Evaluation of Anti Diabetic Activity of Ethanolic Seed Extract of *Datura Innoxia* Mill.

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Abstract The current research has been performed to substantiate the traditional claim of the anti-diabetic activity of seeds of *Datura Innoxia* Mill. in rats. This plant contains scopolamine alkaloid, which has been reported to be used in operations as pre-anaesthesia, used in delivery, as well as in ocular surgeries and for travel sickness prophylaxis. *Datura* is a medicinal and hallucinogenic plant that is used everywhere in the globe. *Datura* is revered in Ayurveda because all parts of the plant have medicinal properties, including the roots, leaves, seeds, and flowers, and have been employed to treat a variety of ailments, including leprosy, rabies, and insanity. Withanolide has recently been found to have potent anti-tumor, anti-inflammatory, anti-bacterial, hepatoprotective, soporific, cytotoxic, and immune suppressant properties. An alloxan-induced diabetes model in rats was used to test the anti-diabetic activity of an ethanolic extract of the seeds of *Datura Innoxia* Mill. (Family-Solanaceae). A comparison was made between both the animals treated with extract from seeds and animals treated with known drug for diabetes i.e., glibenclamide. The extract dose was chosen based on acute oral toxicity tests conducted in accordance with OECD norms. The outcomes revealed the fact that ethanolic extract significantly reduced the glucose levels in blood. The findings supported the use of seeds extract in the treatment of diabetes.

Keywords *Datura Innoxia* Mill, Alloxan Monohydrate, Glibenclamide, Glucose, Glucometer

1. Introduction

Diabetes mellitus (DM) is a worldwide health problem, which is becoming more prevalent as a source of morbidity and mortality [1]. About 5% to 8% of the population has diabetes. Many people are asymptomatic and unaware that they have the condition [2]. Obesity is to blame for much of the recent and expected increase in diabetes diagnoses in the United States. Diabetes is the 3rd greatest illness as a cause of death, the 2nd largest cause of renal fiasco and of blindness, despite the fact that insulin therapy has significantly increased diabetes patients' expectation of life [3].

The prevalence of diabetes mellitus (DM) is rising dramatically in developed countries, owing primarily to lifestyle changes and the associated rise in obesity. The metabolic repercussions of long-term hyperglycemia and dyslipidemia, such as accelerated atherosclerosis, chronic renal disease, and blindness, place a huge burden on diabetic individuals and society. To face this challenge, we must improve our understanding of the pathophysiology of diabetes and its consequences, as well as diabetes prevention and treatment [4].

Because of its cultural acceptance, and compatibility to humans, and lack of negative consequences, herbal medicine is still the primary source of basic health care for around 75-80% of the population of the globe, mostly in

underdeveloped countries [5]. Herbal medicine is also known as nutraceuticals (health foods), with a current market value of \$ 80-250 billion in the United States and Europe [6]. Medicinal plants are found across the world, although they are most numerous in tropical areas. Higher plants are thought to be the source of around 25% of all contemporary medications, either directly or indirectly [7].

Literature survey has revealed that amongst many unexplored plants, *Datura Innoxia Mill* (Solanaceae) is a valuable medicinal plant used traditionally as well as in modern medicine [8, 9]. In Ayurveda, *Datura Innoxia Mill* is called it 'Dhattuura'. *Datura Innoxia Mill* is an annual shrubby plant that typically grows in temperature zones [10]. The native range of *Datura Innoxia Mill* appears to have been Mexico and the U.S. Southwest, the Caribbean Islands, India and China [11]. The plant has anti-viral, anti-cancer activity, wound healing properties, antiperspirant activity, hypoglycaemic effect immunomodulatory effect and anti-ulcer and anti-stress activity [12]. The alkaloid scopolamine is found in the plant and is used as a pre-anesthetic in abscission, parturition, ocular operations, and travel sickness prophylaxis [13, 14, 15]. There has been a recent movement in the worldwide shift from synthetic to natural medicine, which we call a "Return to Nature."

Due to a growing awareness in terms of health risks connected with the aimless application of modern therapy, there is a resurgence of interest in herbal-based treatment these days, and the herbal medicine industry is currently a rapidly expanding sector in the worldwide market [16]. Herbal medication use is on the rise, and the market is expanding at a steady pace [17].

2. Materials and Procedure

2.1. Collection

The dried seeds of *Datura Innoxia* were gathered from Dr.Madhavachetty, Department of Botany, SV.University, Tirupathi.

