Appendix -A

CHEMICALS USED

A

α-amylase enzyme

α-glucosidase

Abscisic acid

ABTS [2,2´ azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)]

Acryl amide

Aluminium chloride

Ammonium per sulphate

Anthrone reagent

Ascorbic acid

B

β-Carotene

Benzidine

Bis-acrylamide

Bovine serum albumin

Bromocresol Green

Bromophenol blue

C

Calcium chloride

1- Chloro-2,4- dinitrobenzene

Chloroform

c-PTIO[2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide]

Copper sulphate

D

Dichloromethane

DNS (3,5-dinitrosalicylic acid) reagent

Dinitrophenyl hydrazine

Dipotassium hydrogen phosphate

Disodium hydrogen phosphate

5,5'-dithiobis-(2-nitrobenzoic acid)

DPPH (2,2-diphenyl-1- picrylhydrazyl)

Dragendroff's reagent

E

Ethanol

Ethyl Acetate

Ethylenediamine tetraacetic acid

Ethyleneglycol-bis(2-aminoethylether)-N,N,N´,N, tetra acetic acid

Evans blue

F

$FeCl_2$

$FeCl_3$

Fehling's solution I (A)

Fehling's solution II (B)

Ferrozine

$FeSO_4$, $7H_2O$

Folin-Ciocalteu reagent

G

Gallic acid

Glacial acetic acid

Glutathione

Glutathione reductase

Glycerol

Glycine

Guaiacol

H

Hydrochloric acid

Hydrogen peroxide

L

Lanthanum chloride

Linoleic acid

Liquid nitrogen

M

Methanol

Methanol-d_4

Methionine

N

Napthylethylenediamine dihydrochloride

Nicotinamide-adanine dinucleotide phosphate (NADPH)

Ninhydrin

Nitro-blue tetrazolium

Nujol

O

Oxidized glutathione

P

Petroleum ether

Phenazine methosulphate

p-nitrophenol-a-D-glucopyranoside

Polyvinyl pyrrolidone

Potassium dihydrogen phosphate

Potassium bromide

Potassium ferricyanide

Potassium hydroxide

Potassium iodide

Potassium persulfate

Potassium sulphite

Proline

Putrescine

Q

Quercetine

R

Rochelle salt

Riboflavin

S

Schiff's reagent

Sodium acetate

Sodium carbonate

Sodium dodecyl sulfate

Sodium dihydrogen phosphate

Sodium hydroxide

Sodium nitroprusside

Spermidine

Spermine

Starch

Sulfanilamide

Sulfosalicylic acid

Sulphuric acid

T

Temed

Thiobarbituric acid

Thiourea

Toluene

Trichloroacetic acid

Tween 20

Trigonelline

Tris base

Tris–Hcl

Triton

X

XTT [3'-[1-[phenylamino-carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzenesulfonic acid hydrate]

Z

Zinc dust

Appendix - B

ABBREVIATION AND SYMBOLS USED

°C	Degree centigrade
•OH	Hydroxyl
$^1O_2$	Singlet oxygen
AA	Alpha amylase
AAE	Ascorbic acid equivalent
abs.	Absorbance
ABTS	2,2′ azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)
AG	Alpha glucosidase
AGE	advanced glycation end-products
ALP	Anti-lipid peroxidation
ANOVA	Analysis of variance
APOX	Ascorbate peroxidase
APS	Ammonium persulphate
ASC	Ascorbic acid content
ATP	Adenosine triphosphate
BCB	β-carotene bleaching
BCE	β-carotene equivalent
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
Ca	Calcium
CaM	Calmodulin
CAT	Catalase
CBL	Calcineurin B-like protein
CC	Calcium chloride
CDPK	Ca^{2+}- dependent protein kinases
CML	Calmodulin like protein
CON	Control
COX	Cytochrome *c* oxidase
COSY	correlated spectroscopy
CP	c-PTIO
c-PTIO	c-PTIO[2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide]
DCM	Dichloromethane
DMRT	Duncan's Multiple Range Test
DNPH	Dinitrophenyl hydrazine
DPPH	2,2-diphenyl-1-pyicrichydrazyl
DR	Degradation rate
DTNB	5-5'- dithiobis-2-nitrobenzoic acid
dS	deciSiemens
EDTA	Ethylenediaminetetraacetic acid
ET	Electron transfer
EG	EGTA
Fe^{2+}	Ferrous ions

