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Neurobehavioral effects of liraglutide and sitagliptin in experimental models

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ABSTRACT

Glucagon-like peptide (GLP-1) receptor agonists and dipeptidyl peptidase 4 (DPP-4) inhibitors are two currently approved therapies for type 2 diabetes mellitus (T2DM). Present study evaluated the effect of liraglutide (a long-acting GLP-1 agonist) and sitagliptin (a DPP-4 inhibitor) on nociception, anxiety, depression-like behavior and cognition in rats or mice.

Nociception was assessed using tail-flick test; anxiety-behavior in open-field test and elevated plus maze (EPM) test while depression-like behavior was evaluated in forced swim test (FST) and tail-suspension test (TST). Cognition was assessed in EPM and Morris water maze (MWM) following memory deficit induced by pentylenetetrazole (PTZ) or scopolamine.

In tail-flick test sitagliptin (6 mg/kg) produced transient nociceptive effect. Liraglutide (200 μg/kg) reduced peripheral square crossings by rats in open field test as well as reduced closed arm entries in the EPM, indicating a decline in exploratory behavior. In FST and TST models for depression, the duration of immobility with sitagliptin (6 mg/kg) was reduced significantly in comparison to control group suggesting its antidepressant effect. Liraglutide did not show any antidepressant action. In EPM test for cognition, liraglutide and sitagliptin ameliorated the increase in transfer latency caused by PTZ in a dose-dependent manner. In MWM liraglutide and sitagliptin prevented the scopolamine-induced increase of the escape latency.

This study shows that sitagliptin has mild antinociceptive effect and anti-depressant effect in the animal models of depression while liraglutide did not have such an effect. Liraglutide showed anxiogenic effects in the animal models. Both liraglutide and sitagliptin produced cognitive improvement in the animal models.

1. Introduction

Modulation of the incretin systems has provided a novel treatment option in type 2 diabetes mellitus (T2DM). Glucagon-like peptide (GLP)-1, an incretin hormone exhibits diverse actions including insulinotropic effects, neogenesis, differentiation and anti-apoptotic preservation of pancreatic β-cells (Baggio and Drucker, 2006; Drucker, 2003). GLP-1 acts through GLP-1 receptors (R). GLP-1R stimulation enhances pancreatic islet beta-cell proliferation, insulin secretion and decreases blood glucose and food intake in patients with type 2 diabetes mellitus.

Endogenous GLP-1 has a half-life of a few minutes as it is broken down by endopeptidase enzymes such as dipeptidyl peptidase 4 (DPP-4) (Vilsboll et al., 2003). Thus it is unsuitable for routine clinical use on account of its short half-life. Drugs with a highly increased half-life acting either by stimulation of GLP-1 receptors (GLP-1 receptor agonists) or by restoring the endogenous GLP-1 pool by inhibiting its DPP-4 mediated breakdown are developed to obtain or maintain high levels of GLP-1 (Drucker, 2003). Growing evidence has shown that GLP-1 is also produced in the brain (Alvarez et al., 1996), particularly from the nucleus of the solitary tract (Larsen et al., 1997), area postrema and caudal brain stem (Hamilton and Holscher, 2009). In the brain, it acts as a growth factor. GLP-1 has been shown to enhance neurite outgrowth and to protect against oxidative injury in cultured neuronal cells (Perry et al., 2007). In addition, GLP-1R is widely expressed in many regions of the CNS and plays an important role in regulating neuronal plasticity and cell survival. Mice overexpressing GLP-1R in the hippocampus developed increased neurite growth and showed improved learning (During et al., 2003).

Substantial amount of evidence supports neurotrophic and neuroprotective potential of GLP-1 and GLP-1R stimulation in an increasing array of cellular and animal neurodegeneration models

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as well as in neurogenesis (Holscher, 2012; Salcedo et al., 2012). Hence, in recent years, research involving GLP-1 and its receptors has shifted from T2DM to focus upon various neurodegenerative disorders (Bertilsson et al., 2008; Martin et al., 2009). Activation of incretin pathway has been shown to stimulate neuronal cell proliferation and prevented cell death (Li et al., 2010). Inhibition of GLP-1 degradation with the DPP-4 inhibitor is also associated with neuroprotection in the diabetic rat, independent of any changes in glycemia (Jin et al., 2009).

