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"Analyses Of The Anti-Diabetic Activity Of Lactuca Sativa (L.) Seeds From Various Geographical Regions"

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Abstract

Through the use of diabetic rats, the current stdies has established that Artemisia indica and Lactuca sativa have the potential to serve as antidiabetic medicines. These plants have been shown to successfully reduce hyperglycemia and guard against metabolic abnormalities that are associated with diabetes. In the treatment of diabetes, Artemisia indica has been used traditionally, and these findings lend credence to that practice. One possible explanation for the reported antidiabetic benefits is that Artemisia indica and Lactuca sativa possess the ability to enhance the quantity of pancreatic β-cells within the islets of Langerhans and to restore their activity. However, additional research is required to analyse various chemical elements of the plant for its hypoglycemic qualities, discover the particular targets and mechanisms of action of these constituents, and evaluate the possibility of combining plant products with manufactured medications. Topical formulations can be designed with a variety of physicochemical properties, such as those that have a solid, semisolid, or liquid consistency. With the help of these studies, we can learn more about the molecular pathways that cause the effects we've seen. After the active compounds have been identified and characterized, the next step is to investigate their potential therapeutic targets. This will allow for the development of a more precise and effective treatment for diabetes.

Keywords: pancreatic β-cells, traditional medicine, anti- diabetic activity, blood glucose levels, ect.

1. Introduction

It is the plant's inherent components that provide medicinal plants their curative effects. Traditional remedies and medicinal plants are relied upon by the majority of poor nations to maintain the health of their citizens [1]. Eighty percent of the population in developing countries relies on traditional medicines, which primarily include herbal plant remedies, for their primary health care, according to the World Health Organisation. The anti-oxidant, antibacterial, and antipyretic actions, among other medicinal qualities, may be attributed to phytochemicals found in plants [2]. Because of this misconception, herbal remedies have long been used by both the general public and medical professionals as a secure substitute for pharmaceuticals. Nobody in the general public or in traditional medicine circles has ever brought up the possibility that plants are poisonous, even though there have been multiple reports in the literature detailing different forms of herb toxicity [3]. The pharmaceutical industry's interest in sourcing raw materials from medicinal plants has been on the rise. The emergence of modern western medicine occurred about the same time as the initial global notion that synthetic chemicals were the best cures for treating and curing disease [4].

Due to a resurgence of interest in health and wellbeing, people are learning about the effectiveness of herbs again. As individuals around the world work to better their health and the planet's condition, herbal treatments are seeing a renaissance in popularity. Particularly so in Western countries. A lot of the harmful side effects of contemporary medications aren't present in herbal treatments, however they nonetheless work [5].

People are turning back to traditional medical techniques in search of more effective alternatives. In the future, people everywhere will choose to get treated via traditional medical methods. Why? Because there are still a lot of things that can limit the uses of modern medicine, even though there is a lot of evidence that it works. Due to the issues with modern medicine, researchers have started investigating alternative systems, including traditional and ancient medicine [6,7].

2. The value of therapeutic plants to people's daily lives [8]

India is home to numerous plant bioresources that have important cultural and medical uses. The usage of therapeutic plants has been documented in numerous varied medical disciplines, including Ayurveda, Siddha, Unani, and the British and American pharmacopoeias [9]. Himachal Pradesh, as one of the original Himalayan states, is rich in medicinal plants. Traditional healing methods and an abundance of medicinal plants can be found in this Himalayan region. Many medicinal plant populations have deteriorated or even disappeared in the last several decades as a result of careless wild collecting and habitat damage. Medicinal plants are a primary source of care for many people living in rural areas of these regions. The demand for medicinal plant species has increased globally due to the growing interest in herbal medicine [10]. The fast increasing demand for plant-based medications is putting a tremendous strain on certain wild populations of valuable medicinal plants due to overharvesting. Many people in the rural sections of these regions rely on medicinal plants to address their basic medical needs[11]. The renewed interest in herbal medicine has led to a rise in demand for medicinal plant species all over the world. Unfortunately, some populations of high-value medicinal plants in the wild are under significant pressure from over harvesting as demand for plant-based pharmaceuticals continues to rise rapidly.