FRAP	Ferric reducing antioxidant power
FWT	Fresh weight tissue
g	gram
GAE	Gallic acid equivalent
GPX	Guaiacol peroxidase
GR	Glutathione reductase
GSH	Glutathione
GSSG	Glutathione oxidized
GST	Glutathione-S-transferase
IR	Infra red
h	Hour
H_2O_2	Hydrogen peroxide
HAT	Hydrogen atom transfer
HC	H_2O_2 content
HETCOR	heteronuclear correlation
HYDRO	Hydroprimed
HL	H_2O_2 localisation
IC50	50% Inhibition concentration
LC	Lanthanum chloride
$LOO^{\bullet}$	Lipid peroxyl
LOOH	Lipid peroxide
LP	Lipid peroxidation
M	Molar
mg	milligram
mM	millimolar
MC	Metal chelating
MDA	Malondialdehyde
METH	Methanol
mg	Milligram
min	Minute
ml	Millilitre
MVSP	Multivariate Statistical Package
NA	Not detected/ not applicable
NADH	Nicotinamide-adanine dinucleotide reduced
NADPH	Nicotinamide-adanine dinucleotide phosphate
NBT	Nitro-blue tetrazolium
NMR	Nuclear magnetic resonance
NO	Nitric oxide
$NO_2^{\bullet}$	Nitrogen dioxide
NOX	NADPH oxidase
NR	Nitrate reductase
$O^{2\bullet -}$	Superoxide
O_3	Ozone
OD	Optical density
OG	octyl gallate
OH	Hydroxyl radical
$ONOO^-$	Peroxynitrite
ORAC	Oxygen radical absorbance capacity
PAGE	Polyacrylamide gel

	electrophoresis
PC	Principal component
PCA	principal component analysis
PG	propyl gallate
PKC	Protein kinase C
PMI	Plasma membrane integrity
PMS	Phenazine methosulphate
pNPG	p-nitrophenylglucopyranoside
POD	Peroxidase
Put	Putrescine
QE	Quercetine equivalent
RNS	Reactive nitrogen species
$RO^{\bullet}$	Alkoxyl
$ROO^{\bullet}$	Peroxyl
ROS	Reactive oxygen species
RP	Reducing power
rpm	Revolutions per minute
RWC	Relative water content
SDS	Sodium dodecyl sulfate
SEE	Standard error of estimates
SL	Superoxide localisation
SNP	Sodium nitroprusside
SO	Superoxide
SOD	Superoxide dismutase
Spd	Spremidine
Spm	Spermine
SPSS	Statistical package for the Social sciences
STI	stress tolerance index
TBA	Thiobarbituric acid
TBARS	Thioburbituric acid reactive substances
TCA	Trichloroacetic acid
TCC	Total carotene content
TEAC	Trolox equivalent antioxidant capacity
TEMED	N,N,N’,N’-Tetramethylethylenediamine
TFC	Total flavonoid content
TLC	Thin Layer Chromatography
TOCSY	Total correlation spectroscopy
TPC	Total phenol content
TPTZ	2,4,6-tripyridyl-*s*-triazine
TRAP	Total radical antioxidant trapping
TRS	Total reducing sugar
TSS	Total soluble sugar
µl	Microliter
µM	Micro molar
UP	unprimed
UV	Ultra violet
XOR	Xanthine oxidoreductase

APPENDIX-C

List of Publications

1. Mandal P and **Gupta SK**. Improvement of antioxidant activity and related compounds in fenugreek sprouts through nitric oxide priming. *International Journal of Pharmaceutical Sciences Review and Research*. 2014; 26(1): 249-257

2. **Gupta SK** and Mandal P. Involvement of calcium ion in enhancement of antioxidant and antidiabetic potential of fenugreek sprouts. *Free Radicals and Antioxidants.* 2015; 5: 74-82

3. **Gupta SK** and Mandal P. Assessment of the effect of nitric oxide and calcium ion on the therapeutic potential and oxidative stress status of fenugreek sprouts. *Asian Journal of Pharmaceutical and Clinical Research*. 2016; 9(2): 271-277

