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

Somnath Kharwar*[1], Prof. (Dr.) B. B. Panigrahi[2], Md. Quammudin[3], Balwan Singh[4] and Khalid Iqbal[5]

[1,2,3]Department of Pharmacology, HIMT College of Pharmacy, Knowledge Park-I, Greater Noida, U.P (India).

[4,5]Department of Pharmaceutics, HIMT College of Pharmacy, Knowledge Park-I, Greater Noida, U.P (India).

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***Corresponding Author**
Somnath Kharwar
Department of Pharmacology, HIMT College of Pharmacy, Knowledge Park-I, Greater Noida, U.P (India).

ABSTRACT

We investigated the effect of freshly prepared chloroform extract of *coccinia indica* fruit and leaf extracton 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 chloroform extracts to each of the animal was given to STZ-induced diabetic rats 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±2) and cold immersion test (10^0C) and paw withdrawal on Eddy's hot plate (45-52 ±2) in diabetics rats. Treatment with these freshly prepared fruit and leaf chloroform 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 chloroform extract of the *coccinia indica.* The higher dose (500 mg/kg/day) of chloroform extract of the *coccinia indica* therapy work better than that of lower dose (250 mg/kg/day) against hyperglycaemia and painful neutopathy in STZ-induced diabetes rats. Therefore the use of these plants as analgesic and antidiabetic is justified. The rosiglitazone (10 mg/kg/day) also knows as thiazolidinediones is an antidiabetic drug. In other member class of medication known as pioglitazone. It is used as a standard drug and maintains the glucose level and diabetic neuropathic pain in diabetic rats.

KEYWORDS: *Cocciniaindica*, rosiglitazone, analgesic, hyperglyceamia,body weight, STZ-induced diabetic rats.

INTRODUCTION

General

Painful diabetic neuropathy (PDN) is a neurological disorder that is a common complication of diabetes mellitus, and can affect many aspects of life and severely limit patient's daily life. Diabetic neuropathy (DN) is a debilitating complication of type-1and type-2 diabetic. Over 20 million Americans are diabetic and the incidence is increasing by 5% per year. The most common complication of diabetes is neuropathy (DN) which occurs in approximately 60% of diabetic patients.

Diabetes mellitus 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). 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 (Rates, 2001). Medicinal plants are frequently considered to be less toxic and free from side effects than the synthetic ones. The world health organization has also recommended that this should be encouraged, especially in countries where conventional treatment of diabetes seems insufficient.

MATERIALS AND METHODS

Plant materials

*CocciniaIndica*commonly known as Kundru belongs to the family: Cucurbitaceae are collected from district Gautambudhnagar of U. P. India. The plant is authenticated with the help of a Scientist 'D' & Head Dr. Sandeep Chauhan, Ministry of Environment, Forest and Climate Change , Botanical Survey of India, BGIR , Sec.-38A Noida 201303.

Preparation of plant extract: CocciniaIndica fruit and leaf collected and air dried in shade at room temperature. The dried fruit and leaf were powdered and sieved using the fine muslin cloth. The fine powdered fruit and leaf were kept with chloroform in soxhlet apparatus to get the crud drugs.

Induction of diabetes

A single dose of freshly prepared STZ (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 STZ injection rapidly produced the characteristic signs of diabetes, such as increased intake of water and food, frequent urination angd increased blood glucose concentration. One week after the STZ 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.

Experimental design

Animals were divided into 5 groups (n = 6 for each) and treated as follows:

Group 1: Control: was given citrate buffer and served as control (without STZ).

Group 2: STZ-induced diabetic rats were divided in to 4 groups (Groups 2 - 5).

Group 3: STZ+ freshly prepared chloroform extract of *Cocciniaindica* fruit and leaf at a dose of 500 mg/kg/day.

Group 4: STZ + freshly prepared chloroform extract of *Cocciniaindica* fruit and leaf at a dose of 250 mg/kg/day.