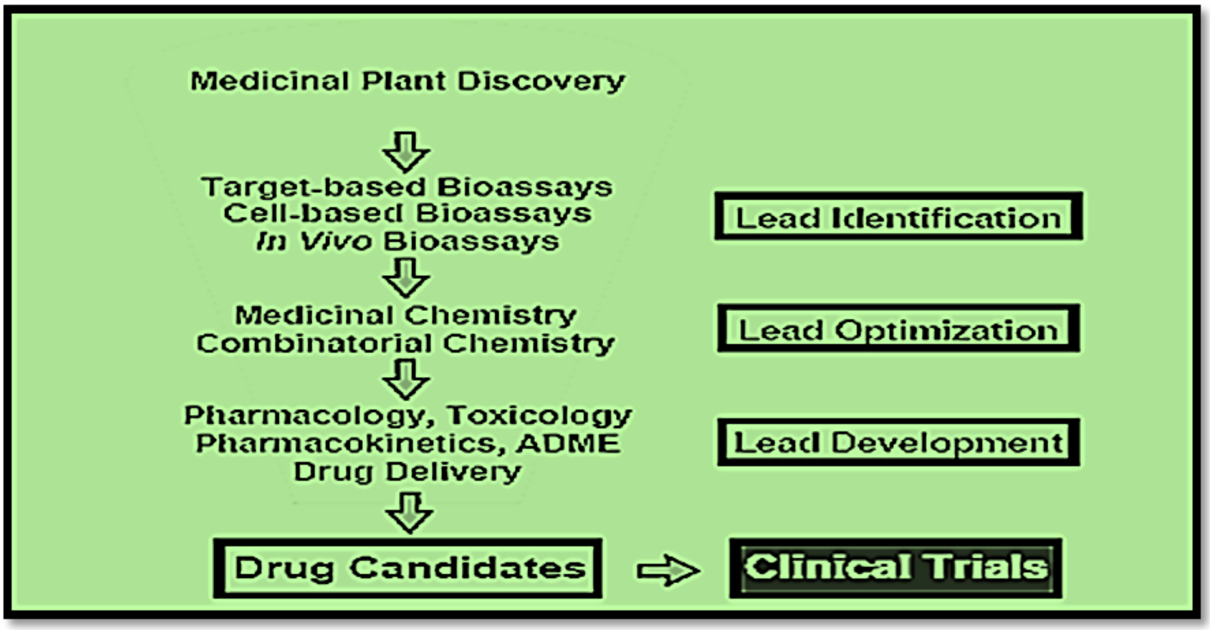

Fig.1 Flow diagram showing the medicinal plant discovery

3. Herbal Remedies from Traditional Practices [12,13]

The term "traditional medicine" describes methods of medical treatment that were in use before the advent of modern medicine. These traditional medical practices have evolved through the years and are as distinct as the countries that practise them, as the name implies. A system is said to be traditional if it has been in use for many generations by the same group. Prior to both the Vedic era and the dawn of human civilization, India has a long history of practicing traditional medicine [14]. Despite many changes throughout the years, it continues to serve as the foundation for medical care for a large portion of the country's population. Pakistan, India, Japan, and China are among the Asian countries that continue to practise traditional medicine. Medicinal plants have been around longer than any other type of medicine.

Traditional Indian medicine, or Ayurveda, has a long history of use [15].

There has likely been continuous practice of Ayurveda, the ancient Indian science of life, for at least five thousand of those years. This is among the most famous forms of modern medicine. In Ayurveda, the five basic elements—space, air, energy, liquid, and solid—are the building blocks of all things. Within the human body, we can only experience a limited kind of vata (space and air), pitta (energy and liquid), and kapha (solid and liquid). "Tridosha" means "three pillars of existence" when combined with vata, pitta, and kapha. When balance is disturbed, illness might develop.

