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Pharmacognostic and Pharmacological Screening of *Tamarindus indica* Seed in Streptozotocin-Induced Diabetic Rats

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Abstract:

Tamarindus indica, commonly known as tamarind, is a versatile tropical tree that has been used for centuries in traditional medicine due to its numerous therapeutic properties. This study aimed to conduct a comprehensive pharmacognostic and pharmacological evaluation of *Tamarindus indica* seeds to ascertain their medicinal potential.

The pharmacognostic evaluation involved macroscopic of the seeds. The seeds were observed for their morphology and phytochemical evaluation; various solvent extracts of *Tamarindus indica* seeds were prepared using increasing polarity solvents, including petroleum ether, chloroform, ethanol, ethyl acetate and water. The extracts were screened for the presence of secondary metabolites using standard qualitative tests. Quantitative estimation of leading phytoconstituents, such as alkaloids, flavonoids, phenolic compounds, saponins, tannins, and glycosides, was performed using established methods.

In the present study, Ethanolic extract of seed of *Tamarindus indica* Linn. was found to have potentiated antidiabetic activity that reduces blood glycemic index in streptozotocin (STZ)-induced diabetic male rats. Supplementation of this Ethanolic extract by gavage at the dose of 80 mg/0.5 ml distilled water/100 g body weight per day in STZ-induced diabetic rats significantly lowered fasting blood sugar level after 7 days. Continuous treatment of this extract for 14 days resulted in no significant difference in this parameter from the control level. Moreover, this treatment significantly elevated liver and skeletal muscle glycogen content; in conclusion, this study's pharmacognostic evaluation provided valuable botanical information; the phytochemical analysis demonstrated that the seeds contain a diverse range of bioactive compounds, suggesting their potential medicinal value.

Keywords: Tamarindus indica, Seeds, Pharmacognostic Evaluation, Phytochemical Screening, Streptozotocin, Antidiabetic Activity.

Introduction:

Tamarindus indica, commonly known as tamarind, is a tropical tree belonging to the family *Fabaceae*. It is widely distributed in various tropical regions of the world and is renowned for its culinary and medicinal applications.[1] While tamarind pulp and leaves have been extensively studied for their medicinal properties, the seeds of *Tamarindus indica* have received comparatively less attention.[2]

Historically, *Tamarindus indica* has been used in traditional medicine for its diverse therapeutic benefits, including its use as an antimicrobial, antidiabetic, anti-inflammatory, and hepatoprotective agent. The seeds, which are usually discarded as waste during the processing of tamarind pulp, have recently emerged as a promising source of bioactive compounds with potential medicinal value.[3]

The pharmacognostic evaluation of medicinal plants is crucial in identifying and authenticating the plant material. In the context of *Tamarindus indica* seeds, pharmacognostic evaluation provides essential information about the seed size, shape, colour, taste, etc., contributing to its botanical characterization.[4]

Phytochemical analysis, on the other hand, aims to identify and quantify the bioactive chemical constituents present in the plant material. *Tamarindus indica* seeds are known to contain a diverse range of secondary metabolites, such as alkaloids, flavonoids, phenolic compounds, saponins, tannins, and glycosides. These phytochemicals have demonstrated various biological activities, indicating the potential medicinal properties of the seeds.[5]

The combination of pharmacognostic and phytochemical evaluation of *Tamarindus indica* seeds can provide valuable insights into their medicinal potential, aiding in developing new drugs or supplements for various health conditions. Moreover, these seeds' sustainable utilization could promote natural and eco-friendly healthcare solutions.

This study aimed to conduct a comprehensive pharmacognostic and phytochemical evaluation of *Tamarindus indica* seeds to explore their medicinal attributes further and shed light on their potential as a valuable source of bioactive compounds for therapeutic applications. The findings of this research could pave the way for the development of novel drugs or herbal formulations, fostering the integration of traditional knowledge with modern evidence-based medicine.