Group 5: STZ + Rosiglitazone 10mg /kg/ day)

Treatments by oral gavage daily were started one week after the single dose of STZ injection and continued for 7 weeks Determination of hyperglycemia preventive effect of *Cocciniaindica*

Dosing of animals

Dosing of Rosiglitazone

The Rosiglitazone was given daily for 7 weeks and maintain the glucose level below 200 mg/dl. The Rosiglitazone was given orally by oral gavage daily after the streptozotocin injection.

Dosing of plant extract

The plant extract was started after one week of the single dose of the STZ injection and continued for 7 weeks.

Pain test

Thermal stimuli: the tail immersion test - warm

The tail of the rat was immersed in a water bath at several temperatures (38, 40. 42, 44 and 46°C) until tail withdrawal orsigns of struggle were observed (cut-off time: 15 set). A minimum10-min interval was maintained between each measurement. Eleven diabetic and 7 age matched control rats were tested. This test afforded each animal the immersion duration necessary to elicit a reaction for each of the 5 temperatures used.

Thermal stimuli: the tail immersion test - cold

The procedure was the same but the water temperature was set at 10 ˚C, a temperature that is normally innocuous six diabetic and 7 age-matched control rats were used for this experiment Shortened duration of immersion indicates allodynia. The cut-off time was 15 sec.

Hind paw analgesic

Pain responses were evaluated in pre- and post-diabetic rats. To quantify thermal sensitivity, rats were placed on a clear Plexiglas chamber and given 5–10 min to acclimate. The radiant heat source delivered a heat stimulus to the plantar surface of the hind paw. The reflective photocell sensor detected when the rat moved or lifted its paw. The time between onset of the stimulus and paw flick was defined as the paw withdrawal latency (PWL). On a given test and for each hind paw, the same procedure was repeated three times at 5 min intervals, the mean withdrawal pressure was computed by averaging the six measurements. The light source was set at 25 °C and the temperature increased to 70 °C over the course of 10 sec. A threshold of 15sec was applied to prevent injury to the rats.

Measurement of MDA and GSH-px levels

All animals were sacrificed after 7^{th} weeks by cervical dislocation under ether anesthesia and kidney tissues of each animal were rapidly excised, weighed and washed twice with cold phosphate buffer solution (PBS). One quarter of the kidney tissue from each rat was weighed, homogenized with Tris-HCl (pH 7.4), and centrifuged. Supernatants were used immediately to assay MDA and glutathione peroxidase (GSH-px) using commercial kits according to the manufacturer’s instructions.

RESULTS

Effect of freshly prepared fruit and leaf chloroform extracts of *Cocciniaindica* on body weight in diabetic rats

STZ-induced diabetic rats showed loss of body weight significantly ($p < 0.05$) at 2 week after the STZ treatment compared to Control rats, which further decreased at 4, 6 and 7weeks after the STZ treatment (Table 1.1).

