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EFFECT OF *COCCINIA INDICA* LEAF EXTRACT ON DIABETIC NEUROPATHY PAIN IN RATS

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ABSTRACT

We investigated the effect of freshly prepared alcoholic extract of *coccinia indica* leaves extract on neuropathic pain, blood glucose level and loss of body weight in hyperglycaemic rats. Streptozotocin (STZ; 60 mg/kg, IP) was injected to albino rats to induce diabetes. Oral administration of freshly prepared alcoholic extracts to each of the animal was given to STZ-induced diabetic rat until 7 weeks after the STZ injection at a dosage of 500 mg/kg/day and 250 mg/kg/day. The glucose level test to follow the changes in blood glucose and body weight measurement to evaluate loss of body weight in diabetic rats. The pain evaluated by stimuli thermal: warm immersion (52 $\pm$2 ^{0}C) and cold immersion test (10 ^{0}C) and paw withdrawal on Eddy's hot plate (45-52 $\pm$ 2 ^{0}C) in diabetic rats. Treatment with these freshly prepared leaf alcoholic extract significantly reduced blood glucose and pain in diabetic rats. Blood glucose lowering effect and analgesic activity of *coccinia indica* decreased the pain in STZ- diabetic rats. STZ-induced loss of body weight was attenuated by the alcoholic extract of the *coccinia indica.* The higher dose (500 mg/kg/day) of alcoholic extract of the *coccinia indica* works better than that of lower dose (250 mg/kg/day) against hyperglycaemia and painful neutopathy. Therefore the use of these plants as analgesic and antidiabetic is justified. Glibenclamide (10 mg/kg/day) is an antidiabetic drug used as a standard drug to maintain the glucose level and diabetic neuropathic pain in diabetic rats.

KEYWORDS: *Coccinia indica*, Neuropathic pain, Streptozotocin (STZ), Glibenclamide, Hyperglyceamia.

INTRODUCTION

Painful diabetic neuropathy (PDN) is a neurological disorder that is a common complication of diabetes mellitus (DM) and can affect many aspects of life and severely limit patient's daily life. Diabetic neuropathy (DN) is a debilitating complication of Type- 1 and Type-2 (DM). Over 20 million Americans are diabetic and the incidence is increasing by 5% per year.[1] DM is caused by inherited and/or acquired deficiency in the production of insulin by the β -cells of pancreas (Type- 1) or by ineffectiveness of insulin produced (Type-2). The Symptoms are Numbness and tingling of extremities, Dysesthesia (abnormal sensation to a body part), Diarrhoea, Erectile dysfunction, Urinary incontinence (loss of bladder control), Facial, mouth and eyelid drooping, Vision changes, Dizziness, Muscle weakness, Difficulty in swallowing, Speech impairment, Fasciculation (muscle contractions), Burning or electric pain etc.[2] Several drugs to increase insulin sensitivity are currently being used. Recently, the search for appropriate hypoglycaemic agents has been focused on plants used in traditional medicine. Medicinal plants are frequently considered to be less toxic and free from side effects than the synthetic ones.[3] *Coccinia indica* belongs to the family Cucurbitaceae. It is a rapidly growing, perennial climber or trailing vine.[4] Traditionally different parts of this plant namely the roots, leaves and fruits are used as medicine for several purposes like antihyperglycemic [5-7], hypolipidemic [8], antioxidant[9], antihepatotoxic [10], larvicidal potential of malarial mosquito *Anopheles stephensi* [11] etc. Research in this area is still unexplored, therefore the present study is directed towards the extraction of *coccinia indica* from leaf with good yield and enhance effect on diabetic neuropathy pain in rats.

MATERIAL AND METHODS

All the chemicals procured from Sigma-Aldrich, CDH and Hi-Media.

Plant materials

Coccinia indica commonly known as Kundru belongs to the family Cucurbitaceae are collected from Sonebhadra district of U.P. India. The plant is authenticated with the help of a Chief scientist & Head raw materials Herbarium & museum (RHMD), Dr: H.B Singh**.**

Preparation of plant extract: *Coccinia indica* leaves collected and air dried in shade at room temperature. The dried leaves were powdered and sieved using the fine

muslin cloth. The fine powdered leaves were kept with 90% alcohol in soxhlet apparatus to get the crud drugs.[12]

Animals used in experiment

8-10 weeks old albino male/ female rats (initially) weighing 200- 250 g were used. Animals were housed 6 per cage, under standard laboratory conditions (Temperature: 25 ± 2 ºC, humidity 30- 70 % and lighting condition 12 hr. dark and 12 hr. light (artificially) and were given food and water ad libitum. The animals acquired from DIPSAR, New Delhi; Jamia Hamdard**,** New Delhi. The experiment is to be performed in animal facility center, RV Northland Institute Chethera Dadri, India. All animal experiment protocols were approved by the Institutional Animal Ethics Committee (IAEC). The rats of both sexes were procured.