Materials and Methods:

Collection and Authentication of *Tamarindus indica* Seeds:

Mature and healthy *Tamarindus indica* seeds were collected from a well-established and authentic source at Hiware Bajar, Ahmednagar district Maharashtra (GPS Location Latitude: 19.093373 & Longitude: 74.620053). The seeds were identified and authenticated by Dr R. K. Chaudhari Biodiversity and Palaeobiology (Plant) at Agharkar Research Institute, Pune, with Voucher no 3/485,2022, Adm:451 dated 08/08/2022 to ensure their accuracy.[6]

Sample Preparation:

The collected *Tamarindus indica* seeds were cleaned to remove extraneous materials such as dirt, debris, and broken seeds. They were then dried under shade at room temperature to maintain the integrity of phytochemical constituents.[7]

Pharmacognostic Evaluation:

The dried seeds were subjected to macroscopic evaluation, including the measurement of seed size, shape, colour, odour, taste and surface characteristics.[8.9]

Preparation of extracts

Tamarindus indicum seeds were dried under the sun's heat. Then good quality whole seeds were then soaked in sodium hydroxide solution (10%) for 30 min, then crushed and washed with water multiple times.[7] The crushed seeds were powdered with the help of an electric grinder till a fine powder was obtained. The ground powder passes through a 50–mesh size.[8] The powder was then divided into two portions and kept in plastic containers at room temperature. 100g of the dried seeds powder was extracted by Soxhlet apparatus (Borosilicate) using solvent petroleum ether (Sigma Aldrich, analytical grade, anhydrous, 90%)%), chloroform (Sigma Aldrich, analytical grade, anhydrous, ≥99.5 %), ethanol (Sigma Aldrich, analytical grade, anhydrous, 99.8%), ethyl acetate (Sigma Aldrich, analytical grade, anhydrous, 99.8%) and water. Each extract obtained was filtered using Whatman filter paper no.42.The solvent was evaporated under reduced pressure using a rotary evaporator (1297, Dolphin, Mumbai, India), yielding the crude extracts.[10]

Proximate Analysis

The macroscopic examination of T. indica seeds was conducted during the standardization process. The study included assessments of total ash, foreign organic matter, water-soluble ash, moisture content, sulphated ash, crude fibre content, acid-insoluble ash, water-soluble extractive values, and ethanol-soluble extractive values, all by established procedures.[11.12]

Phytochemical Analysis

Each solvent extract was tested for the presence of phytochemical constituents using standard qualitative tests. Specific reagents and chemical reactions were employed to identify the presence of alkaloids, flavonoids, phenolic compounds, saponins, tannins, and glycosides.[13]

Selection of Extract for Further Studies

Upon analysis, the extract displaying the highest number of positive results was chosen for more in-depth investigations and studies. This approach ensures that the most chemically rich extract undergoes further scrutiny and potential application.

Proposal Approval for Antidiabetic Activity from IAEC

Project Proposal was approved by IAEC Ref no:1409/PO/RE/s/11/IAEC/2021-22/07/01 dated on 24/07/2021

Selection of Animal

The study was conducted on forty-eight matured Wistar strain male albino rats, weighing 130±10 gm, which were housed in colony cages (four rats per cage) at an ambient temperature of 21±2 ˚C.[14] Rats have free access to standard food and water adlibitum. The principles of laboratory animal care were followed throughout the duration of the experiment, and instruction given by our institutional ethical committee was followed regarding injection and other treatments of the experiment. Normoglycemic animals were selected for this experiment having a fasting blood glucose level of 85 ± 5 mg/dl.

Induction of diabetes mellitus

Streptozotocin-induced diabetes mellitus was produced ina batch of normoglycemic male Wistar strain albino rats bysingle intramuscular injection of streptozotocin at the dose of 7 mg/0.5 ml normal saline/100 g body weight per rat.[15] This single dose of streptozotocin produced type-I Diabetes mellitus after 24 hrs. of injection, and this Diabetic state is maintained throughout the experimental schedule.

Experimental design[16]

Forty-eight rats were divided into six equal groups as follows:

Sr.no	Group	Days	Dose
1	Normal Control-1	7	Rats of this group received a single intramuscular injection of normal saline (0.5 ml/100 g body weight per rat).
2	Diabetic Control-1	7	Rats were made diabetic by a single intramuscular injection of streptozotocin (7 mg/0.5 ml normal saline/100 g body weight per rat).
3	Test Control-1	7	The diabetic rats were forcefully fed with Ethanolic seed extract of *Tamarindus indica* at the dose of 80 mg/0.5 ml distilled water/100 g body weight per rat per day for 7 days by gavage
4	Normal Control-2	14	Rats of this group received a single intramuscular injection of normal saline (0.5 ml/100 g body weight per rat).
5	Diabetic Control -2	14	Rats were made diabetic by a single intramuscular injection of streptozotocin (7 mg/0.5 ml normal saline/100 g body weight per rat).
6	Test Control-2	14	*Tamarindus indica* treatment group (14 days): The diabetic rats were forcefully fed Ethanolic seed extract of *Tamarindus indica* at the dose of 80 mg/0.5 ml distilled water/100 g body weight per rat per day for 14 days by gavage.

