

EVALUATION OF PHYTOTOXIC EFFECTS OF PROPRANOLOL AND AMLODIPINE ON EARLY GROWTH PARAMETERS OF FENUGREEK

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ABSTRACT

Background: Pharmaceuticals play a vital role in enhancing the health and quality of life in both humans and animals. However, their unregulated use has led to the emergence of significant environmental contaminants. Though present at trace levels, they exert greater ecological effects altering growth and reproduction in aquatic organisms, inhibiting plant development and increased mortality among avian species etc.; Given this growing concern, the present study aims to evaluate the phytotoxic effects of selected pharmaceuticals on plant growth. **Materials and Methods:** Two widely used pharmaceutical compounds (Propranolol and Amlodipine) were selected to assess their phytotoxic effects on the early growth parameters such as total germination length, shoot length and root length. Each treatment consisted of six pots, assigned to the respective concentrations (0, 25, 50, 100, 200, and 600 ppb), with 0 ppb serving as the control. The corresponding freshly prepared drug solutions were administered from Day 1. The experiment continued for 14 days, during which plants were regularly monitored. On Day 15, root length, shoot length and total germination length were measured and statistically analyzed. **Results:** Propranolol showed a significant reduction in all germination indices while Amlodipine shows significant reduction in root elongation and total germination length, with root length identified as the most sensitive growth parameter. **Conclusion:** Even at lower traces of concentrations, these commonly used pharmaceuticals can suppress seed development in fenugreek, highlighting their impact on plant health and balance in ecosystem.

INTRODUCTION

Pharmaceuticals and health care products in current modern healthcare management provides many benefits to the consumers and to the communities for control and prevention of disease. Commonly used drugs such as chemotherapeutics, pain killers, antihypertensive agents, antiulcer medications etc.; have significantly improved life expectancy reducing both morbidity and mortality rate thus enhancing the quality of life globally. For years, lifesaving drugs such as antibiotics has controlled many infectious diseases and cardiovascular drugs helped millions in effective management of chronic conditions. They played a very crucial role in controlling endemics, epidemics and pandemics by adopting effective treatment strategies. Development of targeted therapeutic strategies such as personalized medicine

continues to improve better treatment outcomes with lesser side effects.

But these compounds are recognized as current emerging environmental contaminants due to their continuous release into aquatic and terrestrial ecosystems through wastewater outflow, agricultural runoff and improper disposal methods.^[1,2] Due to their widespread and often unregulated use has led to significant environmental concerns. Even at trace level of concentrations, ranging from nanogram to microgram per litre, pharmaceuticals can exert unintended effects on other organisms. Antibiotics have displayed disruption of soil biota,^[2,3] while analgesics such as diclofenac have resulted decline in population of vulture in south Asia due to their accumulation in ecosystem.^[4] Both unchanged and metabolite product of drug which gets excreted can enter surface water and terrestrial ecosystems through animal waste runoff, discharges from

wastewater treatment plants and improper disposal.^[1,2] Studies conducted in the past has detected pharmaceutical residues in surface water, groundwater and even in agricultural soils irrigated with treated wastewater.^[3,17,18]

Wastewater treatment plants are often ineffective in removal of these compounds completely, leading to their detection in rivers, groundwater and even drinking water sources at trace concentration levels.^[17,18] Carbamazepine, atenolol, acetaminophen and various antibiotics are most reported products in Indian municipal wastewater and downstream waters.^[17]

Even at low concentrations, pharmaceuticals can exert untoward effect on non-selective organisms. In plant development, this product may interfere with root and shoot elongation, seed development, and photosynthetic function.^[7,8]

Some pharmaceuticals have been shown to induce oxidative stress, alter gene expression and disrupts hormonal balance in flora.^[9-11] Furthermore, pharmaceuticals such as propranolol and amlodipine which are commonly used cardiac medications have recently gained attention for their phytotoxic potential due to their persistence and bioactivity in ecosystem.^[5,12]