In recent studies, depression is also characterized by enhanced neurodegeneration (Maes et al., 2009) and hence GLP-1 could have a role in depression. In addition, the potential of GLP-1 receptors in animal models of pain (Gong et al., 2014) and DPP-4 in degradation of substance P and its influence on pain pathway is described (Grouzmann et al., 2011). Also, some studies have shown the anxiogenic potential of exogenous GLP-1 (Kinzig et al., 2003).

Liraglutide, a long-acting GLP-1R agonist and sitagliptin, a DPP-4 inhibitor are approved drugs for T2DM. Both liraglutide and sitagliptin have been shown to reverse the deleterious effect on learning and memory in mice fed with high fat diet (Gault et al., 2015; Porter et al., 2010).

However, the neurobehavioral effects of liraglutide and sitagliptin are not clear. Therefore, the present study evaluated the effect of liraglutide and sitagliptin on nociception, anxiety and depression-like behavior and cognition in rats or mice. Nociception was assessed using tail-flick test, anxiety-behavior was observed in open-field test and elevated plus maze (EPM) test while depression-like behavior was evaluated in forced swim test (FST) and tail-suspension test (TST). Cognitive behavior was examined in Morris water maze (MWM) and in EPM following memory deficit induced by scopolamine or pentylenetetrazole (PTZ).

2. Material and methods

The present study was approved by the Institutional Animal Experimentation Ethics Committee of Lady Hardinge Medical College (LHMC) and Smt. SK Hospital (SSKH). The care and use of animals in the present study adhered to the 'CPCSEA [Committee for the Purpose of Control and Supervision of Experiments on Animals], India, Guidelines for Laboratory Animal Facilities'.

2.1. Animals

Male Wistar rats or male Swiss albino mice weighing 100–150 g and 25–30 g respectively were used. The rats were housed individually and mice were housed in groups of four to six per cage under a 12 h light–dark cycle and were fed a standard laboratory diet and water *ad libitum*. Animals were acclimatized for at least 1 week before being used in the studies.

2.2. Drugs

Liraglutide injections (VICTOZA, Novo-Nordisk India Pvt. Limited) were obtained as gift samples from the distributor. Sitagliptin phosphate tablets (JANUVIA, Merck Sharp & Dohme, Italy) were purchased from the market. Morphine, lorazepam and imipramine injections were obtained from the hospital supply. Scopolamine (purity $\geq$ 90%, HPLC) and pentylenetetrazole were purchased from Sigma Chemical Co. (USA).

2.3. Groups

All experiments were performed independently i.e. different set of animals were dosed for each study. The animals (6/group) were intrapertonealy (i.p.) treated as follows: group 1: control (saline), group 2: liraglutide 100 μg/kg, group 3: liraglutide 200 μg/kg, group 4: sitagliptin 3 mg/kg, group 5: sitagliptin 6 mg/kg, group 6: positive control (morphine 2 mg/kg for tail-flick test, lorazepam 1 mg/kg for open-field test and EPM test, imipramine 10 mg/kg for FST and TST). For assessment of learning and memory, the animals (6/group) were treated as follows in EPM tests: group 1: control (saline), group 2: PTZ 200 μg/kg, group 3: PTZ+liraglutide 100 μg/kg, group 4: PTZ+liraglutide 200 μg/kg, group 5: PTZ+sitagliptin 3 mg/kg group 6: PTZ+sitagliptin 6 mg/kg. In MWM test scopolamine 5 mg/kg was used in place of PTZ and only higher dose of liraglutide and sitagliptin was used. Morphine was used as positive control for tail flick test, lorazepam was used as positive control for tests of anxiety (open-field test and EPM test) and imipramine was given as positive control for tests of depression.

2.4. Assessment of nociception

A Techno analgesiometer was used to assess tail-flick latency. The thermal stimulus was applied to the ventral aspect of the mouse's tail and three consecutive readings, at approximately 20 s intervals, were obtained and the average calculated. Care was taken to slightly shift the point of heat application. Animals with tail-flick latency of 4–6 s were included in the study. A cut-off latency of 10 s was set to prevent tissue damage.

2.5. Assessment of anxiety

2.5.1. Open-field test

For open-field test a square open field arena of dimension 68 × 68 × 48 cm divided in 25 square marks measuring 8 × 8 cm each was used. After 30 min of administering saline/test drug, rat was kept in center and its locomotor activity i.e. no. of cental and peripheral squares crossed, was observed for 15 min. Ratio central/total locomotion was calculated to assess anxiolysis.