4. **Gupta SK** and Mandal P. Nitric oxide and calcium signalling in plants under salinity stress and their crosstalk . *NBU Journal of Plant Sciences.* 2016; 10:31-44

5. **Gupta SK** and Mandal P. Elicitation of trigonelline, a hypoglycemic agent in fenugreek sprouts by calcium and nitric oxide priming. *Asian Journal of Pharmaceutical and Clinical Research*. **In Press, 2017.**

APPENDIX-D

Research Article

Improvement of Antioxidant Activity and Related Compounds in Fenugreek Sprouts through Nitric Oxide Priming

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Accepted on: 07-03-2014; **Finalized on:** 30-04-2014.

ABSTRACT

Nitric oxide (NO) is an important signalling molecule in plants. In the present work, the effect of some nitric oxide donors namely, sodium nitroprusside (SNP), sodium nitrite (NN) and potassium ferricyanide (FCN) on the antioxidant activity of *Trigonella foenum-graecum* L. was studied during germination. The treated seeds were germinated in dark for 72 h and the antioxidant activity were determined at interval of 24 h up to 3 days. The *in vitro* antioxidant activity was determined spectrophotometrically by the following methods, DPPH, ABTS, nitric oxide, anti-lipid peroxidation, beta-carotene bleaching, ferric reducing power and metal chelating as well as phytochemicals such as phenol, flavonol and carotene content were also evaluated. The results demonstrated that the seeds treated with nitric oxide donors showed gradual increase in their antioxidant potential from 24 h to 48 h and then declined at 72 h stages. Overall, the seeds treated with sodium nitroprusside and potassium ferricyanide showed higher antioxidant potential particularly at concentration 80 mM and 40 mM respectively, when compared with control. Our data supported the hypothesis that NO is a signalling molecule that plays an important role as an antioxidant component in plants.

Keywords: Antioxidant, Germination, Nitric oxide, Phenolics, *Trigonella foenum-graecum.*

INTRODUCTION

Trigonella foenum-graecum commonly known as fenugreek, being rich in antioxidants and phytochemicals has been traditionally used as a food, forage and medicinal plant.[1-2] The pharmacological and folkloric uses of different plant parts of fenugreek have been reported by different researchers. Its seeds have been reported to have anti-diabetic,[3-4] anti-cancerous,[5] anti-inflammatory[6] and antioxidant activity.[7] Its leaves have been reported to possess potential anti-bacterial activity,[8] anti-diabetic[9] and antioxidant property.[10] Randhir *et al.,*[11] have also reported about the presence of potential antioxidant activity in the sprouts of fenugreek.

Nitric oxide is a short- lived bioactive molecule,[12] which is considered to function as prooxidant as well as antioxidant in plants.[13] Nitric oxide molecule is now recognized as an important signaling molecule and reported to be involved in various key physiological processes such as abiotic stress tolerance,[14] plant defense mechanism,[15] germination,[16] growth and development of plants[17] etc. In the cited study it was also shown that plant response to such stressors like drought, high or low temperature, salinity, heavy metals and oxidative stress, is regulated by NO.[13, 18]

Free radicals, such as reactive oxygen and nitrogen species, are an integral part of normal physiology. An over-production of these free radicals occurs, due to increase in oxidative stress brought by the imbalance of the bodily antioxidant defence system and free radical formation.[19] These highly reactive species on reaction with biomolecules can cause cellular injury and even death. This may lead to the development of several disorders and chronic diseases such as cancers, Alzheimer's and Parkinson's diseases and those related to cardiac and cerebrovascular systems.[20]

Antioxidant compounds play vital role in protecting cell against destructive chemical compounds such as free radicals and reactive oxygen species (ROS) that are constantly produced by the cell metabolism and their concentration increases under stress conditions.[21] Phenolics and flavonoids are considered to be very important antioxidant components, which play very important roles in the prevention of human oxidative damages.[22-23]

In recent times seed sprouting is gaining more significance commercially because it enhances the nutritional value of the seed. A large number of chemical changes occur to mobilize the stored carbohydrates and protein reserve into the germinating sprout.[11,24] Sprouting also removes some anti-nutritive factors such as enzyme inhibitors from the seed that make sprouts safe for consumption. Sprouting in fenugreek is known to improve its soluble protein and fibre content and reduce the phytic, tannic acid and trypsin inhibitors.[24]

The purpose of the present study was to evaluate the effect of nitric oxide donor namely, sodium nitroprusside, potassium ferricyanide and sodium nitrite on the antioxidant activity of *Trigonella foenum-graecum* sprouts germinated under dark condition. To our knowledge, this is the first report on the effect of nitric oxide donors in counteracting oxidative stress in fenugreek sprouts.

