In vitro and in vivo leaf extracts of Gymnema sylvestre involve the protective effects and development of metabolic syndrome in High-Fat Diet Rats

a. Habit; b. A twig with flower; c. Seeds

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More than 1 billion adults worldwide are overweight, among 300 million are clinically obese, and nearly 43 million children are overweight (less than 6 years old) in 2015 (WHO, 2011).

60-80% of diabetic patients are obese, showing that obesity is a strong risk factor for developing type-2 diabetes mellitus (DM).

Insulin resistance initially stimulates the secretion of insulin from the beta cells in the pancreas.

However, over time, the beta cells become exhausted resulting in increased blood glucose levels. As a result, people suffering from both acute and chronic pancreatitis become Type II diabetes.

The administration of certain drugs such as corticosteroids, ACTH, thiazide aggravates the obesity conditions.
Gymnema sylvestre is a woody climber and belongs to the family Asclepiadaceae.

It is well-recognized in traditional medicine as a remedy for diabetes mellitus, stomachic, diuretic and used in folk, Ayurveda and homeopathic systems of medicine.

It maintained blood glucose through increased serum insulin levels provided by repair (or) regeneration of the endocrine pancreas (Shanmugasundaram et al., 1990).

Gymnema sylvestre leaves (or) their extracts have been widely used as health foods in tea bags, tablets, and beverages in recent years in Japan.

The users of these health foods often expect weight reduction (or) improvement of against diabetes because of their ability to suppress the taste of sweetness and inhibit glucose absorption (Nakamura, 1988; Ueno, 1997).
Mode of action of Gymnemic acid

The glycoside of gymnemic acid may block the absorption of sugar from the intestine.

The glycoside of gymnemic acid may block the sweet taste of sugar.

It has also shown the repair or regeneration of insulin producing cells in pancreas and balance insulin level.

“Inhibit sweet taste”
“Gymnemic acid” & “Gurmarin”
Gymnemic acid products - World market

*Gymnema sylvestre* leaves (500kg) used to 1g Gymnemic acid?

*Plant tissue culture* approach?

### Product name (Gymnemic acid) | Price ($)
---|---
Gymnema capsule 60 | 18.95
DIABETES SUPPORT FORMULA | 29.95
Bio-shape | 55.00
Thermo force metabolism 90 capsules | 27.99
Gymnema caps | 6.95
Gymnema Tea | 15.50
DIA-BOTICA | 8.95
Ayurvedic herbal extract | 18.95
Glucobetic | 29.95
SyndRx | 49.95
Diaxinol | 37.35
A to Z | 15.98
Glucose balance (Beta – fast) | 12.29
Glucochrom | 15.00
Ex-Ell | 41.99
Glucosamine plus | 5.55
Shardunikha | 20.95

Objectives

Optimization of culture conditions for *in vitro* callus biomass production and gymnemic acid.

Quantification of gymnemic acid from *in vivo* leaf and *in vitro* raised callus and

Comparative assessment of anti-obesity of methanol extract of *in vitro* raised callus & *in vivo* leaf in High Fat Diet Fed rats.
Callus culture (Optimized Plant Growth Regulators)

Explants (Leaf, Stem and Petiole)

Sterilization (70% ethyl alcohol for 15 sec, 2% Sodium hypochlorite for 3 min, 0.1% HgCl₂ for 3 min)

MS, B5, SH and WPM medium

IAA (Indole-3-acetic acid): 0.5-5.0 mg/l

IBA (Indole-3-butyric acid): 0.5-5.0 mg/l

2,4-D (2,4-dichlorophenoxyacetic acid): 0.5-5.0 mg/l

NAA (1-naphthaleneacetic acid): 0.5-5.0 mg/l

Cytokinins:

BA (6-benzylaminopurine): 0.2-2.0 mg/l

KN (6-Furfurylamino purine): 0.2-2.0 mg/l

Adenine sulphate: 5.0, 10.0 mg/l

Note: In different concentrations and combinations

& growth period

Research Article

Production of Gymnemic Acid Depends on Medium, Explants, PGRs, Color Lights, Temperature, Photoperiod, and Sucrose Sources in Batch Culture of Gymnema sylvestre

