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Pharmacognostical Standardisation and Antidiabetic activity of Artocarpus Heterophyllus Leaves Lam.

Nazli Shahin*, Sanjar Alam, Mohammad Ali**
*Dept. of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India 110062
**Department of Pharmaceutics, Faculty of Pharmacy , Jamia Hamdard, New Delhi 110062

Abstract

The present investigation was carried out to focus on the hypoglycemic effect of the leaves of Artocarpus heterophyllus in normal and streptozocin induced diabetic rats. The Plant was subjected to pharmacognostic, physico-chemical and phytochemical evaluations which will assist in standardization for authenticity, quality and identification of the herbal products. Treatment with extract of the leaves at dose 250 mg/kg to diabetic rats resulted in significant reduction of serum glucose, total cholesterol, whereas significant increased level of high density lipoprotein was observed.The present study clearly demonstrated that the plant is having potential hypoglycemic activity which may be beneficial for the management and treatment of diabetes mellitus.

*Corresponding author, Mailing address:
Sanjar Alam
Tel.: +919718482672
E-mail: nazli5leo@gmail.com
**Corresponding author
Tel : +919999943354
E-mail : sanjaralam10@gmail.com

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MATERIALS AND METHODS

Introduction

Artocarpus heterophyllus Lam (Moraceae) commonly known as jackfruit is native to western ghats of India, Malaysia and also found in central and eastern Africa, southeastern Asia, the Caribbean, Florida, Brazil, Australia, Puerto Rico and many Pacific islands [1]. Although it comes up well at altitudes of 400- 1200m , it is also being cultivated at low elevations and hotter parts throughout India[2].

Jack is monoecious in nature. In India, a good yield is 150 large fruits per tree annually [3]. The plants of Artocarpus species have been used by traditional folk medicine in Indonesia against inflammation, malarial fever, stomachache, ulcers, diarrhoea, dysentery, abscesses, defective urinary secretion, skin disease [4,5] and asthma [6].

The idea of Standardization is to establish consistent potency and to control the full spectrum of bioactive chemical constituents naturally occurring in medicinal plants from batch to batch. Authenticity of the raw drugs based on the morphological characters is lucrative for botanical and species identity. The tests conducted are to determine physico-chemical standards regarding total ash, foreign organic matter, and moisture content.

Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration-hyperglycaemia (fasting plasma glucose >7.0 mmol) or plasma glucose >11.1 mmol/12 hour after a meal), caused by insulin deficiency, often combined with insulin resistance. Hyperglycaemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis [7].

The present study was carried out to determine several pharmacognostic parameters and examine traditional claims for the anti-diabetic activity of the plant. Aqueous extract of *Artocarpus heterophyllus Lam*. leaves was prepared and their anti-diabetic activity was investigated in rats.

EXPERIMENTAL WORK

Drugs and chemicals

Standard drug: Glimepiride prepared in Tween 80 solution; Test drug: plant extract, in CMC (1 %) solution. All chemicals and reagents used were of analytical grade. Streptozotocin (Spectrochem Pvt. Ltd. Mumbai, India) was obtained from Chopra chemicals (New Delhi, India).

Plant authentication and extraction

Artocarpus heterophyllus (leaves) were procured from local market of Patna, Bihar and authenticated by Dr. H.B. Singh, scientist and head (NISCAIR), New Delhi. A voucher specimen (NISCAIR/RHMD/consult/-20-09-10/1322/124) is deposited there. The air dried powdered drug (500 g) was extracted with water in a Soxhlet apparatus for 6 hour. Aqueous extract of the plant was evaporated to dryness under pressure to get solid residue. The residue was stored at 0 - 4 ° C for subsequent experiments.

PHARMACOGNOSTIC EVALUATIONS (STANDARDIZATION)

Morphological properties

Organoleptic properties of the Plant was determined using the reported methods for color, odour, and taste[8,9].

Phyotochemical screening

In order to determine the presence of phytochemical in plant extract, standard identification tests for alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols was performed[10].

Ash Value

Total ash value was determined by taken accurately weighed 2 g of sample extract into ignited tared silica crucible where it was spread as a fine layer on the bottom. At the increasing temperature the sample was burnt up to red hot not exceeding 450°C until free from carbon. Then the crucible was cooled and resultant ash was weighed and thereupon percent total ash value was determined with reference to air-dried extract drug. The obtained ash during the above procedure was taken and boiled with 2N HCl (25 ml) for 5 min respectively for quantitative estimation of acid insoluble ash. Thereafter the insoluble ash was recovered on an ash less filter paper and washed using hot water. Insoluble sample was transferred into a crucible which was again burnt for 20 min and weighed properly. In order to omit

errors the whole step was repeated thrice and the percent acid insoluble ashes were determined with reference to air-dried drug. Water soluble ash was determined by using the recovered ash during the estimation for total ash was taken and boiled with H2O (25 ml) for 5 min interval. Thereafter the insoluble ash was recovered on an ash less filter paper and washed using hot water. Insoluble sample was transferred into a crucible which was again burnt for 20 min and weighed properly. The whole step was repeated thrice in order to omit errors and the percent water soluble ashes were determined with reference to air-dried drug respectively.