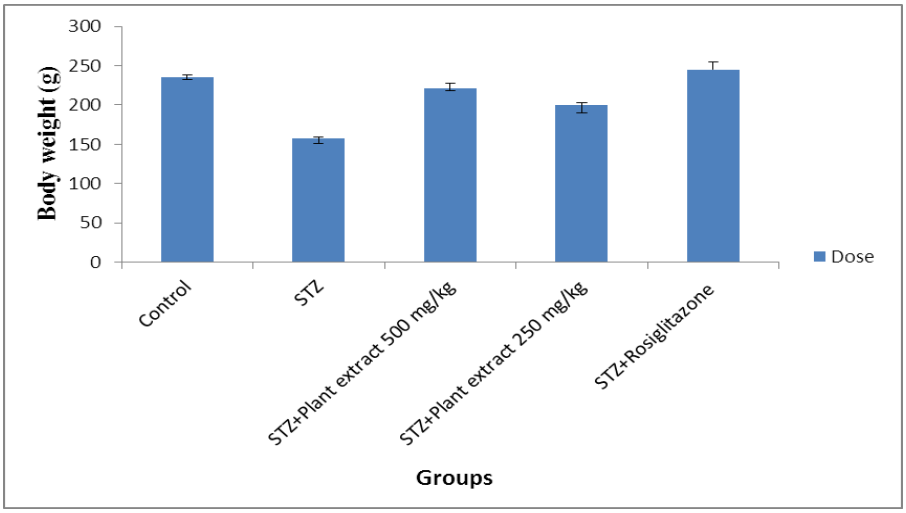

Figure 1.2: Bar graph of body weight in control, STZ-induced diabetic non-treated and treated rats during the 7 weeks after the STZ treatment.Freshly prepared fruit and leaf chloroform extracts treatment significantly improves STZ induced loss of body weight (*p > 0.05).**

Table 1.1: Body weight in control, STZ-induced diabetic non-treated and treated rats during the 0-7 weeks after the STZ treatment. Freshly prepared fruit and leaf chloroform extracts treatment significantly improves STZ induced loss of body weight ($p < 0.05$).

	Body weight (g)					
Weeks	**O wk.**	**1 wk.**	**2 wk.**	**3 wk.**	**5 wk.**	**7 wk.**
Groups Control	228 ± 1.57	224±2.00	228±2.08	230±3.07	236±2.60	235±3.09
STZ	235±4.81 (Before induced) treatmenttrea	222±3.60	197±9.34	180±8.08	159±1.50	157±2.30
STZ+Plant extract 500 mg/kg	252±9.70 (Before induced+ treatment)	243±8.02	234±5.07	229±5.07	224±6.40	221±6.50
STZ+Plant extract 250 mg/kg	243±4.50 (Before induced+ treatment)	234±6.56	220±4.80	210±8.42	201±3.80	200±2.60
STZ+Rosiglitazone	251±10.78(Before induced+treatment)	245±12.87	252±5.70	240±12.60	242±11.01	245±9.80

Antihyperglycemic effect of freshly prepared fruit and leaf chloroform extracts of *Cocciniaindica* plant in diabetic rats

Table 2.1: The blood glucose level in control, STZ-induced diabetic non-treated and treated rats during the 0-7 Weeks after the STZ treatment. Freshly prepared fruit and leaf chloroform extracts treatment significantly improves the blood glucose level.

	Glucose level (mg/ dl)					
Weaks (wk) / **Groups**	**O wk.**	**1 wk.**	**2 wk.**	**3 wk.**	**5 wk.**	**7 wk.**
Control	115±2.85 10	123±1.90	120±3.07 10	122±0.80 + 8	123±1.10	117±0.99 10
STZ	122± 4.30 (Before induced)	475±24.60	429±12.70	474±17.90	435±4.39	435±11.40
STZ+Plant extract 500 mg/kg	120±0.80 (Before induced+ treatment)	432±11.70	316±11.30	283±23.80	245±21.03	208±8.80
STZ+Plant extract 250 mg/kg	120±0.90 (Before induced+ treatment)	446±2.18	294±5.10	332±10.23	297±3.65	285+11.50
STZ+Rosiglitazone	119±2.90 (Before induced+ treatment)	320±2.08	208±0.90	122±2.20	136±5.68	127±6.09

