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Effect of physical and chemical mutagens on antibacterial and hypoglycemic activity of *Aegle marmelos* (L). Corr

Shubhada Walvekar, Dr. Prasanna G. Kaimal

Biotechnology Department, SVKM's Mithibai College, Vile Parle, Mumbai, Maharashtra, India.

Abstract
Aegle marmelos (L). Corr. shoots grown *in vitro* were treated with optimized doses of colchicine, Diethyl Sulphate (DES) and gamma radiations for mutation induction. Effect of these treatments on antimicrobial and hypoglycaemic activity of *Bael* was compared with the untreated control. The shoots treated with colchicine showed increased antibacterial activity while it reduced zone of inhibition was observed in shoots treated with DES and gamma radiations when compared to control. The cytotoxicity and hypoglycaemic activity was analyzed using 3T3 Fibroblast cells. The cytotoxicity level of the treated shoot material was reduced when exposed to DES and gamma radiations while it increased after colchicine treatment. The hypoglycaemic activity of *Aegle marmelos* (L). Corr. shoots was observed to increase in case of DES and colchicine treatment while it reduced when treated with gamma radiations. The uptake of glucose was increased due to exposure to colchicine in the presence and absence of insulin indicating an increase in hypoglycemic activity of the colchicine exposed shoots of *Aegle marmelos* (L). Corr.

Keywords: *Aegle marmelos* (L). Corr, colchicines, gamma radiations, DES, antibacterial activity, hypoglycemic activity

Introduction

Aegle marmelos (L). Corr. Family Rutaceae is an important medicinal plant as well as a fruit plant throughout the tropical countries. All parts of *Aegle marmelos*, such as the roots, bark, leaves, flowers, fruits and seeds are edible and possess medicinal properties. Leaves, stem, roots and fruits at all stages of maturity are used as ethano– medicines against various human ailments.

The leaves of *Aegle marmelos* contain several bioactive compounds including essential oils, phenolics, alkaloids, condensed tannins, anthocyanins and flavonoid glycosides. The leaves are astringent, laxative and an expectorant and are useful in treatment of treatment of inflammations, diarrhoea, dysentery, heart palpitation, and asthmatic complications. It is proved to have antibacterial activity [1], hypoglycemic activity, antialcer, antioxidant, anticancer, radioprotective, anti-inflammatory antipyretic, analgesic and antispermatogenic effects.

Aegle marmelos (L). Corr. plantlets grown *in vitro* were exposed to Diethyl Sulphate (DES), colchicine (chemical mutagens) and gamma radiations (physical mutagen). DES is an alkylating agent and colchicine is a mitotic inhibitor. Gamma rays are high energy ionizing radiations to induce mutations and to improve the medicinal properties of the said plant. The plantlets obtained in M_3 generation were screened for genetic variations induced by mutagenic treatments to make an attempt to produce an improved variety improvements in its medicinal potential.

The effect of mutagen treatment on antibacterial activity and hypoglycemic activity was studied using clinical strains of pathogenic bacteria and using 3T3 Fibroblast cells respectively. The name 3T3 is an abbreviation for the passage scheme of seeding 3 X 10^8 cells on 50 mm dish every 3 days. It is a rapidly dividing (doubling time of ca. 40 hrs), undifferentiated cell line, with sensitivity to contact inhibition.

Materials and Methods

Study of antibacterial activity of control and mutagen treated samples:

a) Preparation of plant extract

1 gm of dried powder of untreated control mutagen treated plant material grown *in vitro* was extracted with 100 ml 80% Methanol at 55 °C for 24 hours. This extract was used to check antimicrobial activity by well diffusion assay.

b) Test organisms

Clinical strains of Gram negative bacteria - *Escherichia coli, Klebsiella pneumonia*; Gram positive bacteria- *Staphilococcus. aureus*, *Bacillus subtilis*.

c) Preparation of inoculums

The inoculums for each bacterial culture (10^8 cfu/ml) was prepared from broth cultures grown in sterile Mueller-Hinton broth at 37 °C for 18 hours.

100µl of extracts for the mutagen treated and control specimens were added in each well with 80% Methanol as negative control and Ciprofloxacin (50 µg/ml) as the positive control. The plates were incubated at 37 °C for 24hrs for bacterial cultures and the zone of inhibition was measured [3]. Clear inhibition zones around the wells indicated the presence of antimicrobial activity. All data on antimicrobial activity were obtained as average of triplicate observations.