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Gymnema sylvestre (L.) Raf. is an important diabetes medicinal plant which yields pharmacologically active compounds called gymnemic acid (GA). The present study describes callus induction and the subsequent batch culture optimization and GA quantification. The callus induction was obtained by inoculating explants in different concentrations and combinations of MS, B5, SH and WPM media. The different PGRs were tested in the present study for the first time. Factors such as light, temperature, sucrose, and photoperiod were studied to observe their effect on GA production. Temperature conditions completely inhibited GA production. Callus, leaf, stem and petiole were used in the present study for the first time. Factors such as light, temperature, sucrose, and photoperiod were studied to observe their effect on GA production. Temperature conditions completely inhibited GA production. Maximum GA production (38.38 mg/g dw) was observed in blue light. The results showed that physical and chemical factors greatly influence the production of GA in callus cultures of G. sylvestre. The factors optimized for in vitro production of GA during the present study can successfully be employed for their large-scale production in bioreactors.

1. Introduction

In vitro techniques are very useful in ensuring sustainable, optimised sources of plant-derived natural products. However, in vitro cultivation should be preceded by proper evaluation of the plants for their ability to produce the required bioactive substances. Therefore, the selection of the right combinations of PGRs is crucial in inducing the technology to potential growers. The ability of plants to produce certain bioactive substances is largely influenced by the physical and chemical environments in which they grow. Plants also produce certain chemicals to overcome abiotic stresses [1]. Plants use light not only as an energy source for photosynthesis but also as an important environmental signal. Plants can detect almost all facets of light such as direction, duration, quantity, and wavelength by using three classes of photoreceptors: the red/far-red (660-730 nm) absorbing phytochromes, and the blue/UV-A (320-380 nm) absorbing cryptochromes. These photoreceptors perceive and transduce light signals, via distinct intracellular signaling pathways, to mediate a broad range of physiological responses to light in addition to cell growth and development [2]. Light can affect morphogenesis and the functioning of plant metabolism as a signal and stress factor [3]. Phytohormones, in most plant cell cultures, secondary metabolites, including the production of phenolic terpenoids, alkaloids, and sesquiterpenoids, is stimulated by light [3]. On the other hand, light had an inhibitory effect on the accumulation of secondary metabolites in the case of nicotine and caffeine [4]. The literature shows that the majority of pharmacologically important compounds of plant origin are produced in response to specific light conditions [3]. Light can also affect the expression of genes involved in the metabolism of these compounds, and the ability of plants to respond to physical and/or chemical stimuli [5]. The use of deciduous and non-deciduous plant materials for the elicitation of pharmacologically active substances is well established. The use of light and dark periods, and the nature of the light source, are the most significant factors influencing the production of secondary metabolites in plant tissue cultures [6].
Callus culture – Under stress conditions

Explant (Leaf)

Sterilization (70% ethyl alcohol for 15 sec, 2% NaOCl for 3 min, 0.1% HgCl₂ for 3 min)

MS + Optimized growth regulators

Different stress conditions:

- Light: Blue, Red, Green, white (16/8h)
- Source: Sucrose-2, 3, 4, 5 and 6%
- pH: 4h/20h, 8h/16h, 12h/12h, 16h/8h, 20h/4h, 24h light; 24h dark conditions

Callus growth curve (15, 25, 35, 45 and 55 days)

Callus: Nature, Fresh & Dry Weight

Phytochemical screening and identification of Gymnemic acid raised callus & in vivo leaf

Extraction with methanol

Callus extract centrifuged in 5000 rpm for 10 minutes

Supernatant was used for further studies

Identification of Gymnemic acid and compare with standard

HPTLC HPLC


HPTLC/HPLC and Gravimetric Methodology for the Identification and Quantification of Gymnemic Acid from Gymnema sylvestre Methanolic Extracts

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Summary. Gymnemic acid (GA), a well known anti-diabetic compound has been detected in methanol extracts of intact leaves and in vitro callus cultures derived from leaf explants of Gymnema sylvestre. Callus biomass was developed in MS medium with optimum plant growth regulators (OPGRs) of 2,4-D (1.5 mg L^-1) + KN (0.5 mg L^-1) under abiotic stress conditions at 45 days determined by growth curve analysis. GA detection and quantification were carried out using thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), and gravimetric techniques. GA detection peak area and their absorption spectra were evaluated through HPTLC and HPLC with the standard GA. Quantification of GA had showed the linearity, accuracy, robustness and precision by HPLC. GA content was significantly higher in gravimetric method than HPLC. All these methods were found to be simple, accurate, selective and rapid and could be successfully applied for the determination of GA. It could have potential as a pharmaceutical drug for Type 1 diabetes mellitus (IDDM) and obesity.

