveolens* (arootha) were collected from Ooty Agriculture department and Kodaikanal (Wild type), Tamil Nadu, India. They were grown in the department nursery under natural conditions without the addition of any fertilizers.

Preparation of plant extract

For extract preparation different parts of the plant viz., stem, leaves, fruit and roots were collected and washed 2-3 times in distilled water. Tissue sterilization was done with Teepol (4% v/v) and rinsed well in sterile distilled water. 1 g of sample was ground in 10 ml of methanol, ethanol and distilled water respectively to prepare various extracts. They were left in shaker overnight at room temperature. The extracts were squeezed through double layered muslin cloth and then filtered through filter paper. The solvents were then evaporated to dryness to obtain residues; these residues were dried, weighed and dissolved in appropriate solvents to prepare extracts of different concentrations. These extracts were stored at 4ºC for further bioassays.

Allium assay

The assay was carried out as suggested by Rank and Nielsen (1993), the bulbs were reeled off their brown scales and their brown root plate was removed leaving the root primordial intact. To achieve sprouting, bulbs were placed in contact with tap water in the test tubes in such a way that the lower portions of bulbs were dipping in the water. The bulbs were placed in the above condition for 2 d (48 h) at 20 ± 1°C and in an incubator in dark, changing water after 24 h. The germinating bulbs with fresh healthy roots were placed in different test tubes containing, ethyl methane sulfonate (EMS) (1mg/ml) to serve as a positive control; rooted onions placed on tap water served as the negative control. Graded concentrations of aqueous extracts, ethanol extracts and methanol extracts were prepared and the bulbs were soaked in them for 48 h in dark. Three roots from each bulb were fixed in Carnoy's fixative (6 parts ethanol: 3 parts chloroform: 1 part glacial acetic acid) after 4 h treatment. Mitotic preparations were made by hydrolyzing the root tips in a mixture of 1N HCl and 2% acetocarmine (1:9) at 50°C for 5 min. After maceration the root tip squashes were prepared in 2% acetocarmine and observed under the light microscope at 100 X magnification. All the experiments were conducted in triplicate.

Microscopic examination and statistical analysis

The microscopic analysis included mitotic index, observation for the presence of micronuclei in interphase cells and chromosomal aberrations in late anaphase and early telophase cells. Mitotic

index was found by counting the cells in different phases of mitosis and dividing them by 1000. Chromosomal aberrations were estimated only if mitotic index is above 10 per 1000. Then the aberrations were scored in the first 100 cells in anaphase or telophase, when observed under different fields of the microscope. The data was expressed as mean ± standard deviation (SD) for percentage of mitotic index and frequency of aberrant cells. The abnormal cells were classified according to the nature of aberrations and the results were tabulated and photographed.

Results and Discussion

The screening of crude plant extracts of *Ruta graveolens* for genotoxic response in *Allium* assay was performed for cytological analysis.

Leaf extracts

The leaf ethanol extracts of *Ruta graveolens* was toxic compared to other extracts and provoked mitotic index at about 36±10.54 and had a statistically significant difference in the relation to the positive control of ethyl methane sulfonate (EMS) which showed about 34.33±3.89. Whereas root tips in negative control (Tap water) showed a mitotic index at about 42.48±4.99, and was found to have a standard break. An analysis of chromosome aberrations showed that most of the fragments detected in different treatments were having both clastogenic and non clastogenic aberrations. The leaf ethanol extract proved to be toxic as it produced maximum clastogenic aberrations such as bridges, laggards and micronuclei.

In addition to chromosome fragments sticky pycnosis and macronuclei were also observed. Aberrations produced by *Ruta graveolens* leaf extracts increased with increase in drug concentration. In cells with membrane damage binulceated cells and nuclear damage were found in various frequencies. Also, apoptotic cells were detected in the group treated with *Ruta graveolens.*

Stem extracts

Ghost cells, were detected in the stem methanol extract treatment, while stem ethanol showed standard breaks and bridges at the anaphase stage, mitotic index was 34.2±8.60 and 40.5±7.46.

