IJPSR (2020), Volume 11, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 April 2019; received in revised form, 21 August 2019; accepted, 01 September 2019; published 01 February 2020

IN-VITRO AND *IN-VIVO* ANTIDIABETIC ACTIVITY OF METHANOLIC EXTRACT OF AERIAL PARTS OF *ALANGIUM SALVIFOLIUM* SUBSPECIE HEXAPETALUM (WANGERIN)

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Keywords:

Antidiabetic, Streptozotocin, Methanolic extract, Metformin hydrochloride, Histopathology

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ABSTRACT: *Alangium salvifolium* is a plant that is traditionally used to treat many diseases like laxative, antiepileptic, jaundice, antiulcer agent, agent to alleviate spasms, anthelmintic, emetic, antiprotozoal agent and hypoglycemic agent. Its subspecies *Alangium salvifolium* subsp. Hexapetalum (Wangerin) is also known for a variety of traditional use like hemorrhoids, rheumatism, and antidote for snake bite. The present work aims for the evaluation of the *in-vitro* and *in-vivo* anti-diabetic activity of methanolic extract of aerial parts of *Alangium salvifolium* subsp. Hexapetalum (Wangerin). The *in-vitro* antidiabetic activity was evaluated by a starch-iodine color assay method. *The in-vivo* method was performed by administering orally the methanolic extract of *Alangium salvifolium* subsp. Hexapetalum in streptozotocin-induced male Albino Wistar rats weighing 200 g. The study was compared using standard metformin hydrochloride (10 mg/kg body weight). The *in-vitro* method showed a dose-dependent anti-diabetic activity that is as the dose increases the percentage inhibition of enzyme activity also increases. The *in-vivo* anti-diabetic activity was accessed by comparing the body weight and blood glucose level on 0^{th}, 5^{th}, 10^{th} and 15^{th} day using a glucometer. Thus, the present study reveals that the methanolic extract of aerial parts of *Alangium salvifolium* subsp. Hexapetalum (Wangerin) was efficient in lowering blood glucose levels.

INTRODUCTION: Plants are widely used in Chinese medicine and Ayurveda-Indian traditional medicine. The traditional system of medicine is still continued to be widely practiced. They emphasize on the use of plants as a source of medicine for various ailments is increasing due to many reasons like an increase in population, side effects of several synthetic drugs, high treatment cost and resistance of many drugs [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(2).700-04

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(2).700-04

Diabetes mellitus is a complex disorder marked by hyperglycemia due to increased hepatic glucose production, decreased insulin secretion and reduced insulin action. Diabetes Mellitus is the leading cause of end-stage renal disease, non-traumatic lower extremity amputations and the affected individuals are susceptible to long term complications affecting the organs like skin, eye, nerves, blood vessels and kidney [2]. Management of diabetes with agents lacking side effects is still a challenge to the medical field. In spite of many advances in the management of this disease, the diabetes-related mortality graph is still increasing. Available synthetic drugs which are used for the treatment of this disease are allied with adverse effect and are unable to control metabolism adequately.

Because of this reason, there is a growing interest in herbal remedies [3]. The literature surveys unveils that *Alangium salvifolium* is traditionally used as laxative, antiepileptic, astringent, antiulcer agent, pungent, purgative, agent to alleviates spasms, anthelmintic, emetic, antiprotozoal agent and hypoglycemic agent. Antidiabetic activity of *A. salvifolium* in aqueous extract of stem and leaves has been reported [4].

The aim of the present work is to scientifically reveal the hypoglycaemic activity of methanolic extract of aerial parts of its subspecie *A. hexapetalum.*

MATERIALS AND METHODS:

Plant Collection and Verification: The aerial parts of *Alangium salvifolium* subsp. Hexapetalum was collected from Tirunelveli district, Tamil Nadu. The plant was identified and authenticated by Mr. Chelladurai, Research officer- Botany, Central Council for Research in Ayurveda and Siddha, Government of India (Accession no. FTN/183/2018).

Preparation of Extract: The plant's aerial parts were desiccated in air shade and ground to coarse powder through a mixer. Using the Soxhlet extraction method, the powdered drug was processed with solvents of increasing polarity. The extracts of petroleum ether, chloroform, methanol and water were subjected to preliminary phytochemical study.