Medicine according to traditional Unani principles [16]

Many people believe that the illustrious Greek philosopher Hippocrates was the founder of the system. "Father of Natural History" Aristotle Golem played a significant role in it. The theoretical underpinnings of this system are the four humours as proposed by Hippocrates and the four proximal characteristics as proposed by Pythagoras. Heat, cold, moisture, and dryness are the four qualities of the four humours, which include blood, phlegm, yellow bile, and black bile. Ground, water, fire, and air represent them. In Unani medicine, the focus is on getting to the root of an illness rather than just

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alleviating its symptoms. For a comprehensive assessment, it is necessary to squat, take the patient's pulse, and have him pee. The patient is treated according to the belief that an imbalance of humours is the fundamental cause of their illness.

Health care centred in homoeopathy [17]

German physician and chemist Samuel Hahnemann laid the groundwork for homoeopathy in the seventeenth century; the practice is considered to be relatively contemporary. One of his ideas was to get to the bottom of why people were sick in the first place. The idea behind his "like cures like" law was that remedies for different ailments are essentially the same. He proved that cinchona can mimic the effects of malaria using that notion. To choose the right medication for each individual, homoeopaths look at their symptoms and overall health.

Medicine from traditional India (Siddha) [18]

In Sanskrit, "Siddha" means "accomplishment," and the "Siddhars" were revered healers who mastered Bhakti and Yoga. This system, whose main emphasis is on medicine, is linked to the pre-Vedic Dravidian culture. This school of thought, similar to Ayurveda, holds that the universe is comprised of the five elements: earth, water, fire, and air. As a diagnostic tool, you can utilise things like pulse readings, body colour analysis, voice analysis, urine analysis, assessments of intestinal health, and tongue analysis.

Alternative Medicine and Yoga[19]

Naturopathy is a system of medicine that emphasises the body as a whole and the need of restoring harmony with nature. The purgative procedures of hydrotherapy, mud packs, baths, massage, etc., and lifestyle aspects like food and exercise are given special attention. The eightfold path of yoga encompasses one's emotional, intellectual, moral, and spiritual health.Starting with the animal condition, it moves on to the typical, and finally reaches the divine in a linear fashion. Samadhi, physical postures, austerity, breathing exercises, contemplation, meditation, and restraint of sense organs are the eight limbs that make up Yoga.