Induction of diabetes

A single dose of freshly prepared Streptozotocin (Sigma chemical Co., St. Louis, MO) in citrate buffer, pH 4.5, was immediately injected intravenously (60 mg/kg) through intraperitoneal (IP) in a volume of 1 ml/kg body weight [13]. Streptozotocin injection rapidly produced the characteristic signs of diabetes, such as increased intake of water and food, frequent urination and increased blood glucose concentration. One week after the Streptozotocin injection, rats having more than 250 mg/dl random blood glucose levels and showing above mentioned characteristic signs of diabetes were selected for this experiment. A drop of blood samples were collected from the tip of the tail by needle puncture for blood glucose measurement on alternate weeks.[14-15]

Analgesic activity[16-17]

Eddy's hot plate and tail flick method

Male/female albino mice were selected and divided into five groups, containing four animals in each group. These animals were fasted for twenty four hours, prior to the experiment. Animal of Group - I considered as Control, was administered with 1% Acacia suspension. Animal of Group- II was treated with Streptozotocin (60 mg/kg). Animal of Group -III and IV were treated with different concentration of *Coccinia indica* (250 mg/kg/day and 500 mg/kg/day) respectively in Streptozotocin induced diabetic animals. Animal of Group - V were treated with Glibenclamide (10 mg /kg/ day) as standard in Streptozotocin induced diabetic animals. The reaction time for each mouse was recorded at time interval of 0 week, 1 week, 3 weeks, 5 weeks and 7 weeks after the administration of test substances by using Eddy's hot plate and tail flick method.

The % analgesic activity (PAA) was calculated by the following formula

$PAA = (T-C)/C \times 100$

C is the reaction time of the control and T is the reaction time of the test compound.

RESULT AND DISCUSSION

Streptozotocin induced diabetic rats showed loss of body weight significantly at 2 week after the Streptozotocin treatment compared to Control rats, which further decreased at 4, 6 and 7 weeks after the Streptozotocin treatment. The results were tabulated as (Table1 and figure 1).

Table 1: Body weight in control, STZ-induced diabetic non-treated and treated rats during the 0-7 weeks after the STZ treatment. Freshly prepared leaf aqueous extracts treatment significantly improves STZ induced loss of body weight.

	Body weight (g)				
Weeks	**O wk.**	**1 wk.**	**3 wk.**	**5 wk.**	**7 wk.**
Groups Control	230.12 ± 3.06	225. 23 ±1.54	233. 76±2.07	237. 65±3.85	236. 45±4.08
STZ	233.12±6.80 (Before induced) treatmenttrea	220.33±5.89	185.34 ±6.80	158.45±1.45	158.45±5.36
STZ+Plant extract 250 mg/kg	240.09±7.16 (Induced+ before treatment)	221.45±4.57	190.56±3.42	185.67±4.08	180.67±4.60
STZ+Plant extract 500 mg/kg	250. 43±11.78 (Induced+ before treatment)	222. 78±9.02	220.54±4.08	200.65±6.23	190.45±3.49
STZ+Glibe-nclamide	250.67±11.78 Induced + treatment	230.45±1.86	224.45±2.78	210.78±3.01	200.56±2.91

The values are given in Mean ±SEM (Standard error of mean).

At 7 week after the STZ treatment, average body weight of Control and STZ (Group 2) rats were 236.45 ± 4.08 and 158.45 ± 5.36 g, respectively .On the other hand treatment with freshly prepared leaf aqueous extracts of indigenous medicinal plants in diabetic rats showed significant prevention of body weight loss .At 7 week after the STZ treatment, average body weight of STZ+ *C. indica 250mg* and STZ +*C. indica* 500mg/ kg, STZ+ Glibenclamide groups were 158. 45± 1.36, 180. 67 ± 4.60, 190.45 ± 3.49 and 200.56±1.91 respectively (Table:1).

