

SCREENING OF METHANOLIC EXTRACT OF *CUSCUTA REFLEXA* ROXB. ON STZ-NICOTINAMIDE INDUCED DIABETIC NEUROPATHY ACTIVITY IN RATS**Abhinav Kumar Shukla*, Vijeta Rajoriya, Dr. Alok Pal Jain****RKDF Collage of Pharmacy, NH-12, Hoshangabad Road, Bhopal (M.P.), 462047***Corresponding Author's E mail: abhinavshukla291995@gmail.com

Received 19 Nov. 2020; Revised 28 Nov 2020; Accepted 17 Dec. 2020, Available online 10 January 2021



Cite this article as: Shukla AK, Rajoriya V, Jain AP. Screening of Methanolic Extract of *Cuscuta reflexa* Roxb. on STZ- Nicotinamide Induced Diabetic Neuropathy activity in rats. Asian Journal of Pharmaceutical Education and Research. 2021; 10(1): 11-19.

<https://dx.doi.org/10.38164/AJPER/10.1.2021.11-19>

ABSTRACT

Neuropathy, is a secondary complication of diabetes mellitus, is generally considered to be related to duration and severity of hyperglycemia. If the blood glucose level is greater than 250mg/dl than it may be cause secondary complication. The main cause of diabetic neuropathy is hyperglycemia. The aim of treatment of diabetic neuropathy should be treatment of diabetic mellitus and followed by prevention of other side effect. *Cuscuta reflexa* is known to contain a number of α -glucosidase inhibitory compounds. *Cuscuta reflexa* is well established drug for diabetic mellitus. Preliminary phytochemical studies of the extract were done according to the published standard methods. Phytochemical analysis revealed the presence of Alkaloids, Glycosides, phytosterols and Triterpenoids, Phenolic and Tannins, flavonoids Methanolic extract of *Cucuta reflexa* were showed significant effect of body weight, because weight gain is the main side effect of anti-diabetic drugs The significant hyperglycemic activity of methanolic extract of *Cucuta reflex* at dose of 200mg/kg as compare to 100mg/kg lowered hyperglycemic and it may be useful for the treatment of diabetic and associated complication Significant increase in pain threshold was observed in diabetic animals treated only with negative control. Methanolic extract of *Cuscuta reflexa* was showed significant effect on thermal pain sensitivity.

Keywords: *Cuscuta reflexa* Roxb, Methanolic Extract, Nicotinamide, streptozotocin, Diabetic Neuropathy activity.

INTRODUCTION

Diabetic Neuropathy is a demonstrable disorder, either subclinical or clinically evident, that occurs in both peripheral and the autonomic nervous systems. Diabetic neuropathy is a peripheral nerve disorder caused by diabetes.¹⁻³ The symptoms of diabetic neuropathy are often slight at first but can occasionally flare up suddenly and affect specific nerves so that an affected individual will develop double vision, drooping eyelids, or weakness and atrophy of the thigh muscles⁴. The main risk factor for diabetic

neuropathy is hyperglycemia. It is important to note that people with diabetes are more likely to develop symptoms relating to peripheral neuropathy as the excess glucose in the blood results in a condition known as Glucojasinogen⁵. The diabetic neuropathies are heterogeneous, affecting different parts of the nervous system that present with diverse clinical manifestations. Painful diabetic peripheral neuropathy (PDN) is a significant cause of pain and distress in patients with Diabetes mellitus (DM) ⁶. It affects patients with both Type 1 and 2 DM. Diabetic peripheral neuropathy (DPN) is an important micro vascular complication of diabetes mellitus⁷ DN is, therefore, diagnosed by exclusion. Tissue injury is usually accompanied by pain and is described as neuropathic if the initiating injury occurs to neural tissue.

Neuropathy, is a secondary complication of diabetes mellitus, is generally considered to be related to duration and severity of hyperglycemia. If the blood glucose level is greater than 250mg/dl than it may be cause secondary complication¹. The main cause of diabetic neuropathy is hyperglycemia. The aim of treatment of diabetic neuropathy should be treatment of diabetic mellitus and followed by prevention of other side effect. *Cuscuta reflexa* is known to contain a number of α -glucosidase inhibitory compounds. A new flavanone-reflexin, tetrahydrofuran derivatives and a coumarin have been isolated from the plant⁸. *Cuscuta reflexa* is well established drug for diabetic mellitus. The plant has medicinal value like stimulant, laxative, antihelminthic, antibacterial, expectorant, blood purifier, diuretic and used in amenorrhoea, dysmenorrheal, helminthiasis, intestinal worms and in wound healing⁹.

