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IN VITRO EFFECT OF 5- AZACYTIDINE AND 50 HZ RADIATIONS ON ENDOGENOUS LEVEL OF ASCORBIC ACID IN *PROSOPIS CINERARIA* L. (DRUCE)

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Biological effect of 5-Azacytidine (a base analoges) and 50 Hz radiations (non-ionising extremely low frequency electromagnetic radiations) (EM) was studied on endogenous level of ascorbic acid *in vitro* cultures of *Prosopis cineraria* L. (Druce). Cultures were initiated from hypocotyl and maintained on MS medium supplemented with different hormones for five months by frequent sub-culturing. Both agents caused diminutive effect on ascorbic acid content. Maximum damaging effect was caused by 50 Hz exposures followed by 5-Azt. Results suggest that safety standards should be followed while using home appliances which generate EM field.

Keywords: Ascorbic acid; 5-Azacytidine; 50 Hz radiations.

Introduction

5-Azacytidine (5-Azt), a base analogues act as a biological toxic or mutagenic agent which modified growth and differentiation by regulating gene expression[1,2]. There are several reports on the biological effect of 5-Azt on microorganisms[3], human beings[4], animals[5] and plants[6]. But scanty information is available on biological effects of 5-Azt on *in vitro*[7,8].

Non-ionising extremely low frequency ((ELF) electromagnetic (EM) radiation having a frequency of 10^{12} emitted by domestic powerlines, mobile phones, pagers, computers, fax, invertors, television and electronic tyos are responsible for various life threatening diseases like cancer, skin diseases and deformities of brain. Few researchers have also studied their possible effect of different plant species *in vivo*[6,9] and *in vitro*[10,11]. Ascorbic acid (Vitamin C) present in almost all the organalles of plant cells, induces resistance to drought and salinity in crop production[12], delays ripening of the seeds, enhancers seedling growth and playing significant role in growth and metabolism[13]. All actively growing and differentiating organs show higher concentration of ascorbic acid[12].

In view of above facts, the study was carried out to determine the effects of 5-Azt and 50 Hz radiations on *in vitro* treated/exposed cultures of *Prosopis cineraria* L. (Druce) by assessing ascorbic acid content.

Material and Methods

The presoaked seeds of *Prosopis cineraria* were surface sterilized with 0.1% $HgCl_2$ and transferred to MS (Murashige and skoog's) basal medium[14] for *in vitro* germination. Hypocotyl explants were excised from one week old seedlings and inoculated for induction of callus on modified, 2 MS (double micronutrients) medium (2,4-D, 2.5mg/l; NAA, 0.5mg/l; BAP, 1.00 mg/l). After about 45 days as one passage of growth, the callus was subcultured on fresh MS medium for another subcultering growth and 5-Azt treatment and 50 Hz exposures.

In one set, static callus cultures were administred with freshly aqueous solution f 5-Azt (5,10,15 and 20 µm), after 3rd, 5th and 7th day for continous supply of 5-Azt, in first weeks of callus growth. Similarly, for another set of experiment of ELFEMR radiations calli were exposed to 50 Hz radiations for various durations (5,7,9 and 13 min) in a specially designed apparatus in first week of callus growth.

For both sets, 5-Azt treated and 50 Hz exposed calli were maintained upto four passages. No further treatment and radiations exposure was provided. Estimation of ascorbic acid was done in all the treated/ exposed cultures including controls by utilizing standard protocol.

Results and Discussion

Ascorbic acid contents in callus cultures steadily decreased with an increase in concentrations of 5-Azt and exposures of 50 Hz radiations. Enhanced effect was observed in 5-Azt treated series at low concentration which is also maintained upto last passages. Exception to this 5 min exposure series where no change in ascorbic acid content was observed (Table 1 and Fig. 1). Deterimental effect was observed in all treated and exposed series till last passage.