Fenugreek (*Trigonella foenum-graecum*), an annual herb that belongs to the Fabaceae family, widely seen across Asia, the Mediterranean, and North Africa for its medicinal, culinary and agricultural uses characterized by trifoliolate leaves, small flowers and yellow brown seeds. Seeds of this plant have been traditionally used in both Ayurveda and Unani medicine for managing metabolic disorders because of its antioxidant, hypolipidemic and hypoglycemic properties.^[13,14] In agriculture and ecological research, legume plants are often studied for understanding early seed sensitivity to the environmental contaminants. Current trends of ecotoxicological studies demonstrate that seeds are very sensitive to pharmaceutical contaminants, showing observable decline in germination length and root elongation when exposed to low concentrations.^[15]

Taking this into consideration our present study investigates the dose-dependent Phyto toxicologic effects of Propranolol and Amlodipine on early growth parameters in fenugreek (*Trigonella foenum-graecum*). In this study we assessed how relevant the concentrations of this pharmaceuticals in the environment can affect shoot length, root length and total germination length and finally contribute toward understanding the environmental risks associated with contamination of pharmaceuticals in our ecosystem.

MATERIALS AND METHODS

Study Design

An in-vitro controlled experimental study that was carried out for 14 days to assess the phytotoxic effect

of pharmaceuticals on plant growth. Pharmaceuticals used in this study were Propranolol and Amlodipine. These pharmaceuticals were tested on growth parameters of Fenugreek (*Trigonella foenum-graecum*). Root length, shoot length and total germination length were assessed.

Study Setting

Study was conducted in experimental pharmacology laboratory, Dhanalakshmi Srinivasan medical college and Hospital, Siruvachur, Perambalur for the duration of 14 days and on the 15th day growth indices were assessed. Before initiating the study, fenugreek seed were authenticated by the botanist from Thiru Vi Ka government arts college, Thiruvarur, Tamil Nadu.

Groups

Two groups consisting of Group I - Propranolol and Group II - Amlodipine which include six treatment solutions (0, 25, 50, 100, 200 and 600 ppb) per group tested on fenugreek (*Trigonella foenum-graecum*)

Seed Germination Method

Fenugreek seeds were first rinsed in fresh water and then soaked for 6 hours and kept in the dark room to induce germination on Day 0. Then seeds were sown in plastic pots which were sterilized and filled with soil. They were allowed to germinate under ambient conditions ($29 \pm 2^\circ\text{C}$; 12-hour light and dark cycle).

Drug Dose and Administration

Fresh aqueous stock solutions of Propranolol and Amlodipine prepared on Day 0. Drugs were diluted in the water to obtain test concentrations of 0, 25, 50, 100, 200, and 600 parts per billion (ppb) where 0 ppb acts as control. The test solutions were applied directly to soil surface for uniform exposure. The test concentrations were chosen based on their levels detected in the ecosystem and from the previous ecological studies.^[5,11]

Study Procedure

Six identical sterilized pots filled with equal quantity of soil were labelled separately as 0, 25, 50, 100, 200 and 600 parts per billion (ppb) to which corresponding stock solutions were administered during the study. Three seeds were sown per pot in uniform spacing. They were allowed to germinate under ambient conditions and each pot received corresponding drug solution daily from Day 1 onward to maintain consistent exposure. On the 15th day root length, shoot length and total germination length were measured and taken for statistical analysis.

Statistical Analysis

The shoot length, root length and total germination length of fenugreek seeds exposed to Propranolol (Group-I) and Amlodipine (Group-II) for different concentrations (0–600 ppb) were expressed as mean \pm SD ($n = 3$). Data was analyzed using ANOVA to compare the mean of the study variables using SPSS software version 24.0. The p -value < 0.05 was considered as statistically significant.

RESULTS

Group I (Propranolol) showed statistically significant changes in shoot elongation ($p=0.0002$), root growth ($p=0.03$) and total germination length ($p=0.002$) as

increase in concentration, whereas Group II (Amlodipine) significantly affected only the root ($p=0.0001$) and total germination length ($p=0.01$), with no statistical change in shoot length ($p=0.50$) which are mentioned in table 1, 2 and 3 respectively.

