The Pharma Innovation Journal 2023; SP-12(8): 1017-1023

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(8): 1017-1023
© 2023 TPI
www.thepharmajournal.com
Received: 23-06-2023
Accepted: 28-07-2023

Pallavi Tiwari
Department of Pharmaceutical Sciences, Shalom Institute of Health and Allied Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad-Prayagraj, India

Dr. Neetu Soni
Faculty, Department of Pharmaceutical Sciences, Shalom Institute of Health and Allied Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad-Prayagraj, India

Mayank Srivastava
Department of Pharmaceutical Sciences, Shalom Institute of Health and Allied Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad-Prayagraj, India

Corresponding Author:
Dr. Neetu Soni
Faculty, Department of Pharmaceutical Sciences, Shalom Institute of Health and Allied Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad-Prayagraj, India

Evaluation of the antioxidant activity and assessment of the hypoglycemia effect of the polyherbal extracts in diabetic rat

Pallavi Tiwari, Dr. Neetu Soni and Mayank Srivastava

Abstract
The present investigation was designed with the preceding information in mind. The objective of the project was to optimize the methodology for screening and determining the efficacy of chosen plants as antidiabetic potentiates by observing whether glucose utilization and insulin secretion increased during treatment or not. The parameters used to validate the models will be used in the future to rigorously identify the hypoglycemic effect (s) and mechanism (s) of the plants, once the best possible methodology has been established through this particular research. The study was aimed in establishing a polyherbal formulation ISDM-007 prepared from hydro alcohol extract of *Azadirachta Indica* leaves, *Ocimum Sanctum* leaves, *Boerhaavia diffusa* flowers and *Aegle marmelos* fruits for its anti-diabetic activity.

Keywords: Polyherbal formulation, antioxidant activity, antidiabetic activity, glucose tolerance test

Introduction
Diabetes mellitus, often known as hyperglycemia, is a long-term, non-infectious endocrine condition that is primarily distinguished by abnormalities in the metabolism of carbohydrates. [1]. It was associated with the onset of significant disorders at the macro vascular (coronary heart disease and peripheral vascular disease) as well as micro vascular levels (nephropathy, retinopathy, and nephropathy) [2-3]. Diabetes was noted to be accompanied by "as sweet urine" and muscle atrophy. The pancreas secretes insulin, a hormone that governs blood glucose levels. When blood glucose levels rise, the pancreas secretes insulin to keep blood glucose levels stable. In diabetic patients, either no or insufficient insulin production results in hyperglycemia [4]. The largest challenge for medical professionals is finding a way to treat diabetes mellitus without having any negative side effects. Throughout medical history, medicinal plants with secondary metabolites like phenolic, flavonoid, and anthocyanin chemicals have been employed as alternative therapeutic agents to treat a variety of ailments. For the potential they have as sources of antioxidants against numerous maladies, different kinds of plant extracts or compounds derived from plants have been researched. Additionally, due to their superior safety profiles, natural antioxidants derived from plants are favored over synthetic ones [5-6]. Finding natural substances that could halt the process of oxidation that drives the pathophysiology of numerous diseases is becoming increasingly crucial [7]. The study's objective is to evaluate how well polyherbal extracts work as antioxidants in diabetic rats produced by streptozotocin. Neem, or *Azadirachta Indica*, has therapeutic benefits and is used in Ayurveda to treat multiple diseases, including diabetes mellitus. It is well recognized to have a number of pharmacological activities, like anti-inflammatory, antipyretic, antibacterial, and anti-diabetic effects. However, it is unclear what molecular process causes A. indica to affect insulin signal transduction and glucose homeostasis [8]. Neem leaves contains Quercetin [9] and Ferulic Acid [10]. *Ocimum Sanctum* contains polyphenols like ferulic acid [11] and Quercetin [12]. *Boerhaavia diffusa* contains Quercetin [14] and polyphenols like ferulic acid [14] and *Aegle marmelos* also contains Quercetin and ferulic acid [15].
Quercetin and ferulic acid both have Antidiabetic activity. Ferulic acid supports the insulin signaling compounds that reduced insulin signaling's negative regulators [16-17]. So, for present study we prepare polyherbal extract and perform it's phytochemical screening and fingerprinting analysis.

Material and Techniques
Chemical agents required for method: Ascorbic acid, DPPH (2, 2-diphenyl-1-picrylhydrazyl) agent, Glibenclamide, and Streptozotocin.

Plant material

Plant parts that are leaves of *Azadirachta Indica,* leaves of *Ocimum Sanctum,* flowers of *Boerhaavia diffusa* and fruits of *Aegle marmelos* collected from local area, then shade dried all procure material then powdered it. By using Soxhlet apparatus extraction procedure perform, for that procedure solvent were used as per polarity basis.