2.2. Identification

The collected seeds were identified and authenticated as *Datura Innoxia Mill* by Dr.Madhavachetty

2.3. Size Reduction

The dried seeds were coarsely powdered.

The extracts of powdered *Datura Innoxia Mill* were used in this study. Sequential soxhalation extraction with ethanol solvent was used to prepare the seeds. The finely powdered *Datura Innoxia Mill* seeds were loaded in the thimble and fit into the soxhlet apparatus. The drug-to-solvent ratio of 1:3 was maintained throughout the

extraction procedure with each solvent (ethanol, chloroform, methyl alcohol) for 24 hours. The Whatmann No. 1 filter paper was used to filter the contents after 24 hours and sterilised muslin cloth, as needed. Filtrate obtained was evaporated on water bath and dried at 37°C and stored at 4°C until used.

3. Preliminary Phytochemical Screening: [18, 19, 20, 21]

The existence of phyto substances like Sterols, Reducing agents, phenolic compounds, Glycosides, Alkaloids, Flavanoids, Saponins, Proteins and carbohydrates were investigated in preliminary assays [22].

4. Pharmacological Studies

4.1. Acute Toxicity Studies [23]

OECD Guidelines for Acute Toxicity Studies:

The extracts were put to the test for acute oral toxicity according to OECD criteria. The Ministry of Social Justice and Empowerment's Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA), provided draught guidelines 423, which were adopted and received. The evaluated animals showed no symptoms of toxicity after receiving stepwise dosages of *Datura Innoxia* extracts ranging from 50 mg/kg body weight to 2000 mg/kg body weight.

Four sets of wistar strain male rats having four rats in each set are taken, of which one of the groups is considered as control and given normal saline. The rest three sets are administered with the extract of *Datura innoxia* seed in graded doses of 50, 300, and 2000 mg/kg sequentially as per OECD guidelines 423. Up to 14 days after receiving the plant extract, the animals were monitored for any changes in behaviour or mortality.

4.2. Test for Glucose Tolerance [24]

The Test for Glucose Tolerance was done on many sets of rats.

Group-I: Standard group (control) animals received glucose, intraperitoneally.

Group-II: Glibenclamide treated animals received glucose intraperitoneally.

Group-III: Extract treated animals received glucose, intraperitoneally.

Procedure:

Fast rats for 16-18 hours. Ensure that they have access to drinking water all the time. On the following day, at

8:00 AM, lay the mice individually in neat cages with only water. Numbers are given to identify the different cages. Arrange the procedure sheet, needle and sticks for determining blood glucose levels and track record the weight of each rat. Measure and track of the amount of glucose needed. (2g glucose/kg) for Intraperitoneal injection as follows: volume of Intraperitoneal glucose injection (ml) = $10 \times \text{body wt (g)}$. Place rats in the confinement cage exposing the tail outside. Use a sterile scalpel blade and scratch the tail's tip. On the test strip, place a drop of blood and check on the glucose meter. It is recorded as the glucose level base line ($t=0$) in the experiment record sheet. Take out the rat from the confinement cage. As previously said (point 4), inject the rat intraperitoneally with the necessary quantity of glucose and mark the time of administration on a record sheet. Using a fresh test strip and a tiny amount of blood, record the readings at 15, 30, 60, and 120 minutes after glucose administration. Remove the clot from the first incision to re-start the flow. The results are written down on the record sheet.

Apply pressure to the incision for 1-2 minutes after each measurement to prevent further blood loss. Place the rats in a clean cage at the end of the experiment and ensure that they have access to plenty of water and food.

4.3. Alloxan Induced Antidiabetic Activity: [25, 26]

4.3.1. Induction of diabetes:

Fasted albino rats were induced diabetic overnight by administering 100mg/kg body weight alloxan monohydrate intraperitoneally. Alloxan was weighed separately for each animal based on its body weight, then it was dissolved in 0.2ml saline solution right before inoculation. After 72 hours the rats with sugar level more than 180mg/dl were utilised in this research.

Design of the study:

Overnight fasted animals were dispersed randomly, and each group consists of five rats.

Group 1: normal rats receiving medium (vehicle).

Group 2: Diabetes treated group (control) which receives alloxan monohydrate and vehicle.

Group 3: The ethanolic extract of seeds of low dose was used to treat this diabetic group.