Analysis of variations in toxicity and hypoglycemic activity in control and treated specimens

a) Preparation of plant extract

Preparation of plant extract (Stock) Plant extracts were prepared with stock concentration of 100µg/ml in sterile Phosphate Buffer Saline (pH 7.4) for control and for mutagen treated plant material. After solubilisation, the stock was

diluted to the concentration of 10µg/ml, 20µg/ml, 40µg/ml and 80µg/ml using sterile PBS. All test samples were prepared fresh just before use.

b) Mouse cell line

Mouse 3T3 Swiss Albino cells are widely used as feeder cells for culturing human epidermis. It is a rapidly dividing (doubling time of ca. 40 hrs), undifferentiated cell line, with sensitivity to contact inhibition.

c) MTT assay for cell viability

The 3T3 fibroblast cells were cultured in DME Medium supplemented with 10% fetal calf serum. 10µl of the plant extracts for control as well as treated samples with concentrations ranging from 10µg/ml to 100µg/ml were added in to the log phase cell culture in triplicates and incubated further for 24 hours. After an exposure of the cells to the plant extracts for 24 hours, 10 µl of MTT reagent (5 mg/ml) was added to each well including blank. The plates were incubated in darkness at 37 °C for 4 hours. The formazan crystals obtaind were eluted in 100 µl of 3.37mM SDS reagent. The plate was kept at room temperature for 30 minutes and shaken for 30 minutes. The absorbance was read at 570 nm [4].

The reading of the test sample was scored on the basis of the comparisons with zero control, which shows 100% viability. The dose response curve was plotted to calculate the IC_{50} value.

d) Study of *in vitro* glucose uptake by 3T3 F442A Fibroblast (3T3 Adipocyte) cells

The 3T3 Adipocyte cells were cultured in serum free DMEM medium to attain serum starved condition. The medium was replaced by 20µl of 2– deoxyglucose mixture containing 130 µl of Glucose free DMEM medium. 10µl of 10µg/ml, 20µg/ml, 40µg/ml and 80µg/ml of each test samples including control and those treated with mutagens were added in respective wells in triplicates and were incubated for 5 hours with and without insulin condition. The cells were observed under microscope after incubation. The supernatant was discarded and the cell were lysed using 3.37mM SDS reagent. The cell lysate was used to determine the glucose content by DNSA method using 10µg/ml, 20µg/ml, 40µg/ml and 80µg/ml of glucose. The plate was read at 570 nm. The readings were scored on the basis of comparison with the zero control (PBS) [5].

Calculations

Percentage viability for the 3T3 fibroblasts was calculated using the following the formula:

$$\% \text{ viability} = \frac{\text{Absorbance of test substance}}{\text{Optical density of the zero control}} \times 100$$

Percentage of glucose uptake by 3T3 fibroblasts was calculated using the following formula:

$$\% \text{ increase in Glucose uptake} = \frac{\text{Absorbance of test substance}}{\text{Absorbance of the vehicle control}} \times 100$$

Results and Discussions

Analysis of antibacterial activity of untreated control and mutagen treated samples of *Aegle marmelos* (L). Corr

The antibacterial activity of untreated control tissue extract of *Aegle marmelos* (L). Corr. showed a zone of inhibition equal to 12.33±0.28mm for *Escherichia coli*. The mutagen treated tissue extracts showed zone of inhibition equal to 14.00±0.00mm for colchicines treatment, 10.33±0.33mm for DES treatment and 11.66±0.33mm for gamma irradiated tissue extract.

The control as well as mutagen treated tissue was ineffective against *Klebsiella pneumoniae*.

The zone of inhibition for *Staphylococcus aureus* was 14.33±0.33mm for the control and 16.00±0.00mm, 13.66±0.33mm and 13.00±0.00mm for colchicines treated, DES treated and gamma irradiated tissue extract respectively. Similarly the zone of inhibition for *Bacillus subtilis* was 14.00±0.00mm, 14.33±0.33mm, 12.00±0.00mm and 11.66± 0.33mm for control, colchicines treated, DES treated and gamma irradiated tissue respectively (Table 1).

It was observed that the extracts of *Aegle marmelos* (L). Corr. were more effective against Gram positive *Escherichia coli* than Gram negative *Staphylococcus aureus* and *Bacillus subtilis* with highest inhibition of *Staphilococcus aureus*, and no inhibition of *Klebsiella pneumoniae*. The antimicrobial action of *Aegle maemelos* (L). Corr. callus with a marginal inhibitory action against *Klebsiella pneumoniae*.

The mutagens had variable effects on antimicrobial activity, colchicine treatment resulted in increase in inhibition of *Escherichia coli*, *Staphilococcus aureus* and *Bacillus subtilis* while exposure to other two mutagens resulted in reduction in the antimicrobial activity.

Similar results showing increase in antimicrobial activity were reported for colchicine induced autotetraploid populations of *Trigonella foenum-graecum* L. against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermalis* and *Enterococcus fecalis* [6].

Cytotoxicity studies in untreated control and mutagen treated samples using MTT

IC_{50} value for the cytotoxicity analysis for untreated control extract was calculated to be 92µg/ml, 72µg/ml for DES treated sample, 85µg/ml for the colchicines treated sample and 95µg/ml for the gamma irradiated tissue (Table 2). The exposure to the chemical mutagen has resulted in significant increase in the cytotoxicity of the plants for the mouse 3T3 cell line.