Key Words: Gymnema sylvestre, gymnemic acid (GA), abiotic stress, HPTLC, HPLC, gravimetric method

Introduction

Type 1 diabetes, or insulin-dependent diabetes mellitus (IDDM), is a common pediatric chronic disease, affecting an increasing number of children every year. IDDM occurs due to autoimmune destruction of insulin-producing β-cells in the pancreas, resulting in low or no production of insulin, a hormone necessary for survival [1]. According to World Health Organization, obesity has reached epidemic proportions globally, with at least 2.6 million people dying each year as a result of being overweight or obese [2].
Experiment groups and given treatment in Wistar rats

Group 1
Normal rats fed with control diet

Group 2
High Fat-fed animals received fat-enriched diet for 40 days

Group 3
High Fat-fed animals treated with standard drug simvastatin (3 mg/kg/day)

Group 4
High Fat-fed + *G. sylvestre* leaf extracts - 200 mg/kg rats body weight (Shanmugasundaram *et al.*, 1990)

Group 5
GCE₁: Blue light treatment callus + MS + (2,4-D 1.5 mg/l) + KN(0.5 mg/l)

Group 6
GCE₂: 5% sucrose treatment + MS + 2,4-D (1.5 mg/l) + KN (0.5 mg/l)

Group 7
GCE₃: 12 h photoperiod with MS + 2,4-D (1.5 mg/l) + KN (0.5 mg/l)

Group 8
GCE₄: 2,4-D (1.5 mg/l) + KN (0.5 mg/l)

Optimum gymnemic acid production treatment

The composition of the experimental diets (g/kg diet)

<table>
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<tr>
<th>Ingredients</th>
<th>Control diet</th>
<th>High-fat diet</th>
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<tr>
<td>Corn starch</td>
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<tr>
<td>Sucrose</td>
<td>100</td>
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<td>Dextrinated starch</td>
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<tr>
<td>Lard</td>
<td>-</td>
<td></td>
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<tr>
<td>Soybean oil</td>
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</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
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<td>Vitamin mixture</td>
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<tr>
<td>Choline</td>
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<td></td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
<td></td>
</tr>
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</table>
Callus culture

↓

Leaf explant

↓

MS medium

Auxins:-

MS + NAA (1.0 mg/l); 2,4-D (1.5 mg/l)

Auxins + Cytokinins:-

MS + 2,4-D (1.5 mg/l) + KN (0.5 mg/l)

MS + NAA (1.0 mg/l) + KN (1.5 mg/l)

MS + NAA (1.0 mg/l) + KN (1.0 mg/l)

Auxins + Cytokinins + Ads:-

MS + 2,4-D (1.5 mg/l) + BA (1.0 mg/l) + 5 mg/l Ads

MS + 2,4-D (1.0 mg/l) + KN (1.0 mg/l) + 5 mg/l Ads

Green callus + Maximum dry weight biomass callus production at 45th day
Callus culture: – Under stress conditions

MS + 2,4-D (1.5 mg/l) + KN (0.5 mg/l)

Stress treatments

- Light – Blue light
- Carbon source – 5 % sucrose
- Photoperiod – 16/8h, 12/12h (light/dark)

Green callus + Maximum dry weight biomass callus at 45th day
Dried leaf and callus

→ Methanol extract

Experiment I.

t value compared with standard Gymnemic acid

Brown spot (Isopropyl alcohol: Chloroform: Methanol: Acetic acid - 5: 3: 1: 0.5) - Gymnemic acid

Experiment II.

C – Leaf and callus extract Rf value compared with standard Gymnemic acid

Experiment III.