2.5.2. Elevated plus maze

For elevated plus maze a plus-maze consisting of two open arms (50 cm × 10 cm) and two enclosed arms (50 cm × 10 cm × 40 cm – height) with an open roof, arranged so that the two open arms are opposite to each other was used. The maze was elevated to a height of 50 cm and rat was placed in the center of maze. After 1 h of the treatment with saline/test drug, entries in closed arm, open arm and motor activity were recorded for a period of 5 min (Pellow and File, 1986).

2.6. Assessment of depression

2.6.1. Forced swim test

In forced swim test mice were individually forced to swim inside a vertical plexiglas cylinder (height: 40 cm, diameter: 18 cm), containing water filled up to 15 cm of height. Animals were observed for a total period of 5 min and duration of immobility in the water was recorded.

2.6.2. Tail suspension test

For tail suspension test the mice were suspended on the edge of a shelf, 58 cm above the ground, by adhesive tape placed approximately 1 cm from the tip of the tail of the mice. Mice were considered immobile when they hung passively and completely motionless. This duration of immobility of the mice was recorded for an observation period of 5 min.

Mice were treated for 7 consecutive days with respective treatment before FST/TST. Further, higher dose of each treatment (sitagliptin 6 mg/kg and liraglutide 200 μg/kg) were tested using inclined plane and rotarod tests to assess any motor side-effects. In

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the inclined plane tests, 10 min after drug administration mice were placed at the upper part of the inclined plane and were given 30 s to hang on or to fall off. The test was repeated every 30 min up to 2 h. In the rotarod tests, the mice were tested prior to drug administration for their ability to remain on the rotating rod for at least 1 min. Ten minutes after drug administration, mice were placed on the rotating rod for 1 min. The test was repeated every 30 min up to 2 h.

2.7. Assessment of learning and memory

2.7.1. Elevated plus maze

Elevated plus maze is used to assess cognitive behavior as described by Itoh et al. (1990). An elevated plus maze consisting of two open arms of dimension 16 cm (l) × 5 cm (b) and two covered arms of dimension 16 cm (l) × 5 cm (b) × 12 cm (h) with an open roof was used. The arms extended from a central platform of dimension 5 cm (l) × 5 cm (b), and the maze was elevated to a height of 25 cm from the floor. On the first day, mice were placed individually at either ends of the open arms and allowed to enter the closed arms. If the animal did not enter the closed arm within 90 s, it was gently pushed in the closed arm. To acquaint with the maze, the animals were allowed to explore it for 10 s after reaching the closed arm and then returned to their home cage. Animals were retrained for five consecutive days after the first day training to test the retention of memory. On test day, the time taken by the animal to move from the open arm to the closed arm was taken as transfer latency.

Memory deficit in mice was induced with PTZ 25 mg/kg/day i. p., administered thrice on 6, 4 and 2 days before the tests (Gupta et al., 2003). Liraglutide and sitagliptin were administered daily over the six days. On days 6, 4 and 2 test drugs were administered 30 min after PTZ.

2.7.2. Morris water maze

A circular water tank of 1.7 m diameter filled with water at 25 °C filled upto 25 cm height was used. The tank was divided into four equal quadrants. A submerged platform (8 cm in diameter and 19 cm in height), was set in one of the target quadrants of the pool. The rats were given two sessions of trial each day for four consecutive days, with inter-trial interval of 15 min. The time taken to find the hidden platform i.e. escape latency was recorded for each trial. If rat failed to find the platform within 120 s, it was guided gently onto the platform and allowed to remain there for 20 s. Significant decrease in latency time from that of 1st session was considered as successful learning (Morris, 1984).

On fifth day, rats were subjected to a probe trial session in which the platform was removed from the pool. A record was kept of the swimming time in the “target quadrant” (TSTQ) where the platform had previously been placed; observation time for TSTQ was 60 s.

Scopolamine causes memory deficit in rats (Flood and Cherkin, 1986). A dose of 5 mg/kg i.p. was administered 30 min before training trials. Liraglutide and sitagliptin were administered daily for 4 days, 30 min before scopolamine to assess their effect on learning and memory.

2.8. Statistical analysis

All values are expressed as mean ± S.D. Repeated-measures anova was used to analyse the effects of drug, time and drug-time interactions on the tail-flick latency in the different groups. Post hoc comparisons of the treatment groups against the control and morphine groups were made withDunnett's t-test (two-sided). Data of elevated plus maze, forced-swim and tail-suspension tests, Morris water maze and probe trial of Morris water maze tests analyzed by one-way analysis of variance (ANOVA) followed by post-hoc tests (Tukey's orNewman-Keuls'). Statistical significance was set at $P < 0.05$.

