A Comparative Study on the antidiabetic activity of Sonchus asper and Sonchus arvensis in Alloxan Induced Diabetic Rats

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***Abstract*: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high level of glucose in the blood resulting from a relative or absolute deficiency of insulin action. DM affects a population of approximately 424.9 million adults (aged 20-79) worldwide in 2017. Worldwide trend towards the utilization of natural plant remedies has created an enormous need for information about the properties and uses of the medicinal plant. The Indian Traditional Medicine like Ayurvedic, Siddha and Unani are predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. The study demonstrated that various doses of Sonchus asper and Sonchus arvensis (100,150 and 200 mg/kg) showed antidiabetic effect against alloxan-induced diabetic animals.It is found that Sonchus arvensis ethanolic leaves extract at high dose (200mg/kg) is more effective than other concentrations after 14 days of treatment.**

***Keywords*: Diabetes mellitus, alloxan, Sonchus asper, Sonchus arvensis, Ayurvedic, Siddha, Unani**

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high level of glucose in the blood resulting from a relative or absolute deficiency of insulin action[1]. DM affects a population of approximately 424.9 million adults (aged 20-79) worldwide in 2017[2]. This disease is associated with micro and macro vascular complications which lead to the development of disability and life-threatening medical conditions[3]. Hyperglycaemia, hyperlipidemia, and oxidative stress are the main important characters of DM and represent a major risk factor for the development of complications of diabetes[4]. To date, the available therapy for diabetes includes insulin and various oral antidiabetic agents such as sulfonylurea, thiazolidinediones, and α-glucosidase inhibitors. These drugs are used as monotherapy or in combination to achieve better glycemic control[5].

Types of diabetes mellitus

Insulin Dependent Diabetes Mellitus (IDDM, Type 1)

Non-Insulin Dependent Diabetes Mellitus (NIDDM Type 2)

Gestational diabetes (Type 3)

Treatment of diabetes mellitus

Insulin and oral hypoglycaemic drugs Insulin therapy should aim to mimic nature, which is remarkably successful both in limiting postprandial hyperglycaemia and preventing hypoglycaemia between meals. Different preparations of insulin are available such as human insulin, beef insulin, pork insulin. Insulin therapy is no free from complications and adverse effects. The most important adverse effect are weight gain and hypoglycaemia when inappropriate dose of insulin is taken and when there is mismatch between meals and insulin injection. They bind to sulfonylurea receptors on the ß-cell plasma membrane, causing closure to ATP sensitive potassium channels, leads to depolarize the cell membrane. Administration of sulfonylurea's to type 2 DM patient's increases insulin release from the pancreas and also may be further increase insulin levels by reduce hepatic clearance of the hormones. It has been shown to increase peripheral uptake of glucose, and to reduce hepatic glucose output by approximately 20-30% when given orally but not intravenously. Impaired absorption of glucose from the gut has also been suggested as a mechanism of action [6].

Herbal medicine medicines used for diabetes therapy

Worldwide trend towards the utilization of natural plant remedies has created an enormous need for information about the properties and uses of the medicinal plant. The Indian Traditional Medicine like Ayurvedic, Siddha and Unani are predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness.The association of medical plants with other plants in their habitat also influences their medicinal values in some cases. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies. Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. Antihyperglycemic effects of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. More than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, cartenoids, etc., that are frequently implicated as having antidiabetic effect. Species will be described in alphabetical order and information about each species will include in sequence: general botanical and taxonomic data, distribution in the world, experimental study and mechanism of action [7].

Sonchus species include annual, biennial and perennial herbaceous plants. They are a class of edible wild plant with 50 known species widely distributed in Europe, Asia, and Africa, only Sonchus asper and Sonchus arvensis are widely used in official medicine. Sonchus species are usually used in infusions or decoction, which are administered orally or externally to treat acute icterohepatitis, cancer, inflammation, rheumatism, diarrhoea, and snake venom poisoning.In recent years, Sonchus species has captured the attention of the international health food industry, and there has been a wide range of research on the nutritional ingredients,chemical constituents,and biological activities of the genus [8].

Materials and Methods

Collection, Authentication and drying the plant material

The leaves of Sonchus asper and Sonchus arvensis were collected from Pathali Pahar area, Lakhimpur district of Assam (A north-eastern part of india) and duly authenticated from Botany Department of Gauhati University,Assam. The plant leaves were shed-dried, pulverized and stored in an airtight container for further extraction.