Preliminary Phytochemical Study: The methanolic extract of aerial parts of *Alangium salvifolium* subsp. Hexapetalum was subjected to preliminary phytochemical screening to find the presence of phytoconstituents like alkaloids, carbohydrates, flavanoids, phenolic content and saponins.

Drugs and Chemicals: For *in-vitro* hypoglycaemic activity different concentrations of methanolic extract of aerial parts of *Alangium salvifolium* subsp. Hexapetalum prepared were 200,400,600 and 800 µg along with α- amylase solution (1 mg/ml), phosphate buffer and 1% starch solution. For the *in-vivo* study the methanolic extract of aerial parts of *Alangium salvifolium* subsp. hexapetalum of 200 mg and 400 mg/kg body weight was prepared in a 10% acacia solution and was administered orally. The study was compared with standard metformin hydrochloride (10 mg/kg body weight) prepared in a 10% acacia solution.

***In-vitro* Antidiabetic Activity:**

Starch-Iodine Colour Assay: Mixed 20 µl of α-amylase solution (1 mg/ml) with 390 µL of phosphate buffer (0.02 M phosphate buffer pH 7.0 containing 0.006 M NaCl, pH 7.0) having different concentration of extracts. The above samples were incubated at 37 °C for 10 min. After incubation added 100 µl of starch solution (1%) and the mixture was re-incubated for another 1 h. To the resulting solution added 0.1 ml of 1% iodine solution and 5 ml distilled water. The absorbance was measured at 565 nm. Blank determinations were carried out under the same reaction conditions. Percentage inhibition of enzyme activity was calculated using the formula [6].

$$\% \text{ Inhibition} = (A\text{-}C) \times 100 / (B\text{-}C)$$

Where, A = absorbance of the sample, B = absorbance of blank (without α-amylase) and C = absorbance of the control (without starch)

Acute Toxicity Study: Acute toxicity study of methanolic extract of the aerial parts of *Alangium salvifolium* subsp. Hexapetalum was determined in Wistar albino rats according to OECD guidelines no. 425 [5]. The overnight fasted animals were orally administered with the methanolic extract 2000 mg/kg body weight. Animals were continuously monitored for the first 3 h and then for 14 days for death, signs of discomfort, general behaviour and nervous manifestations.

Experimental Animals: Healthy male Wistar albino rats (200 g) were used for the study. They were maintained under standard conditions as per CPCSEA guidelines. The experimental protocol was approved by the Institutional Animal Ethics Committee (Registration no. 1118/PO/Re/S/07/ CPCSEA) The Dale View college of pharmacy and Research center, Trivandrum (IAEC approval no. DVCP/2018/005). The animals were habituated for at least one week before use.

Streptozotocin-Induced Anti-diabetic Activity: Prior to the experimental study, animals were fasted by depriving them of food for overnight but allowing free access to water.

Induction of Experimental Diabetes: In experimental animals, diabetes was induced by intraperitoneal (i.p.) injection of nicotinamide (230 mg/kg) 15 min before streptozotocin (65 mg/kg i.p) administration. As STZ induces fatal hypoglycemia due to massive pancreatic insulin release, STZ-treated rats were provided with a 10% glucose solution after 3 h for the next 24 h to prevent fatal hypoglycemia. Different doses of the extracts were selected on the basis of acute toxicity study. Experimental design animals with blood glucose levels above 250 mg/dl were confirmed as diabetic and selected for the study. They were divided into five groups comprising six animals in each group.

Group I: Normal control administered with a 10% acacia solution.

Group II: Negative control, induced with diabetes and no treatment received.

Group III: Treated with reference drug Metformin hydrochloride at (10 mg/kg body weight).

Group IV: Treated with methanolic extract of aerial parts of *A. salvifolium* subsp. Hexapetalum at 200 mg/kg body weight orally for 15 days.

Group V: Treated with methanolic extract of aerial parts of *A. salvifolium* subsp. Hexapetalum at 400 mg/kg body weight orally for 15 days.