Table 1: Ingredients of the Antidiabetic polyherbal composition

S. No.	Plant Botanical name	Common or vernacular term	Family	Part of plant used
1.	*Azadirachta Indica*	Neem	Meliaceae	Leaves
2.	*Ocimum Sanctum*	Tulsi	Lamiaceae	Leaves
3.	*Boerhaavia diffusa*	Punarnava	Nyctaginaceae	Flowers
4.	*Aegle marmelos*	Bael	Rutaceae	Fruit

Polyherbal Preparation

Table 2: Ingredients proportion in Polyherbal composition

Polyherbal formulations	Code
AI-HAE, OS-HAE, BD-HAE and AM-HAE in the ratio of (25:25:25:25)	ISDM-001
AI-HAE, OS-HAE, BD-HAE and AM-HAE in the ratio of (30:20:20:30)	ISDM-002
AI-HAE, OS-HAE, BD-HAE and AM-HAE in the ratio of (20:30:30:20)	ISDM-003
AI-HAE, OS-HAE, BD-HAE and AM-HAE in the ratio of (10:30:30:30)	ISDM-004
AI-HAE, OS-HAE, BD-HAE and AM-HAE in the ratio of (30:10:30:30)	ISDM-005
AI-HAE, OS-HAE, BD-HAE and AM-HAE in the ratio of (30:30:10:30)	ISDM-006
AI-HAE, OS-HAE, BD-HAE and AM-HAE in the ratio of (30:30:30:10)	ISDM-007

HAE: Hydroalcoholic Extract; AI: *Azadirachta Indica*; OS: *Ocimum Sanctum*; BD: *Boerhaavia diffusa*; AM: *Aegle marmelos*

***In vitro* antioxidant activity determination by dpph method** [18-20]

Principle: Using the stable form of 2, 2-di phenyl 1-1-diphenyl picrylhydrazyl (DPPH) radical, the most straightforward approach for determining the antioxidant properties of the extracts (Hydroalcohol and Aqueous) has been developed. The molecular makeup of DPPH and its inhibition by an antioxidant is depicted in Figure 1.

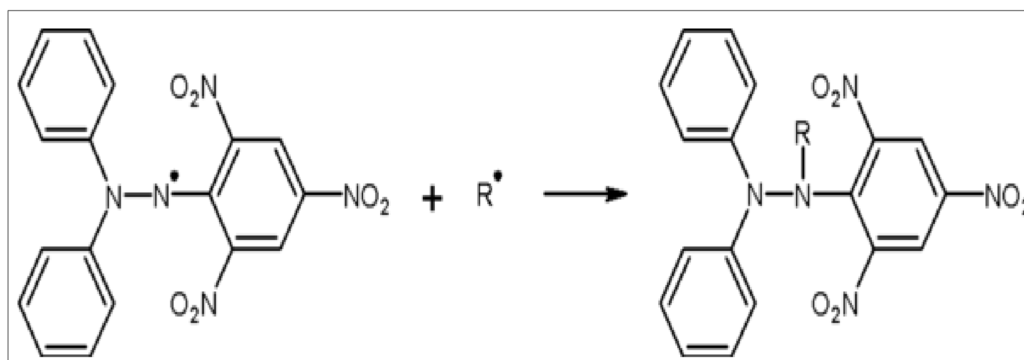

Fig 1: Chemical equation showing the DPPH reduction

DPPH diphenylpicryl hydrazine

DPPH is usually used to measure how well antioxidants get rid of free radicals. DPPH is a stable form of free radical that, by introducing a single electron or a hydrogen radical, can be converted into a diamagnetic molecule that is stable. The ability of antioxidants to get rid of DPPH radicals is measured by how much the absorption at 517nm drops. The stable radical DPPH can be turned into diphenyl picryl hydrazine, which is yellow.

This test measures how well a chemical gets rid of free radicals. The DPPH molecule contains a stable free radical. In the presence of an antioxidant that can give an electron to DPPH, the free radical's purple color fades, and the change in absorbance at 517nm can be measured by spectrophotometry (SPECTRA MAX PLUS®, Molecular device, USA). This simple test can tell you if a compound can donate a hydrogen atom, how many electrons it can contribute, and how antioxidants work. When the structure of the electron source is unknown, like in the case of a plant extract, this method can be used to get information about the sample's reduction potential. This can help compare the reduction potential of unknown materials.