Root extracts produced lesser aberrations, as most of them were bridges and laggards possible on the telophase and anaphase stage, the mitotic index was about 37.7±9.97, 36.3±11.30 for ethanol, methanol extracts. Aqueous extracts of the plant showed minimum aberrations at about 2-3% on the cells as they had very minute effects such as standard breaks and chromosomal bridges

root extracts

Ruta graveolens methanolic leaf extracts showed the strongest genotoxic effects in the root meristem cells. The observation of chromosomal bridges on the anaphase reinforces the hypothesis of toxic effect. Metaphases with sticky chromosome, loses their normal appearance, and they are seen with sticky surface, causing chromosome agglomeration. Most of such damages were found in ethanolic stem extract but were lesser when compared to EMS (positive control). Stickiness has been attributed to the effect of pollutants and chemical compounds.

Effects of ems on roots of Allium cepa

EMS showed lesser mitotic index than that of all the solvent extracts (leaf, stem and root) used for study, this proves that mitotic cell division (normal chromosome) are lesser than the aberrations (clastogenic and non clastogenic) strand breaks ,laggards, pcynocis , macronuclei and micronuclei were the possible aberrations noted and they were comparatively higher than the plant extracts of Ruta *graveolens.* These clastogenic aberrations have been attributed to induction of break at DNA level by the treatment with reagents. Production of bridges at anaphase and telophase can be attributed to breakage and reunion of chromatids or subchromatids. Apoptotic on a treatment with EMS were also seen rarely.

Discussion:

The previous reports on *in vivo* studies of the genotoxic and clastogenic potential of an extract of *Ruta graveolens* and Ruta 200C, a homeopathic preparation showed various types of chromosomal aberrations were noted in bone marrow cells after treatment. The percentage of aberration in cells in 400mg/kg.b.wt extract administered group was found to be 21% and with 1000mg/kg.b.wt it was 31%. Administration of the extract (1000mg/kg.b.wt) over a period of 30

days also resulted in damage to cellular DNA as evidenced by comet formation where the comet parameters such as percentage DNA in tail, tail length, tail moment of the bone marrow cells were increased several fold over control values. These results indicate that *Ruta graveolens* and Ruta 200C may induce genotoxicity in animals. (Korengath *et al*., 2005; Preethi *et al*., 2007). However our bioassay which was done *Invitro* was more effective to read aberration immediately, differing with the percentage of extract in use. Our work describes that the extract in lesser percentage is more than enough to keep normal cells alive and notably minute aberrations.

Taking into account that cytological assays in plants serve as an excellent monitoring systems of detection of substances that may pose a genetic hazard (Grant *et al*., 1978), the test showed that the decoction of *Erythrina velutina* had genotoxic effects at lesser dose and they showed mitotic abnormalities which includes chromosome bridge, lagging chromosome, chromosome fragments and distributed metaphase and showed mitotic index at (29.33±2) which showed comparatively lesser aberrations than our extracts and normal cells were abundant on our extracts.

Azadirachta indica seed extract showed mitotic index at (25.99±66) and kernel extract of *Azadirachta indica* showed (30.88±9), Analysis of the data indicate that all treatments reduced prophase percentage, mean while the percentage of metaphases and ana-telophases rose over those of control. Also, the extracts caused different kinds of chromosome aberrations such as micronucleus and multinucleated cells, bridges, stickiness, non-congression metaphase, laggards. However our extracts showed prevalent normal cells and comparatively less number of chromosomal aberrations.

Whereas, *Inula viscosa* leaf extracts showed the strongest genotoxic effects in the root meristem cells. The observation of sticky metaphase, pcynosis and breaks reinforces the hypothesis of the toxic effect of leaf extracts (Tulay *et al*., 2009). However, our leaf extracts showed extravagant toxicity but there were lesser aberrations, like standard breaks at the anaphase stage.

Ocimum gratissimum showed mitotic index at 67.8% and 33.2 %. (Bernice *et al*., 2009). Standard breaks, bridges and pcynosis where the aberrations recorded, whereas our extracts

showed similar mitotic index and comparatively same kind of aberrations.

Conclusion

In conclusion, the results of this study indicate *invitro* cytotoxic, mitodepressive clastogenic and non clastogenic activity of *Ruta graveolens* largely on the leaf extract but provoked a mitotic index lesser than that of a chemical mutagen (EMS) against *Allium cepa* especially at doses beyond its pharmacological range *in vitro*, suggesting a need for safe dose administration of the plant in human phytomedicine and further *in vivo* safety studies.

