ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. IX: 743-747: 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.com

ANTIDIABETIC ACTIVITY OF *SWERTIA CHIRAYITA* AND *PUNICA GRANATUM* AGAINST STREPTOZOTOCIN INDUCED DIABETES IN RATS

Manoj Kumar *et al.,*

KEYWORDS

Swertia chirayita
Punica granatum
Streptozoticin
Glibenclamide

Proceedings of National Conference on Harmony with Nature in Context of Resource Conservation and Climate Change
(HARMONY - 2016)
October 22 - 24, 2016, Hazaribag,
organized by
Department of Zoology, Botany, Biotechnology & Geology
Vinoba Bhave University,
Hazaribag (Jharkhand) 825301
in association with
National Environmentalists Association, India
www.neaindia.org

The Ecoscan
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES

MANOJ KUMAR*, SUKUMAR DANDAPAT, MANORANJAN PRASAD SINHA
Department of Zoology,
Ranchi University, Ranchi - 834008
e-mail: dr17mk@gmail.com

ABSTRACT

In the present study the aqueous leaf extracts of *Swertia chirayita* and *Punica granatum* were analyzed for antidiabetic activity against streptozotocin (STZ) induced diabetes in albino rats. The blood glucose level was recorded before and after administration of drug. The blood glucose levels of animals of group 4 was recorded before and after administration of glibenclamide. The results pellucidly revealed that the leaf extracts of *Swertia chirayita* and *Punica granatum* act as antidiabetic agent. The blood glucose levels in *Swertia chirayita* and *Punica granatum* treated diabetic rats were 250.62 ± 2.35 mg/dl and 265.58 ± 3.00 mg/dl on day 0, which gradually decremented by the cessation of 21st day to 86.5 ± 2.85 mg/dl and 90.23 ± 2.03 mg/dl respectively. Thus it can be concluded that the aqueous leaf extracts of *Swertia chirayita* and *Punica granatum* act as antidiabetic agents, albeit their impact is less efficacious as compared to the standard drug glibenclamide, but their positive impact against diabetes is a promising development, which may become a substratum of future medicines of plant inchoation for remedying diabetes.

***Corresponding author**

INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Factors responsible for hyperglycemia include reduced insulin secretion, decreased glucose utilization, increased glucose production (Kavita and Dattari, 2013). The no. of diabetics in the world is estimated to cross the 300 million mark by the end of 2025 (Verma *et al.,* 2013). Irregular lifestyle, consumption of energy rich diet, obesity, etc are factors responsible for this rise in diabetic patients. The prevalence of diabetes is more pronounce in third world countries (Alam *et al.,* 2011). In diabetes the oxidative stress increases which is mainly due to an increased production of reactive oxygen species and a sharp reduction of antioxidant defenses occur (Eze *et al.,* 2012).

The management of diabetes without any side effect is still a challenge to medical system. During recent years medicines of plant origin have gained interest, because plants synthesize a variety of secondary metabolites which have antioxidant properties (Bhaskar and Kumar, 2012). *Swertia chirayita* is an important herb, commonly available in India, China, and Nepal. The plant is found at an altitude of 12000-3000 m and available throughout the year (Kumar *et al.,*2015)It has been widely used in ayurvedic and unani medicine as an antihelmintic, febrifuge and stomach and liver tonic. Several studies has been carried out in animals concerning hepatoprotective activity of the genus. *Swertia chirayita* is considered most important for its medicinal properties. The bitterness and antipyretic properties are attributed to amarogentin (most bitter compound isolated till date). It has also been reported that swerchirin, a compound with xanthone structure, has hypoglycaemic properties (Saxena *et al.,* 1991). *Punica granatum* L., commonly known as pomegranate, is a small tree native to Asia. It has been used as traditional medicine in many countries for the treatment of dysentery, diarrhea, and helminthiasis (Choi *et al.,* 2011). Numerous phytochemical constituents have been reported to be present in different parts of *Punica granatum* (Kumar *et al.,* 2013). Therefore owing to the medicinal possibilities of *Swertia chirayita* and *Punica granatum*, the present study was carried out to assess the anti-diabetic activity of aqueous leaf extracts of *Swertia chirayita* and *Punica granatum*.

MATERIALS AND METHODS

Plant Materials

The fresh tender leaves of *Swertia chirayita* and *Punica granatum* were collected. The leaves were washed with deionized water and disinfected with 0.1% $HgCl_2$ solution for 5 min and dried in shade away from light for 15 days and ground to fine powder using electrical grinder and sieved (Kumar *et al.,* 2013; Kumar *et al.,* 2016).