Materials and Methods

1. Spectrophotometer ((Spectra Max Plus®, Molecular device, USA).
2. Micro Pipettes (20-200µl, 200-1000µl).
3. Eppendorf Tubes (20 ml) and stand.
4. 15 ml Falcon tubes.
5. Incubator.
6. Timer.

Reagents / materials

1. 1, 1-Diphenyl-2-picrylhydrazyl: 1mM, pH= 6.8 – 7.0.
2. Methanol.
3. Plant Extract: Test Samples in duplicates.
4. Ascorbic acid (standard).

Pre-Preparation

- Set the incubator at 37 °C and maintain the temperature.
- Make the stock solutions of the plant extracts
- Set the parameters in the spectrophotometer

Procedure

1. A set of Eppendorf tubes are labelled as Blank, Controls

and Samples (different conc.) arranged in the stand.
2. Add the following reagents in the same order
   Plant extracts: x µl
   Methanol: Total vol – (x µll + 140 µl)
   DPPH: 140 µl
3. Mix thoroughly (Vortex) and incubate at 37 ^{0}C for 30 minutes.
4. Blank is maintained with the same total volume without the extract.
5. A set of color control is run for test samples without DPPH, but maintaining the volume with methanol.
6. Read absorbance of all samples at 517 nm.
7. Reaction volume Details for determining the active concentration range

Table 3: It shows preparation of sample for DPPH antioxidant procedure

Reaction volumes for screening assay of plant extracts					
Sample Conc	**Stock Conc**	**Vol of Methanol**	**Vol of extract**	**Amt of DPPH**	**OD 517nm**
µg	µg	µl	µl	µl	
Control	0	860	0	140	
10	1000	850	10	140	
100	1000	760	100	140	
250	1000	610	250	140	
500	2000	610	250	140	
750	2000	485	375	140	

- Color Correction: Includes the sample concentration in methanol lacking DPPH.
- Ascorbic acid is used as the standard.

All stock extracts used for testing were mixed together in DMSO at a concentration of 3 mg/ml (150 g/ml in the final reaction mixer) and serially diluted in DMSO by twofold, which includes the standard solution (Ascorbic Acid) at a strength of 1 mg/ml. DPPH was freshly prepared at a concentration of 4.9mg/25ml in absolute alcohol. In a 96-well plate, the reaction mixture contained 125 µl of DPPH, 100 µl of freshly prepared 0.5mM Tris buffer solution (pH 7.2), and 25 µl of test extracts/standard. After 10 minutes of incubation at ambient temperature, the absorbance of the solution was measured at 517 nm using a UV-visible Spectrophotometer. DPPH results are expressed as the ascorbic acid equivalents (AAE) as a percentage.

Triplicate Reading

- Derive a narrow concentration range with high scavenging activity from the active concentration range.
- A minimum of 6 concentrations are selected and assayed in triplicates.

10 ml of methanol was used to dissolve 0.025 g of dry extract. Before UV measurements, DPPH in methanol (6×10-5 M) was produced daily. 3ml of this solution were mixed with 77µl extracted solution in 1 cm path length disposable micro cuvettes (final mass ratio of extracts with DPPH was 3:1, 1.5:1, 0.75:1). The absorbance decrease was observed after 15 min in the dark at room temperature. Blank samples with the same methanol and DPPH mixture were absorbed. Triplicated experiments were performed.

Calculation

Measure the antioxidant activity using the standard absorbance values for ascorbic acid. Actual absorbance is determined by subtracting the absorbance of the control sample from that of the test sample. This was depicted as a vertical bar graphic with absorbance variation at 517 nm on the y-axis and Sample concentration on the x-axis.

$$\%inhibition = [(A_B - A_A)/A_B] \times 100.$$

Where: AB-absorption of a baseline sample (t=0 min); AA-absorption of an extract solution that has been tested (t=15 min).

Test of Glucose Tolerance (GTT)

Animals were fasted 18 hrs. [21], with free access to water and were separated in 5 groups of 6 rats each. All animals received 2 g/kg D-glucose solution via oral gavage. The second, third, and fourth groups received polyherbal extract by mouth at 200, 400, and 800 mg/kg body weight (P.O.) 30 minutes before the glucose load. Control animals received vehicles. Under ether anesthesia, blood was drawn from each animal's retro-orbital plexus at 0, 30, 90, and 120 minutes following the glucose challenge. Positive control glibenclamide (2 mg/kg) was given to the fifth group. The OPTIMA S Semi Biochemistry analyzer (LABINDIA) and GOD-POD kit assessed blood glucose levels.