LC – Leaf and callus extract retention time compared with standard Gymnemic acid

A - Leaf; B - MS + NAA (1.5 mg/l); C - MS + 2, 4-D (1.0 mg/l); D - MS + 2,4-D (1.5 mg/l); E - MS + NAA (1.0 mg/l) + BA (0.5 mg/l); F - MS + NAA (1.0 mg/l) + KN (1.0 mg/l); G - MS + NAA (1.0 mg/l) + NAA (1.0 mg/l) + BA (1.0 mg/l); H - MS + NAA (1.0 mg/l) + KN (1.5 mg/l); I - MS + 2,4-D + KN (0.5 mg/l).

A - Leaf; B – MS + NAA (1.0 mg/l) + KN (1.5 mg/l); C - MS + 2,4-D (1.5 mg/l) + KN (0.5 mg/l); D - Leaf; E - Effect of blue light; F - Effect of 5% sucrose; G - Effect of 3mM NH₄NO₃; H - Effect of 12h photoperiod; I - Effect of 30°C temperature; J - Effect of red light; K - Effect of green light.
a. Control rats

b. Control rat after 40 days

c. G. sylvestre leaf extracts and callus extract (20 mg/kg) given by oral.

d. Standard drug simvastatin injection (3 mg/kg)

e. Collected pancreas after 40 days

f. Collected liver after 40 days

g. High Fat diet rat after 40 days (obese rat)

h. Standard drug simvastatin after 40 days

i. Gymnema sylvestre leaf treated rats after 40 days
Table 1: Effect of leaf and callus extracts of *G. sylvestre* on Body weight, Blood glucose, and cholesterol in high-fat fed Wister rats.

<table>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>Blood glucose (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
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<td>10th day</td>
<td>205±16.5</td>
<td>87±1.7</td>
<td>86±1.7</td>
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<td>20th day</td>
<td>220±30.2</td>
<td>92.0±2.5</td>
<td>92±2.6</td>
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<tr>
<td>30th day</td>
<td>245±24.6</td>
<td>87±1.9</td>
<td>87±1.2</td>
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<td>40th day</td>
<td>260±14.8</td>
<td>94±2.8</td>
<td>84±3.2</td>
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<tr>
<td>10th day</td>
<td>279±25.4</td>
<td>168±1.2</td>
<td>185±2.5</td>
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<td>20th day</td>
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<td>194±1.8</td>
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<tr>
<td>30th day</td>
<td>330±18.2</td>
<td>215±5.8</td>
<td>220±2.0</td>
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<tr>
<td>40th day</td>
<td>370±20.6</td>
<td>260±1.6</td>
<td>234±1.2</td>
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<td>10th day</td>
<td>200±32.2</td>
<td>135±4.6</td>
<td>110±1.8</td>
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<tr>
<td>20th day</td>
<td>215±34.2</td>
<td>140±5.4</td>
<td>112±3.2</td>
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<td>30th day</td>
<td>230±34.8</td>
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<td>40th day</td>
<td>235±10.6</td>
<td>182±3.2</td>
<td>154±3.2</td>
</tr>
</tbody>
</table>

**Normal; Group II:- High-Fat diet; Group III:- High-fat diet + Drug (simvastatin); Group IV:- High-fat diet + GSLE (200 mg/kg); Group V:- High-fat diet + GSLCE-I (OPGRs + Blue light); Group VI:- High-fat diet + GSLCE-II (OPGRs + 5% sucrose); Group VII:- High-fat diet + GSLCE-III (OPGRs+12 h); Group VIII:- High-fat diet + GSLCE-IV (OPGRs: MS+2,4-D 1.5 mg/l + KN 0.5 mg/l).**

**Conclusions:**

Body weights increased significantly in HFD (Group II) than Control (Group I). Concentration of blood glucose was higher in HFD fed obese rats (Group II) than in normal rats (Group I). Leaf and callus extract supplementation normalized the blood glucose level in Table 1. Simvastatin (Group II), the anti-obesity drug maintained the body weight as well as blood glucose level.
Effect of leaf and callus extracts of *G. sylvestre* on Triglycerides, HDL and LDL in normal and in high-fat fed Wistar rats