3. Results

3.1. Effect of liraglutide and sitagliptin on nociception

Administration of liraglutide 100 μg/kg and 200 μg/kg and sitagliptin 3 mg/kg did not have any anti-nociceptive effect. Higher dose of sitagliptin 6 mg/kg produced a short-lasting anti-nociceptive effect at 60 min [F (5, 30) = 8.12, $P < 0.05$] (Fig. 1).

3.2. Effect of liraglutide and sitagliptin in models of anxiety

Administration of liraglutide 100 μg/kg and 200 μg/kg significantly reduced ($P \leq 0.05$) the number of peripheral square crossings compared to control group, indicating a decline in the exploratory behavior. Number of central and peripheral square crossings in sitagliptin 3 mg/kg and 6 mg/kg groups were similar to control group, indicating a normal exploratory behavior. Lorazepam 2 mg/kg significantly increased the number of central square crossings compared to control group indicating its anti-anxiety effect (Fig. 2).

Similarly in EPM, there was no significant difference in the number of open arm entries following liraglutide (100 μg/kg and 200 μg/kg) or sitagliptin (3 mg/kg and 6 mg/kg) administration as compared to control, indicating no antianxiety effect of these drugs. Lorazepam 2 mg/kg significantly increased the number of open arm entries compared to control group. Liraglutide 200 μg/kg significantly reduced closed arm entries compared to control, indicating a decline in the exploratory behavior (Fig. 3).

3.3. Effect of liraglutide and sitagliptin in models of depression

In both FST and TST, there was no significant difference in the duration of immobility in liraglutide 100 μg/kg and 200 μg/kg groups compared to control. Sitagliptin 3 mg/kg and 6 mg/kg, reduced the duration of immobility in a dose-dependent manner but it was statistically significant ($P \leq 0.05$) with the higher dose only. In both the models, imipramine 10 mg/kg significantly reduced the duration of immobility compared to respective control groups

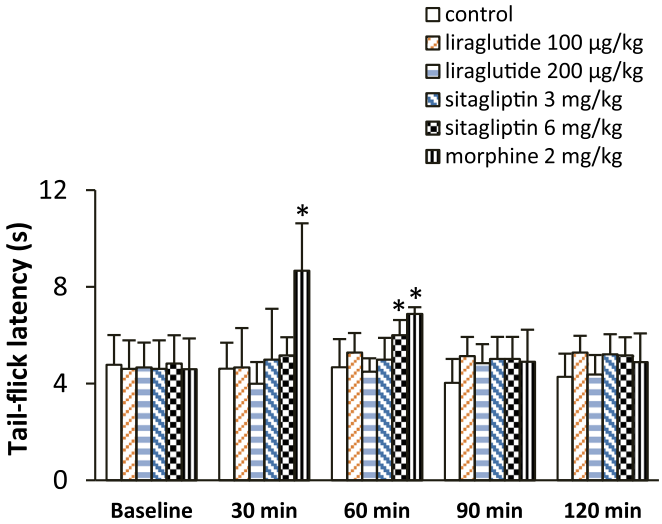

Fig. 1. Effect of liraglutide (100, 200 μg/kg) and sitagliptin (3, 6 mg/kg) on tail-flick latency (mean ± s.d.) using radiant heat in mice, $n = 6$ in each group, $^{*}P < 0.05$ compared to control.

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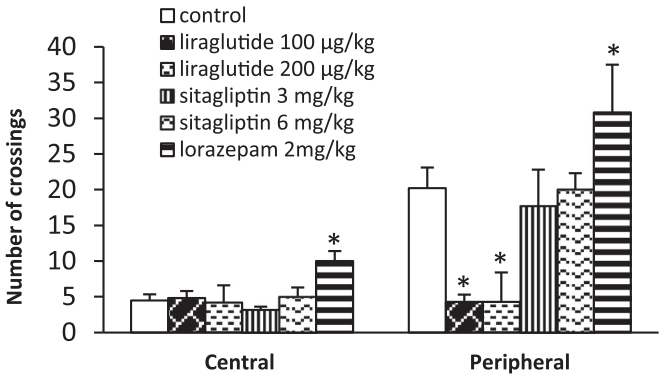

Fig. 2. Effect of liraglutide (100, 200 µg/kg) and sitagliptin (3, 6 mg/kg) in open field test in rats ($n=6$/group). Results are shown as mean ± S.D. $^*P<0.05$ significant in comparison to control.

