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EFFECT OF FLUOXETINE ON PROGRESSION OF DIABETIC NEUROPATHY (MEASURABLE) IN EXPERIMENTAL RATS: A RANDOMIZED CONTROL INTERVENTIONAL STUDY

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ABSTRACT

Introduction: Diabetes mellitus is a chronic disease resulting from defects in insulin secretion, insulin action, or both. About 347 million people worldwide have diabetes. Diabetes doubles the risk of Depression. Fluoxetine lowers the blood sugar level as an adverse effect in reported studies, so the present study planned to see the effect of Fluoxetine on blood glucose levels and diabetic neurophathy in diabetes and associated depression. **Materials and Methods**: Double blind randomized control interventional study performed after clearance from Institutional Animal Ethics Committee at Experimental Pharmacology Laboratory at SMS Medical College, Jaipur. It included 24 rats divided into four groups viz, vehicle control, diabetic control, test group and standard group. Diabetes was induced by a single injection of streptozotocin (70 mg/kg I.p.). Blood sugar levels were evaluated by glucometer. All rats included in the study were observed for 9 weeks. Test group was given Fluoxetine (20 mg/kg, p.o.) whereas standard group received Glibenclamide (0.5 mg/kg p.o.). Unpaired t test and ANOVA test were used to infer the difference in means. **Results:** Results of treatments with Fluoxetine demonstrated significant ($p < 0.05$) decrease in blood glucose levels on 30th day and more significant ($P< 0.01$) decreases in the blood glucose level were observed on 60th day that is comparable to that of standard therapy of Glibenclamide. Also it prevented the progression of Diabetes neuropathy.

Conclusion: From the results it was indicative that treatment of Fluoxetine reduced the blood glucose levels as well as prevented progression of diabetic neuropathy in streptozotocin induced diabetic rats. Fluoxetine also has a well established role in alleviating depression which can be a comorbid condition in diabetes. So it can be concluded that Fluoxetine could offer an alternative role in adjunctive therapy for diabetes with depression.

KEYWORDS: Diabetes mellitus, Blood sugar levels, Diabetic Neuropathy, Fluoxetine, Depression.

INTRODUCTION

About 347 million people worldwide have diabetes. There is an emerging global epidemic of diabetes that can be traced back to rapid increases in overweight, obesity and physical inactivity. Diabetes is predicted to become the seventh leading cause of death in the world by the year 2030. Total deaths from diabetes are projected to rise by more than 50% in the next 10 years. Diabetes is a leading cause of blindness, amputation and kidney failure. Lack of awareness about diabetes, combined with insufficient access to health services and essential medicines, can lead to complications such as blindness, amputation and kidney failure [1]. Effective and reliable instruments, patients can monitor their blood glucose level at home and thus patients can actively participate in management of the disease. The patients can be educated and guided to adopt a more active and healthy living.With the appropriate drug treatment, vast majority of the patients can now achieve excellent glycaemic control with productive and healthy life. Diabetes mellitus is multifactorial disease manifested by hyperglycaemia that results from several deregulated biologic mechanisms [2]. Peripheral neuropathy is a micro vascular complication of diabetes. It develops in 28% to 55% of patients with diabetes mellitus [3]. The risk of developing diabetic neuropathy increases with duration of disease and degree of glycemic control and other contributing factor such as hypertension, dyslipidemia, smoking, body mass index and hyperinsulinemia.

The main risk factor of diabetic neuropathy is hyperglycemia. The excess glucose in the blood results in a condition known as glucojasinogen. This condition results in lack of blood flow to the peripheral intrapectin nerves which govern the movement of the arms and legs. Peripheral neuropathy cause symptoms like tingling, numbness (severe or long-term numbness can become permanent), burning (especially in the evening), pain. Various drugs like pregabalin and gabapentin have been approved by the FDA for the symptomatic relief of neuropathic pain. Selective serotonin reuptake inhibitors (SSRI) and Tricyclic antidepressants

have also been used for the symptomatic pain relief in diabetic neuropathy [3]. Studies suggest that Fluoxetine (SSRI) which is used in the treatment of depression also lowers the blood glucose level as a side effect [4, 5 and 6]. So present study is planned to evaluate the role of 9 week treatment of Fluoxetine on blood sugar and diabetic neuropathy in experimental rats.