Group 4: The ethanolic extract of seeds of high dose was used to treat this diabetic rats group

Group 5: This group of diabetic rats are treated with glibenclamide.

After overnight fasting, the animals receive vehicle and drugs orally using intragastric tubes daily for three weeks.

4.3.2. Blood glucose estimation: [27]

To determine blood glucose level, the samples of blood are collected on 0 day, 7th day, 14th day and 21st day. By rupturing the tail vein, the blood was extracted. One touch

glucometers and blood glucose checkup strips were used to measure blood glucose levels.

5. Results and Discussion

5.1. Phytochemical Investigation

The existence of phytosubstances like Sterols, Reducing agents, phenolic compounds, Glycosides, Alkaloids, Flavanoids, Saponins, Proteins and carbohydrates were carried out in preliminary assays.

In the preliminary investigation on the ethanolic seed extract of the DI, it reveals the existence of steroids, alkaloids, glycosides, flavonoids and tannins.

The result of chemical test of chemical test methanolic seed extract of *Datura innoxia* mill is shown in Table 1.

Table 1. Preliminary phyto chemical screening

Type of phyto constituent	EXTRACT OF ETHANOL
Proteins	AB
Phenolic Compounds	AB
Flavanoids	SP
Triterpenoids & steroids	SP
Tannins	SP
Cardiac glycosides	SP
Alkaloids	MP
Saponins	MP
Carbohydrates	MP
Fixed oils	MP
Glycosides	MP

Absent; SP: Slightly Present; MP: Moderately Present.

5.2. Acute Toxicity Studies:

The rats utilised in the study were chosen using a random sampling procedure. The OECD-423 guidelines were followed for acute oral toxicity testing. The male Wistar strain rats were divided into three groups and are given extract of *datura innoxia* seed with graded doses of 50, 300, and 2000 mg/kg p.o., sequentially, in this toxicity research. The fourth group is considered as control and given normal saline. The drug was administered orally to the rats of three groups. Prior to administration of the medication the rats are fasted overnight with water ad libitum. Animals were maintained under observation for 14 days to see if their behaviour changed or if they died, as shown in Table 2.

5.3. Glucose tolerance test:

The highest amount of glucose in blood climbed quickly compared to the fasting result at 30 minutes after glucose injection, then dropped. The ethanolic extract of

Datura Innoxia Mill at 60 and 120 minutes showed a on extract on glucose tolerance, which is summarized in substantial drop in glucose levels in blood and the effect Table 3.

Table 2. Observations of studies on the acute toxicity of an ethanolic extract of *Datura Innoxia Mill*

S.NO.	Treatment	Dose	Weight of the animal (Day1)	Weight of the animal (Day 14)	Signs of Toxicity	Onset of toxicity	Reversible Or irreversible	Duration
1.	DATURA INNOXIA	50mg/kg	150gm	158gm	NIL	NIL	NIL	14 Days
2.	DATURA INNOXIA	50mg/kg	150gm	152gm	NIL	NIL	NIL	14 Days
3.	DATURA INNOXIA	50mg/kg	150gm	154gm	NIL	NIL	NIL	14 Days
4.	DATURA INNOXIA	300mg/kg	150gm	153gm	NIL	NIL	NIL	14 Days
5.	DATURA INNOXIA	300mg/kg	150gm	151gm	NIL	NIL	NIL	14 Days
6.	DATURA INNOXIA	300mg/kg	150gm	155gm	NIL	NIL	NIL	14 Days
7.	DATURA INNOXIA	2g/kg	140gm	143gm	NIL	NIL	NIL	14 Days
8.	DATURA INNOXIA	2g/kg	140gm	146gm	NIL	NIL	NIL	14 Days
9.	DATURA INNOXIA	2g/kg	140gm	141gm	NIL	NIL	NIL	14 Days

NIL - Indicates no toxicity

Table 3. Observations of glucose tolerance test of ethanolic seed extract of *Datura Innoxia Mill.*

GROUPS	NUMBER OF ANIMALS	Average BLOOD GLUCOSE LEVELS(mg/dl)				
		0min	15min	30min	60min	120min
Control group	3	86±5.567	127±2.64	123.7±11.15	97±2	89±5
Glibenclamide treated group	3	85.67±3.512	122±6.55	128±1	96.67±4.16	91±3.6
Extract treated group	3	86±4.583	113±2	128.67±1.52	101.67±8.02	88.67±3.51