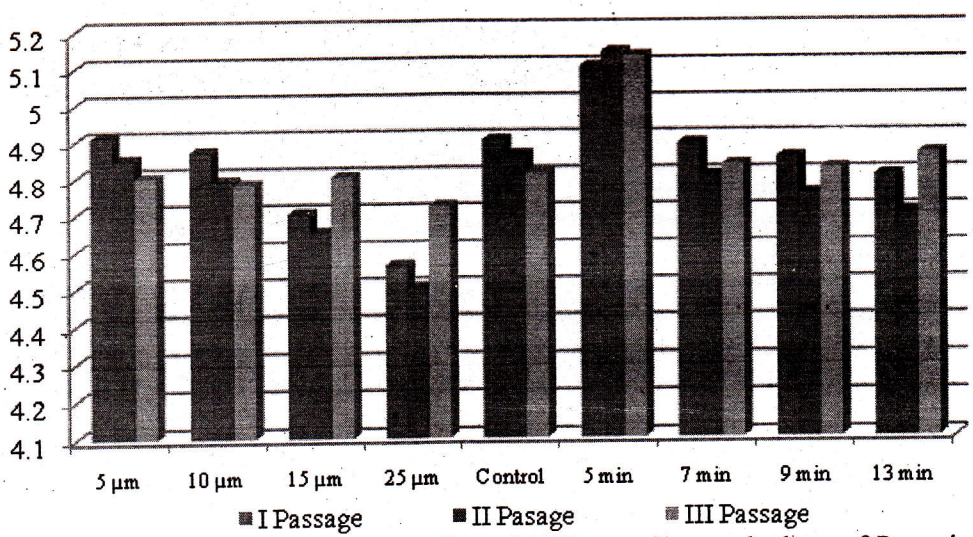

Fig.1. Ascorbic acid content of 5-Azacytidine and 50 Hz treated/exposed cultures of *Prosopis cineraria* L. (Druce) (mg/g/dw).

Table 1. Ascorbic acid content of 5-Azacytidine and 50 Hz treated cultures of *Prosopis cineraria* L. (Druce) (mg/g/dw)

Age/Concentrations/Exposure duration	I - Passage mean ± SE	II -Passage mean ± SE	III - Passage mean ±SE
Control	4.90 ± 0.12	4.86 ±0.10	4.81±0.19
5-Azt			
5 µm	4.91 ±0.27	4.85±0.15	*4.80±0.29*
10 µm	4.87±0.12	4.79±0.21	*4.78±0.17*
15 µm	4.70±0.04	4.65±0.29	*4.80±0.26*
20 µm	4.56±0.39	4.50±0.13	*4.72±0.19*
'r' =	-0.8101	-0.6281	*-0.4320*
Xx =	2.441	3.660	*3.982*
50-Hz exposures			
5 min	5.10±0.09	5.14±0.11	*5.13±0.09*
7 min	4.89±0.07	4.80±0.17	*4.83±0.13*
9 min	4.85±0.21	4.75±0.21	*4.82±0.27*
13 min	4.80±0.11	4.70±0.03	*4.86±0.21*
'r' =	-0.6440	-0.6731	*-0.5562*
Xx =	2.282	3.101	*3.762*

r= karl pearson's coefficient of correlation
Xx= Percentage average of occurance
Reported value are mean of 3 replicates

Comparitively after taking in to account the combined mean values of percentage average of occurrence (Xx), maximum-damaging effects was caused of 50 Hz followed by 5Azt.

In present study, ascorbic acid content either retarded or enhanced due to 5-Azt treatments in short term and its effect decreased and maintained in long term effects. However, in majority of studied parameters short term effect was observed. Previously, researchers have reported only short term effects [8,15]. Similarly, few researchers observed that 5-Azt treatments had short term effect on nicotine accumulation in *Nicotiana tabacum* leaf explants cultures[16].

Scanty information is available, on the *in vitro* effects of ELF radiations as worked out in present study. In the present investigation, all the four exposure durations

of 50 Hz radiations caused determental effect on ascorbic acid contents and is in agreement with previous, researches[8,11] who have also reported determental effect of electric pulses in cultured cells of sunflower.

Therefore, present results further strengthen the belief that it would be worthwhile to follow safety standards while utilizing home, electrical, digital appliences so as to reduce adverse effects of EM fields generated by them.

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