AIMS AND OBJECTIVES

1. To find out the effects of Fluoxetine on blood glucose level.
2. To compare the effect of Fluoxetine with the standard hypoglycemic agent (Glibenclamide) on blood glucose levels.
3. To find out the effect of Fluoxetine on diabetes induced neuropathic pain perception in the rats.

METHODOLOGY

Male Wistar rat weighing 180-220 g were used. The animals were fed with standard laboratory diet, had free access to water under well ventilated condition of 12 h day light cycle. The animals were adapted to laboratory condition for 7 days prior to the experiments. The studies were performed with the approval of Institutional Animal ethics committee (IAEC) of S.M.S. Medical College, Jaipur (Raj.)- order no. of IAEC (38/6.6.2013)

Experimental design:- Experimental model of diabetes was induced by i.p. injection of streptozotocin (70 mg/kg) in Wistar rats (180-220 gm) and the treatment of Fluoxetine (20 mg/kg p.o.) was started after stabilization of blood glucose level from day 15th of streptozotocin injection. Effect of Fluoxetine on blood glucose level and diabetic neuropathic pain were evaluated after 9th week. Induction of diabetic neuropathy was evaluated by grip strength [7, 8] and the neuropathic pain perception by tail flick method [7, 8].

Measurement of DN by behavioural studies: A grip strength determination was used for evaluating neuromuscular strength [7, 8]. The grip strength of animals was measured by simply hanging of animals with their fore limb on fine metal wire which was held at two end of pole. The time taken to hold the metal wire to fall on the surface was considered for the muscle strength determination. The animals whose muscle or nerves got damaged or weak, soon fall 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 perception was done by Tail Flick method [7, 8]. Evaluation of pain threshold in diabetic rats was determined by tail flick time in the tail flick method, Tail of each diabetic rat was exposed to radiant heat. The tail flick time, was the time interval taken by rat to flick its tail after exposure to a source of radiant heat. Cut of time was fixed at 10s.

METHODS

The chemical method was employed here, to study the hypoglycemic activity. streptozotocin was used to induce diabetes. Animals used were male albino rats with body weight between 180-220 gms. Animals were fed with pellet diet and water throughout the experiment. Animals were acclimatized to laboratory conditions before carrying out any experimental work. Glucometer was used to record the blood sugar. For measuring FBS, blood was collected from the rat's tail vein, diabetic neuropathy was measured by using tail flick method and grip strength method.

Induction of diabetes: Animals were injected intra peritoneally with freshly prepared streptozotocin solution dissolved in normal saline, in a dose of 70 mg/kg body weight. Following injection, animals were carefully observed for the first 24 hrs for any evidence of allergic reaction, behavioral changes and convulsions. No untoward reaction was observed in animals. Fasting blood glucose was recorded daily morning, till development of stable hyperglycemia (15^{th} day of Inj. streptozotocin). Rats were divided into 4 groups, each group having 6 animals. The 4 groups were named as Vehicle control, diabetic control, standard group, and test group.

Administration of drugs: Each group of animals was orally fed with the following agents.

GROUP I – VEHICLE CONTROL: 6 rats served as vehicle control and were orally fed with Carboxy Methyl Cellulose.

GROUP II- DIABETIC CONTROL: 6 Streptozotocin induced diabetic rats served as a diabetic control.

GROUP III -TEST: 6 streptozotocin induced diabetic rats were orally fed with fluoxetine 20 mg/kg, p.o. for 60 days.

GROUP IV-STANDARD: 6 streptozotocin induced diabetic rats orally fed with

Glibenclamide 0.5mg/kg. for 60 days.

Thus hypoglycemic activity of Fluoxetine was assessed. Its capacity to reduce the blood sugar level was compared with standard drug Glibenclamide. The results were analyzed by calculating the mean values, the standard deviation, and the analysis of variance (ANOVA). p-value was considered, as follows: $p< 0.05$ considered as significant. $p> 0.05$ considered as unsignificant.

