ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Investigation of Electromagnetic Radiations on Diosgenin Compound of Fenugreek.

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ABSTRACT

Modernization has enormously increased the use of wireless technologies which has lead to an increase in the amount of electromagnetic radiations present in the environment. These radiations have both positive as well as negative effects on the plants and animals including human beings. Therefore the current study was attempted to investigate the effects of electromagnetic radiations from 2G and 3G mobile phones on the morphological parameters and diosgenin content of Fenugreek (*Trigonella foenum-graecum*). The different time durations taken for the exposure of seeds to radiations were ½ hour, 2 hours, 4 hours and 6 hours for both frequencies of radiations. The results of the study showed that the electromagnetic radiations have a negative effect on the germination percentage of fenugreek seeds while the seedling length, fresh weight and dry weight increased. The number of root nodules also increased in the EMR radiated plants as compared to the control plants due to which the root and shoot lengths of the plants increased. The secondary metabolite (Diosgenin) content also showed an increase with the increase in radiation time exposure.
Keywords: Diosgenin, Cell phone radiations, Radiation exposure, Irradiated seeds.

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ISSN: 0975-8585

INTRODUCTION

In the past few years, there has been a massive increase in the use of wireless technologies such as radio, microwave ovens, television, Wi-Fi routers and cell phones and their antennas. Among these, the cell phone technology is used indiscriminately as it provides the convenience of communication without any hindrance to the movement of people. These technologies are continuously emitting a wide range of electromagnetic radiations which has ultimately raised the level of these radiations in the environment.

The extensive use of mobile phones motivated the researchers to study the effect of electromagnetic radiations on both plants and animals (including humans). Various studies have reported that microwaves (300MHz to 300GHz) have an ability to produce changes in the cell membrane's permeability and cell growth rate as well as interference with ions and other molecules, like proteins [1], enzyme activity [2] etc.

2G and 3G cell phone radiations:

A cell phone is duplex device that means two frequencies are used one for talking other for listening. Cell phones communicate by transmitting radio waves. These radio waves are made up of electromagnetic radiations. There are two types of electromagnetic radiations- Ionizing radiations like X-rays & Gamma rays and Non-Ionizing radiations. Ionizing radiations have shorter wavelengths and thus high frequencies and high energies. On the other hand, non-ionizing radiations have longer wavelengths and thus lower frequencies and lower energies. The types of radiations emitted by cell phones are non-ionizing ones. These non-ionizing radiations are called microwaves and they cause some heating effects. Generally, ionizing radiations are much more harmful to living organisms than non-ionizing radiations as ionizing radiations produce ions even at low powers. These ions have potential to cause damage to DNA and lead to denaturation of proteins [3]. While the non-ionizing radiations are harmful to living organisms only in higher proportions and at low powers these radiations are considered as harmless.

The current study is focused on the effect of cell phone (2G and 3G) radiations on the Diosgenin content of Fenugreek by exposing the seeds of the plant for different time intervals to radiations.

MATERIALS AND METHODS

CHEMICALS AND INSTRUMENTS

Methanol, Toluene, Ethyl acetate, Anisaldehyde, Sulphuric acid, Silica gel, Sulphur powder, Formic acid, Soxhlet apparatus, Rotary evaporator, Chloroform, Standard Diosgenin, TLC plates, UV/VIS spectrophotometer.

COLLECTION OF SEEDS

Seeds for the research work were collected from a licensed seed store at Jalandhar in Punjab.

RADIATION TREATMENT

Two types of radiations i.e. 2G and 3G were given to seeds for different time durations. A Nokia 2700 classic phone model with frequency band 850 – 1900 MHz and SAR value 0.22 W/Kg was used for giving 2G phone radiations to the seeds and for 3G phone radiation exposure, Micromax canvas A111 smartphone with frequency band 900 – 2100 MHz and SAR value 0.49 W/Kg was used. The seeds were divided into three different sets; one set of seeds exposed with 2G phone radiations, second set exposed with 3G radiations and third set was taken as a control in which no radiations were given.