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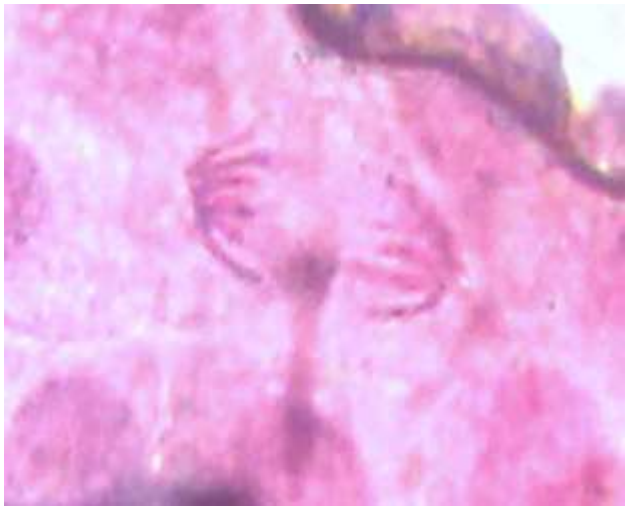
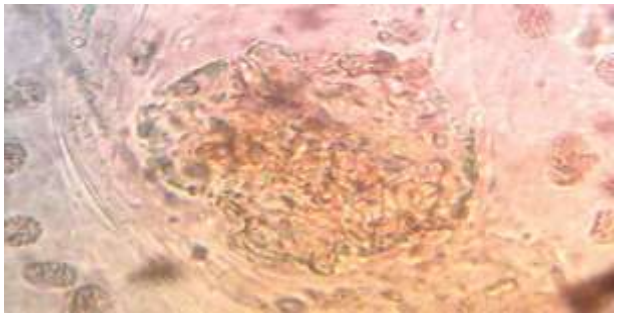
Table 1: Percentage of mitotic index and cytological aberrations in RTCs at anaphase and telophase of *Allium cepa after* treatment with Aqueous, Ethanol, and Methanol extracts of *Ruta graveolens*

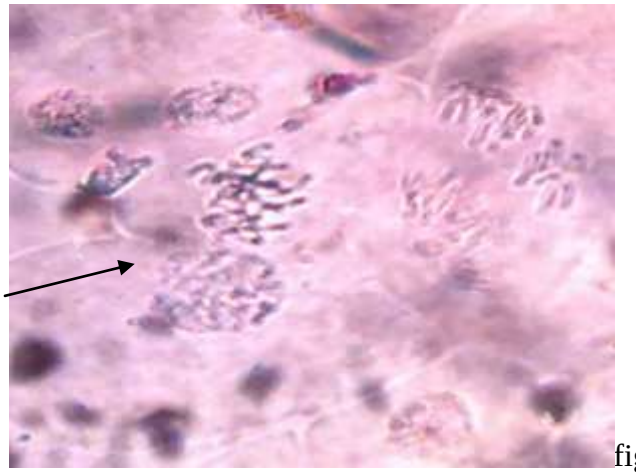
Treatment	Total RTCs studied	Mitotic index (%±SD)	Total cells at Anaphase & Telophase	Aberrant cells			
				Br	Lag	Py	Mn
Ethyl methane sulfonate	3150	34.33±3.89	222	23	11	8	3

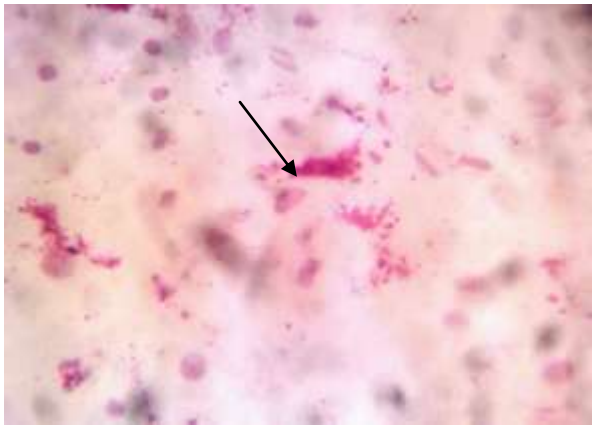
Tap water	4566	42.48±4.99	256	3	-	-	-
Leaf extract							
Ethanol	3220	36.4±10.54	208	11	4	-	3
Methanol	3158	37.9±10.81	229	7	2	-	-
Aqueous	3380	33.3±7.71	243	4	-	-	-
Stem extract							
Ethanol	3003	40.5±7.46	250	9	-	3	-
Methanol	3210	34.2±8.60	249	8	6	4	2
Aqueous	3120	34.7±9.50	230	4	-	-	-
Root extract							
Ethanol	3227	37.7±9.97	234	8	3	-	-
Methanol	3212	36.3±11.30	221	4	2	1	-
Aqueous	3240	30.2±9.26	211	5	-	-	-

