



ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF *STROBILANTHES ASPERRIMUS* IN RATS

Pradeep Kumar Samal*

SLT Institute of Pharmaceutical
 Sciences, Guru Ghasidas
 Vishwavidyalaya, Bilaspur, C.G,
 India

Journal of Global Trends in
 Pharmaceutical Sciences

ABSTRACT

Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Irrespective of so many synthetic drugs, Napralert database lists over 1200 species of plants representing 725 genera in 183 families extending from the marine algae and fungi with antidiabetic activity. Literature survey reveals that *Strobilanthès asperrimus* (Family: Acanthaceae) is used as an antidiabetic agent by the traditional healers of Chhattisgarh. So an attempt has been taken for scientific exploration of this plant as an antidiabetic agent. Preliminary phytochemical test reveals that there is presence of alkaloids, glycoside, flavonoids, phenolic and tannins compounds. Antidiabetic activity was estimated by alloxan induced diabetic model. Ethanolic extracts of *S. asperrimus* have shown significantly decrease ($P < 0.001$) in glucose level for long period of time. *S. asperrimus* at a dose of 200 mg/kg body weight) show low blood glucose level 190.16 ± 5.77 mg/dl as compared to dose of 100 mg/kg body weight shows 208.16 ± 5.13 mg/dl, Control, shows 376.5 ± 14.03 mg/dl and Standard Drug Metformin at the dose 120 mg/kg body weight) shows 183.33 ± 14.06 mg/dl respectively. In oral glucose tolerance test in hyperglycemic rats both extracts dose have shown considerable reduction in blood glucose levels. The maximum possibility is that the presence of flavonoids, tannins and alkaloids may responsible for hypoglycemic activity of ethanolic extracts of *S. asperrimus*.

INTRODUCTION

Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both¹. Without enough insulin, the cells of the body cannot absorb sufficient glucose from the blood; hence blood glucose levels increase, which is termed as hyperglycemia. If the glucose level in the blood remains high over a long period of time, this can result in long-term damage to organs, such as the kidneys, liver, eyes, nerves, heart and blood vessels. Complications in some of these organs can lead to death². Primarily ROS plays an important role in the host defence mechanism against microorganisms, but the increased production of ROS is associated with the

onset of a variety of diseases including cancers, inflammation, neurodegeneration, Parkinson's disease, atherosclerosis and premature ageing. A number of different free radicals and non radicals are produced during normal aerobic metabolism. A collective term, "reactive oxygen species"(ROS), is used for oxygen derived species including oxygen bearing free radicals, as well as certain non radicals. Some non oxygenated radicals are also generated in biological systems, such as carbon-centered free radicals (e.g. alkyl radical, R-H2C. free radicals) and sulphur centered radicals (e.g. thioyl radical, R-S.) which are produced by the attack of free radicals on hydrocarbons and the oxidation of glutathione, respectively^{3,4}. The NAPRALERT database lists over 1200 species of plants representing 725 genera in 183 families extending from the marine algae and fungi with antidiabetic activity⁵.

Address for correspondence

Pradeep Kumar Samal

Email- samalpharmacology@rediffmail.com

Literature survey reveals that *Strobilanthes asperimus* (Family: Acanthaceae) native of India, Japan, Malasia and rest of Asia. It is a high-climbing liana or large shrub, with white blueish flower. Main chemical constituents are flavonoids, alkaloids and tannins. Its also have cardiac glycoside and phenols^{6,7}. Traditional knowledge reveals that the leaves is used as Hypoglycemic. It is also used in goiter, antitumor's , tuberculosis , bactericidal and also in fungicidal. But still there is no scientific exploration of this plant as an antidiabetic agent. So a suitable plan has been designed for the evaluation of anti-diabetic activity.

MATERIAL AND METHODS

Authentication, Collection and Drying of Plant Material:

Fresh leaves of *Strobilanthes asperimus* Nees were collected from Thakur Chhedilal Barrister Agriculture College and Research Centre, Bilaspur, Chhattisgarh in month of September 2011, and air dried at room temperature after wash with tape water. The Plant material was authenticated by Dr. H.B. Singh, Head, Raw Material Herbarium & Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. The specimen voucher no. **(NISCAIR/RHMD/Consult/-2011 12/1830/130)** was deposited in the dept. The crude drug was powdered in hand grinder after drying.