Blood samples were collected from the tail vein of the overnight fasted rats and blood glucose level was determined on 0^{th}, 5^{th}, 10^{th} and 15^{th} day along with body weight. On the 15^{th} day, all the animals were humanely sacrificed using CPCSEA recommended euthanasia procedure (Carbon dioxide inhalation method) and evaluated for the biochemical parameters along with histopathology of the pancreas tissue [7].

Statistical Analysis: All values were expressed as Mean ± SEM. The data was statistically analyzed by Bonferroni Multiple Comparison Test. A P-value of less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION:

Preliminary Phytochemical Screening: Many phytoconstituents present in plants are used to screen and analyze biologically active compounds. Alkaloid-the secondary metabolite present in plants has been investigated for antidiabetic activity. Glycolysis is the center for carbohydrate metabolism where the sugar is converted to glucose by a series of reactions catalyzed by enzymes like hexokinase, pospho fructokinase and pyruvate kinase. Alkaloid amplifies the activity of these enzymes and leads to glucose transport, carbohydrate digestion and absorption. They also cause the regeneration of pancreatic beta cells and insulin secretion. Similarly, flavonoids are also reported to have anti-diabetic activity. They act by targeting glycogen synthesis, glycolysis and gluconeogenesis [8]. The preliminary phytochemical screening of methanolic extract of aerial parts of *A. salvifolium* subsp. Hexapetalum confirms the presence of flavonoids, alkaloids and phenolic content.

Evaluation of *in-vitro* Anti-diabetic Activity: Alpha-amylase is an enzyme present in saliva and pancreatic juice. It breaks down insoluble starch molecules into absorbable molecules. Alpha-amylase inhibitors slow down the breakdown of carbohydrates and thus results in diminishing blood glucose. The methanolic extract of *A. salvifolium* subsp. Hexapetalum shows a dose-dependent action. As the concentration increases the % inhibition also increases.

TABLE 1: INHIBITION OF α-AMYLASE BY METHANOLIC EXTRACT OF *A. SALVIFOLIUM* SUBSP. HEXAPETALUM

Sample	Concentration (µg)	% of inhibition
Control B	-	-
Control C	-	-
Methanolic extract (10 mg/ml)	200	64.8
	400	80.0
	600	82.5
	800	92.9

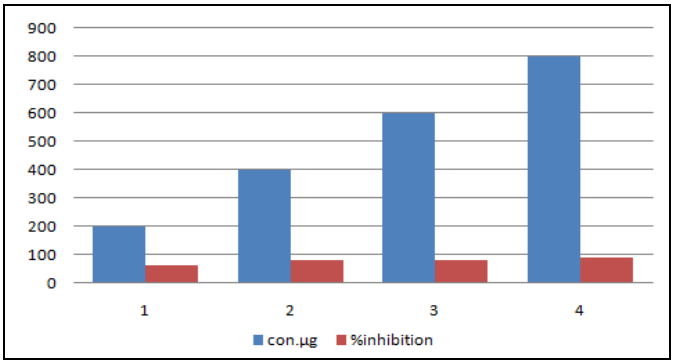

FIG. 1: RELATIONSHIP BETWEEN DIFFERENT CONCENTRATIONS OF METHANOLIC EXTRACT OF *A. SALVIFOLIUM* SUBSP. HEXAPETALUM AND PERCENTAGE INHIBITION

Evaluation of *in-vivo* Anti-diabetic Activity: In this study streptozotocin along with nicotinamide is used to induce diabetes in animals. Streptozotocin enters the B cells through glucose transporter (GLUT2) and causes DNA alkylation.

Damage of DNA leads to activation of poly ADP-ribosylation which causes depletion of cellular NAD+ and ATP. Increased ATP dephosphorylation leads to the formation of superoxide radicals along with hydrogen peroxide and hydroxyl radicals. Streptozotocin release nitric oxide which cause damage of DNA thus results in the destruction of B cells by necrosis [9].

Effect of Extract on Body Weight: Streptozotocin (65 mg/kg) induced experimental animals showed a remarkable (P<0.0001) decrease in body weight compared to the control group.

Standard metformin hydrochloride and the extract-treated animals showed significant (P<0.0001) increase in body weight when compared to vehicle-treated streptozotocin rats.