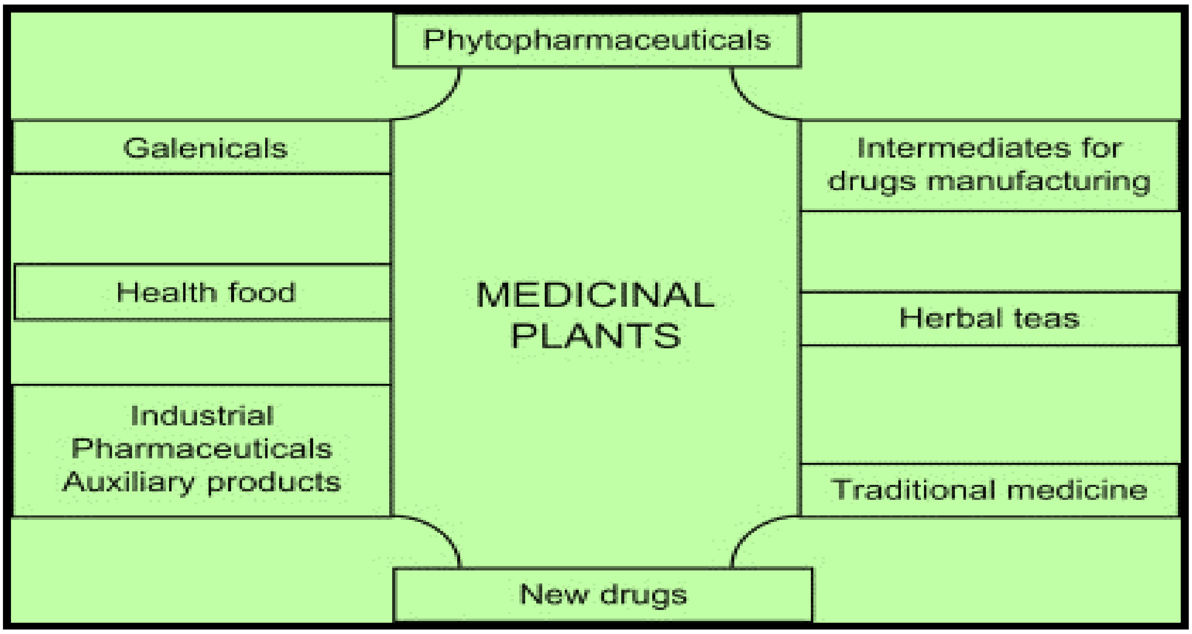


Fig.2 Medicinal plants derived from a wide variety of sources

4. Contemporary applications of herbal remedies [20]

Modernised herbal therapy is nothing more than the rediscovery of ancient, widely disseminated knowledge. Traditional medical practices and surgical procedures failed to alleviate people's pain, so they turned to alternative treatments. Many individuals still use herbal remedies since they are usually believed to be safe due to their all-natural composition. Instead of utilising the full plant, pharmacologists isolate its active components and put them to use. In addition to the active components, other plant chemicals such as minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavonoids, and others are essential for a herb's medicinal actions [21]. These elements also have an important protective role. To be toxic, you need a lot more of a whole plant—including all of its parts—than you would with a single active molecule, whether it be manufactured or isolated. Herbs, on the other hand, are medications and, when taken as directed, can have significant positive effects. They deserve your utmost care.

5. Plant profile and Identification of Lactuca sativa (L.) seeds [21]

on the basis of a study of literature and reports mentioned in the previous chapters Lactuca sativa (L.) seeds (Asteraceae) was selected for a study of its efficacy as an anti- diabetic. To make the study more relevant towards the purpose of standardization, which was the original aim of this work, the plant was sourced from four different geographical regions, viz., Delhi, Amritsar, Chennai and Lucknow. This provided for the recording of any differences in the activity of plants owing to the differences in geographical source and thus arrive at a list of plants with potent antidiabetic activity belonging to any specific geographical location.The plant was identified by taxonomist Prof. Pramod Mishra, Head, Department of Agriculture, Mangalayatan University, Aligarh. A voucher sample of the plant (voucher number 202200107, 202200108, 202200109, 202200110) was deposited in the herbarium of Department of Pharmacy, Mangalayatan University, Aligarh.

Experimental Design
Materials and Methods
General: Sigma Chemical Company of St. Louis, MO, USA, supplied all analytical-grade chemicals and reagents. We used a glucometer (One touch Basic Plus３) that we bought from Johnson & Johnson Inc. (USA) in conjunction with one-touch strips that we bought from Lifescan Inc. (USA) for glucose estimation.
The Animal House at Mangalayatan University in Aligarh provided the adult Sprague Dawley rats used in the experiment. The rats were kept on a 12-hour light/dark cycle and given free access to food and water. Oral administration was used for all of the extracts. The animals were given time to adjust to their new environment before the studies began. The Departmental Animal Ethical Committee met and gave its approval to the study protocol (DAEC/PHARM/2023/10) that involved animals. According to the Unani texts, 100 g of air-dried, powdered plant material was cooked in 250 ml of water for 1 hour and then filtered. For future research, the filtrate was dried and put in the fridge. For the animal investigations, the dried extracts were mixed with water and 1% tween 80.

6. Experimental procedure of Antidiabetic Activity[22]

Materials and methods
Collection of material: *L. sativa* seeds were procured from the markets of Delhi, Amritsar,Lucknow and Chennai and were identified by taxonomist Prof. Pramod Mishra, Head, Department of Agriculture, Mangalayatan University, Aligarh. A voucher sample of the plant (voucher number 202200107, 202200108, 202200109, 202200110) was deposited in the herbarium of Department of Pharmacy, Mangalayatan University, Aligarh.

Fig.3 Plant profile and seeds of Lactuca sativa (L.) seeds (Asteraceae)

Drugs a) Glibenclamide (3mg/kg) was prepared in tween 80 solution.
b) Streptozotocin (50 mg/kg) was prepared freshly in acetate buffersolution (pH 4.5).