Drug and chemicals:

Analytical grade Chemical was used in this study. Metformine drug was obtained from Aarti drugs limited Mumbai as a gift sample. Silymarin (Micro labs, Bangalore) was purchased from local market. Chemical like ethanol (CDH, Mumbai), anesthetic ether (CDH, Mumbai), alloxan monohydrate and CCl₄ (Ranbaxy, Delhi) and other phytochemical reagents were provided by Institute. Glucose kit (GOD/POD)(Span Diagnostics Ltd, Surat) was purchased from local market.

Preparation of extract:

The powdered drug was taken in a soxhlet extractor and subjected for extraction. The extraction was carried out for 16 hrs with ethanol.

Phytochemical Screening:

The Ethanolic extract of *Strobilanthes asperimus* was subjected to Phytochemical screening for the content of alkaloids, glycoside, flavonoids, phenolic and tannins compounds⁸⁻¹⁰.

Animals:

Wistar albino rats (150-200g) purchased from IICB Kolkata was maintained in the department animal house of for experimental purpose. Then all the animals were acclimatized for seven days under standard husbandry conditions, i.e.; room temperature of 25±1⁰ C; relative humidity 45-55% and a 12:12h light/ dark cycle. The animals had free access to standard (Pranav Agro Industries Ltd, Vadodara, India), with water supplied *ad libitum* under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. The approval of the Institutional Animal Ethical Committee (IAEC) of SLT Institute of Pharmaceutical Sciences, Bilaspur (Chhattisgarh) was taken prior to the experiments. All the protocols and the experiments were conducted in strict compliance according to Institutional Animal ethical Committee guidelines(ReferenceNo.IAEC/Pharmacy/2012/51) provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Approval No. 994/a/GO/06/CPCSEA).

Determinations of Acute Oral Toxicity (LD50):

The acute oral toxicity (AOT) of alcoholic extract of fruits of *Strobilanthes asperimus* were determined by using female albino mice (Wistar strains) weighing between 35-50 gm those maintained under standard husbandry conditions.

The animals were fasted 3 hrs prior to the experiment, up and down procedure (OECD guideline no. 425).⁵ Animals were administered with single dose of extracts dissolved in 2% w/v acacia and observed for its mortality during 48 hours study period (short term) toxicity. Based on short-term profile of drug, the dose of the next animals was determined as per as OECD guideline 425. All the animals were also observed for long term toxicity (14 Days). The LD50 of the test extract was calculated using AOT 425 software provided by Environmental protection agency, USA¹¹.

EVALATION OF ANTIDIABETIC ACTIVITY:

Alloxan induced diabetic animals:

Wistar rats, weighing (150-200 gm) were divided into 5 groups consisting of 6 animals in each group. Group I treated as normal control which received normal saline p.o.. Group II treated as diabetic control which received alloxan (120mg/kg i.p). Group III treated as standard which received metformin (80 mg/kg i.p) once a day for one week. Group IV treated as test 1 which received ethanolic extracts of *Strobilanthes asperimus* leaves (EESA) at dose 100 mg/kg body weight p.o..Group V treated as test 2 which received ethanolic extracts of *Strobilanthes asperimus* leaves (EESA) at dose 200 mg/kg body weight p.o.¹².

The treatment of animal began on the 3rd day after alloxan injection and this was considered as 1st day of treatment. Treatment was done for 3 weeks

and Blood samples were collected from the tail vein of each rat at 0, 1, 7, 14 and 21 days after drug administration. Blood glucose levels were determined by using portable glucometer (Johnson and jhonsan). Finally at 21st day's blood sample were collected from cardiac puncture of each rat which was earlier anaesthetized by diethyl ether and measured blood glucose levels through GOD/POD method by using UV spectrophotometer. And antioxidants parameters MDA and CAT also measured. Body weights of the animals were also measured twice a week up to three weeks.

Oral Glucose Tolerance Test:

Oral glucose tolerance test was performed in rats after induction of diabetes. Each group of rats was subjected to oral administration of glucose at a dose of 2g/kg body weight of rat, 5th days after induction of diabetes. Animals were kept on 12 hour fasting before administration of glucose. Finally blood sample of each rat were collected from tip of tail before 1 hour and after 0.5, 1.0, and 1.5 and 2.0 hour after glucose administration and subjected to blood glucose level measurement.

