Available online at www.rajournals.in

RA JOURNAL OF APPLIED RESEARCH
ISSN: 2394-6709
DOI:10.31142/rajar/v4i6.07
Volume: 04 Issue: 06 June -2018

International Open Access
ICV- 74.25
Impact Factor: 5.985
Page no.- 1757-1767

Role of Sitagliptin and Linagliptin in Amelioration of Chronic Mild Stress in Rats

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ARTICLE INFO

Published Online:
11 June 2018

ABSTRACT

Studies show that stress has a pivotal role in the development of diabetes and its complications. Patients with diabetes have an increased prevalence of anxiety and depression as compared to non-diabetic population. The pleiotropic role of Incretins has been a subject of interest in current times, including the anxiolytic and anti-depressant nature of GLP-1 and its analogues. However, no satisfactory reports are available for the role of DPP-IV inhibitors in the same. The role of DPP-IV inhibitors, Sitagliptin and Linagliptin in acute models of anxiety and depression have been investigated in our previous studies. Hence, the present study was carried out to investigate their role in a chronic model of stress (CMS) in rats. Body weight, water intake, sucrose intake, Anhedonia (% preference for sucrose) and cortisol levels were evaluated. Behavioral parameters were evaluated using the light dark box test and Porsolt's forced swim test. Results showed that, Sitagliptin and Linagliptin have a significant role in lowering chronic mild stress in rats as evident from increased sucrose intake and lowering of anhedonia, and cortisol levels in rats treated with Sitagliptin and Linagliptin. This suggests that DPP-IV inhibitors can possibly improve the CNS status of patients of type 2 diabetes.

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KEYWORDS: Diabetes mellitus, DPP-IV Inhibitors, Stress, CMS, Cortisol ,Sitagliptin, Linagliptin

INTRODUCTION

Stress is a nonspecific and physiological mechanism or process by which the organism prepares itself for, and reacts to, demands (called "stressors") that it meets [Alkadhi 2013]. Stress is the emerging as one of the leading cause of cardiovascular disease (CVD) and metabolic disorders like diabetes mellitus and also has a substantial impact on other systems like CNS, GIT, skin and the immune system [Kalia 2002] [Park et al. 2008] [Madamanchi et al. 2005] [Bhatia and Tandon 2005] [Dahlben 2012] [Sterlemann et al. 2008]. Reports suggest that, stress is also implicated in the development of diabetes mellitus and its complications [Radahmadi et al. 2006] [Siddique et al. 2015]. The role of cortisol in stress and diabetes is well studied and documented [Radahmadi et al. 2006] [Detka et al. 2013] [Chiodini et al. 2007]. Cortisol is a primary stress hormone secreted by the adrenal glands in response to inflammation from injury, infection and other allergens and toxins [Nelson and Cox 2008]. High level of cortisol decreases metabolism of glucose and increases mobilization and metabolism of fats. Reports also suggest that elevated levels of cortisol in depression leads to insulin resistance in the brain [Detka et al. 2013]. This contributes significantly to the development of diabetes mellitus and its complications [Cartwell 2014]. Cortisol is being used as a pivotal marker to analyse and assess stress levels in pre-clinical and clinical studies [Kozlov and Kozlova 2014] [Lee et al. 2015]. There are many animal models for the study of acute and chronic stress [Campos et al. 2013] [Sutanto and Kloet 1994]. The chronic mild stress model is a useful and predictable model to study the effect of various drugs in ameliorating chronic stress in experimental animals [Papp 2012] [Papp et al. 2003] [Pochwat 2014] [First et al. 2011] [Pekala et al. 2014]. There are many studies which depict the neuroprotective role of GLP and its agonists in neurodegenerative models in animals [Detka et al. 2013] [Speilman and Klegeris 2014]. Currently, there are insufficient reports of effect of incretins and DPP-IV inhibitors on behavioral and stress related models likes chronic mild stress model in rats. As it is well known, stress is an important factor in the development of diabetes and its complications [Detka et al. 2013], the present study aims at investigating the effect of DPP-IV inhibitors, Sitagliptin and Linagliptin on chronic mild stress model in rats.

EXPERIMENTAL DESIGN

ANIMALS

Wistar albino rats of either sex, weighing 150-250 gm were procured from Zydus Research Centre Ahmedabad. They were housed in groups of six animals each and were fed on standard pellet diet and water ad libitum. Also, they were maintained in optimum conditions of temperature and humidity, at 25± 3 °C and 50±20 % humidity with 12hlight/dark cycle. The experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) and experiments were conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), (Protocol No: LJIP/IAEC/14-15/06).