Fig.1-Sonchus asper plant

Fig.2- Sonchus arvensis plant

Preparation of Extract

The dried leaves of Sonchus asper & Sonchus arvensis were pulverized and passed through 40 mesh size sieve. The coarse powders of both the species were extracted with 95% ethanol at 40˚-50˚ C for 48 hours. The both extracts were filtered, concentrated and dried under reduced pressure by rotating evaporator. The suspension of ethanolic extract was prepared by using 0.5% Tween-80 in saline. Preliminary phytochemical analysis of both the extracts was carried out by different methods of phytochemical analysis.

Animal Husbandry & Maintenance

Healthy adult wister strain albino rats (weighing 210-250 g, 5-8 weeks of age, male) were obtained from animal house facility, NETES institute of pharmaceutical science, Guwahati, Assam, India. The animals were placed in polypropylene cages with free access to standard laboratory diet (Pranav Agro Industries limited, Sangli, Maharastra, India) and provided water ad libitum.Each

individual animal was clinically examined upon arrival and identified by fur marked with picric acid. Animals were grouped and housed in an environmentally-controlled room with temperature of 22±3℃ and 40-70% relative humidity with a 1 h light/dark cycle, and ventilation of 15-21 air changes /h for an acclimation period of 7 days to laboratory conditions prior to the beginning of the experiment in order to adjust the new environment and to overcome stress incurred during their transit [9].Only healthy animals were assigned for these studies. Approval to carry out these studies was obtained from the Institutional Animal Ethics Committee (IAEC) under a subproject and an experiment was performed in compliance with the principles of Laboratory Animal Care (NIH publication 85-23, revised 1985).All of the animal experimental protocols were in accordance with the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA)[10].

Acute oral toxicity studies

The acute oral toxicity studies of extracts were carried out as per the OECD guidelines, draft guidelines 423 adopted on 17 December 2001 received from CPCSEA, Ministry of Social Justice and Empowerment, Government of India. Administration of the stepwise doses of extracts of Sonchus asper and Sonchus arvensis from 50 mg/kg b. wt. up to a dose of 2000 mg/kg b. wt. caused no considerable signs of toxicity in the tested animals.

Experimental Design

Nine Groups of rats, six in each received the following treatment schedule.
Group-I: Normal Control (Saline-10ml/kg b.w)
Group-II: Alloxan treated control (150mg/kg. ip)
Group-III: Alloxan control (150mg/kg. ip) +Glipizide (10mg/kg b.w)
Group-IV: Alloxan control (150mg/kg. ip) + S.asper ethanolic extract (100mg/kg b.w)
Group-V: Alloxan control (150mg/kg. ip) + S.asper ethanolic extract (150mg/kg b.w)
Group-VI: Alloxan control (150mg/kg. ip) + S.asper ethanolic extract (200mg/kg b.w)
Group-VII: Alloxan control (150mg/kg. ip) + S.arvensis ethanolic extract (100mg/kg b.w)
Group-VIII: Alloxan control (150mg/kg. ip) + S.arvensis ethanolic extract (150mg/kg b.w)
Group-IX: Alloxan control (150mg/kg. ip) + S.arvensis ethanolic extract (200mg/kg b.w)

Whole plant extracts and standard drug Glipizide (10 mg/kg b.w) and saline were administered with the help of feeding cannula. Group I serve as normal control, which received saline for 14 days. Group II serve as diabetic group which received alloxan. Group III serve as Diabetic control group which received Glipize(Which previously received Alloxan).Group IV to Group IX are diabetic control rats. Group III to Group VI (which previously received alloxan) are given a fixed dose of Sonchus asper leaves extract (100 mg/kg, p.o), (150 mg/kg, p.o), (200 mg/kg, p.o) and standard drug Glipizide (10 mg/kg body weight) for 14 consecutive days. Group VII to Group IX (which previously received alloxan) are given a fixed dose of Sonchus arvensis leaves extract (100 mg/kg, p.o), (150 mg/kg, p.o), (200 mg/kg, p.o) and standard drug Glipizide (10 mg/kg body weight) for 14 consecutive days.

Induction of Diabetes in Experimental Animals

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg). Alloxan was first weighed individually for each animal according to the body weight and then solubilized with 0.2 ml saline (154 mM NaCl) just prior to injection.

Collection of Blood Sample and Blood Glucose Determination

Blood samples were drawn from tail tip of rat at weekly intervals till the end of study (i.e., 2 weeks). Fasting blood glucose estimation and body weight measurement were done on day 0, 3,7,15 of the study. Blood glucose estimation can be done by one touch electronic glucometer using glucose test strips.

Statistical Analysis

All the values were expressed as mean ± standard error of mean (S.E.M.), data were analysed by one way annova followed by turkey-karmer multiple comparision test, $p<0.001$ compared to diabetic control.

