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AN EVALUATION OF THE PROTECTIVE ROLE OF ESCITALOPRAM IN STREPTOZOTOCIN-INDUCED DIABETIC NEUROPATHY

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Keywords:

Diabetic neuropathy, Diabetes mellitus, Pain perception and Escitalopram

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ABSTRACT: Objective: The aim of the present study was the evaluation of the protective role of escitalopram in streptozotocin-induced diabetic neuropathy. **Materials and Methods:** Diabetes was induced in Wistar rats with streptozocin 70 mg/kg and animals were divided into four groups namely normal control, diabetic vehicle control, glibenclamide control and escitalopram group. After the 4th week of diabetes induction treatment was started for further 28 days (5th to 8th week) with escitalopram 20 mg/kg. Evaluation of diabetic neuropathy was performed after 8 weeks of single injection of streptozotocin (70 mg/kg i.v.) in rats. Blood glucose level, grip strength, locomotor activity, pain sensitivity and threshold in diabetic rats were measured. **Results:** The results of the present study indicate that the 8 weeks treatment of escitalopram demonstrates hypoglycemic effect; it marked decreases the blood glucose level in diabetic treated animals. There was also decrease in the grip strength in diabetic rat indicates to induction of neuropathy or nerve damage. Escitalopram increases the grip strength of diabetic rats. There was also found loss of pain perception in diabetes rats which measured using hot plate and tail-flick methods. Escitalopram increases the licking time, and withdrawal latency in hot plate and tail-flick test respectively indicates the presence of pain perception and prevention of nerve damage demonstrates its protective effect in diabetic neuropathy. **Conclusion:** Our study concludes the chronic treatment of escitalopram significantly decreases the glycemic level as well as it protected from the development of diabetic neuropathy.

INTRODUCTION: Diabetes mellitus is a metabolic disease which damages different body organs, causing kidney failure, vision loss, autonomic and peripheral neuropathy, peripheral vascular disease, myocardial infarction, and cerebrovascular disease with stroke [1]. Diabetes affects the central nervous system and produce disturbances such as behavioral changes, autonomic dysfunctions, altered neuroendocrine functions, and neurotransmitter alterations and thus leading to end-organ damage [2].

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Various pathways involved in the pathogenesis of diabetic neuropathy and degeneration are polyol, hexosamine, protein kinase C, advanced glycation, poly (ADP-ribose) polymerase, oxidative stress, and inflammation. Oxidative stress and inflammation play a crucial role in the development and progression of late-stage complications of diabetes [3, 4]. The antioxidant defenses in humans are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxides. Lipid peroxidation (LPO) was one of the characteristic features of chronic diabetic condition [5]. The pathogenesis of DPN is believed to be multifactorial with hyperglycemia being the primary risk factor.

Suggested theories that postulate the aetiopathogenesis of DN include abnormalities of protein glycation, sorbitol accumulation, polyol

pathway flux, protein Kinase C activation, advanced glycation end products, the receptor for advanced glycation end products, a decrease in neuronal nitric oxide synthase protein, and micro-vascular hypoxia, resulting in oxidative stress [6].

Since DN is not clearly understood, it is hard to make a definitive course of treatment Drugs that have been used in the management of Diabetic neuropathy include tricyclic antidepressants and selective serotonin reuptake inhibitors [7]. Many pharmacological options are available to treat Diabetic neuropathy but still, it is difficult for patients to obtain complete relief because of poor glycemic control. Prevention through strict glycemic control remains the mainstay of therapeutic intervention because effective disease-modifying therapies are yet not available.

Diabetic neuropathy is a common complication of both type 1 and type 2 diabetes, which affects over 90% of diabetic patients. Although pain is one of the main symptoms of diabetic neuropathy, its pathophysiological mechanisms are not yet fully known. It is widely accepted that the toxic effects of hyperglycemia play an important role in the development of this complication, but several other hypotheses have been postulated. The management of diabetic neuropathic pain consists basically in excluding other causes of painful peripheral neuropathy, improving glycemic control as prophylactic therapy and using medications to alleviate pain. First-line drugs for pain relief include anticonvulsants, such as pregabalin and gabapentin and antidepressants, especially those that act to inhibit the reuptake of serotonin and noradrenaline. In addition, there is experimental and clinical evidence that opioids can be helpful in pain control, mainly if associated with first-line drugs.