DRUGS

Sitagliptin and Linagliptin were procured from local sources. Alloxan monohydrate was used to induce diabetes in animals [Chougale et al. 2007] [Misra and Aiman 2012]. Sertraline was used as a reference standard for evaluation of CMS activity [Gomez et al. 2001] [Bilge et al. 2008]. Distilled water was used as a vehicle for the drugs. All the chemicals and reagents used were of analytical grade.

DOSAGE AND ADMINISTRATION

All the solutions were freshly prepared in distilled water before initializing the experiment. Animals were divided into the following groups comprising of 6 animals in each group (table-1). Alloxan was administered Intra-peritoneally to all groups except normal control, 72h before evaluation of anxiolytic activity. Drugs, namely Sitagliptin, Linagliptin and sertraline were given once orally, every day for 21 days, for the entire duration of the study. The doses of Sitagliptin and Linagliptin were optimized in studies performed before the study.

GROUP DESIGN

Table 1: Group Design for evaluation of Sitagliptin and Linagliptin in CMS model in rats

SR NO	GROUP	TREATMENT
1	Normal Control	Vehicle (distilled water)
2	Disease Control	Alloxan (150 mg/kg IP)
3	Sitagliptin	Sitagliptin (10 mg/kg PO)
4	Linagliptin	Linagliptin (3 mg/kg PO)
5	Sertraline	Sertraline (20 mg/kg PO)
6	Sitagliptin + Sertraline	Sitagliptin (5 mg/kg PO) + Sertraline (10mg/kg PO)
7	Linagliptin + Sertraline	Linagliptin (1.5 mg/kg PO) + Sertraline (10 mg/kg PO)

Table 2: Stressor Schedule for CMS study [Kompagnea et al. 2008[[Bhatia et al. 2011][Papp et al 1996][Herrera-Pérez José Jaime et al. 2016]

Day	Morning	Duration	Afternoon	Duration
Day 1	Tail Pinching	5 min	Water Deprivation	12 hrs
Day 2	Tilted cage	4 hrs	Food Deprivation	12 hrs
Day 3	Restrain	120 min	Day-Night Reversal	12 hrs
Day 4	Forced Swimming	15 min	Wet Bedding	18 hrs
Day 5	Cage Rotation	2 hrs	Isolation	18 hrs
Day 6	Tail Pinching	5 min	Food Deprivation	12 hrs
Day 7	Restrain	120 min	Water Deprivation	12 hrs

ANIMAL MODELS

1. **Chronic Mild Stress Model [Kompagnea et al. 2008[[Bhatia et al. 2011][Papp et al 1996]**

   All animals were subjected to a chronic stress procedure for a period of 3 weeks. Baseline parameters were recorded on day 0 and the stressor schedule was initiated. All drugs were administered on day 0 and continued till day 21. Parameters were measured on day 0, 7, 14 and 21. The animals in the control group were maintained during a period of three weeks without stress, but under similar handling and storage conditions to the stressed animals. The stress group was exposed, during a period of three weeks, to several stressors as shown in the table 2.

   Test of Sucrose Consumption [Tsvetan et al. 2016]

   The sucrose preference test (SPT) is a reward-based test, used as an indicator of anhedonia. Anhedonia, or the decreased ability to experience pleasure, represents one of the core symptoms of depression, and hence is used as a pivotal marker in CMS model. Rodents are born with an interest in sweet foods or solutions. Reduced preference for sweet solution in SPT represents anhedonia, while this reduction can be reversed by treatment with antidepressants. The test involved administration of water as well as sucrose solution to rats daily. Water and sucrose solution intake was measured daily, and the positions of two bottles was switched daily to reduce any confound produced by a side bias. Sucrose preference was calculated as a percentage of the volume of sucrose intake over the total volume of fluid intake and averaged over the testing period. After determination of baseline sucrose consumption, young-adultmalerats were randomly

assigned to a control or stress group. The stressor schedule followed in this study is shown in table 2. During the three weeks of CMS, sucrose and water intake were determined weekly, on Day 0, 7, 14 and 21 respectively. The effect of stress on anhedonic state is progressive and the changes in sucrose intake directly establish the onset of anhedonia in the animals.

Light Dark Box Test [Buorin and Hascoet 2003][Vogel 3rd Edition][Bourin et al. 2007]

The apparatus consisted of a Plexiglas box with two compartments (20cm × 20cm each), one of which wasilluminated, while the other remained dark. Each animal was placed at the junction of the light dark, facing theilluminated compartment. The time spent in illuminated and dark chambers, as well as the number of entries in each space, was recorded for 5minutes. The parameters were evaluated one hour after the drugs were administered. This model was evaluated on Day 0, Day 7, Day 14 and Day 21 of the experiment.