Involvement of Calcium ion in Enhancement of antioxidant and antidiabetic Potential of Fenugreek Sprouts

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ABSTRACT

Introduction: Calcium ion (Ca^{2+}) is considered as a key signal transducer in plants which is involved in various physiological processes. The aim of the present study was to evaluate the influence of Ca^{2+} in enhancement of antioxidant as well as anti-diabetic activity of the fenugreek seedlings during developmental phase. **Methods:** The fenugreek seeds primed with Calcium chloride ($CaCl_2$), Calcium chelator EGTA [Ethylene glycol-bis(2-aminoethylether)-N,N,N´, N, tetra acetic acid] and calcium channel blocker $LaCl_3$ (lanthanum chloride) and germinated for 3 days. The sprout extracts were investigated for their antioxidant potential by DPPH, $ABTS^+$, metal chelating, reducing power, nitric oxide scavenging capacity and anti-lipid peroxidation as well beta-carotene bleaching assays along with *in vitro* antidiabetic activity by α-amylase and α-glucosidase inhibition. Along with this, phytochemicals such as phenol, flavonol and carotene were also estimated. **Results:** The results demonstrated that the seeds treated with calcium chloride showed enhanced antioxidant as well as antidiabetic potential over control; on the other hand the action was reversed by EGTA and $LaCl_3$. Similar trend was observed in the phytochemical contents of the sprouts. **Conclusion:** Our data suggested that the improvement in nutraceutical value of fenugreek sprouts by calcium chloride could be due to the involvement of Ca^{2+} in signaling pathways associated with related phenolic compounds.

Key words: Antioxidant, Antidiabetic, Calcium ion, Germination, Phenolics.

INTRODUCTION

Several pharmacological as well as experimental studies have suggested that consumption of foods rich in antioxidant is significantly associated with reduced risk of various disorders and human diseases, including diabetes.[1] Some of the food types such as fruits, vegetables and sprouts and herbal drugs have been found to be very rich in bioactive compounds such as polyphenols, vitamins C and E, β-carotene etc, which posses potential antioxidant activity. Therefore, in recent times the regular consumption of sprout or germinated seeds, fruits and vegetables, is highly recommended as they are considered to provide long term health benefits.[2]

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DOI: 10.5530/fra.2015.2.5

Reactive oxygen species (ROS) or free radicals leaked during the process of metabolism have been the major source for the oxidative stress in the living system. These free radicals are found to be responsible for several chronic diseases and disorders in human body system.[3] The increased level of ROS has been associated with the degradation of pancreatic beta-cells leading to type 1 diabetes and the onset of type 2 diabetes by insulin resistance.[4] The inhibitors of α-amylase and α-glucosidase, which are responsible for post-prandial hyperglycemic conditions have gain more attention by the medical practitioner for the diabetic treatment. The antioxidant compounds present in these food sources and herbal drugs could be attributed to their properties such as hydrogen donors, reducing agents and metal ion-chelators.[5] It has also been reported that the natural sources of antioxidants can play vital role in controlling the post-prandial hyperglycemic conditions via inhibition of the key enzymes α-amylase, α-glucosidase which is a potential approach towards diabetic treatment.[6] *Trigonella foenum-graecum* commonly known as fenugreek, the different plant

Asian Journal of Pharmaceutical and Clinical Research
Innovare Academic Sciences
Knowledge to Innovation
Vol 9, Issue 2, 2016
Online - 2455-3891
Print - 0974-2441
Research Article

ASSESSMENT OF THE EFFECT OF NITRIC OXIDE AND CALCIUM ION ON THE THERAPEUTIC POTENTIAL AND OXIDATIVE STRESS STATUS OF FENUGREEK SPROUTS

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Received: 07 January 2016, Revised and Accepted: 15 January 2016

ABSTRACT

Objective: The aim of the present study was to investigate the effect of priming with exogenous sources of calcium ion and nitric oxide on the antioxidant activity, antidiabetic activity, and related phenolic contents along with the histochemical status of fenugreek sprouts.