Acute toxicity studies

The study of acute toxicity studies was done according to the OECD 420 standards. Standard lab conditions were used to take care of Wistar rats that weighed between 160 and 200 grams. Six animals were used in each group, and each got a single dose of polyherbal drug (2000 and 5000 mg/kg, body weight (P.O.). Before giving the drug, the animals had to fast all night. After the polyherbal drug was given, the recipient didn't eat for 3-4 hours. Each animal was observed at least once within the first thirty minutes after receiving a dose, multiple times within the first twenty-four hours (with a focus on the first four hours), and then regularly for fourteen days. During daily cage-side observations, alterations in the epidermis, fur, pupils, and nose mucous membranes, as well as the rate of breathing, circulation, and autonomic systems, were observed [22].

In vivo Efficacy Screening

The pre-clinical *in vivo* efficacy screening of the polyherbal extracts was performed using a streptozotocin (STZ) -induced diabetes model in wistar rats over 28 days. Briefly, diabetic symptoms were induced in acclimatized wistar rats (body wt. range ~ 200 gm) by intra-peritoneal administration of STZ at 45 mg/kg b.wt. The wistar rats that developed diabetic symptoms (blood glucose level at fasting > 250 mg/dL) were included in the study and divided into specific groups as listed below. (All herbal extracts were administered once daily by oral gavage at three different doses namely, 200 mg/kg. 400 mg/kg and 800 mg/kg).
The *in vivo* efficacy study was comprised of 6 groups of animals (n = 6) as follows:

Group 1: Non-diabetic, normal control animals -Vehicle-treated (0.5% CMC is used as a vehicle to ensure a uniform dispersal of plant extract material during oral administration)

Group 2: Diabetic animals - Un-treated or non-intervened diabetic control

Group 3: Diabetic animals – Administered daily once with 2 mg/kg Glibenclamide (standard anti-diabetic pharmaceutical)

Group 4: Diabetic animals – Administered daily once with 200mg/kg of ISDM-007

Group 5: Diabetic animals – Administered daily once with 400mg/kg of ISDM-007

Group 6: Diabetic animals – Administered daily once with 800mg/kg of ISDM-007

Study Parameters

Histopathological Examination

Tissues were subjected to microscopic examination using standard histopathological procedure. On the termination day of the study the animals were sacrificed and dissected the required organs such as liver, pancreas, kidney, and heart and they were fixed in 10% formalin. The sections of the tissues of 5-6 µm were cut and stained with hematoxylin and eosin stains. The tissue sections were subjected to rehydration by exposing them to decreasing concentration of alcohol i.e., 100-30%. Then the sections were stained with hematoxylin. Later the sections were dehydrated by using increasing concentrations of alcohol and stained with eosin. Finally, the sections were treated with DPX (Diphenylxylene) and mounted on to the slide for examination under microscope [23] .These tissues were embedded in paraffin wax, sectioned at five micrometers and stained with haematoxylin and eosin. A full histopathological examination was conducted on the specified list of tissues including all macroscopically abnormal tissues of all animals from the control and the high dosage level groups, killed at termination. Additional tissues, of animals from other groups, which exhibited gross pathological changes at necropsy, were also subjected to histopathological evaluation. Microscopic examination was not extended to tissues of lower dose levels in absence of any treatment related alterations being detected at the high dose level.

Statistical analysis

Bartlett's test was performed on each set of data to ensure that variance of the sets are homogenous. In case of homogenous set of data ANOVA was performed to determine the treatment effects, and Dunnett's test was employed as appropriate by using origin pro 7.6 statistical software. In case of heterogeneous data, it was transformed using appropriate transformation. The variance was evaluated at 5% level of significance.

Result and Discussion

DPPH is a stable free radical that forms diamagnetic molecules by receiving electrons or hydrogen. *In vitro* experiments measure an extract or compound's scavenging activity using DPPH radicals, which are not found in humans. The polyherbal extracts ISDM-001, ISDM-002, ISDM-003, ISDM-004, ISDM-005, ISDM-006 and ISDM-007 showed free radical scavenging activities of 28.2%, 16.9%, 18.7%, 25.1%, 22.2%, 29.1% and 33.8%, respectively, which is comparable to that of the standard ascorbic acid with 100 percent antioxidant potential. Antioxidants protect B cells against oxidative stress. It lowers glycated hemoglobin like insulin. The study revealed that the extract ISDM-007 possesses a highly potent antioxidant activity, indicating its potential function in diabetes pharmacotherapy.

