Research Article
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PARAMETERS STUDIED FOR DEVELOPMENT OF *GYMNEMA SYLVESTRE* LEAF EXTRACTS: AS INJECTABLE ANTI-DIABETIC

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Received on: 05/01/12 Revised on: 11/02/12 Accepted on: 03/03/12

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ABSTRACT
In the present study a novel attempt has been made to consider the various parameters for development of *Gymnema* leaf extracts for injectable dosage form. Ethanolic and Water extracts of leaves were obtained by decoction and filtered through whatman paper no-1. Extracts were dried in vacuum dryer and stability and solubility was studied. Phytochemical screening and TLC study was carried out. Injections of different strength of dried extracts were made using normal saline and sterilized by autoclaving. The samples were then evaluated for quality control tests and content variation test by TLC and scanning densitometer. Dried extracts were found stable up to 39^0C and 65% relative humidity. Liquid extracts showed presence of triterpenoids, sugars and proteins. By TLC it was confirmed that Gymnemic acid-I found to be major active constituent.
Key words: *Gymnema sylvestre*, Phytochemical screening, Diabetes, Injectables.

INTRODUCTION

Diabetes is the third most dreaded disorder with more than 150 million people suffering from this disorder presently and number is increasing annually by 33% in US alone. It is metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipemia, negative nitrogen balance and sometimes ketonemia. Two major types of diabetes mellitus are Type-I Insulin Dependent Diabetes Mellitus (IDDM) or Juvenile onset diabetes mellitus which is caused by beta cell destruction in pancreatic islets and Type-II Non Insulin Dependent Diabetes Mellitus (NIDDM) or Maturity onset diabetes mellitus caused by abnormality in gluco-receptor of beta cells, reduced sensitivity of peripheral tissues to insulin, excess of hyperglycemic hormones like glucagon. Several synthetic medicines are in use currently such as sulfonylureas (5mg-3g), repaglinides (1.5mg-8mg) and biguanides (25mg-2g) etc[1].

These synthetic drugs suffers from most common adverse effects like hypoglycemia, hypersensitive (photosensitivity) nausea, vomiting, flatulence, diarrhoea or constipation, headache, weight gain, vit B_{12} deficiency, abdominal pain, anorexia, metallic taste, and lactic acidosis. Natural herbal drugs which are used since ancient period for diabetes are comparatively safe and effective. But in case of diabetes most of the drugs are to be used lifelong hence herbal treatment will be best remedy rather than synthetic drugs. *Gymnema sylvestre* is one of the most common plants used for the treatment and prevention of diabetes. It is large climber, rooting at nodes, leaves elliptic, acuminate, base acute to acuminate, glabrous above sparsely or densely beneath. It is found throughout India, in dry forests up to 600m, common throughout in Asia, Tropical Africa, Malaysia and Srilanka[2]. Sushruta describes *Gymnema sylvestre*, as a destroyer of madhumeha (glycosuria) and other urinary disorders. The plant is also reported to be bitter, astringent, acrid, thermogenic, anti-inflammatory, digestive, liver tonic, emetic, diuretic, stomachic, stimulant, anthelmintic, laxative, cardio tonic, expectorant, antipyretic and uterine tonic. It is useful in dyspepsia, constipation, jaundice, haemorrhoids, renal and vesical calculi, cardiopathy, asthma, bronchitis, amenorrhoea, conjuctivitis and leucoderma[3,4]. The literature search reveals that the plant has been studied for antidiabetic and hypolipedemic, antimicrobial, Obesity, In-vitro antioxidant activity[5-8]. The major bioactive constituents of *Gymnema Sylvestre* are a group of oleanane type triterpenoid saponins known as “gymnemic acids”. The latter contain several acylated (tigloyl, methylbutyroyl etc.,) derivatives of deacylgymnemic acid (DAGA) which is 3-O - glucuronide of gymnemagenin (3, 16, 21, 22, 23, 28-hexahydroxy-olean-12-ene).The individual gymnemic acids (saponins) include gymnemic acids I-VII, gymnemosides A-F, gymnema saponins[9-11]. Gymnema is famous ayurvedic drug available in different forms such as Asnadi vati (Ayurved rasashala Pune) Glymine tab (Kerala Ayurveda Ltd), Amree capsule (Aimpl Ltd) Diabecon tab. (Himalaya). Many herbal drugs and ayurvedic formulations are used since time immoral for treatment of diabetes (Madhumeha). Also there is high risk of other disorders in such patients such as cardiac, renal, hepatic disorders. Hence it is very essential to develop the formulation which is safe, fast acting, with maximum curative effect. NIDDM accounts for up to 90% of UK diabetic population and there is an increasing drive to develop novel methods for its treatment to improve either insulin output of β cell or the sensitivity of peripheral tissue to circulating insulin[12]. Since no injectable formulation of gymnema or its constituents is available in the market. In the present work an attempt has been made to study the parameters for development of *Gymnema sylvestre* extract as an injectable antidiabetic.