Other agents, including for topical application, such as capsaicin cream and lidocaine patches, have also been proposed to be useful as adjuvant in the control of diabetic neuropathic pain, but the clinical evidence is insufficient to support their use. In conclusion, a better understanding of the mechanisms underlying diabetic neuropathic pain will contribute to the search of new therapies, but also to the improvement of the guidelines to optimize pain control with the drugs currently available.

The SSRIs are a relatively new class of antidepressants. They differ from tricyclic antidepressants in their specific inhibition of presynaptic reuptake of serotonin, but not of nor-adrenaline, and their lack of postsynaptic receptor blocking effects and quinidine-like membrane stabilization SSRIs like fluoxetine, paroxetine, citalopram, and venlafaxine have been used for the relief of neuropathic pain, with mixed results

In reported case study escitalopram found to shows adverse hypoglycemic effect and it was associated with increased blood insulin levels and also regulate the level of neurotransmitters serotonin and epinephrine [8, 9]. So, by considering the hypoglycemic side effect the present study was aimed at to evaluate the influence of chronic treatment of escitalopram 20 mg/kg (p.o.) on blood glucose level and on progression neuropathy in STZ-induced 8 week diabetic rats.

MATERIALS AND METHODS: This research work is carried out at Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram, Andhra Pradesh and total time duration of research work is 5 months (May 2018 to September 2018).

Materials: Gluc metert (Accu-Chek, Johnson and Johnson, India), Streptozotocin (Nicholas Piramal Pvt. Ltd. Mumbai), Glycosylated Hb Kit. All the reagents and chemicals used in the present study were of analytical grade. Escitalopram (Sun Pharmaceuticals Gujarat, India) was prepared in 0.5% carboxymethyl cellulose using tween 20 (0.2% v/v) as a suspending agent.

Animals and Experimental Protocol: Male Wistar rats (300 ± 50 g) were provided the standard pelleted diet and *ad libitum* water, were kept at environmental temperature (23 °C ± 3 °C) and under 12 h light-dark cycle. The animals were acclimatized to the experimental conditions a week before the study. The animal protocol was approved by the Institutional Animal Ethics Committee and CPCSEA: Regd. no: 439/P0/S/ 01/CPCSEA.

Induction of Diabetic Complication with Treatment Schedule: Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of fresh STZ (60 mg/kg body weight) in citrate buffer (0.1 M, pH 7.4) [10, 11, 12]. After 48 h of

STZ injection, fasting blood samples were withdrawn from the tail vein, and blood glucose level was measured by use of a glucometer (Accu-Chek, Johnson and Johnson, India). The animals having fasting blood glucose level ≥230 mg/dl were randomized in groups. The development of DNN was confirmed by basal nociceptive reaction at the 4th week of STZ injection, and all treatments were started thereafter from 5th to 8th week. Glibenclamide is widely used as second-line therapy in diabetes; several research reports had mentioned the use of Glibenclamide as a positive control. At the end of 8th week, behavioral analyses were done and the blood sample was collected.

The Animals were Divided into Four Groups (n=5)

Group I	**:**	Normal control (NC) animals received saline
Group II	**:**	Diabetic control (DC)
Group III	**:**	Diabetic animal treated with escitalopram 20 mg/kg.p.o
Group IV	**:**	Diabetic animal treated with glibenclamide 2.5 mg/kg.p.o

Measurement of DN by Behavioral Studies: A grip strength determination was used for evaluating neuromuscular strength. The grip strength of animals was measured by using Rotarod apparatus. The time taken to hold the rotating rod to fall on the surface was considered for the muscle strength determination. The animals whose muscle or nerves get damaged or weak it gets fall soon on the floor. The force achieved (in terms of time) by the animal for staying in hanging stage was recorded. Evaluation of the effect of diabetic neuropathy on pain sensitivity and pain threshold was done by Hot Plate and Tail Flick methods. The rats were placed on the hot plate (55-58°) and the time, until either licking or jumping occurs, was recorded by a stopwatch. A cut off time of 10 s was kept to avoid damage to the paw of the animal. Evaluation of pain threshold in diabetic rats was determined by withdrawing latency in tail-flick test. The tail of each diabetic rat was exposed to radiant heat which was given by placing the hot water in glass. The intensity of radiant heat (55-58°) was adjusted to obtained withdrawal latency of not more than 6 seconds in both diabetic and non-diabetic rats [13, 14, 15]. The tail-flick latency is the time interval taken by the rat to flick its tail after exposure to a source of radiant heat. Cut of time was fixed at 10s. Spontaneous locomotor activity was recorded using an actophotometer. Locomotion was recorded in terms of total photobeam count for 5 min per animal