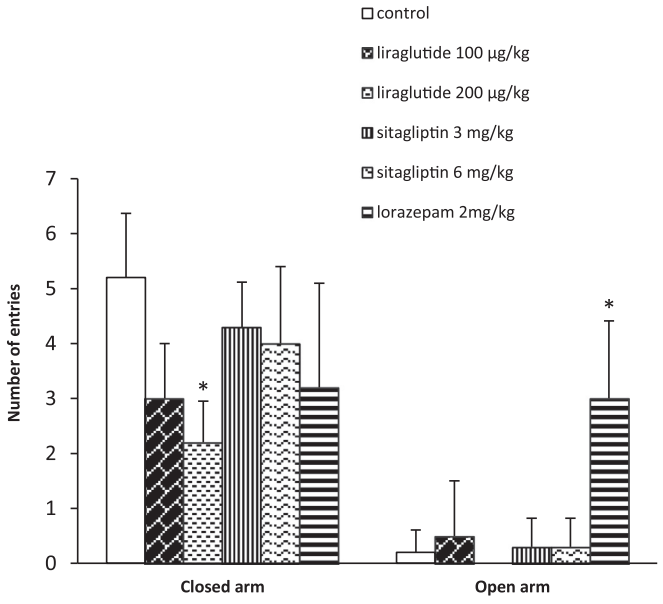

Fig. 3. Effect of liraglutide (100, 200 µg/kg) and sitagliptin (3, 6 mg/kg) in elevated plus maze test for anxiety in rats ($n=6$/group). Results are shown as mean ± S.D. $^*P<0.05$ significant in comparison to control.

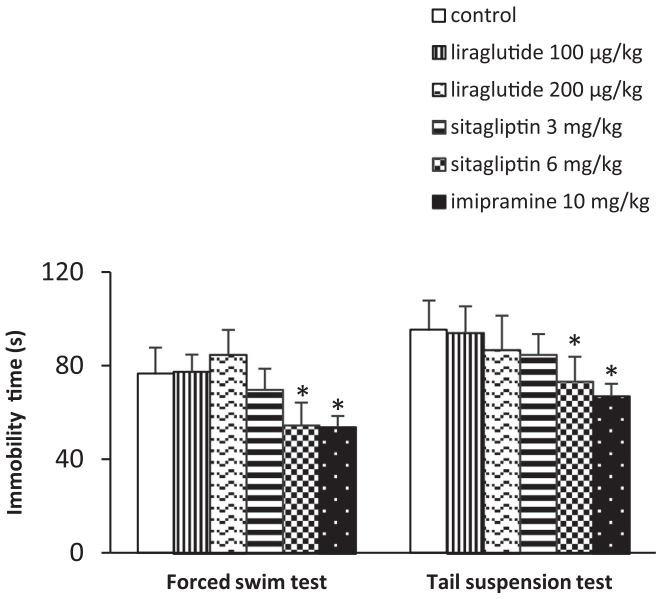

Fig. 4. Effect of liraglutide and sitagliptin in forced-swim and tail-suspension tests in mice ($n=6$/group). 'Immobility times' are shown as mean ± S.D; $^*P<0.05$ significant in comparison to control.

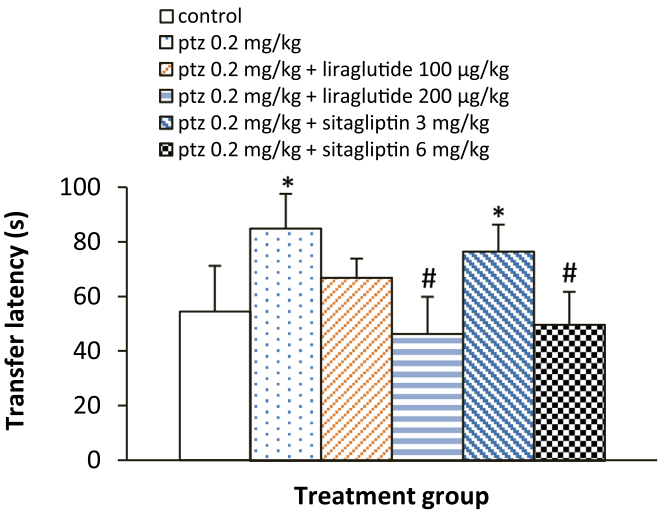

Fig. 5. Effect of liraglutide 100 and 200 µg/kg and sitagliptin 3 and 6 mg/kg on transfer latency of mice in elevated plus maze ($n=6$ in each group). Results are shown as mean ± S.D.; $^*P<0.05$ compared to control, #$P<0.05$ compared to PTZ.