Results & Discussion

Standardization and phytochemical screening

Standardization parameters for Sonchus asper and Sonchus arvensis leaves were determined and all the parameters were found to be within pharmacopoeial standards limit. Crude powder taken for extraction was of green colour with slight bitter taste.

Sl.No	Parameter	Sonchus asper	Sonchus arvensis
1	Losses on drying	2.12%	2.37%
2	Total ash	5.46%	6.12%
3	Acid insoluble ash	3.74%	3.18%
4	Water soluble ash	1.14%	1.04%

Table-1-Physicochemical Parameters

Thin layer chromatography of Sonchus asper & Sonchus arvensis leaves extracts were performed.Sonchus asper leaves extract shows yellow spots/florescence with R_f values 0.51.Sonchus arvensis leaves extract shows yellow spots/ florescence with R_f values 0.55. Phytochemical screening of both the extracts of Sonchus species showed the presence of various phytochemical constituents like Sesquiterpene lactone,Quinic acid ester, flavonoids, carbohydrates, tannins, and traces of alkaloids.

Alloxan-induced diabetic model

The acute oral toxicity study of Sonchus asper and Sonchus arvensis showed no mortality upto 2000 mg/kg. Administration of alloxan (150 mg/kg, i.p.) lead to 1.5- fold elevation of fasting blood glucose levels, which was maintained over a period of 2 weeks. Two weeks of daily treatment of various extract of Sonchus asper and Sonchus arvensis lead to a dose dependent fall in blood sugar levels by 25%–50%. Effect was maximum till 14 days of treatment. Vehicle control animals were found to be slightly increased in their body weight but diabetic rats showed significant reduction in body weight during 14 days. It was observed that the standard drug Glipizide lowered the blood glucose level significantly, bringing it nearly back to normal, whereas plant extract significantly ($P < 0.01$) decreased fasting blood serum glucose in the diabetic rats on 0^{th},3rd, 7th and 15th days as compared to initial (0 hr) blood serum glucose levels.

The study demonstrated that various doses of Sonchus asper (100,150 and 200 mg/kg) and Sonchus arvensis (100,150 and 200 mg/kg) showed antidiabetic effect against alloxan-induced diabetic animals. This shows that the extract contained the antihyperglcemic constituents. The high levels of the antihyperglycemic constituents in the plant could be due to the soil composition and the environmental stress at the sites where the plant was growing and harvested. Bioactive constituents of plants are secondary products produced to help them survive the stress induced to them by the environment.

It is found that Sonchus arvensis ethanolic leaves extract at high dose (200mg/kg) is more effective than other concentrations after 14 days of treatment. Hence the above discussion revels that ethanolic leaves extract at high dose (200mg/kg) is more effective and shows similar curative effect as standard that is, Glipizide (10 mg/kg body weight).

Blood glucose concentration in mg/dl				
Group	0^{th} Day	3^{rd} Day	7^{th} Day	15^{th} Day
Group-I	85±1.6	87.6±2.4	85±2.3	89.3±2.4
Group-II	299±3.6	311.6±2.4	310.2±2.4	301.2±2.4
Group-III	95±1.6	87.6±1.4	89±1.5	90±2.1
Group-IV	245.1±2.1	178.6±1.6	152.2±2.1	112.2±1.1
Group-V	215.1±2.3	163.6±1.3	141.2±2.4	102.2±1.5
Group-VI	201.1±1.4	151.1±1.4	137.2±1.1	95.1±1.3
Group-VII	212.1±1.1	172.6±1.3	150.2±2.2	110.2±1.4
Group-VIII	200.1±1.5	161.6±1.3	140.2±1.2	100.2±1.1
Group-IX	190.1±0.8	143.1±1.2	130.2±1.1	93.1±1.7