Statistical Methods: Blood glucose level was analyzed by One way ANOVA followed by Dunnet test. Data of grip strength, pain sensitivity and threshold were analyzed by Student unpaired t-test. The significant difference was compared at P<0.05. (Graph Pad Prism version 5.0)

RESULTS:

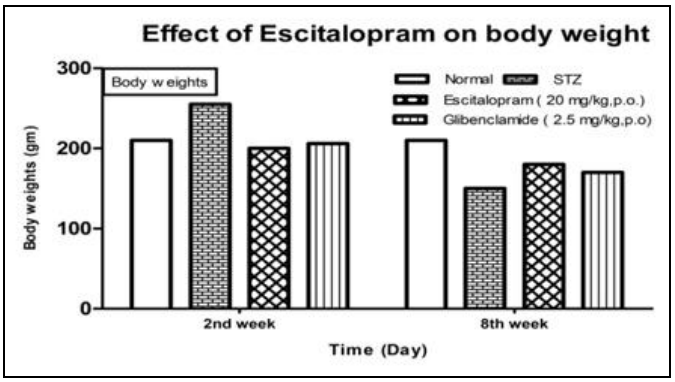

FIG. 1: INHIBITION OF BLOOD GLUCOSE LEVELS IN STREPTAZOCINE INDUCED DIABETES. Diabetic control (STZ 70 mg/kg), Std-Glibenclamide (2.5 mg/kg), Test-Escitalopram (20 mg/kg). Data are expressed as means ± SEM (n = 5). p<0.05 as compared with the vehicle group

Results of chronic treatments with escitalopram 20 mg/kg demonstrate to significant decreases in the blood glucose level at 4th and 8th week while the more significant (P<0.05) effect observed only on 8th week when compared to diabetic control.

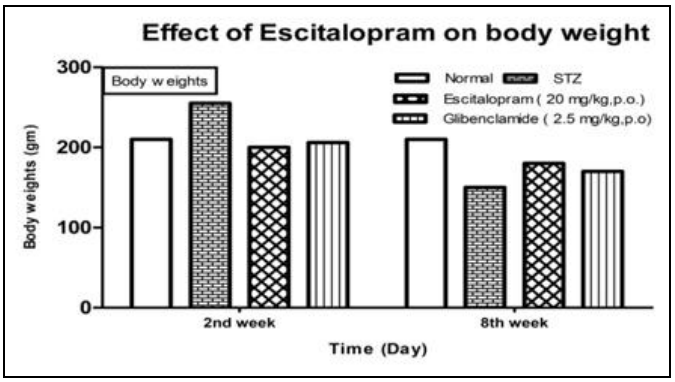

FIG. 2: EFFECT OF BODY WEIGHT IN STREPTAZOCINE INDUCED DIABETES. Diabetic control (STZ 70 mg/kg), Std-Glibenclamide (2.5 mg/kg), Test-Escitalopram- (20 mg/kg). Data are expressed as means ± SEM (n = 5). *p<0.05 as compared with a vehicle group

Improvement in body weight was observed after the treatment with escitalopram. STZ caused a significant decrease in the feed and water intake in the rats.

After treatment with escitalopram for 8th week, a marked increase in the food and water intake was observed in the diabetic rats.