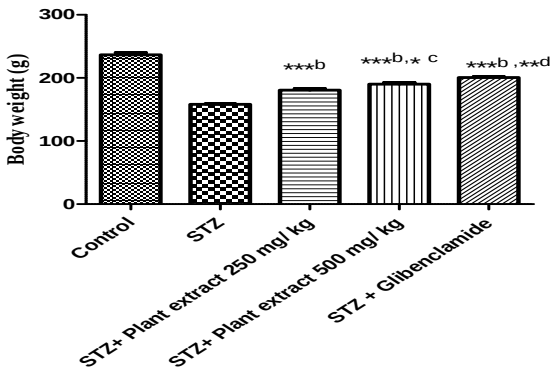

Figure 1: Body weight in control, STZ-induced diabetic non-treated and treated rats during the 7 weeks after the STZ treatment. (*P<0.001, **P<0.01,*P<0.05. a Vs control).**

The anti-diabetic activity of Coccinia indica in Streptozotocin induced diabetic animals showed significant fall in glucose level with compare to standard drug Glibenclamide. The results were tabulated as (table 2 and figure 2).

Table 2: The blood glucose level in control, STZ-induced diabetic non-treated and treated rats during the 0-7 Weeks after the STZ treatment.

	Glucose level (mg/ dl)				
Weeks (wk) / **Groups**	**O wk.**	**1 wk.**	**3 wk.**	**5 wk.**	**7 wk.**
Control	112.23±1.83	118.45±1.92	109.23±0.82	120.34±0.02	113.45±1.99
STZ	104.34± 4.38 (Before induced)	430.80±12.61	473.45±18.92	476.09±3.39	444.35±12.38
STZ+Plant extract 250 mg/kg	108. 12±1.90 (Induced + before treatment)	447.34±1.18	330.23±12.24	315.45±4.08	311.23+13.60
STZ+Plant extract 500 mg/kg	115.45±1.90 (Induced + before treatment)	431.45±12.67	300.23±25.62	285.13±20.04	280.12± 9.81
STZ+Glibe-nclamide	116.02±1.90 (Induced + treatment)	140.06±4.08	173.67±1.18	185.56±6.23	182.67±8.03

The values are given in Mean±SEM.

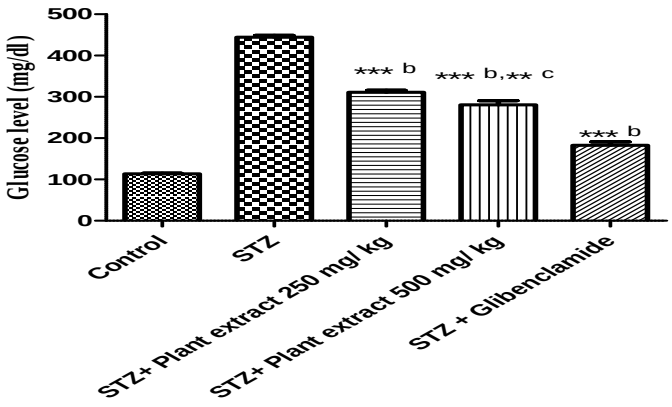

Figure 2: The significant in glucose levels in control, STZ-induced diabetic non-treated and treated rats during the 7 weeks after the STZ treatment. (*P< 0.001, **P<0.01, a Vs. control.**

The analgesic effect of Coccinia indica is very significant with compare to standard drug Glibenclamide. The result of analgesic effect were tabulated in table 3,4 and figure 3,4 with hot plate and cold water.

Table 3: Average pain response in control, STZ-induced diabetic non-treated and treated rats during the 0-7 Weeks after the STZ treatment.

	Thermal stimuli: warm (51±2°C) Response (Sec)				
Weeks(wk.) / **Groups**	**O wk.**	**1 wk.**	**3 wk.**	**5 wk.**	**7 wk.**
Control	10.12±0.83 10	9.23±0.92	8.36±0.82	9.33±0.11	10.23±0.32 10
STZ	11.34± 0.38 (Before induced)	9.56±0.61	6.12±0.92	5.17±0.39	6.13±0.01
STZ+Plant extract 250 mg/kg	8.21±0.90 (Induced + before treatment)	5.13±0.18	5.15±0.24	6.12±0.08	6.33+0.41
STZ+Plant extract 500 mg/kg	9.33±0.90 (Induced + before treatment)	4.66±0.67	5.67±0.62	7.33±0.04	8.33± 0.81
STZ+Glibe-nclamide	10.66±0.50 (Induced + treatment)	8.17±0.08	9.23±0.18	7.16±0.23	8.17±0.92

The values are given in Mean±SEM.