The dose of *Tamarindus indica* supplementation was selected by a dose-dependent study. This is the threshold dose, so the experiment continued using the dose. Before giving the supplement of *Tamarindus indica* extract, the basal blood glucose level was measured in all the groups. On the 8th and 15th days of the experiment, all the animals were sacrificed under light ether anaesthesia. The guidelines of our institutional ethical committee for this purpose were followed strictly. The rats were sacrificed by chloroform method, and necessary organs like liver and skeletal muscles were dissected and stored in 10 % formalin at 20 ^{0}C and used for biochemical assay.[27]

Testing of fasting blood glucose level

Fasting blood glucose level was measured after 7 days and 14 days of aqueous extract of seed of *Tamarindus indica* supplement from the animals of all these groups.[17] Blood was collected from the tip of the tail vein, and

fasting blood glucose level was measured using a single-touch glucometer. The results were expressed in terms of milligrams per deciliter of blood.

Biochemical assay of glycogen level

Glycogen content in the liver and skeletal muscle was measured according to the standard method. Liver and skeletal tissues were homogenized separately in hot 80% ethanol at 100 mg/ml tissue concentration and then centrifuged at 8000 *g* for 20 min. The residue was collected and allowed to dry over a water bath. 5 ml of distilled water and 6 ml of 52% perchloric acid were added to the residue. The extraction was done at 0 ^{0}C for 20 min. The collected material was centrifuged at 8000 gm for 15 min, and the supernatant was collected. From the supernatant, 0.2 ml was transferred to a graduated test tube, and the volume was made up to 1 ml by adding distilled water. Graded standards were prepared using 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working standard solution, and the volume of all these standards was made up to 1 ml by adding distilled water. In all the test tubes, 4 ml of anthrone reagent was added. The test tubes were allowed to heat in a boiling water bath. Then there were allowed to cool at room temperature, and the intensity of the solution's green to dark green colour was recorded at 630 nm. The amount of glycogen was measured from a standard curve prepared with a standard glucose solution. The amount of glycogen in the tissue sample was expressed in micrograms of glucose per milligram of tissue[18.19]

Statistical analysis

Data is represented in Mean±SD. ANOVA was used for statistical analysis of the collected data[20].

Results

Pharmacognostic Evaluation

The table below summarizes the organoleptic characters of the seed of *T. indica*

Table 1: Organoleptic characters of the seed of *T. indica*

Sr. No.	Parameters	Characters
1	Odour	Characteristic, sour
2	Taste	Acidic and sweet
3	Texture	Soft and moist
4	Colour	Reddish-brown

Proximate Analysis

The proximate analysis provides a general profile of the primary components present in a substance. In this context, the proximate analysis offers insights into the fundamental constituents of a seed powder.

Table 2: Proximate Analysis Seed Powder of *T. indica*

Parameter	Values obtained % w/w (Mean ± SEM)
Total ash	4±0.34
Foreign organic matter	0.5±0.16
Water soluble ash	0.7±0.21
Moisture content	6.5±1.13
Sulphated ash	0.3±0.012
Crude fibre content	62±3.23
Acid insoluble ash	0.6±0.17
Water soluble extractive values	10±1.63
Ethanol soluble extractive values	12±2.34

Preliminary Phytochemical Analysis

The preliminary phytochemical analysis provides insights into the plant material's potential therapeutic properties and applications. The ethanolic extract has shown the most test positive for phytochemical analysis[21].