MATERIALS AND METHOD

Collection and Authentication:

The plant *Cuscuta reflexa* Roxb. were collected from SRK University Bhopal and authenticated by Department of botany, Dr. H.S. Gour Central University, Sagar (M.P.)

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade. Diclofenac Sodium (Themis Pharmaceuticals, Mumbai), Carrageenan (Sigma Chemical Co, St Louis, MO, USA) were used in present study.

Extraction method

The shade dried coarse powder of the leaves will be packed in extraction thimble of soxhlet apparatus and subjected to continuous hot extraction with petroleum ether for 18 hours or till the clear extraction will be obtained. The extract will be filtered while hot and the resultant extract will be distilled in vacuum under reduced pressure in order to remove the solvent completely. Mark left after petroleum ether dried below 50°C (hot-air oven) and then packed well in extraction thimble of soxhlet apparatus and subjected to continuous hot extraction with methanol for 18 hours or till clear extraction obtained. The extract filtered while hot and the resultant extract will be distilled in vacuum under reduced pressure in order to remove the solvent completely. It will be dried and kept in a desiccator till experimentation. Obtained extract will be weighed and percentage yield will be calculated in terms of air-dried powdered crude material.¹⁰

Phytochemical screening of the extract

The extract of *Cuscuta reflexa* Roxb. was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids¹¹⁻¹².

Pharmacological activity

Dose Selection: The doses were selected on the basis of OECD guidelines 423, the doses were administered on the difference of 50 under 200, 500 and 1000 & 2000mg/kg. The significant dose was obtained at 1000 and 2000 mg/kg. So the 1/10 dose of these particular dose was 100, 200 mg/kg according to OECD guidelines.

Description of groups

- Group 1 - Positive control (Vehicle treated)
- Group 2 - Negative control (Disease induced)
- Group 3 - Standard (Pyoglitazone 1.25 mg/kg + Glimepiride 0.7 mg/kg + Metformaine 120 mg/kg)
- Group 4 - Test Group I (Methanol extract 100mg/kg)
- Group 5 - Test Group II (Methanol extract 200mg/kg)

The study will performed on one models:

STZ- Nicotinamide induced model for diabetic neuropathy.

Non-insulin dependent diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of 65 mg/kg body weight STZ, 15 min after the intraperitoneal administration of 110 mg/kg body weight of nicotinamide. STZ was dissolved in citrate buffer (0.1M, pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The animals with blood glucose concentration more than 200 mg/dl were used for the study.¹³

Evaluations parameters for diabetic neuropathy

Body weight

Body weight of animals were measured by animal balance

Blood glucose level estimation

Blood samples will have collected by retro-orbital sinus/plexus bleeding method; in which, in anaesthetized animal tip of capillary tube was gently inserted below the eye at approximately a 45-degree angle into the space between the globe and the lower eyelid. At a point; capillary tube was feel rest on the orbit, at that time tube was twisted between thumb and forefinger. After that, sinus/plexus was ruptured and blood will flow through the tube, blood sample was collected and multiple tubes required collecting total volume. Blood glucose level was measured by glucometer. The blood glucose estimation was done weekly after administration of test compound.

RESULT AND DISCUSSION

Preliminary phytochemical studies of the extract were done according to the published standard methods. Phytochemical analysis revealed the presence of Alkaloids, Glycosides, phytosterols and Triterpenoids, Phenolic and Tannins, flavonoids table 1.

In the study methanolic extract of *Cuscuta reflexa* were tested for their diabetic neuropathy activity in Swiss albino rats induced by STZ- Nicotinamide. The body weight of positive control, negative control, standard and different dose of plant extract treated animals were estimated on 0, 7, 14, 21, 28, 35, days respectively as shown in fig no. The body weight of normal control group was normal so, there was not a significant difference found in normal control group (57 vs 175). While negative control group STZ Nicotinamide induced diabetic rats showed a significant decrease in body weight on 21, 28, and 35 days. On day 15 animals treated with standard and (test group I-V) significantly decrease in body weight as compare to normal control groups. On 14, day the treatment started on all groups except positive control and negative control. The methanolic extract of *cuscuta reflexa* showed no weight gain on 35 day (98,

$P > 0.001$) at dose of 200 mg/kg and as compare to negative control group. Methanolic extract of *Cuscuta reflexa* were showed significant effect of body weight, because weight gain is the main side effect of anti-diabetic drugs table 2.