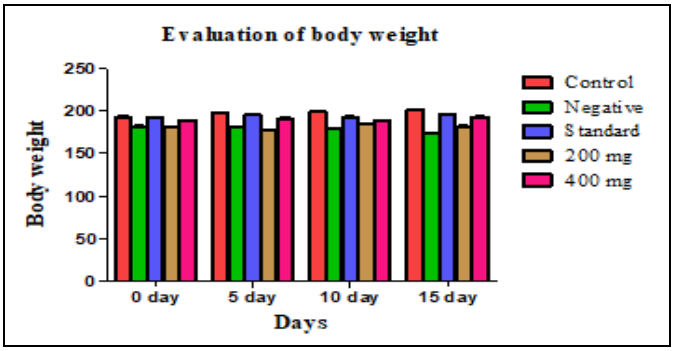

FIG. 2: EFFECT OF METHANOLIC EXTRACT OF *A. SALVIFOLIUM* SUBSP. HEXAPETALUM ON BODY WEIGHT OF STREPTOZOTOCIN INDUCED RATS

Effect of Extract on Blood Glucose: The blood glucose levels were measured in experimental animals on 0^{th}, 5^{th}, 10^{th} and 15^{th} days of treatment. Streptozotocin administered diabetic rats showed a significant (P<0.0001) increase in blood sugar when compared to control group. A significant decrease (P<0.05) (P<0.001) in blood sugar levels was seen in experimental rats orally administered with methanolic extract of *A. salvifolium* subsp. Hexapetalum (200 mg/kg & 400 mg/kg body weight) when compared to negative group.

TABLE 2: EFFECT OF METHANOLIC EXTRACT OF *A. SALVIFOLIUM* SUBSP. HEXAPETALUM ON BLOOD GLUCOSE LEVEL OF STZ-INDUCED RATS

S. no.	Group	Blood glucose at varying days (Mean ± SE)			
		0^{th}	5^{th}	10^{th}	15^{th}
1	Control	100 ± 0.33	106 ± 0.91	103 ± 0.98	103 ± 0.92
2	Negative	368 ± 0.33	349 ± 0.31	355 ± 0.34	347 ± 0.65
3	Standard	242 ± 0.34	225 ± 0.26	158 ± 0.42	120 ± 0.22
4	Extract(200mg)	320 ± 0.50	309 ± 1.8	281 ± 2.2	242 ± 1.0
5	Extract(400mg)	314 ± 0.17	297 ± 2.5	260 ± 1.7	205 ± 1.6