Figure 1. The chart representing the Glucose Tolerance test of *Datura Innoxia Mill*

Table 4. Observations on the anti diabetic efficacy of *Datura Innoxia* Mill ethanolic seed extract

Groups	Number of Animals	Dose	Average weight of animals	Average Blood Glucose Values (mg/dL)			
				0 day	7 th day	14 th day	21 st day
Control group	5	1%	154gms	94.4±14.976	87.8±10.69	92.8±15.34	90.2±8.87
Diabetic treated group	5	100mg/kg	167gms	303.2±36.30	314.4±38.71	319.4±38.13	323.8±41.92
Glibenclamide treated group	5	5mg/kg	145 gms	278.8±8.98	195±31.57	138±15.70	97.8±16.54
Test group (low dose)	5	200mg/kg	149gms	258±17.47	187.2±14.30	158.8±23.004	116.8±11.84
Test group (high dose)	5	400mg/kg	164gms	268.8±18.75	189.4±24.33	155.2±25.91	112.8±16.3003

5.4. Anti-Diabetic Activity

After the diabetes has been induced, the rats, with hyperglycemia were utilised for the study. The animals included in the control group showed normal amounts of glucose in blood whereas the diabetic group (negative control group) animals, and high amounts of glucose in blood. The ethanolic seed extract of *datura innoxia* treated group (DI 200mg/kg, 400mg/kg oral) dose dependently showed significantly ($p<0.01$) (ANOVA test) reduction in the blood glucose levels. The standard anti-diabetic drug glibenclamide showed significantly greater ($p<0.01$) hypoglycemic effect. The findings are summarized in Table 4.

6. Discussion

The medical system still faces a problem in managing diabetes using medications that have no adverse effects. This issue has resulted in a rise in demand for natural anti-hyperglycemic treatments with fewer adverse effects.

Insulin-producing beta cells in the pancreas are destroyed by alloxan, which causes diabetes [28, 29]. Alloxan is preferentially harmful to beta cells in the pancreas, resulting in necrosis of cells, according to in vitro investigations [30, 31]. Alloxan's cytotoxic impact is arbitrated by oxygen reactive species, which causes huge rise in calcium content in the cytosol, causing beta cells to die quickly [32].

The ability of a plant extract to cause β -cells to generate insulin or to stimulate insulin receptors, which allows blood sugar to be absorbed and accelerate glucose consumption in the peripheral tissues could also provide antihyperglycemic effects [33].

The ethanol seed extract of *Datura Innoxia* Mill provided orally for 21 days led in a considerable drop in glucose levels in the blood in an alloxan induced diabetes type and a glucose tolerance test in rats, according to the current research. Similar impact of the plant extracts on glibenclamide could indicate a similar mechanism of action. As a result, the current study backs up the traditional assertion that *Datura Innoxia* Mill has

anti-diabetic properties [34].

The preliminary assays, proved that *Datura Innoxia* contains saponins, alkaloids, cardiac glycosides, flavonoids. The presence of these elements could possibly explain the extract's anti-diabetic properties.

Anti-diabetic properties of the extract could be related to effects such as encouraging -cell regeneration or protecting them from damage, limiting glucose load, and promoting unconstrained endogenous insulin action. The ability of plant extract on β -cells to generate insulin or stimulate insulin receptors to absorb blood glucose and accelerate glucose consumption in periphery could also be responsible for the anti-hyperglycemic effect.

The plant extract has shown no toxic effects on animals when treated maximum dose. It proves that it has a safe and broad therapeutic index. It is also shown from the glucose tolerance studies that the drug is not elevating the glucose levels. The main activity has proved that the drug has anti diabetic activity, with very minimal side effects or no side effects and most important and it is safe with maximum dose.

7. Conclusions

In conclusion, the present study systematically investigated the anti-diabetic potential of ethanolic seed extract of *Datura Innoxia* Mill., and supports its traditional use as anti-diabetic medicine.

Therefore, it means that *Datura Innoxia* have anti-diabetic activity and because of the anti hyperglycemic properties, when compared to manufactured medicines, medicinal plants have fewer negative effects, tremendous expense of contemporary medicines, and many rural communities in underdeveloped nations lack access to modern medicines, which could be considered a substitute for conventional diabetes mellitus treatment. The findings suggest that *Datura Innoxia* Mill seed extracts have anti-diabetic effect.

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