<table>
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<tr>
<th></th>
<th>Normal</th>
<th>Group II: High-Fat diet</th>
<th>Group III: High-fat diet + Drug (simvastatin)</th>
<th>Group IV: High-fat diet + GSLE (200 mg/kg)</th>
<th>Group V: High-fat diet + GSLE (200 mg/kg) + OPGRs + Blue light</th>
<th>Group VI: High-fat diet + GSLCE-II (OPGRs + 5% sucrose)</th>
<th>Group VII: High-fat diet + GSLCE-III (OPGRs+12 h Photoperiod)</th>
<th>Group VIII: High-fat diet + GSLCE-IV (OPGRs (MS+2,4-D 1.5 mg/l + KN 0.5 mg/l))</th>
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<td>Triglyceride (mg/dl)</td>
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</tbody>
</table>

Table 2. The leaf and callus methanol extracts showed significant improvement in total cholesterol, triglycerides and LDL-cholesterol were significantly elevated and HDL-cholesterol level was significantly decreased in the HFD obese rats (Group II), when compared to the normal controls animals (Control I).

However, HFD obese rats when treated with *G. sylvestre* leaf and callus methanol extracts showed significant improvement.
Thank you

Short communication

**In vitro** callus and **in vivo** leaf extract of *Gymnema sylvestre* stimulate regeneration and anti-diabetic activity in Wistar rats

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**ABSTRACT**

A methanolic extract of *Gymnema sylvestre* leaf and callus showed anti-diabetic activity by regulating β-cells. Optimum callus was developed under stress conditions of low light and I2N (0.5 mg/l), which induced maximum biomass of green compact callus by growth curve analysis. Leaf and optimus callus extracts contained gymemic acid using TLC, HPLC and HFC methods. The research reported here deals with leaf, callus, which significantly increased the weight of the whole body, liver, pancreas content in alloxan-induced diabetic rats (Wistar rats). The gymnemic acid in leaf significantly increases the regeneration of β-cell in treated rats, when compared with rats, it could have potential as a pharmaceutical drug for insulin-dependent diabetes.

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**Introduction**

Type 1 diabetes, or insulin-dependent diabetes mellitus (IDDM), is a common pediatric chronic disease, affecting an increasing number of children every year. IDDM occurs due to autoimmune destruction of insulin-producing β-cells in the pancreas, resulting in lower production of insulin, a hormone necessary for survival (International Expert Committee 2000).

*Gymnema sylvestre* (syn. Periplaca sylvestris Retz) is a traditional medicinal plant, with reported use as a remedy for diabetes mellitus, stomachic and diuretic problems. The plant extracts are also used in folk, ayurvedic and homopathic systems of medicine (Mitra et al., 1991). The extract of *G. sylvestre* plays a major role in blood glucose homoeostasis through increased serum insulin levels through regeneration of the endocrine pancreas (Shanmugam et al., 1991; Shanmugam et al., 1980). *G. sylvestre* occurs mainly in the Deccan peninsula of western India, Tropical Africa, Vietnam, Malaysia, Sri Lanka and is widely available in India, Germany and the USA as a health food (Ye et al., 2000). Within the last 10 years, a number of Gymnema products, including Gymnema capsules, Gymnema tea, BioShape, and Diamino® have appeared on the world market.

In the past few decades, secondary metabolite plant tissue culture has been identified as a new drug development and clinical research pharmacology and medicine. Plant cell culture has been used widely in the form of fraction compounds as potential bioactive molecules (Gupta et al., 1999). However, external factors like growing speed, p.i. and medium play an important acid production in suspension cultures (Dewey, 1978), sucrose, inulinum density, auxins, and cytokinins play a very crucial role in the production of gymnemic acid, the growth study of cell growth (Lee et al., 2002). Studies were undertaken to identify the *G. sylvestre* extract molecules that contribute to the promotion of anti-diabetic effects. In addition, we report that gymnemic acid plays an anti-diabetic role in anti-diabetic experiments. In the pancreas weight and glycogen content were of alloxan-induced diabetic Wistar rats, further is linked to β-cell regeneration and the determination.

**Materials and methods**

**Plant material and sterilization**

*G. sylvestre* plants (GS) were collected from hills and maintained in the plant science garden at Bharathidasan University, Tiruchirappalli, Tamil Nadu.