Impact of medication aqueous extract on normally fasting rats[23]
Before the trial began, the animals were fasted for one night. Each of the seven groups consisted of six rats. The car was given to Group I, which acted as control. Glibenclamide (3 mg/kg), the gold standard, was administered to Group II. The aqueous plant medicine extracts were administered to Groups III–VI, with doses of 200 mg/kg each. Oral administration was used for all of the extracts. Two and four hours before treatment, as well as immediately thereafter, the blood glucose level was measured.
Pharmacological effects of drug aqueous extracts on normal, fasting rats' oral glucose tolerance tests There were seven groups of animals, with six rats in each. The car was given to Group I, which acted as control. Glibenclamide (3 mg/kg), the gold standard, was administered to Group II. The aqueous plant medicine extracts were administered to Groups III–VI, with doses of 200 mg/kg each. When albino rats were fasted for 16 hours, they were given 1.5 g glucose/kg body weight orally (dissolved in water for injection) through a canula-fitted needle linked to a syringe. This was part of an oral glucose tolerance test (GTT). Oral administration of a single dose of the reference drug glibenclamide (3 mg/kg) and plant extracts (200 mg/kg) were given to the appropriate groups immediately following glucose feeding in order to examine their effects on GTT. Before, 30 minutes, 60 minutes, and 90 minutes after glucose injection, blood glucose levels were assessed.

The impact of medication aqueous extract on diabetic rats' blood glucose levels[24]
In order to induce diabetes in rats, a single intraperitoneal injection of streptozotocin (50 mg/kg in acetate buffer 0.1M, pH 4.5) was administered by Sigma, St. Louis, USA. Testing non-fasting blood glucose levels 48 hours following streptozotocin injection confirmed diabetes. Oral administration of the appropriate medicines was performed on the animals that were chosen after excluding those with glucose levels below 250 mg/dl. The first group, which served as the control, got the car. The diabetic control group, Group II, got the car. Glibenclamide (3 mg/kg), the reference medication, was administered to the third group. Oral administration of the extracts (200 mg/kg) was administered to groups IV–IX. We checked blood sugar levels soon before, 2 hours after, and 4 hours after extract delivery.
Statistical analysis: The mean plus or minus the standard error of the mean is used to express the values. Using one-way analysis of variance and Dunnett's t-test, we determined if the results were statistically significant. A p-value of less than 0.05 was used to determine whether the values were substantially different.

Result and Discussion

Effect of Aqueous Extract of Drugs on the Blood Glucose Level of Normal Fasted Rats

Table 1. Effect of aqueous extract of drugs from Delhi region on the blood glucose level of normal fastedrats

Groups	**Treatment**	**Blood glucose level mg/100 ml**		
		Initial	**2 Hrs**	**4 Hrs**
I	Control	86.16± 3.10	83.83± 3.54	84.11± 3.42
II	Glibenclamide (3 mg/kg)	84.83 ± 2.85	46.83± 4.57[1]	48.50± 4.64[1]
III	*Delhi region* (200 mg/kg)	81.83 ± 2.81	68.41± 3.30[2]	69.33± 3.17[2]
IV	Amritsar region (200 mg/kg)	83.81 ± 1.28	67.33± 3.10[2]	66.12± 2.54[2]
V	Lucknow region (200 mg/kg)	85.14 ± 2.42	67.45± 3.67[2]	64.36± 3.82[1]
VI	Chennai region (200 mg/kg)	84.35± 2.26	61.85± 3.48[1]	60.56± 3.11[1]

All values are Mean ± SEM [1] $p<0.01$, [2] $p<0.05$, [ns] not significant when compared with control

Effect of Aqueous Extract of Drugs on the Oral Glucose Tolerance Test of Normal Fasted Rats

Table 2. Effect of aqueous extract of drugs from Delhi region on the oral glucose tolerance test of normalfasted rat