Preparation Of Plant Extract

The fine powders (50g) of *Swertia chirayita* and *Punica granatum* were made into thimble for loading in Soxhlet apparatus and extraction was done using distilled

water. The extraction was done continuously for 72 hours. The extracts thus obtained were concentrated under vacuum rotary evaporator and extracts were kept in desiccators until used (Dandapat *et al.,* 2015).

Phytochemical Screening

Preliminary phytochemical screening were conducted on *Swertia chirayita* and *Punica granatum* leaf sample according to previously published standards (Kumar *et al.,* 2013).

Animals

Albino rats weighing about 175-200 g were used in the study. They were maintained under standard laboratory conditions and ambient temperature of 25 ± 2ºC and relative humidity at 50 ± 15%, with dark-light cycle of 12h. Animals were fed with a commercial pellet diet and water *ad libitum* (Choudhury *et al.,* 2013). The experiment was performed after prior approval of Ethics Committee of Ranchi University, Ranchi (Proceeding No. 46, Page no. 137)

Acute Toxicity Tests

The acute toxicity tests were carried out as per stair case method (Mallory and Evelyn, 1956). 50 albino mice of either sex were used to determine LD_{50} of extracts. The mice were divided into 3 groups of 10 mice each as follows for *Swertia chirayita* and *Punica granatum* aqueous leaf extract.

Group A: received 1 ml of distilled water orally

Group B: received 0.1 ml/kg/day i.p. of Streptozotocin

Group C: received 200 mg/kg body weight of extract orally

Group D: received 500 mg/kg body weight of extract orally

No mortality was observed until 500 mg/kg body weight of both extracts.

Drugs

Sterptozotocin (Stz)

Streptozotocin solution was prepared in 0.1M sodium citrate buffer of pH 4.5. STZ was administered at a dose of 5-60 mg/kg by intraperitoneal route (Ghosh, 2008).

Sodium Citrate Buffer (Scb)

Composition of 100 ml of 0.1M citrate buffer of pH 4.5.

Glibenclamide

Glibenclamide was used as standard drug at a dose of 0.5mg/kg body weight by oral route and results were compared with test drug (aqueous leaf extracts of *Swertia chirayita* and *Punica granatum*)

Glucometer

the glucometer used was Accu-check-Active™. Roche Group, Germany for measuring blood glucose level.

Induction Of Diabetes

After 18 hrs of fasting, diabetes was induced in rats by intraperitoneal (i.p.) injection of STZ dissolved in 0.1 M sodium citrate buffer (pH 4.5) at a dose of 50-60 mg/kg body weight. Animals were observed for first 24 hrs following the injection of STZ for any evidence of allergic reactions, behavioural changes and convulsions. Animals were fed with 5% glucose solution to overcome STC induced hypoglycaemia. No reactions were observed in any animal

After 72 hrs of STZ injection, blood glucose levels were recorded. Only those animals whose blood glucose levels were between 200-300 mg/dl with glycosurea were selected for study and were divided into 6 groups as follows. The control group was not given STZ.

Group 1 (Normal control group), Group 2 (Diabetic control group), Group 3 (Extract test group), Group 4 (Glibenclamide standard group).Group 1 received 0.5 ml of normal saline daily for 21 days by oral route. Blood glucose levels were recorded before the administration of normal saline of day 0 at 10 am., then on 3rd, 7th, 14th and 21st day at 10 am.Group 2 received 0.5 ml of normal saline daily orally for 21 days. The animals were observed for evidence of any behavioural changes, hyperglycaemia and convulsions. The blood glucose levels of this group was recorded at 9 am on day 0 before administering normal saline.Group 3 received dose of leaf extracts at 200 mg/kg concentration orally for 21 days daily. The blood glucose level were recorded at 10 am on day 0 before administration of drug. Then after administration of drug the blood glucose level was recorded on 3rd, 7th, 14th and 21st day at 10 am. The animals were observed for evidence of any hypoglycaemia and convulsions. The blood glucose levels of animals f group 4 was recorded at 10 am on day 0 before administration of glibenclamide at a dose of 0.5 mg/kg body weight daily orally in the morning for 21 days. Their blood glucose levels were recorded on 3rd, 7th, 14th and 21st day at 10 am. The animals were observed for evidences of any hypoglycaemia and convulsions.

RESULTS AND DISCUSSION

Diabetes is said to be one of the most important and critical health problem in the future. Adequate treatment of diabetes is thus very important. Medicinal plants play an important role in the management of DM. The results obtained is represented in figure 1, which shows the blood glucose levels of group1, group 2, group 3A, group3B and group 4. The results clearly revealed that the aqueous leaf extracts of *Swertia chirayita* and *Punica granatum* act as antidiabetic agent by reducing the increased blood glucose level in the blood of the induced diabetic rats. The blood glucose levels in *Swertia chirayita* and *Punica granatum* treated diabetic rats were 250.62 ± 2.35 mg/dl and 265.58 ± 3.00 mg/dl on day 0, which gradually decreased by the end of 21st day to 86.5 ± 2.85 mg/dl and 90.23 ± 2.03 mg/dl respectively.