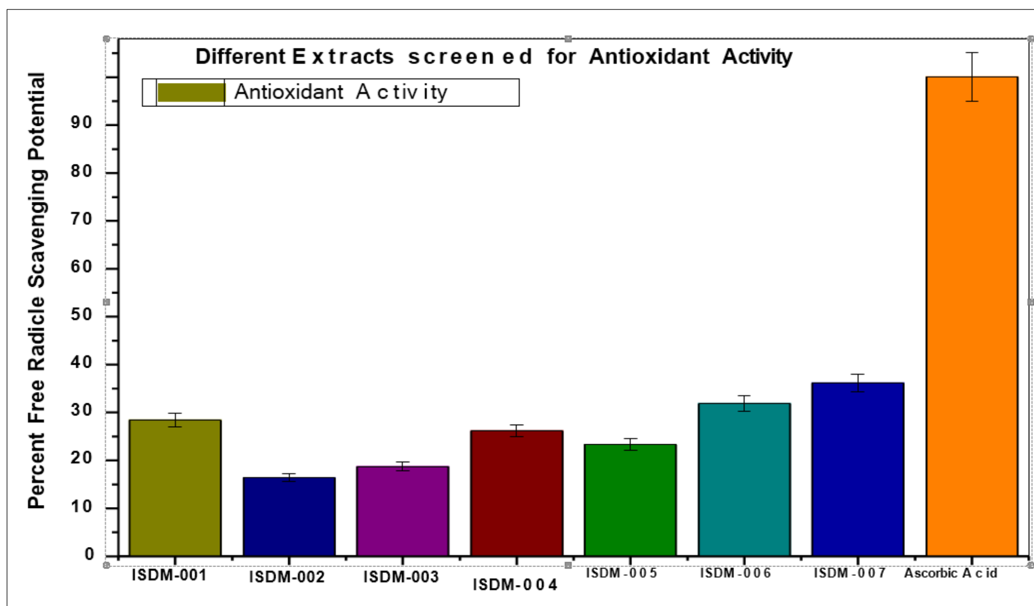

Fig 2: Herbal extract antioxidant activity vs. ascorbic acid, Two studies with three replicates produced the Mean S.E. values.

Table 4: Polyherbal herbal formulation (ISDM-007) and blood glucose in diabetic rats.

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
I	90.67±1.77	91.33±1.21	93.34±0.88	93.5 ± 6.75	92.00±2.05
II	89.33±1.86	298.67±1.21	299.33±3.48	305.01 ± 4.14	312.03±2.66
III	89.34±2.19	301.00±2.52	192.01±1.75	141.33±5.45	103.23±0.88
IV	91.67±1.46	302.64±6.67	215.00±1.16	163.4±2.67	132.04±0.12
V	90.36±1.21	297.33±5.24	206.34±1.22	155.01± 3.23	123.11±0.18
VI	88.24±1.19	300.00±5.67	197.34±2.02	144.3 ± 1.42	105.23±0.55

N=6 rodents) Data expressed as mean standard error. Blood glucose levels were significant at $p<0.01$