Estimation of plasma Glucose:

Blood glucose was estimated by glucose oxidase/peroxidase (GOD/ POD) method. The red colour so developed was measured spectrophotometrically at 505 nm. The intensity of red colour is proportional to the concentration of the glucose present in the specimen¹³.

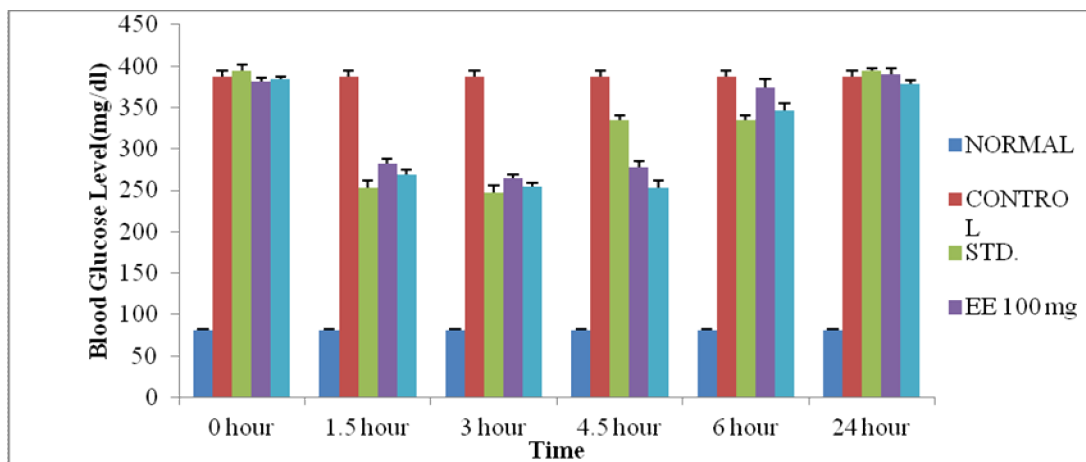


Fig.1: Effect of Ethanolic extracts of *S. asperrimus* on Blood glucose level in alloxan induced diabetic rats (in hour)

RESULTS AND DISCUSSION

Phytochemical screening reveals that there is presence of adequate amount of alkaloids, glycoside, flavonoids, phenolic and tannins compounds in *S. asperrimus* ethanolic extract. After the treatment of crude drug and standard the blood glucose level was measured in every 1.30 hour. The result was shown in figure-1. The extracts of *S. asperrimus* have shown significantly decrease ($P < 0.001$) in glucose level for

long period of time. Group V EESA (at dose of 200 mg/kg body weight) show low blood glucose level 190.16 ± 5.77 mg/dl as compared to Group IV EESA (at dose of 100 mg/kg body weight) shows 208.16 ± 5.13 mg/dl, Group II (control) shows 376.5 ± 14.03 mg/dl and Group III (Standard Drug Metformin at the dose 120 mg/kg body weight) shows 183.33 ± 14.06 mg/dl respectively. The results are shown in Fig. 2.

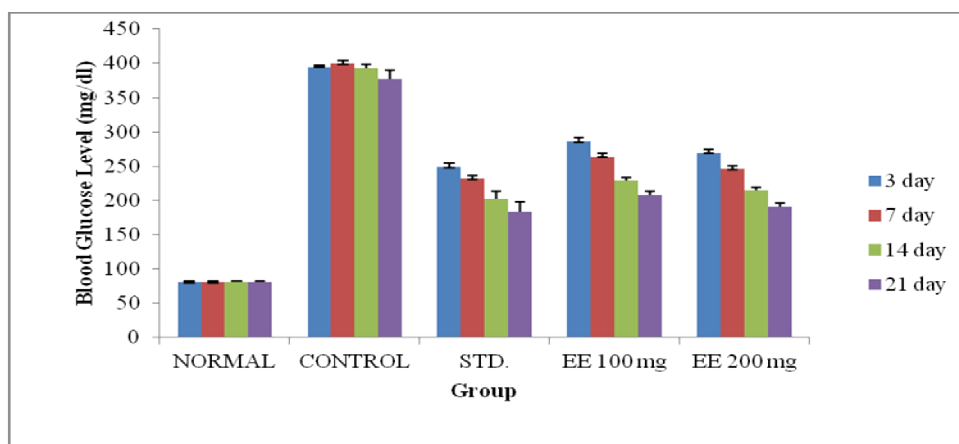


Fig. 2: Effect of Ethanolic extracts of *S. asperrimus* on Blood glucose level in alloxan induced diabetic rats (in days)