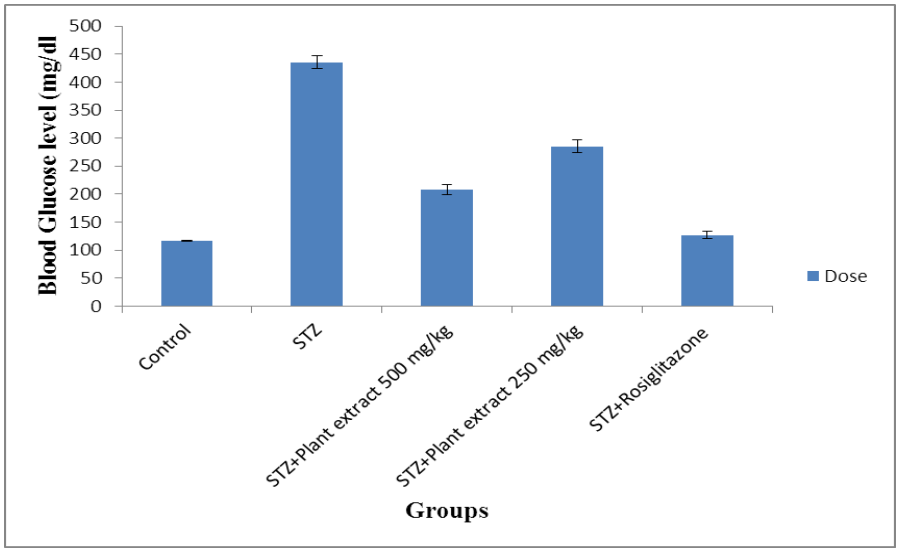

Figure 2.1 Blood glucose levels in control, STZ-induced diabetic non-treated and treated rats during the 7 weeks after the STZ treatment. Freshly prepared fruit and leaf chloroform extracts treatment significantly improves STZ induced alevation of blood glucose level (*p> 0.05).**

Effect of freshly prepared fruit and leaf chloroform extracts of *Coccinia indica* on reaction to pain stimuli in diabetic rats.

I Thermal stimuli: warm (51±2°C)

Table 3.1: Average pain response in control, STZ-induced diabetic non-treated and treated rats during the 0-7 Weeks after the STZ treatment. Freshly prepared fruit and leaf chloroform extracts treatment significantly improves the pain response.

	Thermal stimuli: warm (51±2°C) Response (Sec)					
Weeks (wk.) / **Groups**	**O wk.**	**1 wk.**	**2 wk.**	**3 wk.**	**5 wk.**	**7 wk.**
Control	10±0.80 10	8±1.90	11±1.70 10	9±0.83 + 8	8±0.20	10±0.39
STZ	10± 0.50 (Before induced)	10±1.05	7±0.08	5±1.80	5±0.45	6±0.30
STZ+Plant extract 500 mg/kg	10±0.95 (Before induced+ treatment)	5±0.68	6±1.30	6±0.65	8±1.04	9± 1.83
STZ+Plant extract 250 mg/kg	8±0.82 (Before induced+ treatment)	5±0.20	5±1.09	6±1.25	6±0.08	7+1.80
STZ+Rosiglitazone	11±1.50 (Before induced+ treatment)	9±0.80	10±0.36	9±0.20	8±1.32	9±0.95

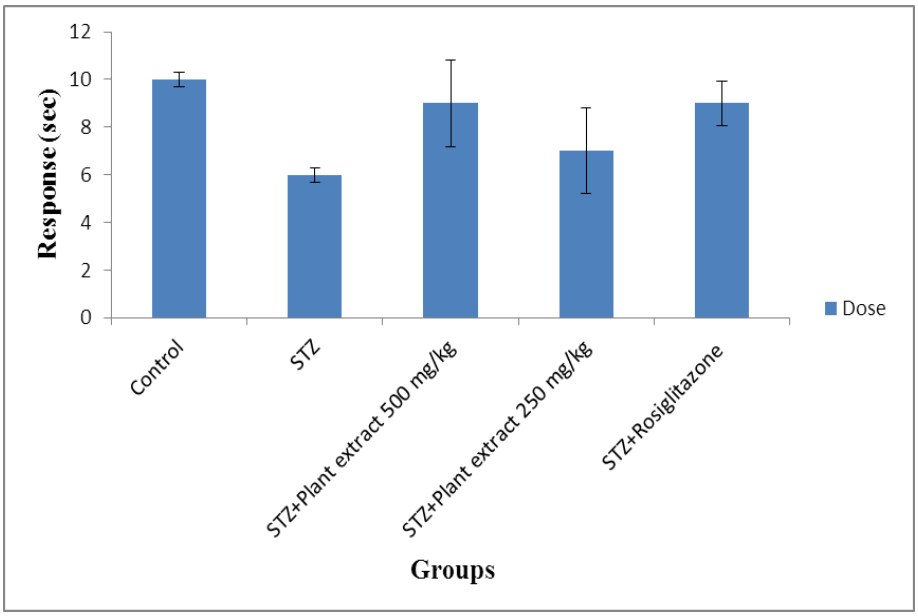

Fig 3.1: Time course of pain thresholds of control, diabetic, diabetics + plants extract treated rats submitted to the tail immersion test in hot (51±2°C) water. Scores were determined at the 7th week of diabetes and plant extract (p> 0.05).