Table 1: Root length expressed in cm

Growth Indices	Dose	Propranolol (Group -I)	Amlodipine (Group -II)
Root length	0 PPB	6 ± 0.82	6 ± 0.84
	25 PPB	4.8 ± 0.68	5.5 ± 0.72
	50 PPB	4.5 ± 0.60	4.8 ± 0.69
	100 PPB	3.5 ± 0.57	3.5 ± 0.52
	200 PPB	4 ± 0.60	3.3 ± 0.53
	600 PPB	2.2 ± 0.42	2.5 ± 0.46
<i>P value</i>		0.0002*	0.0001*

* *p value of < 0.05 was considered to be statistically significant*

Table 2: Shoot length expressed in cm

Growth Indices	Dose	Propranolol (Group -I)	Amlodipine (Group -II)
Shoot length	0 PPB	5.5 ± 0.79	6.2 ± 0.86
	25 PPB	5 ± 0.74	6 ± 0.85
	50 PPB	5 ± 0.75	5.6 ± 0.71
	100 PPB	5.5 ± 0.76	5.5 ± 0.75
	200 PPB	3.5 ± 0.55	5.4 ± 0.77
	600 PPB	4.3 ± 0.65	5 ± 0.74
<i>P value</i>		0.03*	0.5

* *p value of < 0.05 was considered to be statistically significant*

Table 3: Total Germination length expressed in cm

Growth Indices	Dose	Propranolol (Group -I)	Amlodipine (Group -II)
Total Germination length	0 PPB	11.5 ± 1.35	11.6 ± 1.37
	25 PPB	9.8 ± 1.18	11.5 ± 1.35
	50 PPB	9.5 ± 1.16	11 ± 1.30
	100 PPB	9 ± 1.11	8.9 ± 1.09
	200 PPB	7.5 ± 0.96	8.5 ± 1.04
	600 PPB	6.5 ± 0.84	8.3 ± 1.02
<i>P value</i>		0.002*	0.01*

* *p value of < 0.05 was considered to be statistically significant*

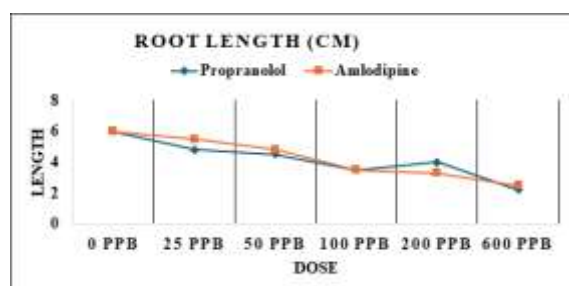


Figure 1: Root length (expressed in centimeter (cm)) of Fenugreek compared between Group I (Propranolol) and Group II (Amlodipine)



Image 2: Group II – Amlodipine



Image 1: Group I - Propranolol

DISCUSSION

This current study demonstrates dose-dependent inhibition effect on shoot length, root length and total germination length, following exposure to two commonly used drugs, propranolol and amlodipine. Even at lower concentrations of 25–50 ppb, both the drugs produced observable reductions in plant growth, showing that these pharmaceuticals, when released into soil or water, possess phytotoxic effects.

Propranolol, a non-selective first generation β -adrenergic receptor blocker showed a significant reduction in root elongation ($p = 0.0002$) and total germination length ($p = 0.002$), with observable growth reduction notable at 25 ppb, becoming more intensifying at 600 ppb. The inhibitory effect on root length was more than on shoot length, showing that root act as the primary site of toxicity and accumulation. The observed phytotoxic effect could be due to propranolol's lipophilic character, allowing its uptake thus altering ion homeostasis, cell elongation, and oxidative balance. Previous studies have reported similar stress responses, including reactive oxygen species (ROS) generation, mitochondrial dysfunction, and enzyme inhibition in plants exposed to β -blockers.