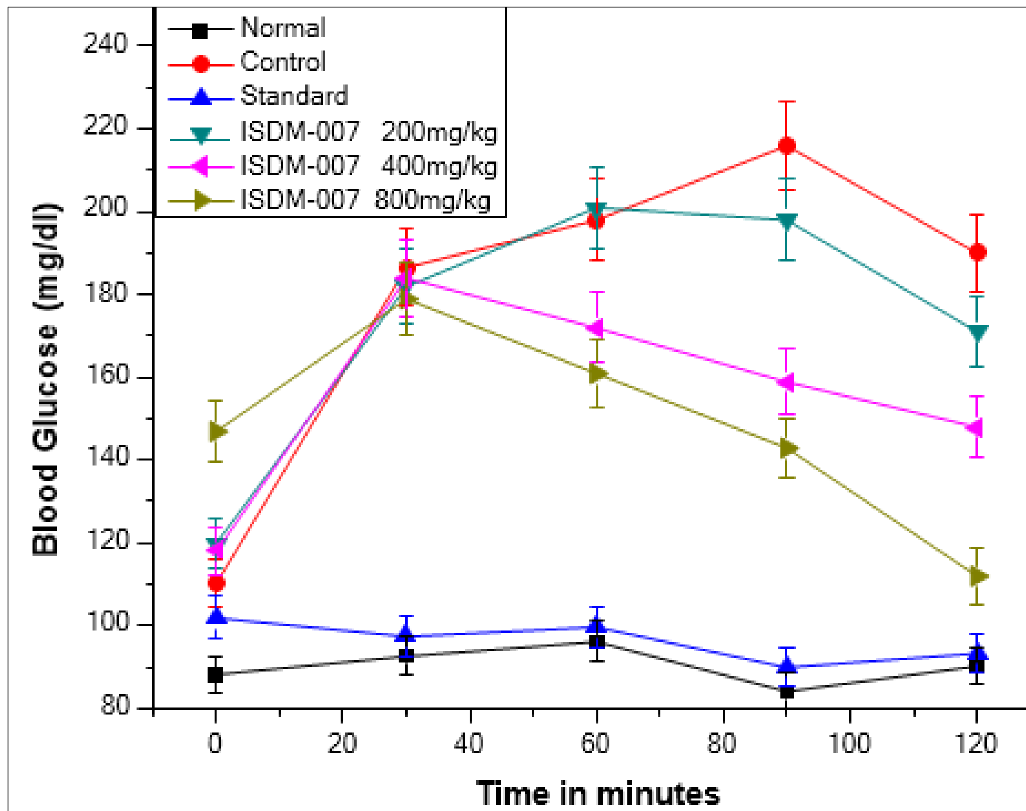

Fig 3: The effects of the polyherbal extract ISDM-007 on the glucose tolerance test, values are Mean± S.E, (N=6) and P Values are significant at $p<0.05$.

Histopathology

All the microscopic changes noticed in this study appeared to be incidental as their frequency and severity remained identical for the control and the treated animals. Thus it was concluded that ISDM-007, at and up to the dose level of 800 mg/kg body weight did not produce any histopathological effects in rats under the said experimental conditions. The major organs of highest concentration were dissected and compared with control for any microscopic abnormalities (Figure 4-5).

The various microscopic changes noticed in several organs have been appended in the individual animal pathology data. The summary of these changes has been presented in a summary table. Results of this study showed that all the microscopic changes noticed in this study appeared to be incidental as their frequency and severity remained identical for the control and the treated animals. Thus it was concluded that ISDM-007, at and up to the dose level of 800 mg/kg body weight did not produce any Histopathological effects in rats.

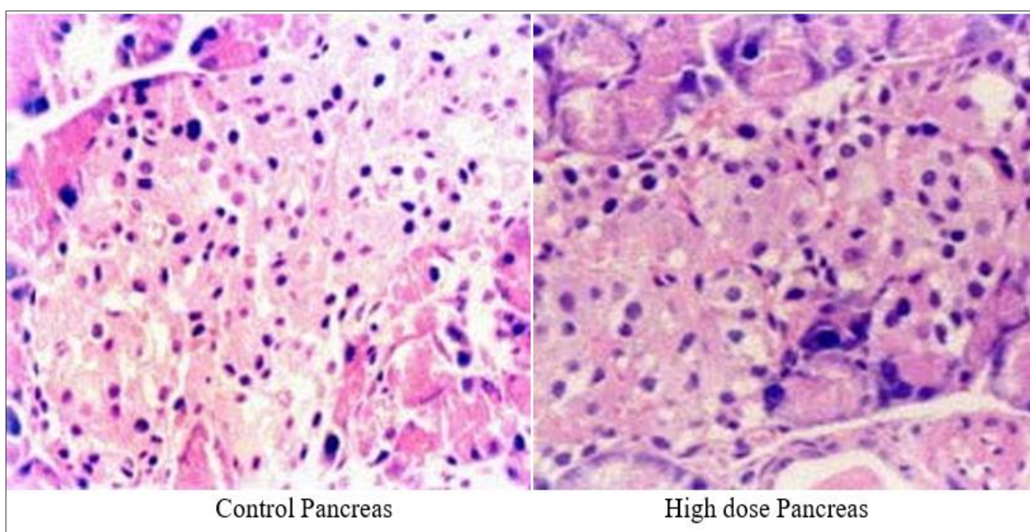

Fig 4: It shows regeneration in the pancreatic cells of the diabetic rats.