Groups	**Treatment**	**Blood glucose level mg/100 ml**			
		Initial	**30 min**	**90 min**	**180 min**
I	Control	82.18± 2.32	128.43± 3.35	120.61± 3.67	111.23± 3.94
II	Glibenclamide (3 mg/kg)	84.32± 2.12	108.15± 3.36	96.34± 3.62[1]	89.45± 2.81[1]
III	*Delhi region* (200 mg/kg)	79.33± 2.33	114.51± 4.03	106.15±4.56[2]	96.82± 4.12[2]
IV	Amritsar region (200 mg/kg)	85.25± 2.64	116.36± 3.69	108.61±3.45[ns]	97.28± 4.82[ns]
V	Lucknow region (200 mg/kg)	84.86± 2.21	114.31± 4.18	106.62±4.12[2]	98.19± 3.91[2]
VI	Chennai region (200 mg/kg)	82.46± 2.21	115.45± 4.01	108.36±3.78[ns]	99.20± 4.15[ns]

All values are Mean ± SEM [1] p<0.01, [2] p<0.05, [ns] not significant when compared with control

Effect of Aqueous Extract of Drugs on the Glucose Level of Diabetic Rats

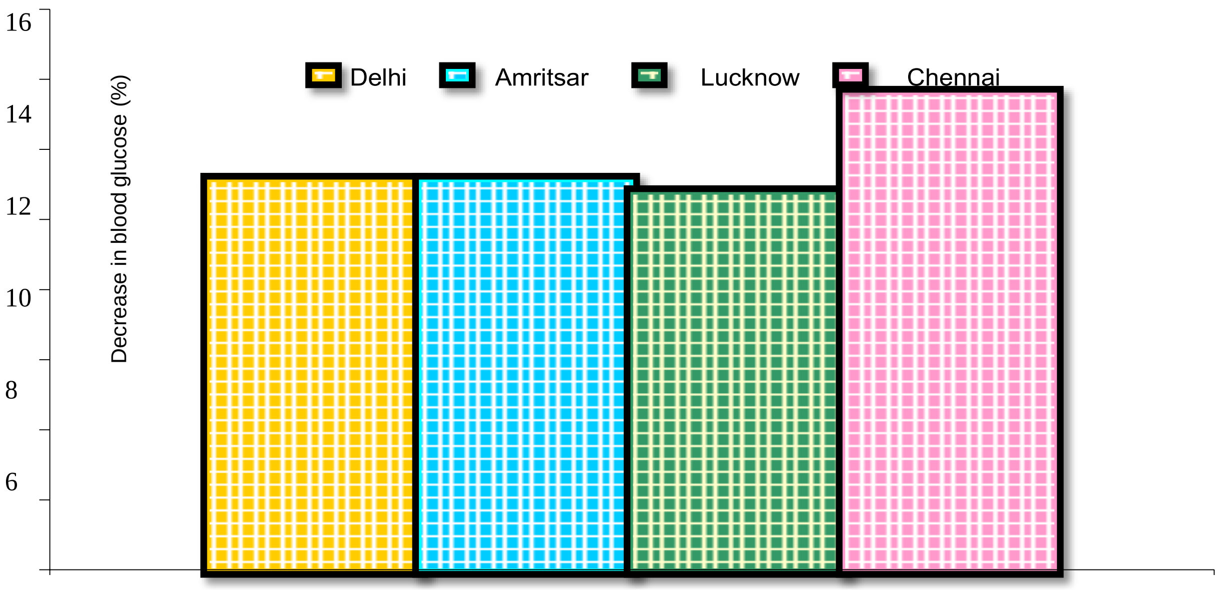
Table 3. Effect of aqueous extract of drugs from Delhi region on the glucose level of diabetic rats

Groups	Treatment	Blood glucose level mg/100 ml		
		Initial	**2 Hrs**	**4 Hrs**
I	Control (Normal)	82.24± 3.64	81.63± 2.84	82.47± 2.35
II	Diabetic control	281.56± 8.25[1]	276.36± 7.58[1]	280.12± 8.64[1]
III	Glibenclamide (3 mg/kg)	283.85± 8.75	244.38± 10.21[3]	218.15± 2.64[2]
IV	*Delhi region* (200 mg/kg)	288.44± 10.12	255.71± 13.45[ns]	234.86± 3.25[3]
V	Amritsar region (200 mg/kg)	285.14± 9.26	268.89± 13.35[ns]	253.48± 13.51[3]
VI	Lucknow region (200 mg/kg)	280.83± 9.42	265.36± 12.27[ns]	250.62± 13.15[3]
VII	Chennai region (200 mg/kg)	289.46± 10.62	269.56± 12.84[ns]	250.11± 13.45[3]