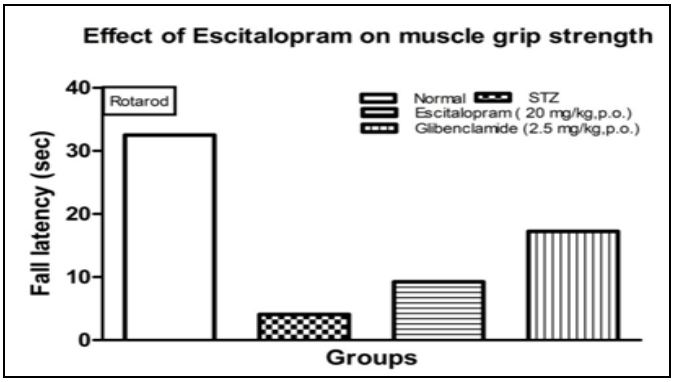

FIG. 3: EFFECT OF GRIP STRENGTH BY USING ROTA-ROD APPARATUS IN STREPTAZOCINE INDUCED DIABETES. Diabetic control (STZ 70 mg/kg), Std-Glibenclamide (2.5 mg/kg), Test-Escitalopram-(20 mg/kg). Data are expressed as means ± SEM (n = 5). *p<0.05 as compared with the vehicle group

The results suggest that escitalopram 20 mg/kg treated animals after 8th weak show greater muscle grip strength when compared to diabetic control.

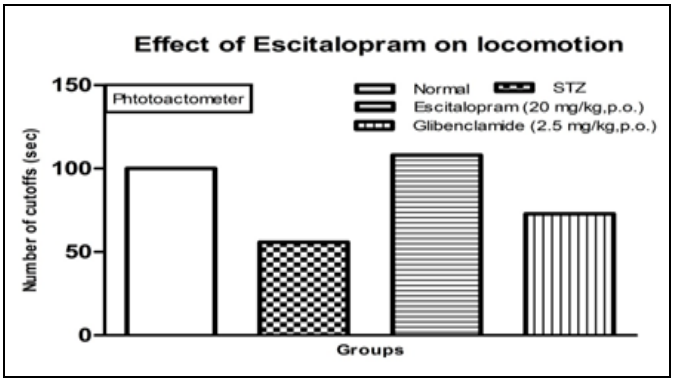

FIG. 4: EFFECT OF LOCOMOTION BY USING ACTOPHOTOMETER APPARATUS IN STREPTA-ZOCINE INDUCED DIABETES. Diabetic control (STZ 70 mg/kg), Std-Glibenclamide (2.5 mg/kg), Test-Escitalopram-(20 mg/kg). Data are expressed as means ± SEM (n = 5). *p<0.05 as compared with the vehicle group

Results suggest that escitalopram 20 mg/kg treated animals after 8th show greater locomotor activity when compared to diabetic control.

The escitalopram 20 mg/kg treated animals show an increase in jump time when compared to diabetic control so results suggest that escitalopram show analgesic activity.

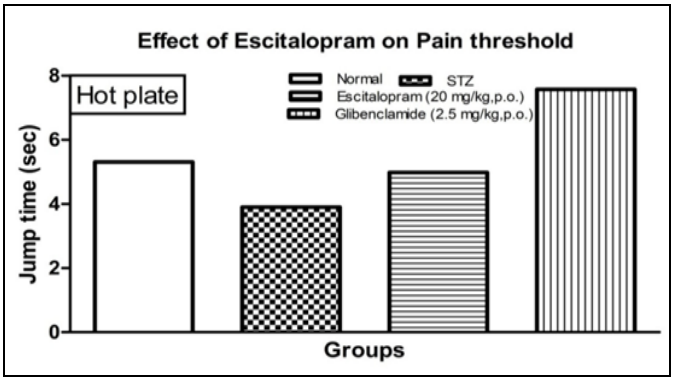

FIG. 5: ANALGESIC ACTIVITY BY USING HOT PLATE APPARATUS IN STREPTAZOCINE INDUCED DIABETES. Diabetic control (STZ 70 mg/kg), Std Glibenclamide (2.5 mg/kg), Test-Escitalopram-(20 mg/kg). Data are expressed as means ± SEM (n = 5). *p<0.05 as compared with vehicle group

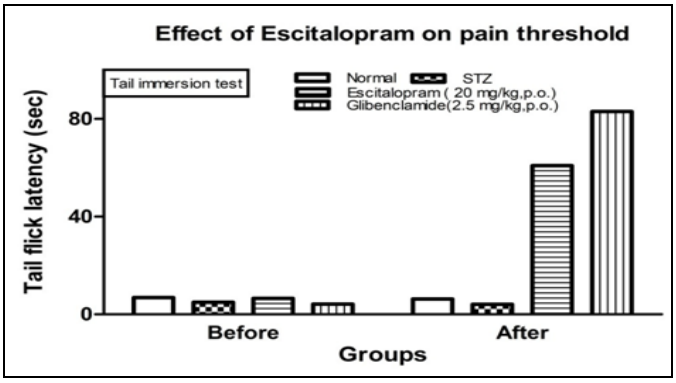

FIG. 6: ANALGESIC ACTIVITY BY TAIL IMMERSION METHOD IN STREPTAZOCINE INDUCED DIABETES. Diabetic control (STZ 70 mg/kg), Std-Glibenclamide (2.5 mg/kg), Test-Escitalopram-(20 mg/kg). Data are expressed as means ± SEM (n = 5). *p<0.05 as compared with vehicle group