RESULT

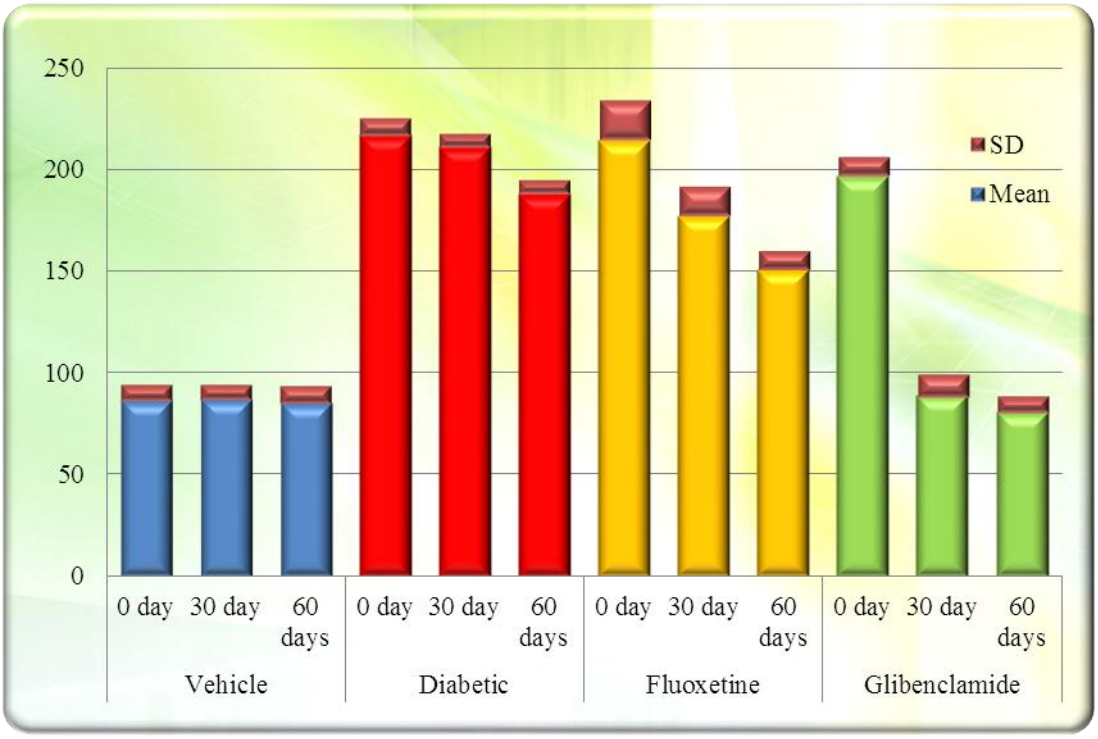
Table no. 1 Blood glucose levels (Fasting and Post prandial) on 0^{th}, 30^{th} and 60^{th} day

GROUP	Rat no.	Fasting blood sugar			Post prandial sugar		
		0^{th} day	30th day	60th day	0^{th} day	30th day	60th day
VEHICLE CONTROL	1	80	78	74	115	120	116
	2	92	94	88	122	116	123
	3	85	88	91	107	110	127
	4	74	78	74	106	112	109
	5	88	92	88	110	116	122
	6	98	94	95	116	118	107
DIABETIC CONTROL	1	210	212	178	380	378	350
	2	217	210	192	356	350	340
	3	219	208	190	405	392	356
	4	205	200	194	378	356	322
	5	222	216	182	390	378	318
	6	228	220	193	392	356	302
TEST GROUP	1	222	182	156	392	308	182
	2	218	192	166	402	312	192
	3	248	156	148	380	318	162
	4	202	189	142	356	298	168
	5	192	162	152	398	292	172
	6	205	182	140	368	305	159
STANDARD GROUP	1	192	107	79	397	112	122
	2	199	92	77	393	122	102
	3	211	78	74	405	113	110
	4	205	88	87	383	118	128
	5	188	89	72	387	133	105
	6	187	77	94	401	127	127

Table and Diagram No. 2: Fasting blood glucose (Mean ± S.D.)

		Vehicle	Diabetic	Fluoxetine	Glibenclamide
0 day	Mean	86.16	216.83	214.5	197
	SD	8.54	8.28	19.71	9.69
30 day	Mean	87.33	211	177.16	88.5
	SD	7.55	6.89	14.72	10.93
60 day	Mean	85.00	188.17	150.67	80.50
	SD	8.90	6.59	9.61	8.41