(Fig. 4).

In inclined plane tests all six mice treated with liraglutide 200 µg/kg and sitagliptin 6 mg/kg hung on till the cut-off time of 30 s at all time-points. In rota rod tests, 5 of the six mice treated with liraglutide 200 µg/kg and all six treated with sitagliptin 6 mg/kg held on till the cut-off time of one min, showing no motor side effects.

3.4. Effect of liraglutide and sitagliptin on cognitive behavior

In elevated plus maze for learning, comparison of transfer latency amongst the groups showed significant differences [F (5, 35)=9.4; $P<0.01$] on day 6. PTZ treated animals showed significantly increased transfer latency as compared to control group ($P<0.01$). Administration of liraglutide 100 µg/kg and 200 µg/kg and sitagliptin 3 mg/kg and 6 mg/kg decreased the transfer latency as compared to PTZ alone group, which was statistically significant with higher doses of both the drugs ($P<0.01$) (Fig. 5).

In MWM, a significant difference in the escape latency time (ELT) was seen between first day sessions and 4th day sessions [F (1, 40)=38.6; $P<0.001$] and owing to groups*days interaction [F (3, 40)=5.6; $P<0.01$] (two way ANOVA). In control group a significant decrease in the ELT on 4th day sessions as compared to first day sessions ($P<0.01$) was observed, indicating a normal acquisition (Fig. 6A). In probe trial on day 5, mice preferentially stayed in the target quadrant (TSTQ > 50% of observation period) (Fig. 6B). It indicates normal retrieval of memory in the control mice.

In scopolamine-treated mice ELT was significantly increased as compared to control mice on day 4 (Fig. 6A). These mice also spent significantly less time in the target quadrant as compared to the control mice on day 5 ($P<0.05$) (Fig. 6B). These observations suggest that scopolamine impairs the process of acquisition of new memory which subsequently affects retrieval of memory implying lack of acquisition.

Administration of liraglutide 200 µg/kg and sitagliptin 6 mg/kg prevented the scopolamine-induced increase of the ELT on day 4, indicating amelioration of scopolamine-induced impairment of acquisition (Fig. 6A).

Further, treatment with liraglutide 200 µg/kg and sitagliptin 6 mg/kg also attenuated scopolamine-induced decrease TSTQ in

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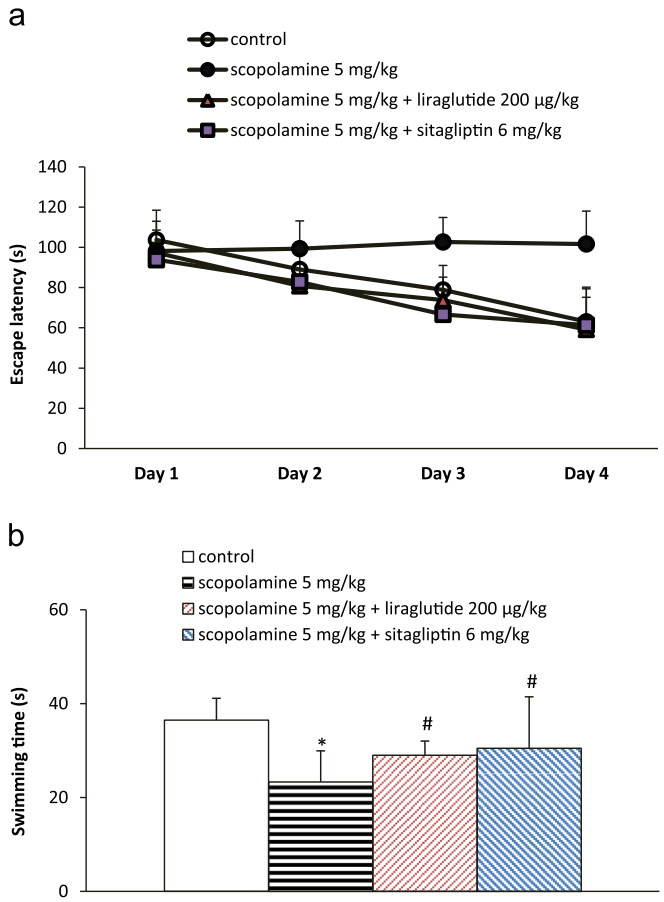