Punica granatum has been reported to have antioxidants higher than green tea, cranberries and even red wine (Radhika *et al.,* 2011). STZ induced hyperglycaemis in animals is considered to be a good model for the preliminary screening of agents active against type II diabetes. STZ is a potent DNA methylating

Table 1: Showing the results of preliminary phytochemical screening of aqueous leaf extracts of *Swertia chirayita* and *Punica granatum*

Phytochemicals	*Swertia chirayita*	*Punica granatum*
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+
Quinones	+	-
Saponins	+	+
Tannins	+	+

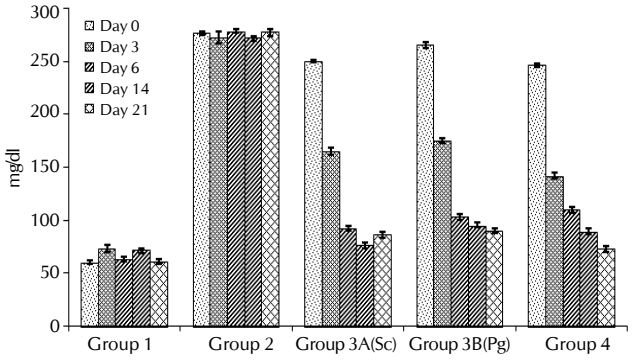

Figure 1: Showing the impact of aqueous leaf extract of *Swertia chirayita* (Group 3A) and *Punica granatum* (Group 3B) on blood glucose levels of diabetic rats, in comparison with the standard medicine Glibenclamide (group 4), Group 1 is control receiving 0.5 ml of normal saline

agent and acts as nitric oxide donor in pancreatic cells. Beta cells are particularly sensitive to damage by nitric oxide and free radicals because of their low levels of free radical scavenging enzymes. Administration of STZ results in increased blood glucose levels (Bhaskar and Kumar, 2012).

The preliminary phytochemical screening (table – 1) shows that both *Swertia chirayita* and *Punica granatum* aqueous leaf extracts contain several phytochemicals such as alkaloids, flavonoids, phenols, quinones, saponins tannins etc., which have been reported to possess strong antioxidant and radical scavenging activity (Kumar *et al.,* 2013; Dandapat *et al.,* 2014: Kumar *et al.,* 2015;). Antioxidants are effective against diabetic complications, and may be beneficial either by ingestion of natural antioxidants or through dietary supplementation (Bajaj and Khan, 2012). There are multiple sources of reactive oxygen species (ROS) production in diabetes including those of mitochondrial and non-mitochondrial origins. Oxidative stress results from an imbalance between radical-generating and radical scavenging systems (Bajaj and Khan, 2012). Mishra *et al*. (2015) reported that the phytochemicals such as alkaloids, flavonoids, phenols, saponins and tannins can be used as curative agents against diabetes. Antioxidants are effective in reducing diabetic complications, indicating that they may be beneficial in diabetes. Thus the antidiabetic activity of leaf extracts of *Swertia chirayita* and *Punica granatum* can be attributed to the presence of phytochemicals which act as antioxidant and radical scavenging agents. Thus on the basis of present work it can be concluded that the aqueous leaf extracts of *Swertia chirayita* and *Punica granatum* act as antidiabetic agents although their impact is less effective as compared to the standard drug glibenclamide, but their positive impact against diabetes is indeed a promising development, which may become a basis of future medicines of plant origin for curing diabetes.

ACKNOWLEDGEMENT

The authors acknowledge the facilities made available by Department of Zoology, Ranchi University, Ranchi. The authors are further thankful to the Principal, St. Xavier's College, Ranchi and Head, Department of Zoology, St. Xavier's College, Ranchi for their continuous support and motivation, without which this work may not have been completed.

REFERENCES

Alam, K. D., Ali, M. S., Mahjabeen, S., Hassan, R.. Rahman, F. and Chowdhury, R. M. A. A. 2011. Potential hypoglycemic effect of *Swertia chirayita* - an Indian subcontinent herb with important medicinal value. *Pharmacology online*. **2:** 642-647.

Bajaj, S. and Khan, A. 2012. Antioxidants and diabetes. *Indian J. Endocrinological Metabolism*. **16(Supplement 2):** S267-S271.