Methods: The fenugreek seeds were primed with calcium chloride (CC), calcium chelator ethylene glycol-bis (2-aminoethylether) -N, N, N´, N tetra acetic acid (EG), sodium nitroprusside (SNP) and 2-(4-carboxyphenyl) -4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (CP) and germinated for 72 hrs. The sprout extracts were investigated for their antioxidant potential by 2,2-diphenyl-1-picrylhydrazyl, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid, reducing power as well as beta-carotene bleaching assays along with *in vitro* antidiabetic activity by α-amylase and α-glucosidase inhibition. Along with this, phytochemicals such as phenolics, flavonoids, and carotene content were also estimated, and the histochemical detection of reactive oxygen species in roots was performed.

Results: The results demonstrated that the seeds pre-treated with CC and SNP showed enhanced antioxidant as well as antidiabetic potential over control; on the other hand, their action was reversed by their antagonists, EG, and CP. A similar trend was observed in the phytochemical contents of the sprouts. Furthermore, it was evident from the histochemical detection of H_2O_2 and superoxide localization as well as lipid peroxidation and plasma membrane integrity that the exogenous supply of calcium ion and nitric oxide exhibited protective role in the germinating seedlings.

Conclusions: The study suggests the active involvement of the signal molecules, Ca^{2+}, and nitric oxide in signaling pathways associated with related phenolic compounds and oxidative stress management.

Keywords: Antidiabetic, Antioxidant, Calcium ion, Germination, Histochemical localization, Phenolics, Nitric oxide, *Trigonella foenum-graecum*.

INTRODUCTION

The fact that the consumption of antioxidant-rich food is extensively associated with reduced risk of various disorders and diseases has been suggested by several researchers [1]. Natural resources such as fruits, vegetables, and also sprouts have been found to be a potential source of bioactive compounds such as polyphenols, ascorbic acid, β-carotene, etc., which possess high antioxidant activity. Therefore, the habitual intake of sprouts, fruits, and vegetables is highly recommended by the nutritionists, as they provide long-term health benefits [2].

Free radicals, such as reactive nitrogen species (RNS) and reactive oxygen species (ROS), are considered to be a fundamental part of normal physiology. Oxidative stress occurs as a consequence of the imbalance in the antioxidant defense system and free radical production [3,4]. These free radicals are mainly responsible for several oxidative stresses mediated chronic diseases and disorders in the human body system, including diabetes [5]. Excessive production of ROS leads to degradation of the pancreatic β-cells, thus causing Type 1 diabetes and Type 2 diabetes with insulin resistance. The inhibiting agents of α-amylase and α-glucosidase (AG), which are responsible for post-prandial hyperglycemia, have gained more attention for diabetic treatment [6]. Antioxidant compounds play a vital role in preventing the cellular damages, against the highly unstable chemical components such as free radicals and ROS, which are constantly produced by the cell metabolism and their concentration increases under stress conditions [7]. It has also been reported that the natural sources of antioxidants can efficiently control the post-prandial hyperglycemia via inhibition of α-amylase and AG without any negative effects [8].

Trigonella foenum-graecum commonly known as fenugreek has been reported to possess several pharmacological and folkloric applications. Its leaves have been reported to show potential antioxidant property, antimicrobial as well as antidiabetic activity. The *in vivo* hypoglycemic activity of fenugreek seeds has been established in various animal model systems. In addition, fenugreek seeds possess potential hypocholesterolemic effect, antioxidant property and are also very effective in the treatment of diabetic disorders [9].

Nitric oxide is a bioactive molecule, which functions both as a pro-oxidant as well as antioxidants in plant system [10]. The chemical properties of nitric oxide make it a versatile signaling molecule that functions via interactions with several cellular components [11]. It is also considered as an RNS and its concentration-dependent impacts on different systems were reported to be either protective or toxic [12].

Calcium (Ca^{2+}) is another important secondary messenger and signaling molecule which is actively involved in various physiological and developmental processes. In the cited literature, Ca^{2+} has also shown a protective effect against stress by mitigation of oxidative damages and membrane stabilization [13].