II Thermal stimuli: cold (10 °C)

Table 3.2: Average pain response in control, STZ-induced diabetic non-treated and treated rats during the 0-7 Weeks after the STZ treatment. Freshly prepared fruit and leaf chloroform extracts (500 mg/kg and 250 mg/kg) treatment significantly improves the pain response (p > 0.05).

	Thermal stimuli: Cold (10 °C) Response (Sec)					
Weeks (wk.) / **Groups**	**O wk.**	**1 wk.**	**2 wk.**	**3 wk.**	**5 wk.**	**7 wk.**
Control	10±1.80 10	10±1.90	14±1.30	12±0.85	11±0.44	12±0.70
STZ	14±1.55 (Before induced)	11±0.36	9±0.73	7±0.40	8±1.03	9±0.01
STZ+Plant extract 500 mg/kg	13±0.82 (Before induced+ treatment)	10±0.85	9±1.07	8±0.40	10±1.03	11± 0.63
STZ+Plant extract 250 mg/kg	12±0.82 (Before induced+ treatment)	8±0.20	10±1.05	8±0.82	9±0.10	10+0.95
STZ+Rosig-litazone	9±0.36(Before induced+ treatment)	8±0.63	10±0.14	10±0.40	9±0.50	13±1.82

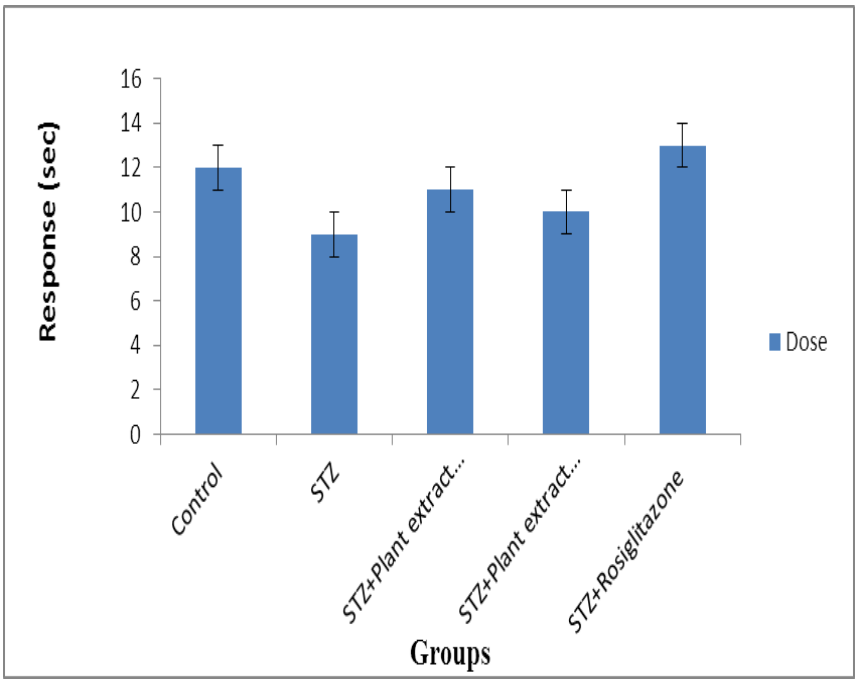

Figure 3.2: Time course of pain threshold of control, diabetic; diabetics + plants extract treated rats submitted to the tail immersion test in cold (10 °C) water. Scores were determined at the 7th week of diabetes and plant extract.