Fig. 6. Effect of liraglutide 200 μg/kg and sitagliptin 6 mg/kg on (A) escape latency of rats in Morris water maze in trial sessions from days 1 to 4 (B) "time spent in target quadrant TSTQ" ($n=6$ in each group). Results are shown as mean ± S.D.; $^{*}P<0.05$ compared to control, $\#P<0.05$ compared to scopolamine.

search of missing platform during retrieval trial in a significant manner on day 5 ($P<0.05$ for both), reflecting a reversal of scopolamine-induced impairment of memory (Fig. 6B).

4. Discussion

In the present study, we examined the effect of liraglutide and sitagliptin on nociception, anxiety and depression-like behavior and their effects on cognition in rats or mice. Tail flick test was used in normal mice to evaluate the effect on nociception. Anxiety like behavior was evaluated using open field test and elevated plus maze. The open field test may provide a good measure of the approach response toward novelty, that is, exploration (Prut and Belzung, 2003) and the elevated plus maze has face validity, construct validity and predictive validity for anxiety-related behaviors of rodents (Walf and Frye, 2007).

Depression-like behavior was measured by increased duration of immobility in the forced swim and tail suspension tests. FST and TST are increasingly being used to measure a specific depressive-like behavior, behavioral despair. Cognition was assessed in EPM and MWM following memory deficit induced by PTZ and scopolamine, respectively.

The important finding of our study is that administration of liraglutide and sitagliptin (3 mg/kg) did not have anti-nociceptive effect in tail flick model; higher dose of sitagliptin 6 mg/kg produced a short-lasting anti-nociceptive effect at 60 min. This result is somewhat similar to a study showing that intrathecal GLP-1R agonists and exenatide alleviated neuropathic, cancer and diabetic pain by 60–90%, without affecting acute nociceptive responses in tail flick and hot plate tests in rats and mice (Gong et al., 2014).

The other finding of this study is that in anxiety tests sitagliptin did not influence the central squares crossings in the open field test and open arm entries in the EPM, indicating that it has neither anti-anxiety nor anxiogenic effect in rats, whereas liraglutide (200 μg/kg) administration reduced the peripheral square crossings in open field test and reduced closed arm entries in the EPM, suggesting an anxiogenic action of liraglutide in rats. Here, it is pertinent to mention that 200 μg/kg dose of liraglutide did not produce any effect on inclined plane test or rota rod test, indicating no general CNS depressant effect. Thus in our study DPP-4 inhibitor had no effect on anxiety while GLP-1 agonist, particularly at high dose, decreased exploratory behavior which could represent anxiety-related behavior.

Next important finding is that in FST and TST models for depression, the duration of immobility with sitagliptin (6 mg/kg) was significantly reduced suggesting its antidepressant effect and duration of immobility with liraglutide 200 μg/kg was not reduced significantly in comparison to control group indicating a minimal/no anti-depressant effect of the drug. Other antidiabetic drugs have similarly shown antidepressant effect in preclinical studies (Eissa Ahmed et al., 2009; Salehi-Sadaghiani et al., 2012).

The differential action of liraglutide and sitagliptin in nociceptive, anxiety and depressive behavior in our study are somewhat intriguing. DPP-4 inhibitors increase active GLP-1 concentrations by two or three times the concentrations at baseline, whereas the stimulation of GLP-1 receptor activity with liraglutide is estimated to be several times higher than with DPP-4 inhibitors (Degn et al., 2004). Anti-depressant effect and mild anti-nociceptive effect of sitagliptin could be due to increasing the endogenous peptide GLP-1 concentration by inhibiting DPP-4 enzyme as compared to activation of higher number of GLP-1 receptors by liraglutide simultaneously; one study has demonstrated that GLP-1 crosses the blood–brain barrier (Kastin et al., 2002) and either inhibition of the degrading enzyme DPP-4 or high doses of GLP-1 to saturate the enzyme enhance the penetration of GLP-1 across the BBB. Further, it is important to note that other peptides and substrates of DPP-4 with reported neuroprotective and neurogenic actions, such as pituitary adenylate cyclase-activating polypeptide, glucose-dependent insulinotropic peptide and stromal cell-derived factor -1α may also be involved in the antidepressant action mediated by sitagliptin.