The Blood glucose level was reduced considerably within 1st day and maximum within 21 days of the drug administration. The EESA (at dose of 200 mg/kg body weight) show maximum effect 49.53 % ($P < 0.001$) as compared to EESA (at dose of 100 mg/kg body weight) 44.71 % ($P < 0.01$). In oral glucose tolerance test in hyperglycemic rats both extracts

dose have shown considerable reduction in blood glucose levels. The EESA (at dose of 200 mg/kg body weight)) have shown significant decrease ($P < 0.001$) in glucose level. Group V EESA (at dose of 200 mg/kg body weight) show low blood glucose level 196.66 ± 26.03 as compared to Group IV EESA (at dose of 100 mg/kg body weight) shows 230.16 ± 12.99 , Group II

(control) shows 455 ± 31.69 and Group III (Standard Drug Metformin at the dose 120 mg/kg body weight)

shows 210.39 ± 31.09 respectively. The results are shown in fig. 3.

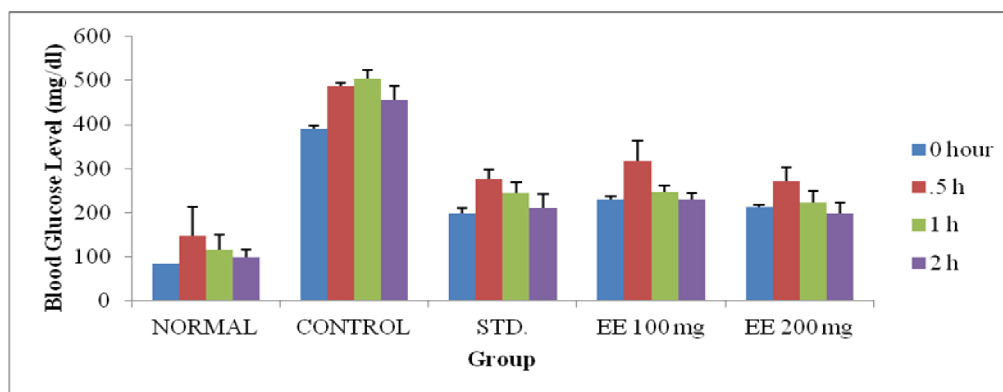


Fig.3: Effect of Ethanolic extracts of *S. asperrimus* on Blood glucose level in alloxan induced diabetic rats (in OGTT) The reduction in glucose level is significant ($P < 0.001$) in the treated animals at 0, 0.5h, 1h and 2h after drug administration. The maximum percentage reduction in blood glucose level was found to be in ethanolic extract (200mg/kg body weight) shows 56.77% , while ethanolic extract (100mg/kg body weight) shown 49.41% blood glucose level. Treatment of the diabetic rats with Metformin (120 mg/kg body weight) shown 53.84 % fall of blood glucose after 2 h

treatment. In alloxan- induced diabetic rats both extracts dose show loss of body weight. The results are shown in fig.4. In ethanolic extracts shown significantly increase the body weight as compared to control group. The maximum percentage increase in body weight was found to be in ethanolic extract (200mg/kg body weight) shows 25.87 % , while ethanolic extract(100mg/kg body weight) shown 24.89 %^{14,15}.