Table 3: Preliminary Phytochemical Screening of All Extracts of T. indica seed

Phytochemical Test	*T. indica* seed Extracts				
	Petroleum ether	**Chloroform**	**Ethanol**	**Ethyl acetate**	**Aqueous**
Tests for Alkaloids					
Meyers Test	+	-	+	-	-
Wagner's Test	+	-	-	-	+
Hager's Test	-	-	-	-	-
Dragendorff Test	+	+	+	+	-
Test for Tannins & Phenolic compounds					
FeCl3	-	-	+	-	-

Lead acetate	-	-	+	+	-
Tests for Flavonoids					
Sod-hydroxide Test	+	+	+	-	-
Lead acetate Test	+	+	+	-	-
Shinoda test	-	+	+	-	-
Test for Saponin					
Foam test	+	-	-	-	+
Test for Glycosides					
Killer- Killani Test	-	+	+	-	-
Borntrager's Test	+	-	-	+	-
Tests for Steroids					
Libermann reaction	+	-	-	-	-
Libermann Burchard reaction	-	-	-	-	-
Salkowaski reaction	-	+	+	+	-
Test for Proteins					
Biuret test	-	-	-	-	-
Millions test	-	-	-	-	-
Test for Non-reducing polysaccharides					
Iodine Test	-	-	-	-	-
Tests for carbohydrates					
Molish Test	-	-	+	-	-
Fehling Test	-	-	+	+	-
Benedict Test	-	-	+	+	-
Test for Monosaccharide					
Barfoed's Test	+	-	-	-	-

Note: + Indicates presence of phytoconstituents, - Indicates absence of phytoconstituents

Body weight

There was a significant diminution in the body weight of the animals in the diabetic group compared to the control. After aqueous seed extract of *Tamarindus indica* supplementation for 7 days, the body weight recovered significantly but not to the control level. After 14 days of this supplementation,[25] the body weight of all the animals were insignificantly different from the control level [Table no 4]

Table 4: Effect of ethanolic seed extract of *Tamarindus indica* on body weight in streptozotocin-induced diabetic male albino rats

Groups	Body weight (gms)
Normal Control-1 (7 days)	135.5 ± 10.2
Diabetic Control-1 (7 days)	110.4 ± 10.4***
Test Control-1 (7 days)	124.6 ± 9.6*
Normal Control-2 (14 days)	134.4 ± 10.3
Diabetic Control-2 (14days)	100.2 ± 9.8***
Test Control-1 (14 days))	132.3 ± 8.9***

The data is represented in Mean±SD, n=8. One-way ANOVA followed by Sidak. Normal control was compared with Diabetic Control, and Diabetic control was compared with Test control at 7 and 14 days, respectively. The p-value represents *** p>0.001, ** p>0.01, * p>0.05 and ns p< 0.05.

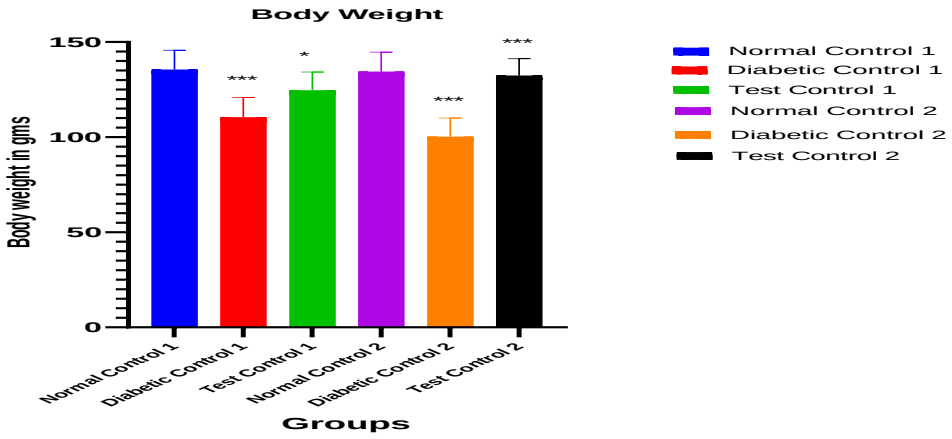

Figure 2: Effect of ethanolic seed extract of *Tamarindus indica* after 7 days and 14 days treatment on blood sugar level in streptozotocin-induced diabetic male albino rats.

Fasting blood glucose level

All animals' fasting blood glucose levels before treatment were within the normal range. Fasting blood glucose level was significantly elevated after 24 hrs. of streptozotocin injection with respect to the control level.[22.23] Supplementation of aqueous seed extract of *Tamarindus indica* for 7 and 14 days, fasting blood glucose level was insignificantly different from the control level shown in [Table No 5]

Table 5: Effect of aqueous seed extract of *Tamarindus indica* after 7 days and 14 days treatment on blood sugar level in streptozotocin-induced diabetic male albino rats.