The escitalopram 20 mg/kg treated animals show an increase in Tail flick latency when compared to diabetic control, so results suggest that Escitalopram show analgesic activity.

DISCUSSION: STZ-induced diabetes is characterized by a severe loss in body weight, [16] which could be due to poor glycemic control or the excessive catabolism of proteins to provide amino acids for gluconeogenesis during insulin deficiency, resulting in muscle wasting and weight loss in diabetic, untreated rats.[17] Improvement in body weight was observed after the treatment with escitalopram. STZ caused a significant decrease in the feed and water intake in the rats. After treatment with escitalopram for 8th week, a marked increase in the food and water intake was observed in the diabetic rats [18, 19]. This decrease may have occurred due to the decreased concentration of glucose in circulating blood, causing a reduction of

osmotic effects in the urine. Insulin deficiency resulting from STZ causes unrestrained lipolysis and proteolysis, leading to extreme hunger, which was allayed by escitalopram.

Results of chronic treatments with escitalopram demonstrate to significant decreases in the blood glucose level at 4^{th} and 8^{th} week while the more significant ($P<0.05$) effect observed only on 8^{th} week. The observed effect of escitalopram was comparable to standard hypoglycemic agent glibenclamide.

STZ injected rats had nociceptive threshold significantly lower than Normal control rats as observed by tail immersion test and hot plate assay. Escitalopram treated diabetic rats exhibited a rise in the tail-flick latency as compared to diabetic control rats. Escitalopram treated group showed significantly improved pain threshold as compared to diabetic control rats. Diabetic animals showed reduced locomotion (lo) ability as observed in a number of cut off significantly different from NC rats (100.2 ± 2.03). Escitalopram treated diabetic animals showed a significant rise in lo time as compared to diabetic control rats. The rota-rod test experiment demonstrated the impairment of the motor function and coordination in the diabetic rats with a significant reduction in fall off time as compared to normal control rats. Escitalopram treated diabetic rats showed a significant increase in fall off time as compared to diabetic animal.

For over 30 years, antidepressants and anticonvulsants have been used in the management of neuropathic pain. Which of the two drug classes should be the first-line choice remains unclear. Empirically, antidepressants have been used for burning pain, Antidepressants have often been prescribed as first-line rather than anticonvulsants because of a perceived lower incidence or severity of adverse effects. Two systematic reviews'[22] however, appeared to show no difference in either efficacy or in the incidence of adverse effects between antidepressants and anticonvulsants for diabetic neuropathy. Given the increased use of selective serotonin reuptake inhibitors (SSRIs) for pain management and the advent of newer anticonvulsants, such as gabapentin, we wanted to revisit the question of which drug class was best for neuropathic pain.

Based on the above question, this work is planned and evaluate the diabetic neuropathy of escitalopram. This study reveals that the SSRI s is best drugs for the treatment of neuropathy in future aspects.

CONCLUSION: From the results, it indicates that chronic treatment of escitalopram reduced the blood glucose level as well as prevents progression of diabetic neuropathy in streptozotocin-induced diabetic rats. So, it can be concluded that escitalopram can be used as ideal drug which could offer a better choice in the curative therapy for diabetic neuropathy. This can act by, either preventing the nerve damage or by providing symptomatic pain control along with good glycemic control. Hence, it could be helpful in treating the diabetic patient having the complication like diabetic neuropathy.

Significance Statement: "This study discovered the escitalopram that can be beneficial for the treatment of diabetic neuropathy and this study will help the researchers to uncover the critical areas of diabetic neuropathy that many researchers were not able to explore. Thus, a new theory on diabetes and diabetic neuropathy may arrive.

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CONFLICTS OF INTEREST: Nil

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