For radiation exposure, cell phone was placed in an air tight box with about 50-60 seeds around it. During radiation exposure the cell phone was kept on talking mode and the exposure durations given to both the sets of seeds (2G and 3G) were ½ hr, 2 hr, 4 hr and 6 hr [4].

SOWING OF SEEDS

The radiated and control seeds of fenugreek were sown in separate pots filled with soil in the first week of February. For every radiation exposure two replicas were grown. All the pots were watered regularly for 45 days.

MORPHOLOGICAL ANALYSIS

The shoot and root lengths of *in-vivo* (pots) grown plants were taken after 40-45 days of sowing and noted to check the effects of radiations on the growth of plants. The numbers of nodules formed were also counted for each reading to check the effects of radiations on nodule formation of fenugreek plant. The germination percentage of seeds was checked *in-vitro* by placing 10 seeds of each reading on separate petri-plates having a filter paper soaked in distilled water. Two replicates were taken for each reading namely R1 and R2. The germination percentage was checked after 3 days by counting the number of seeds germinated in each petri-plate. After 7 days, the mean length of plumule and radical was noted and fresh weight of three morphologically similar seedlings from each petri-plate was taken. These seedlings were first air dried for 24 hours and then allowed to dry in a hot air oven at 50˚C for 5-10 minutes and after that their dry weight was taken.

BIOCHEMICAL ANALYSIS

Collection and drying of leaves:

The leaves were collected from the plants after 40-45 days of sowing. Then the leaves were washed under tap water and allowed to air dry for 1-2 days. Further drying of leaves was done in hot air oven at 50-60˚C temperature. The dried leaves were powdered manually.

Extraction and purification of extract:

About 5g of leaf powder was taken in a Soxhlet apparatus and extracted with 100ml of methanol for 4 h at 60-80˚C temperature [5]. The extract was then cooled at room temperature, collected and purified with the help of rotary evaporator with vacuum control.

Preliminary screening of the extract:

Preliminary tests for saponins-

a) **FOAM TEST:** 1 ml of plant extract was shaken vigorously for 10 minutes with distilled water. Formation of stable froth indicated the presence of saponins [6].
b) **SULFUR POWDER TEST:** Sulfur powder test was performed by taking small amount of sulfur powder in 1 ml of plant extract and it was observed whether it sinks to the bottom or not. If the powder sinks at the bottom then steroidal saponins are present [7].

Thin layer chromatography:

Diosgenin was separated by TLC and identified on the basis of the Rf value. The stationary phase used for thin layer chromatography was silica gel precoated TLC plates. The extract was applied on TLC plates. The mobile phase was prepared by taking Toluene: Ethyl acetate: Formic acid in 5:4:1 ratio [8]. Spray reagent was prepared by anisaldehyde: sulfuric acid (0.5 ml p-anisaldehyde in 50 ml glacial acetic acid and 1 ml 97% sulfuric acid). The plates were first sprayed with anisaldehyde: sulfuric acid spray and then the plates were heated until maximum visualization of spots and Rf was measured.

Spectrophotometric Analysis:

The absorbance of the extract was measured at wavelength of 430 nm in a UV- Visible Spectrophotometer [9] against the blank of methanol and compared with the standard Diosgenin (1 mg in 1 ml of chloroform).

ISSN: 0975-8585

RESULTS

Germination percentage, seedling length and fresh weight:

The germination percentage of unstressed seeds was higher than the stressed seeds. The data in table no. 1 shows that 2G phone radiations negatively affected the germination of the seeds while these radiations were positively affecting the other parameters like seedling length and fresh weight. The dry weight of seedlings showed almost similar results. This indicated that the plants surviving in the stress of EMR are better in terms of growth and thus the seedling length and fresh weight in comparison to control.

Table 1: showing mean and standard deviation of 2G radiations exposed seeds.