The findings of cognitive behavior show that both liraglutide and sitagliptin ameliorated PTZ induced cognitive impairment in EPM and scopolamine induced cognitive impairment in MWM test in a dose-dependent manner. In water maze task, during the acquisition trials, rat locates the hidden platform in water using spatial cues. Spatial cues help in locating the platform in subsequent trials. In this model, the memory is developed progressively with repetitive trials in few days. In our experiments, control rats exhibited well-formed reference memory.

In EPM and MWM tests of cognition, cognitive impairment was induced by administration of PTZ and scopolamine, respectively. PTZ causes cognitive impairment by increasing oxidative stress. (Gupta et al., 2003). There is a possibility that improvement in cognitive impairment by liraglutide and sitagliptin could be on account of decrease in oxidative stress, which is also shown by other studies. It has been shown that liraglutide and sitagliptin improved memory and cognitive dysfunctions by reducing oxidative stress in mice with high fat dietary-induced obesity and insulin resistance (Gault et al., 2015; Porter et al., 2010).

Further, scopolamine induces cognitive impairment by blocking cholinergic receptors as it is an anticholinergic agent; as well as by increasing the products of oxidative stress (Jeong et al., 2008). GLP-1 has role on cholinergic neurons—activation by exendin-4

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increased preservation of cholinergic neurons in the spinal cord of mouse model of amyotrophic lateral sclerosis (Li et al., 2012). GLP-1 and exendin-4 were shown to restore cholinergic marker function (choline acetyltransferase immunoreactivity) following excitotoxic-induced damage in the basal forebrain (Perry et al., 2002). Although liraglutide and sitagliptin have no direct effect on cholinergic neurons and receptors yet in the present study, their beneficial effect in scopolamine-induced cognitive impairment could be caused by the modulating/increasing effect of GLP-1 and GLP-1R on cholinergic activity/tone of neurons responsible for learning and memory.

In another study peripherally injected liraglutide prevented memory impairments in object recognition and water maze tasks, and also prevented synapse loss and deterioration of synaptic plasticity in the hippocampus in an Alzheimer mouse model, APP/PS1 mice (McClean et al., 2011). In rat models, bilateral intracerebroventricular streptozotocin (ICV-STZ) administration was used to produce impaired insulin signaling in the brain. Fourteen days following ICV-STZ injection, rats treated with twice-daily exendin-4 had better learning and memory performance in the Morris water maze test compared with rats treated with saline. Additionally, histopathological evaluation confirmed the protective effects of exendin-4 treatment on hippocampal neurons against degeneration (Chen et al., 2012). In a recent study, sitagliptin protected against cognitive impairment and brain damage in a murine (C57BL/6 mice) model of chronic cerebral hypoperfusion through suppressing oxidative stress and inflammatory reaction (Tsai et al., 2015). Thus, in the current study liraglutide and sitagliptin might have produced the cognitive benefits by reducing the oxidative stress.

In clinical practice incretin mimetic therapies are increasingly being prescribed for the treatment of T2DM. Current research has shown that CNS GLP-1 is produced in a discrete population of neurons in the nucleus of the solitary tract (Hamilton and Holscher, 2009). GLP-1-producing neurons project to multiple brain areas, including many that are critical to stress responses. In addition, GLP-1 signaling does not affect blood sugar levels in non-diabetic people and therapies that affect GLP-1 signaling have a good safety profile. Chronic patients of DM frequently develop depression (Holt et al., 2014) and cognitive deficits (Kodl and Seaquist, 2008). Anti-depressant activity of sitagliptin and cognitive benefits shown by both liraglutide and sitagliptin will be a desirable attribute. Thus, it is conceivable that the above modulating effects of liraglutide and sitagliptin may be relevant in depression, anxiety and cognitive behavior.

In conclusion, this experimental study shows that sitagliptin has anti-depressant and mild anti-nociceptive effect. However, liraglutide showed anxiogenic effects in the animal models. Both liraglutide and sitagliptin produced cognitive improvement in the animal models. Whether DPP-4 inhibitors and GLP-1 analogues offer additional therapeutic benefits by reducing the co-mobidities in patients of T2DM needs to be evaluated in clinical studies.

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