The blood glucose level of rats in experimental groups, except the normal control group, increased significantly after the STZ Nicotinamide administration on 14th day. On 21th, 28th, and 35th days post induction significantly increases in blood glucose level in negative control groups. On 14th day the treatment started on all group except positive control and negative control. On repeated administration of standard drug and methanolic extract of *Cuscuta reflexa* for 21th, 28th, and 35th days, a sustained and significant decreased in blood glucose level of diabetic rats was observed in dose dependent manner s compare to diabetic control group. The methanolic extract of *Cuscuta reflexa* showed decrease on 35th day ($P > 0.001$) at dose of 200mg/kg as compare to negative control group. In this study the different fraction of methanolic extract of *Cuscuta reflexa* reduced blood glucose level significantly in the hyperglycemic rats. The significant hyperglycemic activity of methanolic extract of *Cuscuta reflexa* at dose of 200mg/kg as compare to 100mg/kg lowered hyperglycemic and it may be useful for the treatment of diabetic and associated complication table 3.

The paw jumping response was measured by Eddys hot plate. In normal control group there was not a significant difference found in thermal pain sensitivity. But in negative control group, were increased in paw jumping response (5.12 vs 4.12). In standard group there were significant increase in paw jumping response of (test group I-V) decrease significantly on 21, 28, and 35 days as compare to negative control groups. The methanolic extract of *Cuscuta reflexa* showed decrease on 35 days (5.12 vs 0.15 $P > 0.001$) as compare to negative control group. Hyperalgesia is a constant feature of sensory dysfunction in spontaneously and experimental model of diabetic neuropathy, we observed hyperalgesia and a significant improvement in hot plate response that was pain- threshold of diabetic animals with *Cuscuta reflexa* treatment. The response with dose of 200 mg/kg was found to be better than 100 mg/kg doses. The analgesic action was found to be near normal. Significant increase in pain threshold was observed in diabetic animals treated only with negative control. Methanolic extract of *Cuscuta reflexa* was showed significant effect on thermal pain sensitivity table 4.

Table 1. Phytochemical tests of methanolic extract of *Cuscuta reflexa*
AJPER Jan- Mar 2021, Vol 10, Issue 1 (11-19)

S. No.	Chemical Test	Observation	Inference
1.	Tests for Alkaloids		
	Mayer's reagent:	Yellow cream ppt	positive
	Dragendroff's reagent:	Raddish brown ppt	positive
	Wagner's reagent	Brown ppt	positive
	Hager's reagent	Yellow ppt	positive
2.	Tests for carbohydrates		
	Molisch's test	Red –dull violet colour	negative
	Barfoed's test	Reddish ppt	negative
	Fehling's test	Red ppt	negative
	Benedict's test	Raddish brown ppt	negative
3.	Tests for Glycosides:		
	Modified Borntrager's test	Red colour	negative
	Keller killiani's test	Bluish Green colour	positive
	Legal test	Pink colour	positive
	Baljet test	Yellow colour	negative
4.	Tests for Protein and Amino acids:		
	Millon's test	White ppt-red colour	negative
	Ninhydrin test	Precipted	negative
5.	Tests for phytosterols and Triterpenoids		
	Liebermann's Test	Blue colour	positive
	Libermann-Burchard test	Ring t the junction	positive
	Salkowaski Test	Colourless	positive
6.	Test for Phenolic and Tannins		
	Ferric Chloride Test	Yellow ppt	positive
	Lead acetate Test	Blue-Green ppt	positive
7.	Test for flavonoids		
	Shinoda's test	Purple colour	positive
	Alkaline reagent test	Yellow colour to colourless	positive
8.	Tests for Saponins		
	Foam Test	Foam formation	negative

Table 2. Effect of MECR on body weight in STZ induced diabetic rat

S. No.	Groups	Body weight (gms)					
		0 day	7 th day	14 th day	21 th day	28 th day	35 th day
1	Positive Control(Vehicle treated)	57	141.5	152.5	164	169	175
2	Negative control(Disease induced)	74.5	92.5	80.5	96	99	100
3	Standard (Pyo+Gli+Met)	82	76	86.5	94	97	99
4	Test I(Methanol extract 100mg/kg)	74.5	90	94	74.5	70.5	99
5	Test II(Methanol extract 200mg/kg)	65.5	100	105	91.5	98	85.5

Table 3. Effect of MECR on Blood Glucose estimation in STZ induced diabetic rat

S.No.	Groups	Glucose estimation(mg/dl)					
		0 day	7 th day	14 th day	21 th day	28 th day	35 th day
1	Positive Control(Vehicle treated)	96	94	96	95	95	96
2	Negative control(Disease induced)	97	110	115	167	210	251
3	Standard(Pyo+Gli+Met)	96	98	210	165	154	135
4	Test I (Methanol extract 100mg/kg)	94	145	210	198	172	176
5	Test II (Methanol extract 200mg/kg)	96	156	234	189	140	143

