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Evaluation of behavioural and cognitive effects against diabetic model in young and adult rats

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Abstract
The present work was carried out to study the behavioural and cognitive effects in young and adult diabetic rat models. Diabetes was induced in young and adult rats by using two different doses of STZ (40mg/kg & 60 mg/kg. Behavioural studies were carried out on 1st , 14th and 28th day by using various psychopharmacological tests like elevated plus maze, hole board test, forced swim test. The body weight and fasting blood glucose level were also assessed. The STZ induced groups showed a decrease in body weight after the induction of diabetes. The fasting blood glucose level was found increased in whole groups irrespective of the doses. The time spent and the number of entries in the open arm of elevated plus maze was decreased during the course of the study with an increase in the transfer latency in adult group than that in young groups. The light dark test too showed similar results to that of elevated plus maze with a decrease in the time and number of entries to the light chamber. The hole board test showed a decrease in number of poking in those groups administered with STZ.The forced swim test showed a significant increase in the immobility time in adult groups whereas the younger groups didn't show any significant changes.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action or both .Type 1 diabetes (idiopathic or immune mediated) or Type 2 diabetes (combination of progressive insulin resistance and β-cell failure). Complications of diabetes includes cardiovascular disease ,nerve Damage (neuropathy) kidney damage (nephropathy),eye disease (retinopathy), oral Health, osteoporosis ,diabetic Foot cognitive and memory defects etc. slowing of information processing ,psychomotor efficiency. Some other deficits were also noted such as deficits in motor speed, general intelligence, attention, somato sensory examination, motor strength, memory, executive function, increased number of mental subtraction errors, loss of inhibitions and focus, impaired speed of information processing, decreased attention and impaired working memory has all been noted during acute hyperglycaemia in patients with type 1 & type 2 DM. Streptozotocin and alloxan induces diabetes in animals. The DNA alkylating property of STZ is due to the methyl nitrosourea moiety at the O_6 position of the guanine molecule after reaching inside the cell, the nitrosourea moiety is released and actively poisons the cell by cross-linking with various essential structures present inside the cell..STZ induces DNA alkylation and DNA damage results in the necrosis of the beta cells, induce β cell toxicity and diabetogenecity. Behavioural studies were carried out using various psychopharmacological tests like elevated plus maze, hole board test, forced swim test.

MATERIALS AND METHODS

Young and Adult male wistar rats weighing 50-150g were obtained, 45 days old young male wistar rats, with weight of 50-100 gm. and , 5months old adult rats with weights of 120-180 gm were selected for the study. STZ was used to induce diabetes. Overnight fasted rats were injected with STZ (40mg/kg and 60 mg/kg, ip), dissolved in citrate buffer (pH 4.5) to induce experimental diabetes. Studied the behavioural and cognitive effects in the animals using elevated plus maze ,the hole board apparatus, forced swim test. Statistical analysis was done by means of one way ANOVA followed by Dunnet's test. Results were expressed as mean ±SEM.

RESULTS

Changes in body weight in STZ induced adult diabetic rats

Groups	Changes in body weight					
	Adult group			Young group		
	Day1	Day 14	Day28	Day1	Day14	Day28
Control (vehicle)	144.33±9.91	151.66±9.99	160.50±9.93	102.33±6.08	108.00±6.16	114.33±5.88
Streptozotocin (40mg/kg)	120.83±6.36**	116.66±7.33***	115±10.98***	95.50±.43ns	93.66±7.99**	93.33±8.14***
Streptozotocin (60 mg/kg)	133.33±5.16*	131.00±9.52**	129±12.93**	_	_	_

Table :1

Changes in Blood GlucoseLevel in adult and young rats before and after treatment with STZ

Groups	Changes in Blood Glucose			
	Adult group		Young group	
	Initial	final	Initial	final
Control (vehicle)	87.50±8.87	93.83±7.93	93.33±5.46	93.83±7.93
Streptozotocin (40mg/kg)	90.66±7.96	292.16±178.73*	90.66±7.96	309.16±168.59
Streptozotocin (60 mg/kg)	91.16±7.35	318.33±196.59*	-	-

Table:2

Assessment of behavioral and cognitive parameters by Elevated plus maze on adult group