Fluorescence analysis

Many herbs fluorescence when cut surface or powder is exposed to UV light and this can help in their identification method. The fluorescence character of the plant powders (40 mesh) was studied both in daylight and UV light (255 and 366 nm) and after treatment with different reagents like sodium hydroxide, picric acid, acetic acid, hydrochloric acid, nitric acid, iodine, ferric chloride etc .

Loss on drying

This parameter determines the amount of moisture as well as volatile components present in a particular sample (i.e. water drying off from the drug).The powdered drug sample (10 gm) without preliminary drying was placed on a tarred evaporating dish and dried at 105ºC for 6 hours and weighed. The drying was continued until two successive reading matches each other or the difference between two successive weighing was not more than 0.25%. Constant weight was reached when two consecutive weighing after drying for 30 minutes in a desiccator, showed not more than 0.01 gm difference .

FOREIGN MATTER

It is the matter present in the drug. Its presence may be due to faulty collection of crude drug or due to deliberate mixing. It was separated from the drug so that results obtained from analysis of the drug gives accuracy. Its percentage in the crude drug was calculated.

HYPOGLYCEMIC EVALUATION

Preparation of the extracts

The air dried powdered drug (500 g) was extracted with water in a Soxhlet apparatus for 6 hour. Aqueous extract of both the plants were evaporated to dryness under pressure to get solid residue. The residue was stored at 0 - 4 ° C for subsequent experiments.

Animals

Wistar albino rats (150-200 g) were obtained from Central Animal Facility, Jamia Hamdard University and maintained in 25 ± 1°C, with 55 ± 5 % humidity with 12 hr light/dark cycle. The animals were given standard pellet diet (Lipton rat feed, Ltd., Pune) and water *ad libitum* throughout the experimental period. The Institutional Animal Ethics Committee approved the experiments. All the extracts and the standard drugs were administered orally.

Chemicals

All chemicals and reagents used were of analytical grade. Streptozotocin (Spectrochem Pvt. Ltd. Mumbai, India) was obtained from Chopra chemicals (New Delhi, India).

Drugs

Standard drug: Glimepiride prepared in Tween 80 solution; Test drug: plant extract, in CMC (1 %) solution.

Induction of diabetes

The animals were fasted for 16 hour prior to the induction of diabetes. STZ freshly prepared in citrate buffer (pH 4.5) was administered i.p. at a single dose of 50 mg/kg. Development of diabetes was confirmed by polydipsia, polyurea and by measuring blood glucose concentrations 72 hour after injection of STZ. Rats with blood glucose level of 250 mg/dl or higher

were considered to be diabetic and selected for experiment [11]

Diabetic animals were randomly assigned to groups. Group I contained normal animals and served as normal control. Group II served as diabetic control (toxic). Groups I and II received vehicle during the experiments, while the Group III received the reference standard drug glimeperide (0.1 mg/kg) and groups from IV to V received the aqueous extract of the leaves of *Artocarpus heterophyllus* (250 mg/kg, and 500 mg/kg) respectively.

Biochemical estimation

Initial, 7th 14th and 21st day non fasting blood glucose levels were determined just before administering the drugs. On the last day of experiment, blood samples were collected from tail vein from each animal. Serum was separated from the blood by centrifuging at 3000 rpm for 20 minutes for biochemical estimations of TC and HDL-C [12,13,14].

Estimation of blood glucose

The blood glucose level was estimated with One Touch Basic Glucometer (Accu Chek Active, Roche, Germany). Serum total cholesterol (TC), high-density lipid cholesterol (HDL-C), were estimated by using standard enzymatic colorimetric kits (Span diagnostic Ltd. Surat, India).

Statistical analysis

Values are expressed as mean ± standard error of the mean. Statistical significance was calculated by using one-way analysis of variance (ANOVA) followed by Dunnetts't' test. The values were considered statistically significant when the P- value was less than 0.05. ($P<0.05$)

RESULTS

Morphological characters of the Plant

It grows to a height of 22.8 m. Jackfruit is an evergreen with glossy leathery oblong leaves. Male and female flowers are on the same tree but separate. Jackfruit is monoecious, while the fruits are 91.4 cm long and 50.8 cm wide. The fruit can weigh to 27.2 kg and sometimes even more and it contains from 100 – 500 oval seeds. The viability time of the seeds are short (not more than a month). The fruit is yellowish and composed of hard cone-like points. All parts contain a white latex. The taste is somewhat between banana and pineapple.

Preliminary Phytochemical Screening

The results of preliminary phytochemical investigation of methanolic extract of Artocarpus heterophyllus showed the presence of various constituents as are shown in Table 1.