Table 4 Effect of MECR for Diabetic Neuropathy

S. No.	Groups	Eddy's Hot Plate					
		0 day	7 th day	14 th day	21 th day	28 th day	35 th day
1	Positive Control(Vehicle treated)	6.24	4.14	4.12	2.10	1.42	2.12
2	Negative control(Disease induced)	5.12	4.52	2.13	4.12	0.19	4.12
3	Standard(Pyo+Gli+Met)	3.41	0.85	0.21	0.13	0.16	5.45
4	Test I(Methanol extract 100mg/kg)	8.45	1.45	1.20	0.12	0.16	0.13
5	Test II(Methanol extract 200mg/kg)	5.12	2.15	2.12	4.12	2.13	0.15

CONCLUSION

Cuscuta reflexa Roxb. (Cuscutaceae a division of Convolvulaceae) is an extensive climber parasite. It occurs throughout the plains of India. It is more often called dodder in English. Traditional healers called in Hindi Akash bel. It is concluded the plant has medicinal value like diabetic mellitus, stimulant, laxative, antihelminthic, antibacterial, expectorant, blood purifier, diuretic and used in amenorrhoea, dysmenorrhoea, helminthiasis, intestinal worms and in wound healing. The plant contains Quercetin, Cuscutine, and Cuscutamide etc. *Cuscuta*'s seed and stem are highly medicinal values which are responsible for diabetic Neuropathy Diabetic neuropathy is a long-term complication of diabetes that develops early in the course of the disease and is observed in 60–70% of all diabetic patients. It is known that diabetic neuropathy is a nerve degenerative disease characterized by axonal degeneration, nerve fibers demyelization, and a reduction in the number of medium to large diameter nerve fibers, particularly in peripheral nerves.

Reference:

1. Gale EAM and Anderson JU. Diabetes mellitus and other disorders of metabolism. In: Kumar P, Clark M. Clinical Medicine. 5th ed. London: WB Saunders. 2002;1069-101.
2. Raghavan VK. Treatment of dyslipidemia in patients with type 2 diabetes. Lipids Health Dis.2010; 9: 144.
3. Gandjbakhch I, Leprince P, D'Alessandro C, Ouattara A, Bonnet N, Varvous S and Pavie A. Coronary artery bypass graft surgery in patients with diabetes. Bull Acad Natl Med. 2005; 189:257-66.
4. Ansari A. The Efficacy of Newer Antidepressants in the Treatment of Chronic Pain: A Review of Current literature. Harv Rev Psychiatry. 2000; 7: 257-277.
5. Ardid D and Guilbaud G. Antinociceptive Effects of acute and Chronic Injection of Tricyclic antidepressants Drugs in a New Model of Mononeuropathy in Rats. Pain. 1992; 49: 279-287.
6. Pirart J. Diabetes Mellitus and Its Degenerative Complications: A Prospective Study of 4,4000 Patients Observed Between 1947and 1973. Diabetes Care. 1978; 1:168-88.
7. Mimi O, Teng Cl and Chia Yc. The Prevalence of Diabetic Peripheral Neuropathy in an Outpatient Setting. Med J Malaysia. 2003; 58:533-8.
8. Vinik AI, Mitchell BD, Leichter SB, Wagner AL, O'brian JT and Georges LP. Epidemiology of The Complications of Diabetes. In: Leslie Rdg, Robbins Dc, Eds. Diabetes: Clinical Science in Practice. Cambridge, United Kingdom: Cambridge University Press; 1995;221-287.
9. Bennett M. The Lanss Pain Scale: The Leeds Assessment of Neuropathic Symptoms and Signs. Pain. 2001; 92: 147–57.

10. Roopashree TS, Dang R, Rani SRH and Narendra C. Antibacterial activity of anti-psoriatic herbs: *Cassia tora*, *Momordica charantia* and *Calendula officinalis*. International Journal of Applied Research in Natural Products. 2008; 1(3): 20-28.
11. Obasi NL, Egbuonu ACC, Ukoha PO and Ejikeme PM. Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* pods. African journal of pure and applied chemistry. 2010; 4(9): 206-212.
12. Audu SA, Mohammed I and Kaita HA. Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae). Life Science Journal. 2007; 4(4): 75-79.
13. Birgani GA, Ahangarpour A, Khorsandi L and Moghaddam HF. Anti-diabetic effect of betulinic acid on streptozotocin nicotinamide induced diabetic male mouse model. Braz. J. Pharm. Sci. 2018;54(2): e17171.
14. Wolfe G. The evaluation of the analgesic action of pethidine hydrochloride. J Pharmacol Exp Ther. 1944; 80:300-307.