Group	Number of entries in open arm -adult group(I)			Number of entries in closed arm- adult group(II)		
	Day1	Day 14	Day28	Day1	Day14	Day28
Control (vehicle)	1.83±1.16	5.33±3.55	6.33±3.61	8.50±1.87	6.50±2.16	7.33±1.63
Streptozotocin (40mg/kg)	23.66±14.58ns	1.16±1.16*	1.00± 1.09**	6.50±1.51ns	4.33±0.81*	5.00±1.41*
Streptozotocin (60 mg/kg)	0.83± 0.40ns	0.83±0.98*	0.83±0.40**	5.83±3.54ns	3.00±2.76*	2.33±1.86**

Table:3 Number of entries in open and closed arm(adult group)

Groups	Number of entries in open arm young group(I)		
	Day1	Day14	Day28
Control (vehicle)	3.83±1.94	5.16±1.60	7.33±2.33
Streptozotocin (40mg/kg)	3.50±1.04ns	4.33±1.03ns	3.83±1.16**

Table:4 Time spent in open and closed arm (adult group)

Groups	Transfer latency (seconds)		
	Day 1	Day14	Day28
Control (vehicle)	21.33±11.23	10.83±4.99	12.83±5.45
Streptozotocin (40mg/kg)	23.66±14.58ns	27.83±13.01*	34.83±11.95**
Streptozotocin (60 mg/kg)	20.16±8.40ns	30.83±11.49**	34.00±9.79**

Table:5 Transfer latency(adult group)

Assessment of anxiety by hole board apparatus -adult diabetic and Young diabetic group

Group	Time spent in open arm adult group			Time spent in closed arm		
	Day 1	Day 14	Day 28	Day 1	Day 14	Day 28
Control (vehicle)	12.16 ±11.49	29.50 ±23.27	33.50 ±22.00	266.50 ±14.50	259.66 ±25.63	253.66 ±23.80
Streptozotocin (40mg/kg)	9.50 ±9.20ns	7.16 ±6.11*	5.83 ±8.79*	266.83 ±17.29ns	265.00 ±15.69ns	259.33 ±15.04ns
Streptozotocin (60 mg/kg)	9.16 ±7.02ns	6.00 ±7.12*	6.50 ±4.59*	272.33 ±14.40ns	263.16 ±14.27ns	259.50 ±9.64ns

Table:6 Number of entries in open and closed arm(young group)

Assessment of behavioral and cognitive parameters by Elevated plus maze on young group

Groups	Time spent in open arm young group		
	Day 1	Day14	Day 28
Control (vehicle)	11.00±9.5	22.50±14.02	31.33±10.76
Streptozotocin (40mg/kg)	9.66±6.08^{ns}	12.83±4.07^{ns}	15.33±4.67**

Table:7 Time spent in open and closed arm (young group)

Groups	Transfer latency (seconds)		
	Day 1	Day14	Day28
Control (vehicle)	21.33±11.23	10.83±4.99	12.83±5.45
Streptozotocin (40mg/kg)	23.66±14.58^{ns}	27.83±13.01*	34.83±11.95**

Table:8 Transfer latency(young group)

Assessment of depression by Forced swim test on adult diabetic group and Young diabetic group.

Groups	Number of head dipping- adult group(I)			Number of head dipping – young group(II)		
	Day1	Day14	Day28	Day1	Day14	Day28
Control (vehicle)	14.6666±1.21	16.5000±3.83	21.1667±2.56	14.00±2.52	15.66±2.33	19.33±3.14
Streptozotocin (40mg/kg)	11.333±1.03***	11.8333±1.83*	9.6667±2.80***	12.33±3.98^{ns}	11.16±4.02*	9.50±3.93**
Streptozotocin (60 mg/kg)	9.5000±2.16***	9.333±1.75**	7.6667±2.42.42***	-	-	-

Table:9 Number of head dippings

Groups	Duration of Immobility(sec) adult group(I)				Duration of Immobility(sec) young group(II)	
	Day1	Day14	Day28	Day1	Day14	Day28
Control(vehicle)	144.16±8.13	15.33±14.10	149.83±7.54	102.16±12.20	107.00± 6.35	102.00±9.95
Streptozotocin (40mg/kg)	14.333±15.70^{ns}	177.16±7.11**	176.83±9.80***	99.66±16.83^{ns}	102.16± 14.82^{ns}	107.66± 10.63^{ns}
Streptozotocin (60 mg/kg)	147.33±18.10^{ns}	186.00±12.96**	182.16±17.03***	-	-	-

Table:10 Duration of Immobility

Statistical comparison applicable to all the above tables: One way ANOVA, followed by Dunnett's comparison was performed. Group II and Group III were compared with vehicle control group(Group I) (*** P<0.001, ** P<0.01, * P< 0.05, ns -non significant).