The tissue treated with chemical mutagens showed lesser IC_{50} value than the untreated control indicating increased cytotoxicity levels after mutagen treatment whereas the gamma irradiation resulted in a higher IC_{50} value indicating a marginal decrease in cytotoxicity levels.

Table 1: Analysis of antibacterial activity of untreated control and mutagen treated samples of *Aegle marmelos* (L). Corr

Sample	Zone of inhibition (mm) ± S.E. Mean			
	Gram Negative organisms		Gram Positive organisms	
	Escherichia coli	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Bacillus subtilis*
Control	12.33±0.28	No inhibition	14.33±0.33	14.00±0.00
Colchicine	14.00±0.00	No inhibition	16.00±0.00	14.33±0.33
DES	10.33±0.33	No inhibition	13.66±0.33	12.00±0.00
Gamma	11.66±0.33	No inhibition	13.00±0.00	11.66±0.33
80% Methanol Negative control	No inhibition	No inhibition	No inhibition	No inhibition
Ciprofloxacin Positive control	16.00 ± 0.00	20.00 ± 0.00	14.00 ± 0.00	27.00 ± 0.00
Diameter of the well = 8 mm				

Uptake of glucose in untreated control and mutagen treated samples

Table 2: Cytotoxicity studies of untreated control and mutagen treated samples using MTT

Sample	IC_{50} value
Control (Untreated)	92µg/ml
DES treated	72µg/ml
Colchicine treated	85µg/ml
Gamma treated	95µg/ml

Comparative Glucose uptake for control and treated samples with the standard control

The uptake of glucose was observed to 15.13 percent and 96.17 percent for untreated control sample for 20µg/ml glucose. The plant material treated with colchicine showed the increase in the value to 34.51 percent when insulin was absent and 103.06 percent increase was noted in the presence of insulin. The exposure of *Aegle marmelos* (L). Corr. to DES resulted in 41.59 present increase in glucose uptake in the absence of insulin and 121.22 percent increase in the presence of insulin, which was the highest value among all the samples. Gamma irradiation resulted in reduction in the uptake, with a value of 8.24 percent and 89.14 percent in the absence and presence of insulin respectively. These values were lesser than the values for the untreated control.

Withania somnifera was used as a standard antidiabetic drug [7]. The leaf extract (WSL – Std.) prepared in a similar way as the other extracts was used as the standard control, which is a known anti diabetic drug. The percentage increase in glucose uptake in the presence of the standard control without insulin was 60.00 and it was 162.34 percent in the presence of insulin (Graph 1).

The study of glucose uptake indicated that glucose uptake was typically increased in the absence of insulin, and this effect was additive to that of insulin rather than potentiating it [8, 9].

Aegle marmelos (L). Corr. treated with mutagens showed an increase in glucose uptake in case of DES and colchicine treatments, but gamma irradiation showed lesser glucose uptake in the presence and absence of insulin. It could be stated that chemical mutagens DES and colchicine used in the present study could improve the hypoglycaemic action of *Aegle marmelos* (L). Corr.

Conclusion

Effect of mutagen treatment on antibacterial activity of *Aegle marmelos* (L). Corr

The treatment with colchicine has enhanced the antibacterial activity in all pathogens except *Klebsiella pneumonie.* DES and gamma treatments have resulted in decrease in the antibacterial potential of *Aegle marmelos* (L). Corr.

The treated shoot material treated with DES and gamma radiations was observed to have lesser cytotoxicity and showed more uptake of glucose than the control indicating improved hypoglycaemic activity as a result of the treatment. Cytotoxicity was more due to colchicine treatment along with increase in hypoglucemic activity. In summary, exposure to colchicine enhanced the antibacterial and hypoglycemic activities of *Aegle marmelos* (L). Corr.

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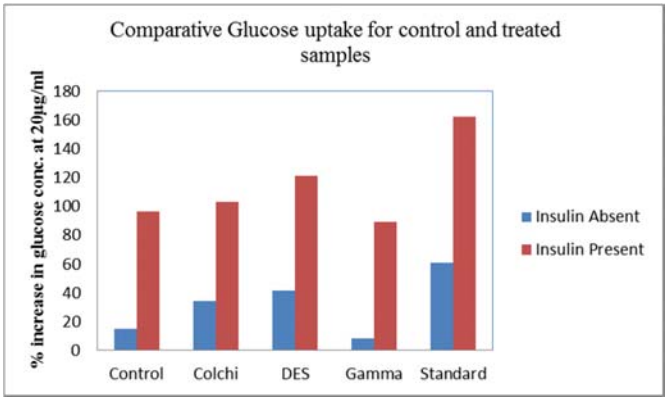

Fig 1: Comparative Glucose uptake of untreated control and mutagen treated tissue of *Aegle marmelos* (L). Corr

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