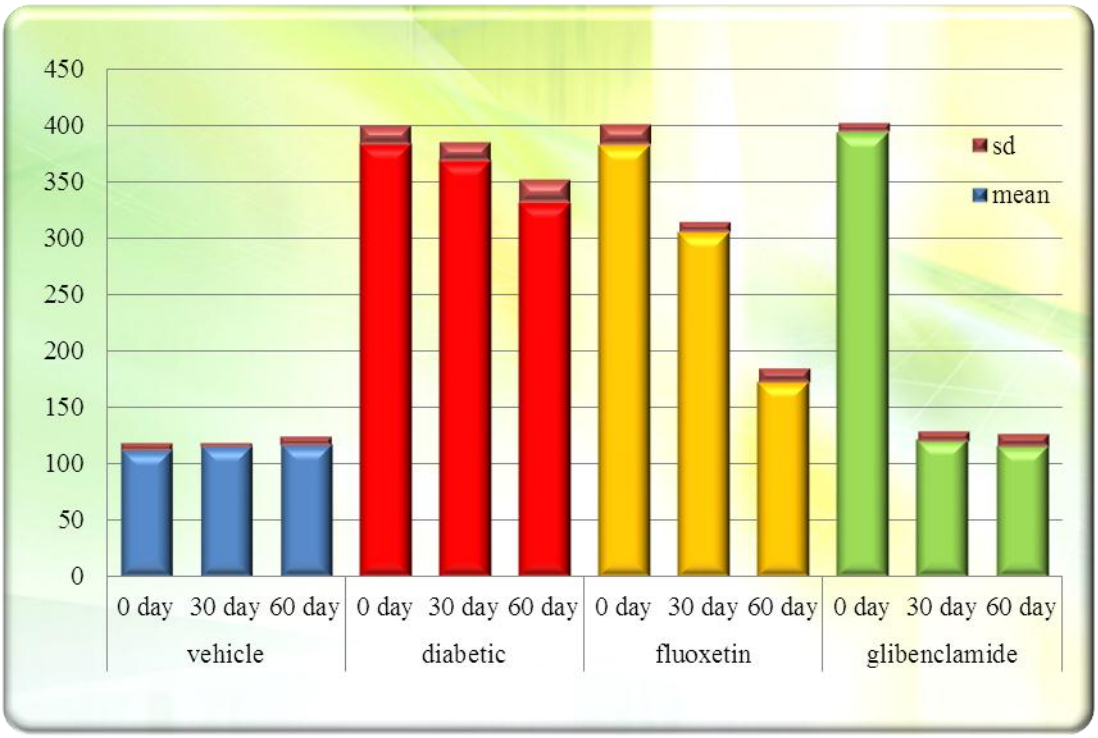
Bar Chart: Fasting blood sugar



Data expressed as mean ± S.D., n=6 the data statistically, (ANOVA test) indicates significant (p value= <0.001) induction of diabetes compared to vehicle control at 0 day. Fluoxetine significantly ($p<0.05$) decreased blood glucose level at 30th day as compared to 0 day reading and significantly ($p < 0.01$) decreased blood glucose level at 60th day compared to 0 day reading.

Table and Diagram No.3: Post prandial blood Sugar

		Vehicle	Diabetic	Fluoxetine	Glibenclamide
0 day	Mean	112.67	383.50	382.67	394.33
	SD	6.12	16.59	18.05	8.36
30 day	Mean	115.33	368.33	305.50	120.83
	SD	3.72	16.66	9.42	8.18
60 day	Mean	117.33	331.33	172.50	115.67
	SD	8.07	20.77	12.52	11.43



Data expressed as mean ± S.D., n=6 the data statistically, (ANOVA test) indicates significant (p value= <0.001) induction of diabetes compared to vehicle control at 0 day. Fluoxetine significantly ($p<0.05$) decreased blood glucose level at 30th day as compared to 0 day reading while more significantly ($p<0.01$) decreased blood glucose at 60th day.