III Thermal hyperalgesia: hind paw withdrawal (PWL)

Table 3.3: Average pain response in control, STZ-induced diabetic non-treated and treated rats during the 0-7 Weeks after the STZ treatment. Freshly prepared fruit and leaf chloroform extracts (500 mg/kg and 250 mg/kg) treatment significantly improves the pain response (p > 0.05).

	Thermal hyperalgesia: hind paw withdrawal (PWL) Response (Sec)					
Weeks (wk.) / **Groups**	**O wk.**	**1 wk.**	**2 wk.**	**3 wk.**	**5 wk.**	**7 wk.**
Control	10±0.43	8±1.90	13±1.09 10	14±0.73	10±0.20	13±0.30 10
STZ	14± 0.50 (Before induced)	8±1.36	9±1.05	10±0.80	8±0.30	9±0.09
STZ+Plant extract 500 mg/kg	14±2.80 (Before induced+ treatment)	9±1.80	8±0.36	10±1.62	10±0.30	11± 1.85
STZ+Plant extract 250 mg/kg	12±1.92 (Before induced+ treatment)	9±1.20	8±1.09	8±0.36	9±1.80	10+0.86
STZ+Rosig-litazone	13±0.44(Before induced+ treatment)	8±0.92	10±0.40	10±0.23	11±1.20	12±1.82

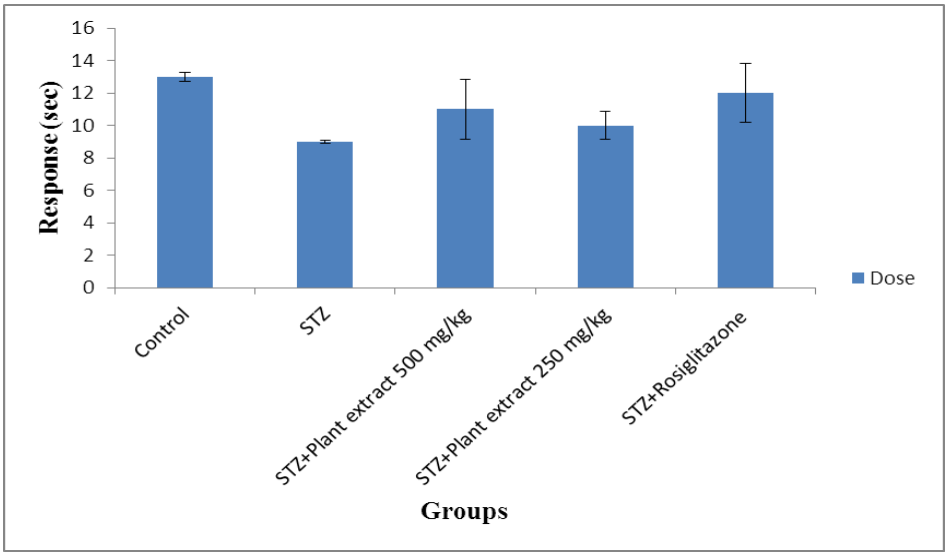

Figure 3.3: Pain thresholds values of control, diabetic; diabetics + plants extract treated rats submitted on the Eddy's hot plate (Analgesiometer). Scores were determined at the 7th week of diabetes and plant extract (p > 0.05).

Change in renal function related parameters

Table 4.1: Changes in Renal Function Parameters in control, STZ, STZ + Plant extract treated animals and STZ + Rosiglitazone in rats.These parameters were taken after 7th weeks (p > 0.05).

Groups / Parameter	Control	STZ	STZ+ Plant extract 500 mg/ kg	STZ+ Plant extract 250 mg/ kg	STZ + Rosiglitazone
Water intake (mL/d)	31.4±3.9	122.6±10.2	52.5±2.3	73.8±4.5	35.07±6.4
Urine production (mg/24 hrs)	20.98±1.30	35.60±2.80	25.05±2.92	25.36±3.44	20.14± 1.40
KW/BW	4.30±1.18	5.36±0.81	4.69±0.24	6.10±1.29	4.80± 1.82

KW/BW, kidney weight/body weight.

The values are means±SEM (standard error of mean) of six rats per group.