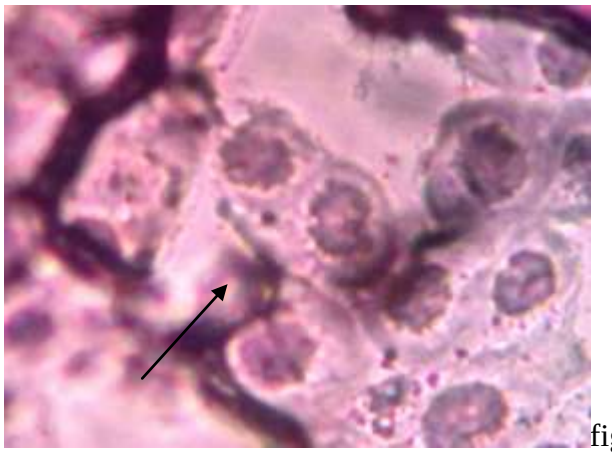
Br- bridges, Py- Pcynosis, Lag- Laggards, Mn- micronuclei

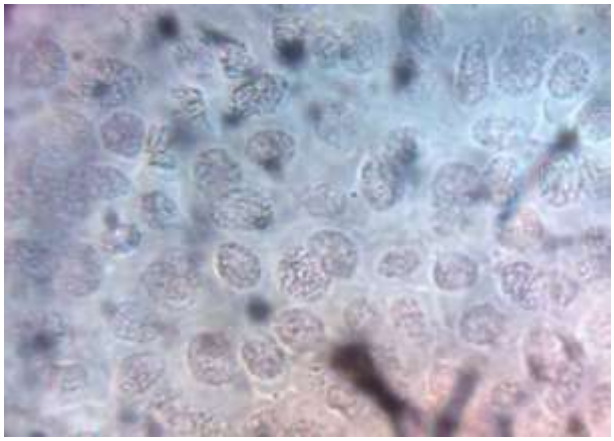
1.  fig1:Normal anaphase –tap water

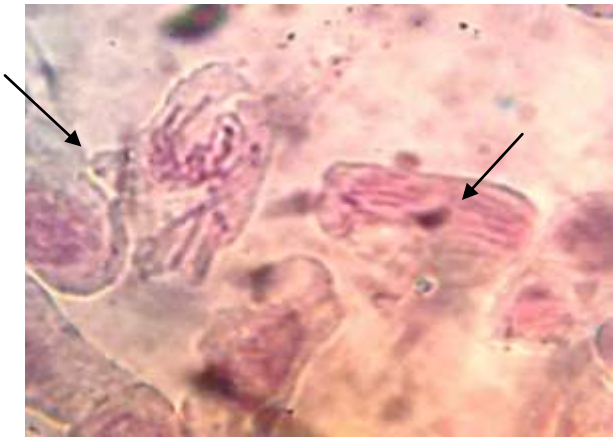
2.  fig2: Ghost cells-EMS

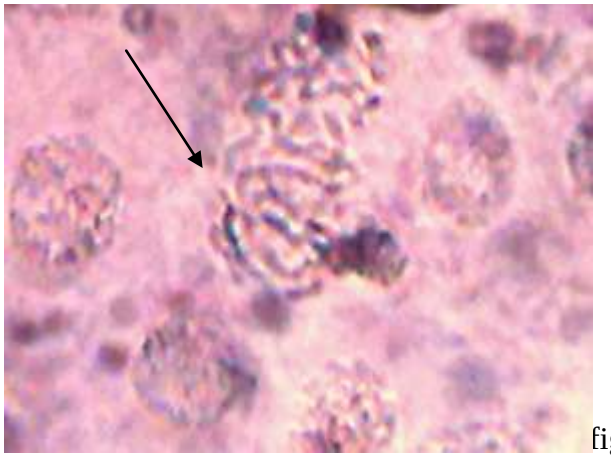
3.  fig3:Bridges (disruption in anaphase stage)-EMS

4.  fig3:Pynosis-leaf ethanol

5.  fig5:Micronucleai-leaf methanol

6.  fig6:Normal –leaf aqueous

7.  fig 7:Bridges-stem ethanol

8.  fig8:Laggards-stem methanol