Seed priming is a pre-sowing technique in which seeds are subjected to the low external water potential that limits hydration which does not allow the protrusion of radicle through the seed coat. This technique is known to enhance the primary development of seeds under unfavorable environment [14,15]. The priming of seeds with various substances such as water, inorganic salts, osmolytes, and hormones has been successful and reported as a cost-effective strategy to enhance tolerance under saline conditions [16].

Sprouting has been considered as the effective means by which the nutritional quality of the seeds is enhanced. During germination process, mobilization of complex macromolecules such as stored

NBU Journal of Plant Sciences Vol. 10, No. 1, March 2016 p. 31-44

ISSN No. 0974-6927

Review Article

Nitric oxide and calcium signalling in plants under salinity stress and their crosstalk

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Abstract

Salinity is considered as one of the major factor affecting the crop production throughout the world. The oxidative stress induced by salinity can retard plant growth and yield as major part of energy is wasted on conserving water and improving ionic balance. The free radicals produced during stress are considered to be a major factor for most of the damages as these free radicals attack vital biomolecules such as lipids, protein and carbohydrates which are the basic requirement of almost all physiological and developmental processes. Understanding the mechanism of stress tolerance along with the involvement of important signalling molecules in stress signalling network is essential for crop improvement. Likewise, the two signalling molecules nitric oxide and calcium ion have been reported to be actively involved in upregulation of various stress response mechanisms thus indicating the existence of a possible cross talk among these molecules and other associated pathways. In this review, emphasis was given on the impact of salinity and oxidative stress mediated damages on plant system. Additionally, the role of nitric oxide and calcium ion as signalling molecules in response to stress signals and their implication in mitigation of salinity stress has also been discussed.

Keywords: Calcium ion, Free radicals, Nitric oxide, Salinity, Signalling.

Introduction

Salinity is considered as one of the major factor affecting the crop production throughout the world. Salinity either in water or soil represents one of the major abiotic stresses especially in arid and semi-arid regions, which can severely limit the agricultural production (Shanon, 1998). High concentration of salt creates ionic imbalance and hyper osmotic stress in plant system which consequently leads to oxidative damages. Such drastic changes in plant system cause retardation of growth, molecular damages, membrane disruption and even death. For the plant to be tolerant to salinity stress: their homeostasis must be re-established along with detoxification mechanism must be boosted (Zhu, 2001). Most of the cellular damages caused by salinity are usually associated with ROS mediated oxidative stress (Parida and Das, 2005).

Nitric oxide and calcium both are considered as highly versatile signalling molecules. Various literatures have reported the significant involvement of both of these molecules in wide range of physiological and developmental processes in plants. Additionally, these molecules have found to mitigate the adverse effect of varied environmental stresses including salinity (Wilson *et al.*, 2008; Sirova *et al.*, 2011; Lecourieux *et al.*, 2006).

Effect of salinity on plant system

The two major consequences of salinity on plant system are osmotic stress and ionic toxicity; these physical conditions affect all other physiological, biochemical and developmental processes in plants (Yadav *et al.*, 2011). High salt content in the substratum creates rise in osmotic pressure of the substratum thus, affecting the water uptake capacity of plants. Furthermore, decrease in the turgor pressure of the plant cells cause closing of stomata which leads to reduced carbon fixation but increase in ROS production. These highly reactive and unstable free radicals disrupt various cellular processes by damaging the major biomolecules like lipids, proteins, and nucleic acids (Parida and Das, 2005). Ionic toxicity is the physiological state

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AJPCR_16910_RA

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ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

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Vol 10, Issue 5, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

ELICITATION OF TRIGONELLINE, A HYPOGLYCEMIC AGENT IN FENUGREEK SPROUTS BY CALCIUM AND NITRIC OXIDE PRIMING

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Received: 03 January 2016, Revised and Accepted: 09 February 2017

ABSTRACT

Objective: This work was performed to evaluate the effect of priming with exogenous sources of calcium ion and nitric oxide on the antidiabetic activity and the alkaloid contents of fenugreek sprouts along with isolation and identification of trigonelline, a bioactive alkaloid responsible for hypoglycemic property of fenugreek.