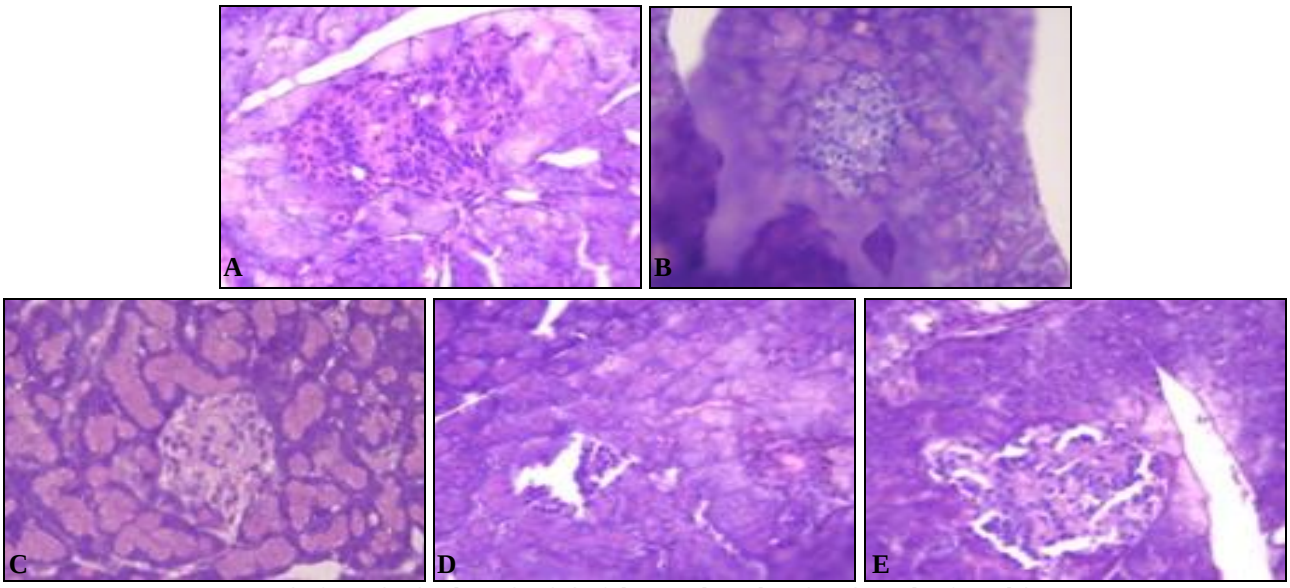

FIG. 3: HISTOPATHOLOGICAL EVALUATION OF PANCREAS

A) Pancreas of control group, B) Streptozotocin induced pancreas, C) Streptozotocin (65 mg/kg) + metformin HCl (10 mg/kg), D) Streptozotocin (65 mg/kg) + Extract (200 mg/kg), E) Streptozotocin (65 mg/kg) + Extract (400 mg/kg).

CONCLUSION: The present study deals with the pharmacological and preliminary phytochemical analysis of a methanolic extract of aerial parts of *Alangium salvifolium* subspecie Hexapetalum (Wangrein). According to the phytochemical evaluation, it shows the presence of phyto-constituents like alkaloids, phenolic contents and flavanoids. Studies have reported that alkaloids [10] and flavanoids [11] play a key role in alleviating diabetes mellitus. Both *in-vitro* and *in-vivo* method of anti-diabetic activities were evaluated. The methanolic extract of *Alangium salvifolium* subspecie Hexapetalum showed a significant *in-vivo* anti-diabetic activity which was supported by the *in-vitro* study. This may be due to the presence of alkaloids and flavonoids present in the methanolic extract.

ACKNOWLEDGEMENT: For the excellent and helpful support to enable the successful completion of this work, the authors are thankful to Athmic Biotec Solutions Pvt. Limited, present at kalliyoor, Kerala, India. For presenting the opportunity to carry out the anti-diabetic *in-vivo* study the authors are indebted to The Dale View College of Pharmacy & Research Centre, Trivandrum, India.

CONFLICTS OF INTEREST: We declare no conflicts of interest.

REFERENCES:

1. Narayanaswamy N and Balakrishnan KP: Evaluation of some medicinal plants for their antioxidant properties. International J of Chem Tech Res 2011; 3(1): 381- 85.
2. Ranjit Singh, Rajasree PH and Sankar C: Screening for anti-diabetic activity of the ethanolic extract of *Barleria cristata* seeds. Int J of Pharm & Life Sci 2012; 3(10): 2044-47.
3. Hegde K and Jaisal KK: Anti-diabetic potential of ethanolic extract of *Holarrhena antidysenterica* Linn. leaves. International Journal of Pharma Sciences and Res 2014; 5: 429-35.
4. Kalarani DH, Dinakar A and Senthilkumar N: Hypoglycemic and antidiabetic activity of *A. salvifolium* wang in alloxan induced diabetic rats. Asian Journal of Pharmaceutical and Clinical Research 2011; 4(1): 130-33.
5. OECD, Guidelines for testing of chemicals, Acute oral toxicity, Environmental Health and Safety Monograph Series on Testing and Adjustment No. 425, 2001, 1.
6. Sheikh JH, Iyo, Tsujiyama MT, Ashabul IM, Rajat SB and Hitoshi: A total phenolic content, anti-oxidative, antiamylase, anti-glucosidase and antihistamine release activities of Bangladeshi fruits. Food Sci Technol Res 2008; 14: 261-68.
7. Gogoi N: *In-vivo* anti diabetic activity evaluation of the bark of *Cascabela thevetia* L. in streptozotocin-induced diabetic rats. Int J Pharm Pharm Sci 2017; 9(6): 48-53.
8. Bharti SK, Krishnan S, Kumar A and Kumar A: Anti diabetic phytoconstituents and their mode of action on metabolic pathways. Therapeutic advances in Endocrinology & Metabolism 2018; 9(3): 81-100.
9. Szkudelski T: The mechanism of alloxan and STZ action in B cells of the rat pancreas. Physiol Res 2001; 50(6): 537-46.
10. Agarwal R, Sethiya NK and Mishra SH: Antidiabetic activity of alkaloids of *A. lanata* roots on streptozotocin- nicotinamide induced type-II diabetes in rats. Phar Bio 2013; 51(5): 635-42.
11. Sarian MN: Anti oxidant and anti diabetic effects of flavanoids: a structure – activity relationship based study. Bio Med Res Int 2017; 1-15.
12. Kavitha KN and Dattatri AN: Experimental evaluation of antidiabetic activity of *Swertia chirata* – aqueous extract. J Pub Health Med Res 2013; 1(2): 1-75.
13. Unnikrishnan R, Anjana RM and Mohan V: Diabetes mellitus and its complications in India. Nat Rev End 2016; 12: 357-70.
14. Anand P, Murali KY and Tandon V: Preliminary studies on antihyperglycemic effect of aqueous extract of *Brassia nigra* in streptozotocin-induced diabetic rats. Indian J Exp Biol 2007; 45: 696-70.
15. Swaroopa P, Reddy VJS, Koshma M, Sudharani Y, Basha SJ and Adithya TN: Review on antidiabetic activity on medicinal plants. International Journal of Pharmacological Research 2017; 7(12): 230-35.
16. Khan MF, Rawat AK, Khatoon S, Hussain MK, Mishra A and Negi DS: *In-vitro* and *in-vivo* antidiabetic effect of extracts of *Melia azedarach*, *Zanthoxylum alatum*, and *Tanacetum nubigenum*. Integrative Medicine Res 2018; 7(2): 176-83.
17. Arika WM, Nyamai DW, Agyirifo DS, Ngug, MP and Njagi ENM: *In-vivo* antidiabetic effect of aqueous leaf extract of *Azardirachta indica*, A. juss in alloxan induced diabetic mice. J Diabetic Complications Med 2016; 1(2): 1-6.
18. Ishnava KB and Motisariya DM: *In-vitro* study on α-amylase inhibitory activity of selected ethnobotanical plant extracts and its herbal formulations. Int J Pharmacogn Chinese Med 2018; 2(3): 1-11.
19. Nair SS, Kavrekar V and Mishra A: *In-vitro* studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. European J of Exp Bio 2013; 3(1): 128-31.
20. Rajeswari R, Lalitha V, Korah MC, Rahman PVJ and Kumar AS: Phytochemical and pharmacological evaluation of ethanolic extract of the *Artocarpus hirsutus* Lam. Leaves. IJPSR 2019; 10(4): 1972-80.
21. Sucharitha E and Estari M: Evaluation of antidiabetic activity of medicinal plant extracts used by tribal communities in rural areas of Warangal district, Andhra Pradesh, India. Biology and Medicine 2013; 5: 20-25.
22. Sharma AK and Gupta R: Anti-Hyperglycemic activity of aqueous extracts of some medicinal plants on Wistar rats. J Diabetes Metab 2017; 8(7): 1-7.
23. Sathiavelu A, Sangeetha S, Archit R and Mythili S: *In-vitro anti*-diabetic activity of aqueous extract of the medicinal plants *Nigella sativa*, *Eugenia jambolana*, *Andrographis paniculata* and *Gymnema sylvestre*. Int J Drug Dev & Res 2013; 5(2): 323-28.
24. Shravya S, Vinod BN and Christudas S: Pharmacological and phytochemical studies of *Alangium salvifolium* Wang. – a review. Bulletin of faculty of pharmacy Cairo University 2017; 55(2): 217-22.

How to cite this article:
Seena H, Kannappan N and Kumar PM: *In-vitro* and *in-vivo* anti diabetic activity of methanolic extract of aerial parts of *Alangium salvifolium* subspecie Hexapetalum (Wangerin). Int J Pharm Sci & Res 2020; 11(2): 700-04. doi: 10.13040/IJPSR.0975-8232.11(2).700-04.