MATERIAL AND METHODS

Plant leaves were collected from Gaganbavada region of Kolhapur district in the month of December and authenticated by Dr. S. R. Yadav Taxonomist Shivaji University, Kolhapur. Leaves were dried under shade and

coarse powder (# 44) was obtained using electric blender. Powder sample was extracted using 70% ethanol and chloroform water IP (0.25%) by boiling on water bath for 1 hour. TLC study was made using silica gel G and injectable was formulated in normal saline using dried potassium salt of extracts. The formulated injections were then tested for various quality control tests using standard reagents and chemicals. Standard gymnemagenin and gymnemic acids were isolated and confirmed by spectral characters.

Experimental

Coarse leaf powder (500g each) was extracted[13] with 70% ethanol and chloroform water (IP) by boiling on water bath for 1 hour and cooled extracts were filtered through vacuum filtrations unit ((Hi speed appliances-HSV-2), evaporated to dryness on rotary film vacuum evaporator (Dolphin). The dried extracts (45g and 112g respectively) were kept in refrigerator for future use.

Identification of crude drug by TLC

Sample was prepared as per the method described in the literature[14] supplied by Natural Remedies Bangalore. 500 mg of *Gymnema sylvestre* leaf powder was dissolved in 10ml of 50% (v/v) ethanol and then 2ml of KOH was added and heated on a boiling water bath under reflux for an hour and then cooled. To this 1.8ml of 12N HCl was added and heated on water bath. After cooling the pH was adjusted to 7.5-8.5 with 11% KOH. This solution was dissolved with 50% (v/v) ethanol and filtered. 10µl was applied on TLC plates (Figure 1)

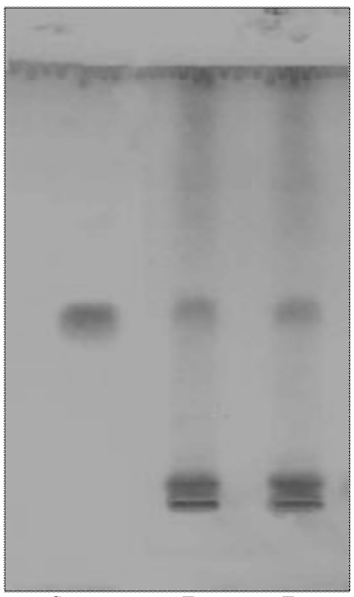
Solvent system

Chloroform: methanol: acetic acid (5: 1: 1)

Solvent front: Run up to 8 cm

Detecting agent

Vanilline sulphuric acid reagent (Heating at 100^0C for 5-8 min)



S_1 T_1 T_2

Figure 1: TLC of alcoholic and aqueous extracts of Gymnema leaf with standard

S_1 - Gymnemagenin,
T_1 – Alcoholic extract of *Gymnema sylvestre*
T_2 - Aqueous extract of *Gymnema sylvestre*

Identification of extract by TLC

Sample preparation before hydrolysis 500 mg of *Gymnema sylvestre* extract was dissolved in 50ml of methanol. 10µl was applied on TLC plates.

After hydrolysis 100 mg of *Gymnema sylvestre* extract was dissolved in 10ml of 50% (v/v) ethanol, and then 2ml of KOH was added and heated on a boiling water bath under reflux for an hour and then cooled. To this 1.8ml of 12N HCl was added and heated on water bath .After cooling the pH was adjusted to 7.5-8.5 with 11% KOH. This solution was dissolved with 50% (v/v) ethanol and filtered. 10µl was applied on TLC plates (Figure 2)