Methods: The fenugreek seeds were pre-treated with calcium chloride (CC), lanthanum chloride (LC) a calcium channel blocker; ethylene glycol-bis (2-aminoethylether) -N, N, N′, N tetra acetic acid (EG) a calcium chelator; sodium nitroprusside (SNP) and 2-(4-carboxyphenyl) -4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (CP) a nitric oxide scavenger and germinated for 72 hrs. The sprout extracts were evaluated for their *in vitro* antidiabetic potential by α-amylase and α-glucosidase inhibition along with their trigonelline content**.** Trigonelline was isolated from fenugreek sprouts and identified by Infrared analysis and nuclear magnetic resonance (NMR) spectroscopy.

Results: The results revealed that sprouts pre-treated with CC and SNP exhibited enhanced antidiabetic potential as well as alkaloid content over control; on the other hand, their action was reversed by their antagonists, EG, LC, and CP. The sprouts pre-treated with 2mM CC showed the best elicitation of alkaloid content and antidiabetic activity followed by SNP-20 mM.

Conclusions: The study suggests probable involvement of the signaling molecules, calcium ion, and nitric oxide in pathways associated with biosynthesis of bioactive compounds responsible for hypoglycemic activity of fenugreek sprouts one of which being trigonelline.

Keywords: Antidiabetic, Calcium, Fenugreek sprouts, Nitric oxide, Priming, Trigonelline, Nuclear magnetic resonance.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i4.16910

INTRODUCTION

Plants are the basic source of knowledge of modern medicine. Almost all the parts of a plant, namely leaves, flowers, fruits, bark, roots, stem, and seeds are known to have various medicinal properties [1]. The trend of using natural products has increased, and the active plant extracts are frequently screened for new drug discoveries and for the presence of potential bioactive components [2,3].

India with the highest number of people suffering from diabetic disorders has been considered as the diabetic capital of the world by the "International Journal of Diabetes in Developing Countries." There is an alarming rise in diabetes patients in India; approximately 3.4 million deaths occur due to complication related to high blood sugar [4].

Diabetes mellitus is a serious metabolic disorder that leads to hyperglycemic condition due to decreased insulin production or inefficient insulin utilization. It is usually characterized by hyperglycemia, lipoprotein abnormalities, high basal metabolic rate, impairment in the activity of important enzymes, and oxidative stress which damages the pancreatic beta cells. It is the most common endocrine disorder which disrupts glucose homeostasis causing severe diabetic associated complications in major organs such as eye, blood vessels, and brain [5,6]. Multiple risk factors responsible for the disease to occur include persistent stress and depression, obesity, environmental pollutants, and sedentary lifestyle [7].

Some of the synthetic antidiabetic components such as metformin, acarbose, biguanides, and voglibose are found to be used clinically in combination with another diet to control diabetes, but moreover, they exhibit adverse side effects after long term use [8-10]. To prevent or overcome the side effects of these synthetic drugs and also to create other safer alternative drug choices, it has become essential to seek other inhibitors for further drug development. Thus, in recent years, several efforts have been made for increasing the availability of glucosidase inhibitors from natural sources [11,12].

Likewise, fenugreek besides having several pharmacological properties it is also reported to possess potent antidiabetic property both *in vitro* and *in vivo* system. Fenugreek is known to be a rich source of various bioactive components having a different therapeutical property such as sapogenins, fenugreekine, nicotinic acid, phytic acid, and trigonelline [13,14]. Trigonelline is a pyridine alkaloid known to be mostly found in Fabaceae members and is reported to be metabolically active as a hypocholesterolemic agent along with potential hypoglycemic effect [15-18]. Fenugreek has been successfully implemented as antidiabetic remedy for both types I and II diabetes [19]. Moreover, fenugreek has been reported to be enriched with wide spectrum of pharmacological and folkloric significance [20].

This study deals with the isolation and identification of trigonelline by 1-D and 2-D nuclear magnetic resonance (NMR) from fenugreek sprouts. Further, the elicitors of calcium and nitric oxide were applied for the enhancement in the alkaloid content of fenugreek sprouts along with the *in vitro* antidiabetic property. This study may provide an insight in the role of these signaling molecules in modulating the biosynthesis of alkaloids, a potential hypoglycemic agent; in addition, the utility of 2-D NMR spectroscopy in identification of bioactive compounds.