Bhaskar, A. and Kumar, A. 2012. Antihyperglycemic, antioxidant and hypolipidimic effect of *Punica granatum* L. flower extract in streptozotocin induced diabetic rats. *Asian Pacific J. Trop. Biomed.,* pp. s1764-s1769.

Choi, J. G., Kang, O. H., Lee, Y. S., Chai, H. S., Oh, Y. C. and Brice, O. O. 2011. *et al*. In-vitro and in-vivo antibacterial activity of *Punica granatum* peel ethanol extract against *Salmonella,* Evid. Based complement. *Alternative medicine*. pp. 1-8.

Choudhury, S., Sharan, L. and Sinha, M. P. 2013. Antidiarrhoeal potentiality of leaf extracts of *Moringa oleifera*. *British J. Appl. Sci. Tech.* **3(4):** 1086-1096.

Dandapat, S., Kumar, M. and Sinha, M. P. 2015. Assessment of Bioactivity of *Cinnamomum tamala* (Buch.-Ham.). *Turkish J. Agriculture – Food Science and Technology*. **3(3):** 121-125.

Dandapat, S., Kumar, M. and Sinha, M. P. 2014. Pharmacological and phytochemical screening of *Aegle marmelos* (L.) and *Cinnamomum tamala* (Buch.-Ham.) leaves for therapeutic efficacy. *Middle-East J. Scientific Research*. **22(5):** 626-632.

Eze, E. D., Dawud, F. A., Zainab, A. A., Jimoh, A., Malgwi, I. S. and Isa, A. S. 2012. Preliminary studies of effects of Vitamin C and zinc on some liver enzymes in alloxan - induced diabetic wistar rats. *Asian J. Med. Sci.* **4(1):** 17-22.

Ghosh, M. N. 2008. Some standard drug & salt solutions & some useful information. *Fundamentals of Experimental Pharmacology, (2008); 4th Edition. Kolkata.*

Kavitha, K. N. and Dattari, A. N. 2013. Experimental evaluation of antidiabetic activity of *Swertia chirayita*-aqueous extract. *J. Public Health and Medicinal Research*. **1(2):** 71-75.

Kumar, M., Dandapat, S., Kumar, A. and Sinha, M. P. 2013. Determination of Nutritive value and Mineral elements of Five-leaf Chaste Tree (*Vitex negundo* L.) and Malabar nut (*Adhatoda vasica* Nees). *Academic J. Plant Sciences*. **6(3):** 103-108.

Kumar, M., Dandapat, S., Sinha, M. P. 2016. Antioxidant activity of Chiraita (*Swertia chirayita*) and Anar (*Punica granatum*). *European J. Pharmaceutical and Medical Research*. **3(2):** 267-269.

Kumar, M., Dandapat, S. and Sinha, M. P. Phytochemical analysis and growth inhibitory impact of *Swertia chirayita* aqueous leaf extract against some human pathogens. World Journal of Zoology, (2015); **10(3):** 188-190.

Kumar, M., Dandapat, S. and Sinha, M. P. 2015. Phytochemical screening and antibacterial activity of aqueous leaf extract of *Punica granatum*. *Balneo Research J.* **6(3):** 168-171.

Kumar, M., Kumar, A., Dandapat, S., Sinha, M. P. 2013. Phytochemical screening and antioxidant potency of *Adhatoda vasica* and *Vitex negundo*. *The Bioscan*. **8(2):** 727-730.

Mallory, H. T., Evelyn, E. A. 1956. The Determination of Bilirubin with Photoelectric Colorimeter. *J. Biol. Chem.* **119:** 481-485.

Mishra, C, Singh, B., Singh, S., Siddiqui, M. J. A., Mahdi, A. A. 2015. Role of phytochemicals in Diabetes lipotoxicity: an overview. International *J. Research and Development in Pharmacy and Life*

Sciences. **4(4):** 1604-1610.

Radhika, K. H., Smila, K. H. and Muthezhilan, R. 2011. Antidiabetic and hypolipidemic activity of *Punica granatum* Linn on alloxan induced rats. *World J. Medical Sciences.* **6(4):** 178-182.

Saxena, A. M., Bajpai, M. B. and Mukherjee, S. K. 1991. Swerchirin induced blood sugar lowering of streptozotocin treated hyperglycaemic rats. *Indian J. Exp. Biol.* **29:** 674-675.

Verma, V. K., Sarwa, K. K. and Zaman, K. 2013. Antihyperglycemic activity of *Swertia chirayita* and *Andrographis paniculata* plant extracts in streptozotocin-induced diabetic rats. *International J. pharmacy and Pharmaceutical Sciences.* **5(3):** 305-311.