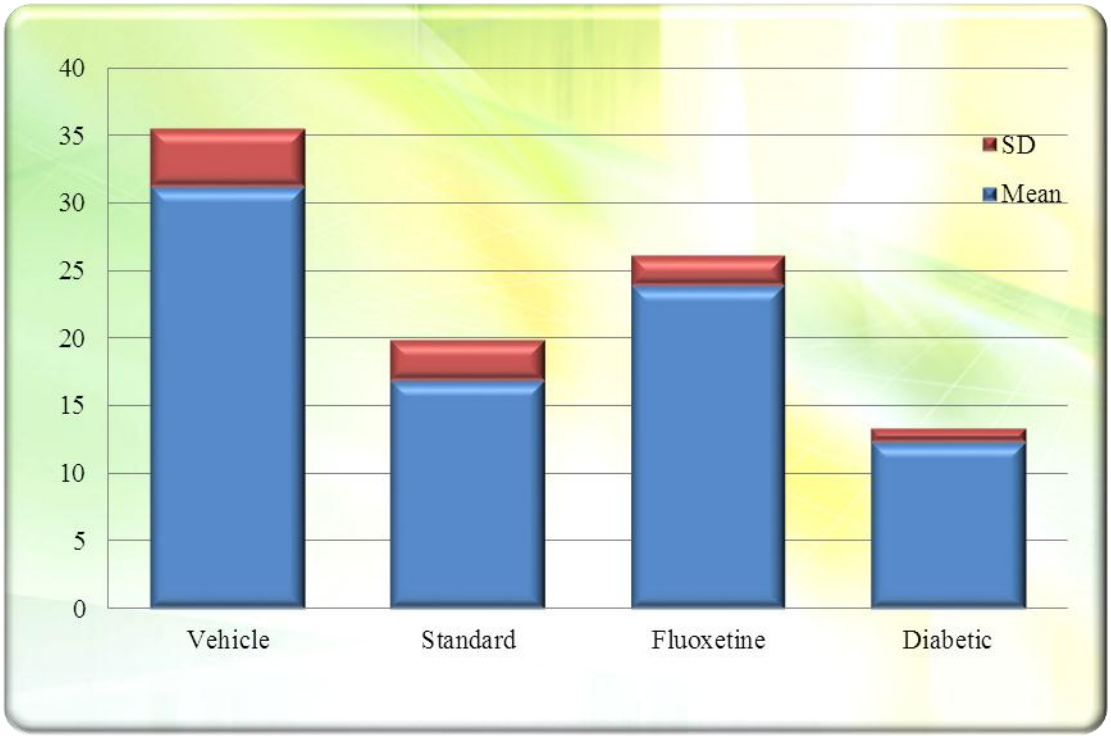
Table No. 4: Grip strength time in seconds on 60th day

Group	Rat No.	Grip Strength on 60th Day
Vehicle control	1	26
	2	38
	3	32
	4	29
	5	34
	6	28
Diabetic control	1	12
	2	11
	3	14
	4	12
	5	13
	6	12
Test Group	1	23
	2	22
	3	27
	4	24
	5	26
	6	21

Standard Group	1	17
	2	18
	3	15
	4	22
	5	13
	6	16

Table and Diagram No. 5: Grip strength (Mean ± S.D.)

	Vehicle	Standard	Fluoxetine	Diabetic
Mean	31.16	16.83	23.83	12.33
SD	4.40	3.06	2.31	1.03



Effect of Fluoxetine on grip strength after 60 days in diabetic rats. Data expressed as mean ± S.D.

t-test unpaired

diabetic compared to vehicle- P value <0.05 significant

Fluoxetine compared to diabetic- P value <0.05 significant

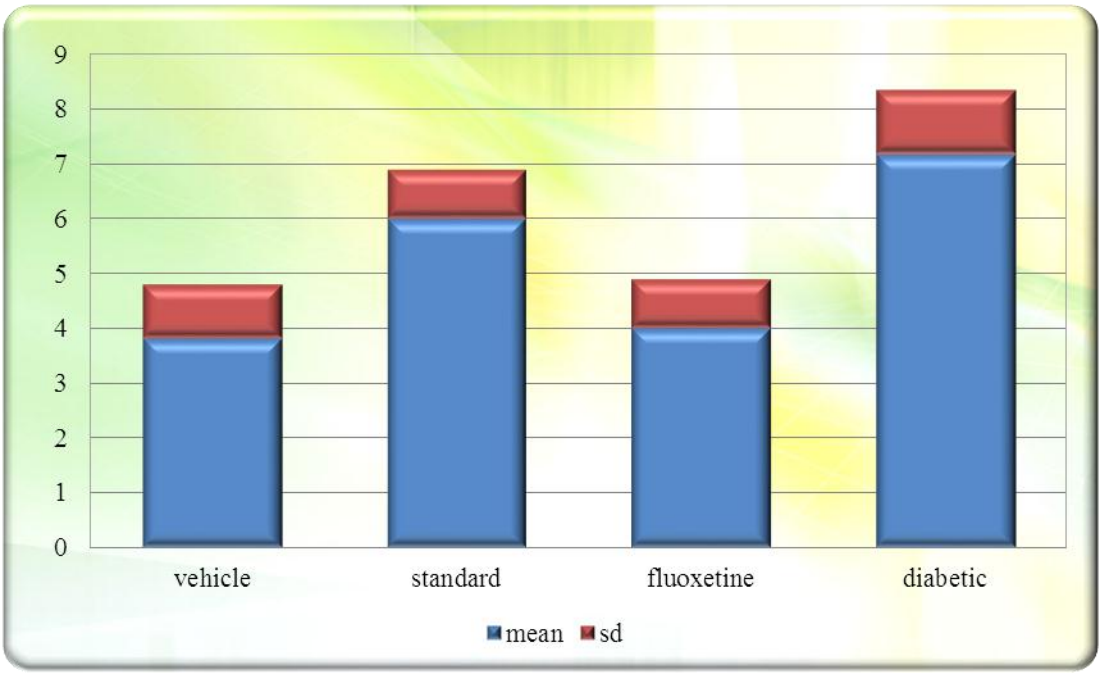
Table No. 6: Tail Flick time in seconds on 60th day