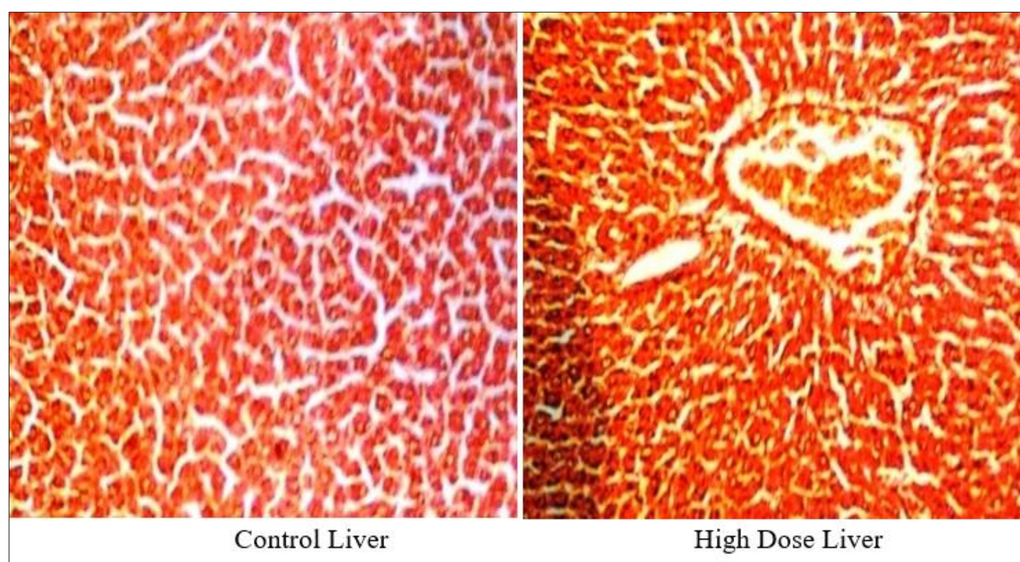

Fig 5: It shows regeneration in the hepatic cells of the diabetic rats

In traditional system of Indian medicine, plant formulation and combined extracts of plants are used as drug of choice rather than individual or single herb. Various herbal formulations such as Diamed [24] Coagent DB [25] and hyponid, are well known for their antidiabetic effects individual and combined effects and their significance. The animal models are more appropriate for screening antidiabetic activity. Study reported with *in vitro* evaluations warrants for further assessment by using *in vivo* techniques which will help in understanding the mechanism of action. The present study was performed to assess the antidiabetic effects of ISDM-007 on streptozotocin induced diabetic rats, and the possible morphologic changes in the liver and kidney.

The present study was planned considering the above facts. The objective of project was to optimize the methodology to screen and determine the effectiveness of the specific plants as antidiabetic potentiates, through observing if there is increases in glucose utilization and insulin secretion occurred during treatment. Once best possible methodology is achieved through this specific research, the parameters used to validate the models in the future will be applied to scientifically establish the antidiabetic effect (s) and mechanism (s) of the plants. The study was aimed in establishing a polyherbal formulation ISDM-007 prepared from hydro alcohol extract of *Azadirachta Indica, Ocimum Sanctum, Boerhaavia diffusa* and *Aegle marmelos* for its anti-diabetic activity.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Arumugam G, Manjula P, Paari NA Review: Anti Diabetic Medicinal Plants Used for Diabetes Mellitus. J. Acute Dis. 2013;2(3):196-200. https://doi.org/10.1016/s2221-6189(13)60126-2.
2. Upendra Rao M, Sreenivasulu M, Chengaiah B, Jaganmohan Reddy K, Madhusudhana Chetty C. Herbal Medicines for Diabetes Mellitus: A Review. Int. J Pharm Tech Res. 2010;2(3):1883-1892.
3. Kumar A, Goel MK, Jain RB, Khanna P, Chaudhary V. India towards Diabetes Control: Key Issues. Australas. Med. J. 2013;6(10);524-531. https://doi.org/10.4066/AMJ.2013.1791.
4. Bordoloi R, Dutta KN. A Review on Herbs Used in the Treatment of Diabetes Mellitus. J Pharm. Chem. Biol. Sci. 2014;2(2):86-92.
5. Duh PD, Tu YY, Yen GC. Antioxidant Activity of Water Extract of Harng Jyur (Chrysanthemum Morifolium Ramat). LWT - Food Sci. Technol. 1999;32(5):269-277. https://doi.org/https://doi.org/10.1006/fstl.1999.0548.
6. Hyun TK, Kim HC, Ko YJ, Kim JS. Antioxidant, α-Glucosidase Inhibitory and Anti-Inflammatory Effects of Aerial Parts Extract from Korean Crowberry (*Empetrum Nigrum* var. Japonicum). Saudi Journal Biol. Sci. 2016;23(2):181-188. https://doi.org/10.1016/j.sjbs.2015.02.008.
7. Barut B, Barut EN, Engin S, Özel A, Sezen FS. Investigation of the Antioxidant, α-Glucosidase Inhibitory, Anti-Inflammatory, and DNA Protective Properties of *Vaccinium Arctostaphylos* L. Turkish J. Pharm. Sci. 2019;16(2):175-183. https://doi.org/10.4274/tjps.galenos.2018.28247.
8. Satyanarayana K, Sravanthi K, Shaker I, Ponnulakshmi R. Molecular Approach to Identify Antidiabetic Potential of *Azadirachta Indica*. J Ayurveda Integr. Med. 2015;6(3):165-174. https://doi.org/10.4103/0975-9476.157950.
9. Tatke P, Desai S, Gabhe SY. Isolation of Quercetin-3-O-β-D-Glucoside from *Azadirachta Indica*. Am. J. Phyto medicine Clin. Ther. 2014;2(7):870-876.
10. Singh UP, Maurya S, Singh DP. Phenolic Acids in Neem (*Azadirachta Indica*). Journal Herb. Pharmacother. 2005;5(1):35-43. https://doi.org/10.1080/J157v05n01_05.
11. Priya S, Peddha MS. Physicochemical Characterization, Polyphenols and Flavonoids of Different Extracts from Leaves of Four Varieties of Tulsi (*Ocimum* Sp.). South African J. Bot. 2023;159:381-395. https://doi.org/10.1016/j.sajb.2023.06.025.
12. Garg P, Garg R, Praveen Garg C. Phytochemical Screening and Quantitative Estimation of Total Flavonoids of *Ocimum Sanctum* in Different Solvent Extract. ~ 16 ~ Pharma Innov. J. 2019;8(2):16-21.
13. Nayak P, Thirunavoukkarasu M. A Review of the Plant Boerhaavia diffusa: Its Chemistry, Pharmacology and