All values are Mean ± SEM[1] p<0.001, when compared with normal control (group I) [2] p<0.01, [3] p<0.05, [ns] not significant when compared with diabetic control (group II)

Table.4. Comparison of percent decrease in blood glucose level of STZ diabetic rats on treatment withsame plant from different geographical sources

Drug	Percent decrease in blood glucose level			
	Delhi	**Amritsar**	**Lucknow**	**Chennai**
Lactuca sativa	11.09	11.10	10.75	13.59



Lactuca sativa

Fig.4. Histogram showing percent decrease in blood glucose levels of STZ diabetic rats on treatment with the plants from different geographical regions

Discussion

In the test for effect of extract of the *L. sativa* plant from on the glucose level of normal fasted rats, the aqueous extract of Delhi region decreased the glucose levels significantly at 2 hrs (p<0.05) and 4 hrs (p<0.05). The reduction in the glucose level produced by the extracts from Amritsar regionwas also significant at 2 hrs (p<0.05) and 4 hrs (p<0.05). When plants from Lucknow were givento the normal fasted rats to observe their effect on their blood glucose levels, it was found that extract of *L. sativa* also brought down the blood glucose levels significantly at 2 hrs (p<0.05) and4 hrs (p<0.01) on comparison with control rats. Extracts from Chennai region exhibited significant reduction in glucose levels at 2 hrs (p<0.01) and 4 hrs (p<0.01).

The effect of aqueous extracts from all the four locations was also observed on the animals in theGTT where the animals had been fed glucose externally (1.5 g/kg) and then administered with the standard drug as well as plant extracts immediately. The results obtained are discussed in the following paragraphs. When extracts of plants sourced from Delhi were given to animals, it was found that the Extract of *L. sativa* also brought down the blood glucose levels significantly at 90 min ($p<0.05$) and 180min ($p<0.05$) on comparison with control rats. From Amritsar region the glucose levels were notreduced significantly upon administration of the extracts of *L. sativa*. Aqueous extract of *L. sativa* exhibited significant reduction in glucose levels at 90 min ($p<0.05$) and 180 min ($p<0.05$). FromChennai extracts of *L. sativa* could not cause a decrease in glucose levels significantly either at 90min or at 180 min.The extracts were finally tested for their efficacy as an antidiabetic agent by administering them to STZ diabetic rats. Blood glucose level was measured just prior to and after 2 hrs and 4 hrs of extract administration. The extract of *L. sativa* obtained from Delhi showed that the initial reduction in the blood glucoselevels was not significant but the reduction at 4 hrs was found to be significant ($p<0.05$). For thee xtract from Amritsar, *L. sativa* was found to decrease the level of glucose significantly at 4 hrs. ($p<0.05$) on being compared to the diabetic control. The extract of *L. sativa* obtained from Lucknow produced an initial reduction in the blood glucose levels that was not significant but thereduction at 4 hrs was found to be significant ($p<0.05$). The extract of *L. sativa* from Chennai, was found to decrease the level of glucose significantly at 4 hrs ($p<0.05$) on being compared to the diabetic control. An overview of the above discussion reveals that the aqueous extracts of *L. sativa* decreased the blood glucose of normal animals.
Administration of streptozotocin (STZ) destroys β-cells of the islets of Langerhans in pancrea. Destruction of β-cells in the pancreas causes marked decrease in serum insulin levels. However, the aqueous extracts exhibited antihyperglycaemic activity in STZ-induced diabetic rats.. From the overall results it is evident that extracts of *L. sativa* exhibited hypoglycaemic activity innormal rats as well as STZ-induced diabetic rats. When the percent decrease in blood glucose levels in the STZ diabetic rats produced by extracts of the same plant sourced from different places was compared to see whether the difference in the geographical source also led to a difference inthe antidiabetic activity of the plant, it was found that there was no specific or considerable difference in the activity of the drug from different regions in any of the models studied.

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Conflict of Interests

The authors have no conflict of interests.

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