Group	Rat No	Tail Flick time on 60th day
Vehicle control	1	3
	2	5
	3	3
	4	4
	5	5

	6	3
Diabetic control	1	7
	2	6
	3	8
	4	9
	5	7
	6	6
Test	1	4
	2	5
	3	3
	4	3
	5	4
	6	5
Standard	1	7
	2	5
	3	6
	4	7
	5	5
	6	6

Table and Diagram No. 7: Tail Flick

	Vehicle	Standard	Fluoxetine	Diabetic
Mean	3.83	6	4	7.16
SD	0.98	0.89	0.89	1.16



Effect of Fluoxetine on pain sensation using tail flick method. Data expressed as mean ± S.D.

t-test unpaired

diabetic compared to vehicle- P value <0.05 significant

Fluoxetine compared to diabetic- P value <0.05 significant.

Comparison of the effect of Fluoxetine with Glibenclamide on blood glucose level

Table No. 8: Mean reduction in FBS

		Fluoxetine	Glibenclamide	P value
At 30th day	Mean	37.33	108.5	<0.001 Significant
	SD	28.2	16.24	
At 60th day	Mean	63.83	116.5	<0.001 Significant
	SD	20.18	14.27	

Table No. 9: Mean reduction in post prandial Glucose

		Fluoxetine	Glibenclamide	P value
At 30th day	Mean	77.17	273.5	<0.001 Significant
	SD	19.19	13.66	
At 60th day	Mean	210.2	278.7	<0.001 Significant
	SD	12.69	14.32	

DISCUSSION

In this study, the effect of Fluoxetine (SSRI) on blood glucose levels and on diabetic neuropathy was investigated in streptozotocin induced diabetic rats. In 1996 M A Deeg and E W Lipkin reported that symptomatic hypoglycemia was associated with increased blood insulin levels during a 72 hour fast, when the patient was taking fluoxetine [9]. In 2008 Derijks HJ, Heerdink ER, De Koning FH, Janknegt R, Klungel OH, Egberts AC. reported SSRIs and tricyclic antidepressants, had been found to interfere with blood glucose metabolism, increasing the risk of hypoglycemic episodes [10]. In 2012 Paul Zammit posted a case report which reports a rare case of recurrent hypoglycemia following SSRI use in a non diabetic elderly woman [11].

In 2001 Ryan J. Anderson, Kenneth E. Freedland, Ray E. Clouse, MD and Patrick J. Lustman, concluded that the presence of diabetes, doubles the odds of co morbid depression.[12]

In 1999 Sawynok J1, Esser MJ, Reid AR stated that 5-hydroxytryptamine (5-HT) reuptake inhibitor had recently been demonstrated to produce a peripheral antinociceptive action in an inflammatory (formalin test) and a neuropathic pain mode [13].

In 2004 Duby JJ, Campbell RK, Setter SM, White JR, Rasmussen KA JJ, cam Jan concluded that Diabetic neuropathy was a many-faceted complication of diabetes that can be managed symptomatically with an array of drugs like fluoxetine etc. [14]

So, by considering the hypoglycemic side effect, the present study was aimed to evaluate the influence of 9 week treatment of fluoxetine on blood glucose levels and on progression of diabetic neuropathy in STZ-induced diabetes rats.