Effect on MDA and GSH-px activities

Table 5.1: Effects on the Content of Antioxidant Enzymes and MDA in diabetic Rats.

Groups / Parameter	Control	STZ	STZ+500 mg/kg Plant Extract	STZ+250 mg/kg Plant Extract	STZ + Rosiglitazone
GSH-px (U/mg protein)	35.63±1.70	25.64+1.70	30.50±0.70	27.10±1.68	28.75±1.55
MDA (nmol/mg)	10.36±0.80	17.33±2.90	12.40±0.50	14.30±0.39	12.65±0.40

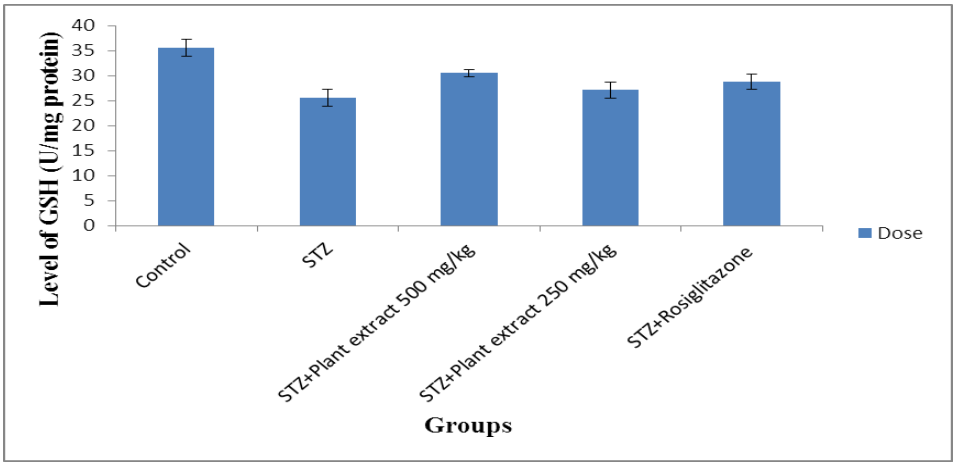

Figure 5.1: The effect of GSH- pxlevel in control, diabetics rats and *Cocciniaindica* treated animals (p>0.01).The significant in control and diabetic rats (*p> 0.01).**

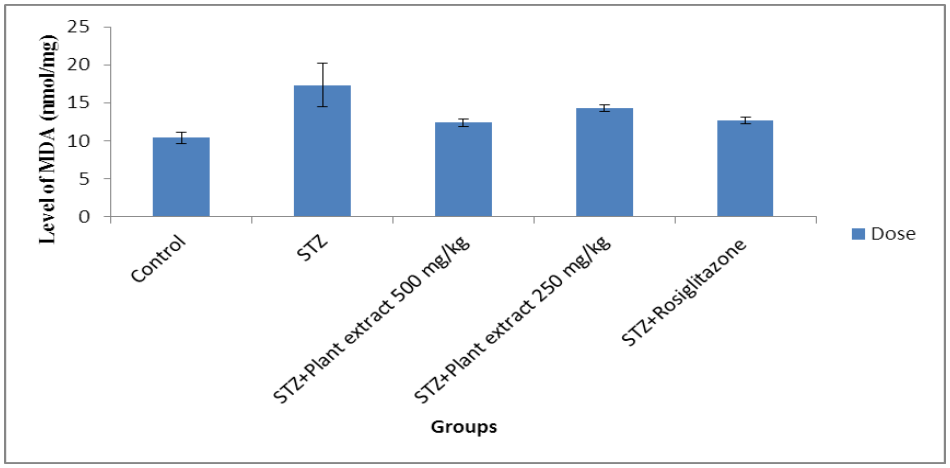

Figure 5.2: The effect of MDA level in control, diabetics rats and Cocciniaindica treated animals (p> 0.01).The significant in control and diabetic rats (*p> 0.01).**

CONCLUSION

Experimental data showed that freshly prepared fruit and leaf of *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 Chloroform 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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