Exposure time	Germination percentage	Seedling length	Fresh weight	Dry weight
Control	100±0	4.86±0.01	0.06±0.0152	0.001±0
½ hour	90±14.142	5.315±0.502	0.055±0.007	0.001±0
2 hour	70±14.142	5.895±0.615	0.07±0.0141	0.002±0
4 hour	80±0	6.015±0.120	0.065±0.007	0.0025±0.00071
6 hour	70±14.142	6.33±0.184	0.075±0.007	0.003±0

Table 2: showing mean and standard deviation of germination percentage, seedling length and fresh and dry weight of 3G radiations exposed seeds.

Exposure time	Germination percentage	Seedling length	Fresh weight	Dry weight
Control	100±0	4.86±0.01	0.06±0.0152	0.001±0
½ hour	70±14.142	5.315±0.502	0.05±0.0141	0.002±0
2 hour	60±28.284	5.895±0.615	0.055±0.0007	0.002±0
4 hour	60±0	6.015±0.120	0.06±0.0141	0.0025±0.00071
6 hour	50±14.142	6.33±0.184	0.07±0.0141	0.0035±0.00071

Similar results were found in case of 3G phone radiations also. On comparing the data obtained from 2G and 3G irradiated seeds, it was observed that 3G radiated seeds are better in seedling length and fresh weight and dry weight of seedlings as compared to 2G radiated seeds. While on the other hand the germination percentage of 2G radiated seeds is better than 3G radiated seeds.

Morphological parameters of fenugreek plant:

In morphological analysis an increase in the mean number of nodule formation was observed as the time durations used to expose the seeds to radiations were increasing. The mean of root and shoot length also showed an increase and this may be due to the fact that number of nodules are increasing leading to an increase in nitrogen fixation to plants.

Table 3: showing effect of 2G phone radiations on the shoot and root length and nodule formation of fenugreek

Time exposure	Shoot length (in cm)	Root length (in cm)	Nodule formation
Control	4.7	6.1	8
½ hour	4.9	7	12
2 hour	6	9	26
4 hour	5.2	7.7	22
6 hour	5.6	8.5	25

ISSN: 0975-8585

Table 4: showing effect of 3G phone radiations on the shoot and root length and nodule formation of fenugreek plant

Time exposure	Shoot length (in cm)	Root length (in cm)	Nodule formation
Control	4.7	6.1	8
½ hour	5.1	6	18
2 hour	5.6	6.5	24
4 hour	6	6.4	27
6 hour	6.3	9.1	30

Table 3 & **4** clearly shows an increase in the number of root nodules of the plants emerging from 2G & 3G radiated seeds respectively as compared to the control and thus lengths of roots and shoots.

Biochemical analysis:

The preliminary screening of extract showed the presence of diosgenin in all the samples. The results of thin layer chromatography also showed the presence of diosgenin in all plant extracts and it was done on the basis of color resemblance of spot with standard diosgenin.

The Rf value of standard diosgenin was calculated as 0.85 while for control it was 0.78. Rf (Retention factor) value of Diosgenin present in Fenugreek extracts irradiated with 2G and 3G radiations is shown below in **table 5**.

Table No. 5

Time Duration	Rf of Diosgenin	
Standard	0.85	
Control	0.78	
	2G	3G
½ hour	0.78	0.79
2 hours	0.79	0.81
4 hours	0.78	0.83
6 hours	0.81	0.84

SPECTROPHOTOMETRIC ANALYSIS:

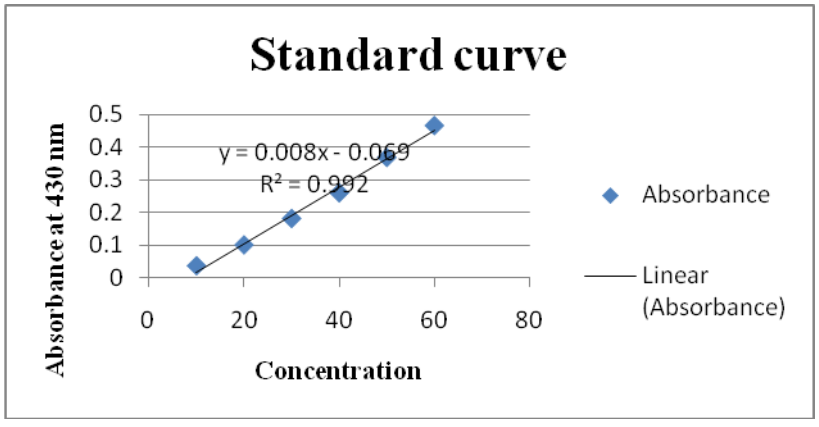

Fig 1: Standard curve of Diosgenin

The spectrophotometric analysis of plant extracts of both the sets of radiations indicated that the absorbance is increasing with an increase in exposure time. If the absorbance of 2G radiations irradiated plant extracts is compared with the absorbance of 3G radiations irradiated plant extracts then 3G irradiated are better in terms of diosgenin content. Thus electromagnetic radiations had a positive effect on the secondary

metabolite production of the plants. The spectrophotometeric results of 2G and 3G samples are mentioned in **table 6**. The absorbance of control sample was found to be 0.592 at 430 nm.

Table 6: Absorbance at 430 nm and concentration of diosgenin in plant extracts.

Time Duration	Absorbance at 430 nm		Concentration (µl/ml)	
Control	0.592		60.034	
	2G	3G	2G	3G
½ hour	0.625	0.647	63.827	66.356
2 hours	0.687	0.695	70.954	71.873
4 hours	0.712	0.738	73.828	76.816
6 hours	0.754	0.774	78.655	80.954

DISCUSSION

Radiations have different effects on both human beings and plants and these effects may be positive as well as negative. The purpose of present study was to examine the effects of mobile phone radiations on the germination percentage of seeds, fresh weight and dry weight of seedlings, seedling length and various morphological and biochemical parameters of Fenugreek plant. The study concluded that microwave radiations from mobile phones cause changes in the morphology and biochemistry of the plant.

The previous research shows that there was an increase in the germination percentage, dry weight, seedling length, proteins, lipid and guaiacol content in comparison to control seeds [10]. Also they reported an increase in the number of root nodules formed by the irradiated plants as compared to the control plants. While in present study decrease in germination percentage and increase in seedling length and fresh weight and dry weight is observed in comparison to control seeds. The increasing trend of the number of root nodules formed by the plants is similar to the previous study. A decrease in germination percentage of irradiated seeds in comparison to control seeds was observed [11] which is similar to the present study. But in previous study the seedling length and fresh and dry weights are also decreasing which is opposite to the present study.

Current study showed an increase in the production of secondary metabolites in radiation stressed plants as compared to the control plants. In one previous research similar results were found when plants and *in-vitro* cultured plant cell and tissue cultures were exposed to UV radiations [12]**.** The capacity of plants and cell and tissue cultures to produce secondary metabolites enhanced after exposure to UV radiations.

So in this study the radiations from mobile phones influenced the plant growth and production of secondary metabolites positively. The effects of mobile phone radiations on plant growth and secondary metabolite production should be further evaluated and investigated.

CONCLUSION

The present work concluded that the mobile phone radiations lead to a decrease in the germination percentage of the seeds of fenugreek. While the radiations showed a considerable increase in other morphological parameters of the Fenugreek seedlings like seedling length, fresh weight and dry weight.

From the results of morphological parameters of Fenugreek plants it can be concluded that the electromagnetic radiations increased the number of root nodules in Fenugreek plant which in turn increased the root and shoot length of the plant by supplying sufficient amount of nitrogen.

The results of biochemical tests showed an increase in the secondary metabolite content of the plants emerging from the radiation treated seeds. The spectrophotometric analysis of the radiations treated plant extracts showed an increase in the Diosgenin content when compared with the control plant extract. But in comparison to standard Diosgenin the concentration was less. This may be due to the reason that the plant produced more secondary metabolites under the stress of electromagnetic radiations for defense purpose.

So from this study it can be concluded that the mobile phone radiations increased the production of secondary metabolites (Diosgenin) in Fenugreek.

ISSN: 0975-8585

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