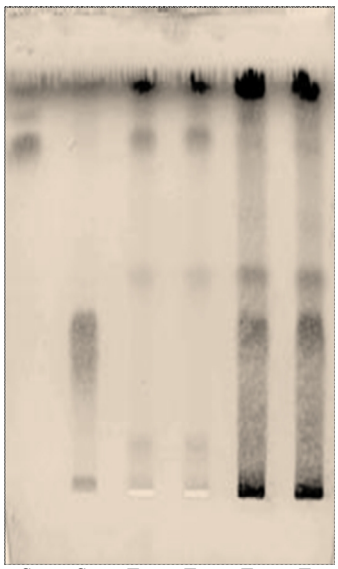
Solvent system

Isopropyl alcohol: methanol: Chloroform: acetic acid (5: 1: 3: 0.5)

Solvent front: Run up to 8 cm

Detecting agent

Modified Vanillin Sulphuric Acid reagent (Heating at 100^0C for 5-8 min)



S_1 S_2 T_1 T_2 T_3 T_4

Figure 2: TLC analysis of Gymnema extracts before and after hydrolysis

S_1 - Gymnemagenin,
S_2 - Standard Gymnemic acid,
T_1 - Alcoholic extract of Gymnema after hydrolysis
T_2 - Aqueous extract of Gymnema after hydrolysis
T_3 - Alcoholic extract of Gymnema before hydrolysis
T_4 - Aqueous extract of Gymnema before hydrolysis

Estimation of gymnemic acid in extract by gravimetric method

Alcoholic extract (3g) was taken in beaker and dissolved in 50ml of double distilled carbon dioxide free water. Solution was filtered and 10% HCl was added till the pH was adjusted to 1.5. Sample was allowed to stand for 30 minutes at room temperature and filtered on Whatman No.1 filter paper. Residue was washed with 20 ml of distilled water and the filtrate was discarded. Precipitate was dissolved in 20 ml of 80% v/v ethanol thrice and combined filtrate and washings were evaporated in pre-weighed beaker and dried in oven under vacuum at 70^oC to constant weight. Percentage of total gymnemic acid was calculated.

Stability study of extract

Dried extracts were kept in stability study chamber at room temperature, 38 and 45°C and at 65 ± 2% relative humidity for one month. The extracts were found stable and tested by TLC analysis.

Formulation of injectables 5% and 10% v/v solution of alcoholic extract of *Gymnema sylvestre* were prepared using normal saline solution and filtered through membrane filter (G-4). Filtrate was then filled in 5 ml amber colored ampoules and sterilized by moist heat sterilization method. Sterile samples were subjected for quality control tests[15].

a) **Sterility test** sterile sample was inoculated in sterile Fluid thioglycolate media at about 40-45°C under strict aseptic condition and incubated at 35°C for 14 days and frequently checked the growth on 3rd, 5th, 8th and 14th day.
b) **Particulate matter** Sterile sample was subjected to clarity test apparatus followed by microscopic examination to check particles.
c) **Leaker test** Ampoule was submerged entirely in a deeply colored dye solution (1% methylene blue solution) and observed for color change of ampoule solution.

Estimation of total gymnemic acid content in formulation

After formulation of injection in bulk (5% of 500 ml) of ethanolic extract of *Gymnema sylvestre* the total content of acids was calculated by gravimetric method as described earlier.

RESULT AND DISCUSSION

Alcoholic extract gave about 45g of solid extract while aqueous extract yields 112g of solid residue. Crude extract has shown presence of Gymnemagenin (R_f – 0.47) with comparison of reference sample in both alcoholic and aqueous extracts. After alkaline hydrolysis both extracts have shown gymnemagenin (R_f –0.83) but gymnemic acid was shifted to higher R_f – 0.49 while before hydrolysis it appears at R_f–0.46. Total gymnemic acid was found to be 8.78 and 7.42% respectively in ethanolic and aqueous extracts. The potassium salt of gymnemic acid was soluble in normal saline and injectables were found stable, sterile, leak proof and free from particulate matter. This was a novel attempt made and further studies are needed to establish pyrogen testing and biological response on animal and human models to study the pharmacokinetics of dosage form in comparison with present marketed dosage forms.

ACKNOWLEDGMENT

Authors are thankful to Dr. H. N. More, Principal Bharati Vidyapeeth College of Pharmacy Kolhapur for providing facilities to carry out the above research. Also we are thankful to Dr. S. R. Yadav, Taxonomist Shivaji University, Kolhapur for identification and authentication of plant.

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Source of support: Nil, Conflict of interest: None Declared