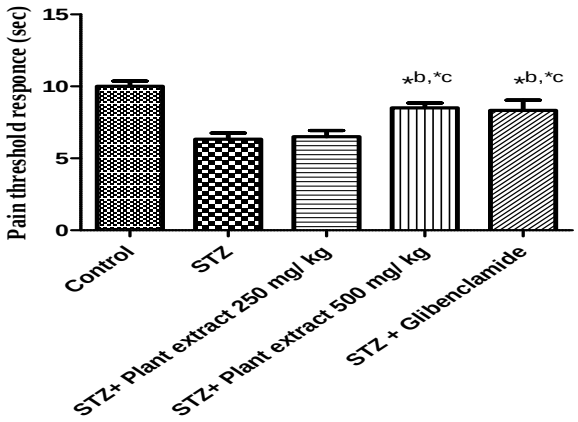

Figure 3: Time course of pain thresholds of control, diabetic, diabetics + plants extract treated rats submitted to the tail immersion test in hot (51±2°C) water (*P<0.001, *P<0.05, a Vs control).**

Table 4: Average pain response in control, STZ-induced diabetic non-treated and treated rats during the 0-7 Weeks after the STZ treatment

	Thermal stimuli: Cold (10 °C) Response (Sec)				
Weeks(wk.) / **Groups**	**O wk.**	**1 wk.**	**3 wk.**	**5 wk.**	**7 wk.**
Control	11.66±0.61 10	9.23±0.92	13.27±0.82	9.54±0.11	10.33±0.32
STZ	13.12± 0.51 (Before induced)	10.24±0.61	6.37±0.92	6.12±0.39	5.23±0.51
STZ+Plant extract 250 mg/kg	11.13±0.90 (Induced + before treatment)	8.12±0.18	5.10±0.24	6.09±0.08	7.08+0.91
STZ+Plant extract 500 mg/kg	12.15±0.90 (Induced + before treatment)	9.33±0.67	7.17±0.62	9.68±0.04	8.66± 0.81
STZ+Glibe-nclamide	10.45±0.50 (Induced + treatment)	8.46±0.08	9.87±0.18	7.89±0.23	9.07±0.92

The values are given in Mean±SEM.

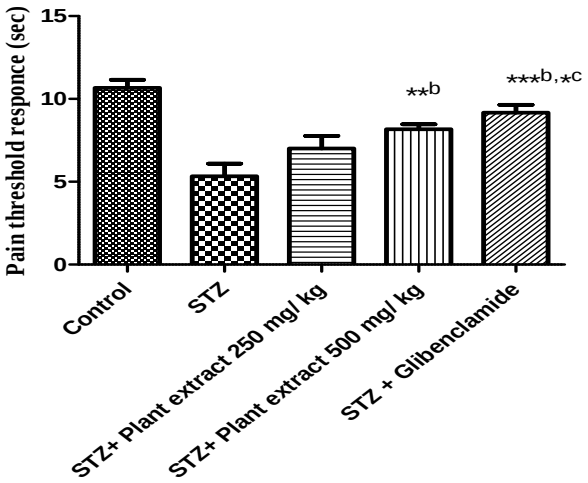

Figure 4: Time course of pain threshold of control, diabetic; diabetics + plants extract treated rats submitted to the tail immersion test in cold (10 °C) water. Scores determined at the 7 th week of diabetes and plant extract treatment (*P<0.001, **P<0.01,*P<0.05, a Vs control).**

CONCLUSION

Experimental data showed that freshly prepared *Coccinia indica* attenuates hyperglycemia and diabetic neuropathy in STZ-induced diabetic rat, due to antihyperglycemic and analgesic activity. We also showed that higher doge of *Coccinia indica* plant extracts (500 mg/kg/day) have more blood glucose lowering effects when treatment started one week after the STZ injection.

The higher doge (500 mg/ kg / day) of alcoholic extract of the *Coccinia indica* therapy works better than that of lower dose (250 mg/ kg / day) against hyperglycaemia and painful neuropathy in STZ-induced diabetes rats.

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