Groups	Days of Treatment		
	0 day	7 days	14 days
Normal Control	86.4 ± 3.2	86.4 ± 3.4	85.9 ± 3.4
Diabetic Control	86.5 ± 3.9^{ns}	340.6 ± 10.3***	367.4 ± 10.4***
Test Control (*Tamarindus indica* supplement)	86.8 ± 3.7^{ns}	105.6 ± 8.8***	99.4 ± 10.6***

Data is represented in Mean±SD, n=8. Two-way ANOVA followed by Dunnett's test. Normal control was compared with Diabetic Control, and Diabetic control was compared with Test control at 7 and 14 days, respectively. The p-value represents *** p>0.001, ** p>0.01, * p>0.05 and ^{ns} p< 0.05.

Figure 2: Effect of ethanolic seed extract of *Tamarindus indica* after 7 days and 14 days treatment on blood sugar level in streptozotocin-induced diabetic male albino rats.

Glycogen level in tissue

After 7 days of aqueous seed extract of *Tamarindus indica* supplementation to the diabetic rats, there was a significant elevation in liver and skeletal muscle glycogen levels with respect to the diabetic group. This parameter must still be resettled to the control level [Table No 6]. After 14 days of this supplementation, the above parameter was resettled to the control level.[24, 25]

Table 6: Effect of ethanolic extract of *Tamarindus indica* seed after 7 days and 14 days treatment on glycogen in liver and muscle in streptozotocin-induced diabetic male albino rat**s.**

Groups	Glycogen in Liver Days of Treatment		Glycogen in Muscle Days of Treatment	
	7 days	14 days	7 days	14 days
Normal Control	26.8 ± 1.43	26.4 ± 1.24	25.7 ± 1.32	26.2 ± 1.23
Diabetic Control	18.3 ± 1.32***	14.8.4 ± 1.24***	17.32 ± 1.35***	13.34 ± 1.11***
Test Control (*Tamarindus indica* supplement)	23.16 ± 1.47***	25.4 ± 1.36***	22.44 ± 1.39***	24.67 ± 1.22***

Data is represented in Mean±SD, n=8. Two-way ANOVA followed by Dunnett's test. Normal control was compared with Diabetic Control, and Diabetic control was compared with Test control at 7 and 14 days, respectively. The p-value represents *** p>0.001, ** p>0.01, * p>0.05 and ^{ns} p< 0.05.

Discussion

The present paper discussed the antidiabetic effect of an aqueous extract of the seed of *Tamarindus indica* on streptozotocin-induced diabetic rats in a duration-dependent fashion. Physicochemical analysis of the seed extract indicated the presence of various phytochemicals, including alkaloids, flavonoids, phenolics, tannins, and saponins. These secondary metabolites are known for their potential therapeutic properties and may contribute to the antidiabetic effects of Tamarindus indica seed extract. Treatment with Tamarindus indica seed extract significantly reduced blood glucose levels in diabetic rats compared to the untreated diabetic group. The dose-dependent decrease in blood glucose levels suggests the potential of the seed extract to improve glycemic control. Streptozotocin injection resulted in diabetes mellitus, possibly due to the destruction of β cells of Islets of Langerhans as proposed by others. After 7 days and 14 days, supplementation of aqueous extract of seed of *Tamarindus indica* resulted in a significant diminution of fasting blood glucose level concerning diabetic rats, but no significant alteration of fasting blood glucose level to the control, which further strengthened the antidiabetogenic action of this extract.

Conclusion

This study aims to provide scientific evidence for the potential antidiabetic properties of Tamarindus indica seed through pharmacognostic and pharmacological evaluations. If the results show promising anti-hyperglycemic, antioxidant, anti-inflammatory, and organ-protective effects, Tamarindus indica seed extract may be a valuable therapeutic option for managing diabetes. However, further research will be needed to identify and isolate specific active compounds responsible for the observed effects and determine the seed extract's long-term safety and efficacy in diabetic patients.

List of Abbreviations

STZ: Streptozotocin, *T. indica*: *Tamarindus indica*, IAEC: Institutional Animal Ethical Committee, mg/dl: milligram deciliter, gm: Grams, ml: milliliter, SD: Standard Deviation, ANOVA: Analysis of Variance

Conflict of Interest

Authors declare no conflict of interest.

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