Amlodipine, a dihydropyridine calcium channel blocker, exhibited a milder effect compared to propranolol, showing statistically significant inhibition of root length ($p = 0.0001$) and total germination length ($p = 0.01$). But shoot length remained relatively unaffected ($p = 0.50$), progressive suppression of root growth was evident at concentrations ≥ 100 ppb. As we know, calcium plays a vital role in cell division and signalling mechanisms, disrupting calcium transport may interfere with the cell function. Amlodipine contains aromatic rings and polar groups, which may cause adsorption to root surfaces, hindering nutrient uptake, disrupting the growth.

An J, Zhou Q, Sun F, Zhang L,^[11] conducted a controlled ecotoxicological assessment of paracetamol on early growth of wheat (*Triticum aestivum*). Seed-germination, growth indices (percentage of germination, shoot elongation, root length) and biochemical markers were assessed. The study plant was exposed to a range of drug concentrations and this study was conducted as seven days acute and 21 days chronic thus allowing to differentiate immediate phytotoxic impact from chronic and subacute effects. This study concludes significantly decreased wheat root and shoot elongation as increase in concentration with root as the most sensitive marker and lower concentrations producing measurable biochemical alteration in the plant after chronic exposure.^[11]

Bellino and colleagues studied the four antibiotics effect on early developmental stages of tomato using standard seed-germination and assays. Seeds were exposed to a series of test drug concentration of antibiotics. This study uncovers that percentage of germination was largely unaffected but there was a significant reduction of root elongation in concentration dependent manner.^[7] A study by De Mastro F, Brunetti G, Traversa A, Cacace C, Coccozza C^[5] investigates the twelve pharmaceuticals effect on *Ocimum basilicum* L. This study concludes dose dependent inhibition of plant growth showing root as most sensitive indicator identifying propranolol as highly phytotoxic to root elongation.

The study conducted by Abdullah HS, Chia PW, Omar D, Chuah TS,^[16] concludes that calcium acts as

a major key signal in growth of plant.^[16] Many research conducted till now on pharmaceutical effects on plant shows that plant cell depends more on calcium regulation mechanism for germination of seed, root elongation and stress signalling thus giving new insight on phytotoxic effect of drugs with calcium channel blocker as emerging ecologically toxic xenobiotic. In our study, calcium channel blocker amlodipine has shown negative impact on growth parameters which could be due to disruption of calcium influx in root cells inhibiting growth. Calcium channel inhibitors may block calcium uptake in plant roots and arrest cell division.

Gorovits R, Sobol I, Akama K, Chefetz B, Czosnek H^[9] studied molecular and biochemical stress effects of commonly found pharmaceuticals in treated wastewater on tomato plants. Lower concentration shows measurable changes in stress marker where higher concentration displays phenotypic impact on plants.

Balakrishna K, Rath A, Praveenkumarreddy Y, Guruge KS, Subedi B,^[17] reviewed the study evidences on the presence of pharmaceuticals and personal care products such as antipsychotic, cardiovascular agents, antimicrobials and analgesic products that are frequently detected at micro to nano gram per litre in Indian water ecosystem and connects it to the emerging global challenge applying expanded nationwide monitoring of drug disposal and its impact on aquatic environment.^[17]

A review done by Bu Q, Wang B, Huang J, Deng S, Yu G raises the global concern of environmental hazard due to the presence of trace level of personal care products and pharmaceuticals in chinese aquatic environment and sediments.^[18]

Previously conducted studies have amplified our study findings that pharmaceuticals used in humans and for veterinarian purposes often persist in treated wastewater even at lower concentration used for irrigation has negative impact on plant growth with root length act as sensitive indicator suggesting it as primary site for uptake and accumulation.

CONCLUSION

This study shows that root length acts as the most sensitive indicator of drug-induced phytotoxicity effect compared to shoot length, which shows disruption in root development at early growth stages. Though therapeutically beneficial to humans, they act as non-target xenobiotics with adverse effects on the plant. Integrated studies of both transcriptomics and metabolomics could reveal whether these drugs can modulate genetic expressions related to growth, cell signalling and stress response in plant growth. Designing biodegradable ecofriendly drug molecules possessing minimal ecotoxic potential should be incorporated in drug development. Implementing screening of ecotoxicological effects of drug in regulatory submissions would promote the safety of not only

consumers but also ecosystems exposed to pharmaceutical residues.

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