Table1: Detection of Phytoconstituents in leaves of *Artocarpus heterophyllus*

Constituents	*A. heterophyllus*
Alkaloids	-
Carbohydrate	+
Glycoside	+
Phenolic compound and tannins	+
Flavonoids	+
Proteins and Amino Acids	+
Saponins	+
Sterols	+
Acidic compound	-
Mucilage	-
Resin	-
Lipids/ fats	+

(+)=Present; (-) = Absent

Ash Value

Table 2: Ash value of *A. heterophyllus*

Drugs	Total ash (%)	Acid insoluble Ash (%)	Water soluble Ash (%)
A. heterophyllus	0.84	0.11	0.34
	0.83	0.13	0.35
	0.85	0.12	0.36
Mean	0.84	0.12	0.35

Loss on drying

The result of loss on drying is given in Table **3** for *Artocarpus heterophyllus*

Table 3: Loss on drying in *Artocarpus heterophyllus* leaves (10 g)

S. No.	*Artocarpus heterophyllus*
1	0.58
2	0.53
3	0.59
Mean	**0.56**

Foreign matter

The result of foreign matter is given in Table **4** for *Artocarpus heterophyllus*

S. No.	Wt. of Drug (g) (A)	Wt. of Drug After removal of F.M. (g) (B)	Wt. of foreign matter(g) (A-B)	% F. M.
1	200	196	4.00	2
2	200	198	2.00	1
3	200	197	3.00	1.5
Mean	200	197	3.00	1.5

Fluorescence Analysis:

The behavior of *Artocarpus heterophyllus* powder with different chemicals was studied. The results are shown in **Table 5**

Drug powder as such	Light green	Light green	Dark green
Distilled Water	Dark green	Dark green	Black
KOH (1%)	Brown	Dark green	Black
Conc. HCl	Yellowish green	Light green	Black
Conc. H_2SO_4	Grey	Grey	Black
HNO_3 (50%)	Light grey	Dark grey	Dark grey
NaOH (1N) in water	Brown	Dark green	Black
GAA	Grey	Dark grey	Light green
Chloroform	Light green	Dark green	Black
Ethanol	Light green	Dark green	Red

ANTIDIABETIC ACTIVITY

Effect of 21 days treatment of extracts on blood glucose of streptozotocin-induced diabetic rats

Extracts of *A.heterophyllus* leaves decreases the blood glucose levels statistically significantly (P<0.01) which is shown in Table 6.1

Groups	Treatment	Dose	Blood glucose level in mg/dl			
			1st day	8st day	14st day	21st day
I	Normal	Normal saline	112.83± 4.79*	114.67± 1.02*	115.50± 1.56*	118.50± 1.15*
II	Diabetic control (streptozotocin)	50 mg/kg	368.33± 5.05	372.50± 1.76	368.33± 3.22	373.33± 4.46
III	Standard (Glimepiride)	0.1 mg/kg	373.67± 5.06	161.33± 2.21*	123.67± 1.80*	105.50± 3.12*
IV	Aq. extract of *A.heterophyllus*	250 mg/kg	367.67± 5.90	180.50± 3.25*	161.07± 3.80*	128.67± 2.88*
V	Aq. extract of *A.heterophyllus*	500 mg/kg	366.67± 9.99	167.67± 3.58*	141.12± 2.75*	119.17± 2.28*

Table: 6.1 Antidiabetic activity of aqueous extract of *A. heterophyllus* leaves
All values are Mean ± SEM; n=6 * P<0.01 compared with diabetic control (group II)

Effect of 21 days treatment of extracts on serum lipid profile of streptozotocin-induced diabetic rats

Treatment with extracts of *A. heterophyllus* leaves decreases total cholesterol (TC) and increases high density lipid cholesterol (HDL) significantly (P<0.01) and increases body weight shown in Table 6.2.

Table: 6.2 Effect of aq. extracts of drugs on lipid profile of streptozotocin-induced diabetic rats

Groups	Treatment	Lipid Profile		Body weight	
		TC	HDL-C	Initial	Final
I	Normal	114.17±2.84*	47.50±1.18*	1.67.83±3.28	178.83
II	Diabetic control (streptozocin) 50 mg/kg	251.83±2.70*	35.67±0.42*	167.67±2.45	156.50
III	Standard (Glimepiride) 0.1 mg/kg	112.00±2.37	44.32±0.95	171.00±2.94	184.50
IV	aq. extract of *A .heterophyllus* 250 mg/kg	133.33±2.28*	36.67±1.33*	168.83±3.54	186.67
V	aq. extract of *A.heterophyllus* 500 mg/kg	126.33±2.09*	39.33±0.84*	164.83±2.68	195.83

Values are Mean ± SEM; n=6 *P<0.01 when compared with normal control group

DISCUSSION

These herbal drugs were standardized as per WHO guidelines. All these parameters, which are being reported, could be useful in identification of distinctiveness features of the drug and also valuable in manufacturing as raw material or in prescription medicine. The aqueous extract of *A. heterophyllus* supplementation for 21 days showed antidiabetic and antihyperlipidemic potential as shown by restoration of blood glucose level and biochemical profiles. We can further explore *A. heterophyllus* for the important leads for antidiabetic drugs. Detail biological studies are further required to establish the MOA.

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