DISCUSSION

Diabetes mellitus is one of the major metabolic disorders characterized by hyperglycaemia resulting from defects in secretion of insulin, insulin action or life threatening complications that mainly affects the heart, blood vessels, renal system, eye, nerves etc. Subjects with type I and type II diabetes mellitus have been reported to have cognitive dysfunction but is paid less attention. The central nervous system is seriously affected by the deleterious effects of diabetes which results in behavioral and cognitive decline. The present thesis was aimed to explore and compare the behavioral and cognitive effects against diabetic models in adult as well as young diabetic rats. Diabetes was induced using streptozotocin at two different doses for adult rats and with lower dose to younger rats such that, the dose dependent alterations in behavior and cognitive parameters could be determined.

Body weight:

In the course of 28 days study, all the rats were weighed periodically and compared with the control group. In the adult groups, rats administered with 40 and 60 mg/ kg STZ showed a reduction in weight with p<0.001 and p<0.01 respectively. In case of younger group induced with STZ showed a significant decrease in weight on14th (p<0.01),

and 28th day (p<0.001) of the study period. Animals showed significant increase in the blood glucose level. shown in table no.1 and 2

Elevated plus maze: In adult diabetic rats (STZ 40 mg/kg and 80 mg/kg) showed a significant decrease in the number of entries to open arm on 14th (p<0.05) and 28th day (p<0.01) of the study as shown in table no.3 When we consider the groups induced with STZ (40 mg/kg) showed a significant decrease in the time spent in open arm on day 14 (p<0.05) and 28th day (p<0.05) similarly group (STZ 60 mg/kg) also showed a significant decrease in the time spent in the open arm on 14th (p<0.05) day of the study.

The transfer latency significantly increased in adult group as shown in table no:5. The groups induced with STZ (60 mg/kg showed a higher TL, throughout the 28 days study than 40 mg/kg induced groups. The TL was significantly increased on day 14 (p<0.05), day and day 28(p<0.01). In younger groups with diabetes, STZ(40 mg/kg) showed a significant decrease in the number of entries towards open arm on 28th day (p<0.05) of the study where as no significant changes was on 14th day as shown in table no.6.Animals didn't show any significant changes in time spent in open arm on day 14.There was a significant decrease in time spent in the open on 28th day (p<0.01) .

Hole board test: Both adult and young diabetic rats were subjected to hole board test to analyse the anxiety state during diabetes. In adult diabetic the number of head poking was found to be decreased significantly throughout the 28 day study with a greater decrease in the group induced with higher the dose of STZ (group III) as shown in the table no.9. The younger diabetic group didn't showed any significant changes in head dipping on day 1, later on showed significant decrease in the number of head dipping on day 28(p<0.01) which is shown in table no.9.

Forced swim test: Forced swim test was carried out in order to find out the state of depression during diabetes condition. There were no significant changes in the duration of immobility on day 1 incase of adult diabetic group. The groups induced with STZ(40 mg/kg and 60 mg/kg) showed a significant increase with a higher value in the immobility time on day 14(p<0.01) and on day 28(p<0.001)**.** The younger diabetic groups didn't show any significant changes in the duration of immobility table no.10.

CONCLUSION

In the present study, the diabetes was induced using streptozotocin alterations in behavior and cognitive parameters could be determined and alterations between age groups like young and adult rats were compared. The results from behavioural study indicated the altered behavioural patterns of the animals.There was an abundant increase in anxiety were observed in all the models against STZ, there was no significant changes in the transfer latency between young and adult diabetic rats.The results from behavioural studies like hole board test, forced swim test locomotions, head dipping /anxiety, immobility/depressive behaviour respectively in the adult rats but there was no significant altered behavioural changes were observed in young diabetic rats which indirectly reveals that younger animals (45 days of age) are suitable for behavioural performance/alteration based studies in relation with diabetes

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