1. This study suggest that 9 week treatment of Fluoxetine caused significant ($p<0.05$) decrease in the blood glucose level at 30th day and more significant ($p<0.01$) effect was observed only on the 60th day.
2. This study also suggests that treatment of Fluoxetine when compared to Glibenclamide showed a decrease in blood glucose levels at 30th day and 60th day but less as compared to Glibenclamide.
3. The diabetic animals demonstrated a significant ($p<0.05$) decrease in the grip strength as compared to normal rats. This indicates muscle weakness and induction of neuropathy, whereas 9 week treatment with Fluoxetine indicated a rise ($p<0.05$) in the grip strength in diabetic rats indicating protective effect of Fluoxetine on grip strength in diabetic condition.
4. The diabetic animals demonstrated significant ($p<0.05$) increase in tail flicking time (time interval taken by rats to flick its tail after exposure to source of radiant heat) as compared to normal rats indicating loss of pain perception in diabetic rats. This could be attributed to nerve damage resulting, due to development of DN in 9 weeks diabetic rats. While 9 week treatment with Fluoxetine decreased tail flicking time significantly ($p<0.05$), indicated presence of pain perception. Thus it concludes that Fluoxetine protects from the nerve damage in the diabetic animals.

CONCLUSION

Various classes of antidepressant agents that help in regulation and treatment of depressive emotions and neuropathic pain by sustaining balanced level of two neurotransmitters serotonin and norepinephrine. Serotonin and norepinephrine are implicated in modulating descending inhibitory pain pathways in the central nervous system, and are known to help in regulating emotions as well as sensitivity to pain [15].

In diabetes, there is loss of pain perception and it is thought to be due to nerve damage and

induction of peripheral neuropathy. Painful diabetic neuropathy significantly affects the quality of life; and so far, no ideal drug has been available for its management. In the absence of curative therapy, the main aim of the management is to provide symptomatic pain control along with good glycemic control. In reported case studies, Fluoxetine was found to show hypoglycemic side effects [4,9]. So, by considering the hypoglycemic side effect, the present study was undertaken to evaluate the influence of 9 weeks treatment of Fluoxetine (20 mg/kg p.o.) on blood glucose level and on progression of diabetic neuropathy in STZ-induced diabetic rats.

Results of 9 week treatment with Fluoxetine, demonstrates to significant decrease in the blood glucose level at 30th and 60th day while the more significant ($P<0.01$) effect is observed only on 60th day. The observed effect of Fluoxetine was comparable to standard hypoglycemic agent Glibenclamide. Previously it was reported to show hypoglycemic reaction in one of the case report of patient administered with Fluoxetine. The results was also similar with the earlier findings of sertaline which belonged to same antidepressant category [4, 9].

In the present study, induction of diabetic neuropathy was evaluated in terms of muscle strength by measuring grip strength in 9 weeks STZ-induced diabetic rats. The diabetic animals demonstrates significant ($P<0.05$) decrease in the grip strength as compared to normal rats indicating muscle weakness and induction of neuropathy at the end of 9 weeks, whereas 9 week treatment with fluoxetine indicated to raise ($P<0.05$) the grip strength in diabetic rats. Thus the results of the present study demonstrates the protective effect of fluoxetine on grip strength in diabetic condition.

In present study, the pain threshold measured by tail flick method of analgesia, indicated significant ($P<0.05$) increased time in the tail flicking (time interval taken by rats to flick its tail after exposure to source of radiant heat) in tail flick test in diabetic rats. These results indicated a loss of pain perception in diabetic rats in tail flick method, which could be attributed to nerve damage, resulting due to the development of DN in 9-weeks diabetic rats. While 9 week treatment with Fluoxetine caused decrease ($P<0.05$) in the tail flick time in tail flick method. The decrease in tail flick time indicates presence of pain perception in animals. Thus it concludes that Fluoxetine protected from the nerve damage in the diabetic animals. The prevention or management in the DN in the present study may be due to controlling of the glycemic level in diabetic rats.

Finally, from the results, it indicates that 9 week treatment of Fluoxetine reduced the blood glucose level as well as prevented progression of diabetic neuropathy in streptozotocin induced diabetic rats. Hence, it could be helpful in treating the diabetic patient having the complication like diabetic neuropathy.

CONFLICTS OF INTEREST

None.

ACKNOWLEDGEMENTS

None.

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