Therapeutical Potential. J. Phytopharm. 2016;5(2):83-92. https://doi.org/10.31254/phyto.2016.5208.

14. Juneja K, Mishra R, Chauhan S, Gupta S, Roy P, Sircar D. Metabolite Profiling and Wound-Healing Activity of Boerhavia Diffusa Leaf Extracts Using *in vitro* and *in vivo* Models. J Tradit. Complement. Med. 2020;10(1):52-59. https://doi.org/10.1016/j.jtcme.2019.02.002.
15. Rahman S, Parvin R. Therapeutic Potential of *Aegle marmelos* (L.): An Overview. Asian Pacific J Trop. Dis. 2014;4(1):71-77. https://doi.org/10.1016/S2222-1808(14)60318-2.
16. Vessal M, Hemmati M, Vasei M. Antidiabetic Effects of Quercetin in Streptozocin-Induced Diabetic Rats. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 2003;135(3):357-364. https://doi.org/10.1016/S1532-0456(03)00140-6.
17. Narasimhan A, Chinnaiyan M, Karundevi B. Ferulic acid exerts its antidiabetic effect by modulating insulin-signalling molecules in the liver of high-fat diet and fructose-induced type-2 diabetic adult male Rat. Appl. Physiol. Nutr. Metab. 2015;40(8):769-781. https://doi.org/10.1139/apnm-2015-0002.
18. Brand-Williams W, Cuvelier ME, Berset C. Use of a Free Radical Method to Evaluate Antioxidant Activity. LWT - Food Sci. Technol. 1995;28(1):25-30. https://doi.org/10.1016/S0023-6438(95)80008-5.
19. Aqil F, Ahmad I, Mehmood Z. Antioxidant and Free Radical Scavenging Properties of Twelve Traditionally Used Indian Medicinal Plants. Turkish Journal Biol. 200630(3):177-183.
20. Belinky PA, Aviram M, Fuhrman B, Rosenblat M, Vaya J. The Antioxidative Effects of the Isoflavan Glabridin on Endogenous Constituents of LDL during Its Oxidation. Atherosclerosis. 1998;137(1):49-61. https://doi.org/10.1016/S0021-9150(97)00251-7.
21. Sumalatha G, Vidya Sagar J, Ragini V, Suresh K. Extraction and Evaluation of Roots of *Decalepis Hamiltonii* for Antidiabetic Activity. Journal Pharm. Phytochem. Res 2010;2(3):20-25.
22. No OT. 423: Acute Oral Toxicity-Acute Toxic Class Method. OECD Guidel. Test. Chem. Sect. 2002;4:14.
23. Jelodar Gholamali A, Maleki M, Motadayen MH, Sirus S. Effect of Fenugreek, Onion and Garlic on Blood Glucose and Histopathology of Pancreas of Alloxan-Induced Diabetic Rats. Indian J Med. Sci. 2005;59:64-69.
24. Pari L, Ramakrishnan R, Venkateswaran S. Antihyperglycaemic Effect of Diamed, an Herbal Formulation, in Experimental Diabetes in Rats. J Pharm. Pharmacol. 2001;53(8):1139-1143.
25. Pari L, Saravanan G. Antidiabetic Effect of Cogent Db, a Herbal Drug in Alloxan-Induced Diabetes Mellitus. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 2002;131(1):19-25.