Table-2: **Blood glucose concentration in mg/dl**

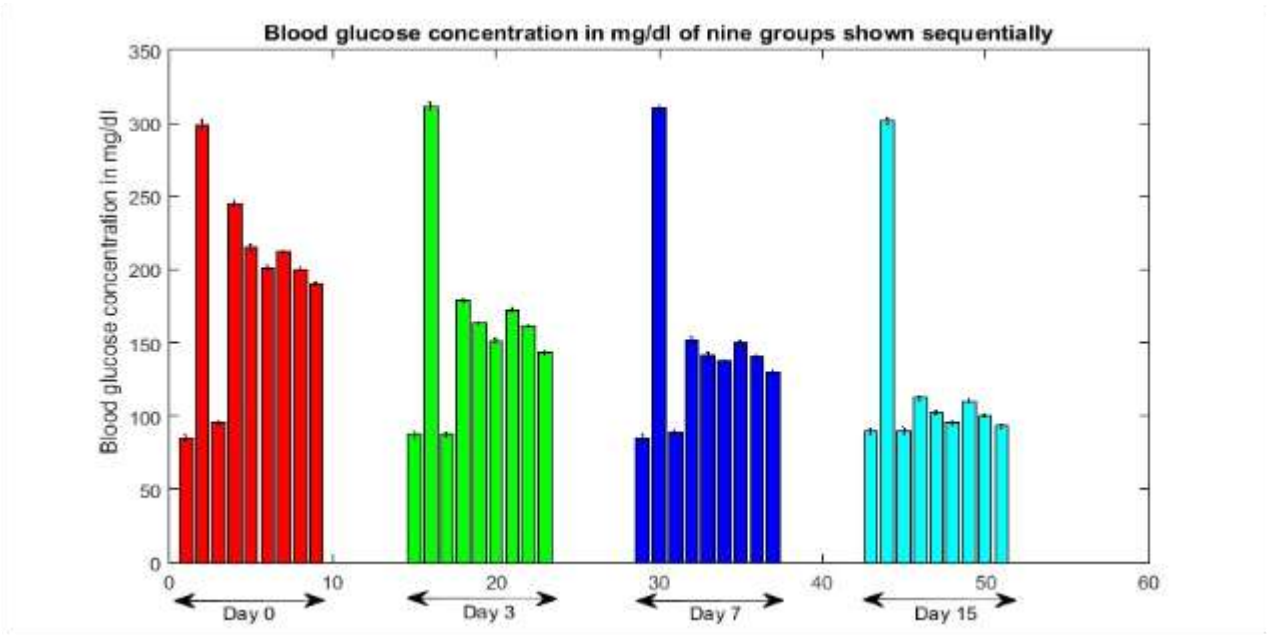

Figure-3: **Blood glucose concentration in mg/dl**

Conclusion

In conclusion, the ethanolic extract of Sonchus asper and Sonchs arvensis were found to exhibit a significant anti hyperglycemic activity in alloxan induced diabetic rats. Phytochemical screening of the extract of Sonchus species showed the presence of various phytochemical constituents like Sesquiterpene lactone,Quinic acid ester, flavonoids, carbohydrates, tannins, and traces of alkaloids. Further histopathology studies are also required to examine the toxicity profile of the plant.

Conflict of Interest

The authors declare no conflict of interest

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References

[1] World Health Organization, *Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications*, Swizerland: Geneva, 1999.
[2] International Diabetes Federation, *Diabetes Atlas*, Brussels, Belgium, 8th edition, 2017,
[3] American Diabetes Association, "Diagnosis and classification of diabetes mellitus," *Diabetes Care*, vol. 37, supplement 1, pp. S81–S90, 2014.
[4] V. A. Kangralkar, S. D. Patil, and R. M. Bandivadekar, "Oxidative stress and diabetes: a review," *International Journal of Pharmaceutical Applications*, vol. 1, no. 1, pp. 38–45, 2010.
[5] S. A. Nabi, R. B. Kasetti, S. Sirasanagandla, T. K. Tilak, M. V. J. Kumar, and C. A. Rao, "Antidiabetic and antihyperlipidemic activity of piper longum root aqueous extract in STZ induced diabetic rats," *BMC Complementary and Alternative Medicine*, vol. 13, no. 37, 2013.
[6]. Cinmay D. Deshumukh and Anurekha Jain. Diabetes Mellitus: A Review, *International Journal of Pure and Applied Bioscience*,2015; 3(3):227-228.
[7]Dutta Nandan Koushik,Chetia Purbajit,Herbal plants used as diuretics:A comprehensive review, *Journal of Pharmaceutical, Chemical and Biological Sciences*,2014;2(1):27-32
[8]. Xiu-Mei Li and Pei-Long Yang, Research progress of Sonchus species, *International journal of food properties*, 2018;vol.21(1):147–157
[9] Banerjee S,Chattopadhyay P,Ghosh A, Acute dermal irritation, sensitization and acute toxicity studies of a transdermal patch for prophylaxis against (±) Anatoxin-A poisoning, *Int J Toxicology*,2013;32:308-313
[10] Dutta, K.N., Chattopadhyay, P. & Banerjee, S. Exploration of *Mucuna pruriens* (Linn) starch powder formulations as a natural non-lethal riot control agent. *Toxicol. Environ. Health Sci.* (2020). https://doi.org/10.1007/s13530-020-00044-8