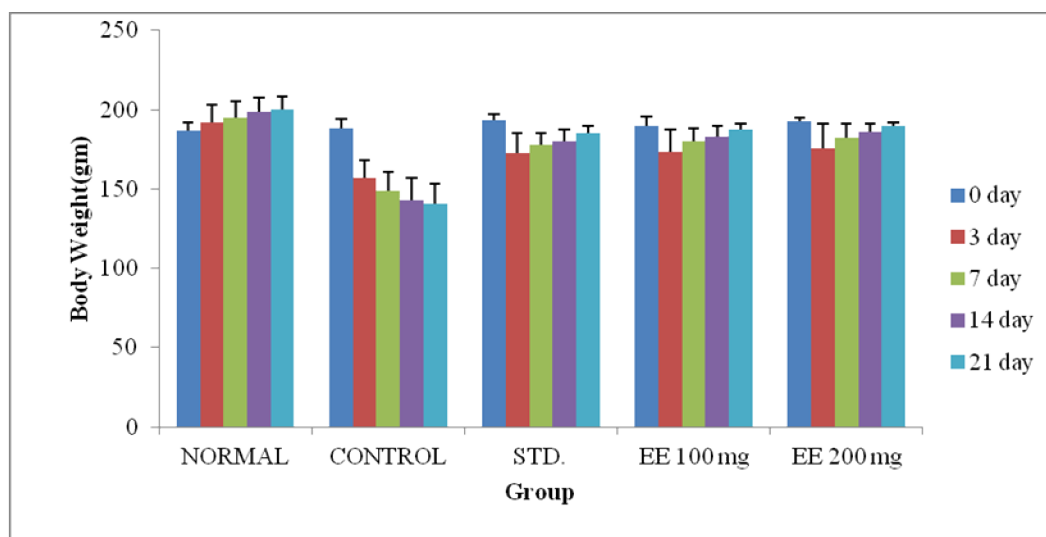


Fig. 4: Effect of Ethanolic extracts of *S. asperrimus* on Body weight in alloxan induced diabetic rats (in g)

CONCLUSION

In the present study, phytochemical investigation of ethanolic extracts of *S. asperrimus* showed the presence of alkaloids, glycoside, flavonoids, phenolic and tannins compounds. The

maximum possibility is that the presence of flavonoids, tannins and alkaloids may responsible for hypoglycemic activity of ethanolic extracts of *S. asperrimus*.

REFERENCES

1. Barcelo A, Rajpathak S, Incidence and prevalence of diabetes mellitus in the Americas, *American Journal of Public Health*,10: (2001) 300-308.
2. Pari L, Saravanan R, Antidiabetic effect of diasulin, a herbal drug, on blood glucose, plasma insulin and hepatic enzymes of glucose metabolism in hyperglycaemic rats, *Diabetes Obesity and Metabolism*, 6:(2004)286–292.
3. Ivanova E, Ivanova B, Mechanisms of the extracellular antioxidant defend, *Experimental Pathology and Parasitology*, 4:(2000) 49-59.
4. Cheeseman KH, Slatter TF, An Introduction to free radical biochemistry. *British Medical Bulletin*, 49: (1993) 481-493.
5. World Health Organisation: 1985. Diabetes mellitus: Report of a WHO Study Group. WHO Technical Report Series 727.
6. Tiwari SKS, Ojha BM, Singh A, Sahu RK, Book of Chattisgarh ayurvedic traditional plant collections, Agriculture College and Research Centre, Bilaspur (CG), Page no. 47.
7. Subramoniam A, Pushpangadan P, Rajasekharan S, Evans D A, Latha P G, Valsaraj R, Effects of *Artemisia pallens* Wall. on blood glucose levels in normal and alloxan-induced diabetic rats, *Journal of Ethnopharmacology*,50: (1996) 13-17.
8. Trease GE., Evans MC. Text book of Pharmacognosy London, BailliereTindall, 12:193(1983), 336.
9. Khandelwal K R. Practical Pharmacognosy. Techniques and Experiments Pune, Nirali Prakashan, 2: (2000)149-155.
10. Kokate CK. Practical Pharmacognosy. New Delhi, Vallabh Prakashan, 4: (1994)110-111.
11. OECD 2001-guideline on acute oral toxicity (AOT) Environmental health and safety monograph series on testing and adjustment No.425.
12. Danielle M.O. Aragao, Lyvia Guarize, Juliana Lanini, Juliana C. da Costa, Raúl M.G. Garcia, Elita Scio, Hypoglycemic effects of *Cecropia pachystachya* in normal and alloxan-induced diabetic rats, *Journal of Ethnopharmacology* ,128: (2010) 629–633.
13. Barham, D, Trinder P, An improved color reagent for the determination of blood glucose by the oxidase system. *Analyst*. 97, (1972)142–145.
14. Muslim N S, Ng KW, Itam A, Nassar Z D, Ismail Z, Majid A, Evaluation of Cytotoxic, Anti-angiogenic and Antioxidant Properties of Standardized Extracts of *Strobilanthes crispus* Leaves, *International Journal of Pharmacology*,6(5): (2010)591-599.
15. Hanoon N A N, Asmah R, Rokiah MY, Fauziah O, Faridah H, Antihyperglycemic, Hypolipidemic and Antioxidant Enzymes Effect of *Strobilanthes crispus* Juice in Normal and Streptozotocin- Induced Diabetic Male and Female Rats, *International Journal of Pharmacology*, 5(3): (2009) 200-207.