2. **Porsolt's Forced Swim Test [Porsolt et al. 1997]**

The behavioral despair test (or Porsolt's forced swimming test) is a test, centered on a rodent's response to the threatof drowning. It is commonly used to measure the effectiveness of antidepressants. Animals are subjected to varioustrials during which they are forced to swim in a glass cylinder filled with water, and from which they cannot escape. The first trial lasts 15 minutes. Then, after 24-hours, various trials are performed that lasts 5 minutes each up to 3hrs. The time that the test animal spends without making any movements beyond those required to keep its headabove water is measured. This immobility time is described as passive mobility and the time it spends moving ortrying to swim is known as active immobility. The parameters were evaluated one hour after the drugs were administered. This model was evaluated on Day 0, Day 7, Day 14 and Day 21 of the experiment.

STATISTICAL ANALYSIS

All results were expressed as Mean± SEM or Percentage based on parametric or non-parametric data. Statistical analysis was done using ANOVA followed by Tukey's multiple comparison tests. Non- parametric data were analyzed using Wilcoxon's Signed rank test. $P<0.05$ was considered to be significant and $P<0.01$ was considered to be highly significant.

RESULTS

1. **PHYSIOLOGICAL PARAMETERS**

a. **Body Weight:** Stress and its effect on body weight have always been debatable. Some recent studies show that rats subjected to CMS do not show a loss of body weight, but a significant decrease in weight gain, thereby appearing as weight loss [Herrera-Pérez José Jaime et al. 2016] [Willner 2017] [Willner et al. 1996] [Gianluigi G et al. 2013]. Results of the study show that, there was a significant difference in the body weight of animals in the disease control group as compared to normal control group as the study progressed, indicating the onset of stress in the animals ($P<0.05$). Furthermore, there was a significant difference in the body weight of animals of the disease control group as compared to the treatment groups ($P<0.05$). The animals treated with a combination of Sitagliptin or Linagliptin with sertraline showed a significant difference in body weight as compared to individual treatment groups ($P<0.05$) (fig.1a).

b. **Sucrose Intake (ml):** Sucrose intake is a measure of reward system studied in rats. The preference for sucrose or sucrose solution is decreased with the onset of stress or depression in the chronic mild stress model in rats. The sucrose intake was significantly decreased in disease control animals as compared to normal control animals ($P<0.05$). This indicates onset of anhedonia in these animals. Sucrose intake was observed to be significantly higher in animals treated with Sitagliptin or Linagliptin as compared to disease control animals ($P<0.05$). The sucrose intake was also observed to be significantly higher in animals treated with a combination of Sitagliptin or Linagliptin with sertraline as compared to individual treatment groups ($P<0.05$) (fig-1b).

c. **Anhedonia (% Preference for sucrose):** The sucrose preference test (SPT) is a reward-based test, used as in indicator of anhedonia. Anhedonia, or the decreased ability to experience pleasure, represents one of the core symptoms of depression. Rodents are born with an interest in sweet foods or solutions. Reduced preference for sweet solution in SPT represents anhedonia. Sucrose preference is calculated as a percentage of the volume of sucrose intake over the total volume of fluid intake and averaged over the testing period [Tsvetan et al. 2016]. Results of the study show that the % sucrose preference was significantly lowered in disease control animals as compared to normal control group ($P<0.05$). Furthermore, it was significantly higher in animals treated with Sitagliptin or Linagliptin as compared to disease control group ($P<0.01$). Animals treated with a combination of standard drug Sertraline with Sitagliptin or Linagliptin also showed significant reduction of anhedonia as compared to individual treatment groups ($P<0.05$) (fig-1c).

d. **Water Intake (ml):** Water Intake is inversely proportional to the sucrose intake or sucrose

preference. Thus, water intake is an indirect indicator of anhedonia indicating the extent of stress- induced in rats. Results of the study show that water intake was observed to be significantly higher in disease control animals as compared to treatment groups (P<0.05). Interestingly, the water intake was found to increase gradually with the progression of the experiment in the disease control animals, whereas the opposite was observed in animals treated with Sitagliptin and Linagliptin. A similar pattern was observed in the animals treated with the standard drug sertraline (fig-1d).

e. **Cortisol Levels:** The role of cortisol in stress and diabetes is well studied and documented [Radahmadi et al. 2006] [Detka et al. 2013] [Chiodini et al. 2007]. Reports also suggest that elevated levels of cortisol in depression leads to insulin resistance in the brain [Detka et al. 2013]. Results of the study show that cortisol levels are significantly increased in disease control animals as compared to normal control group, indicating onset of stress and depression in these animals (P<0.01). Additionally, cortisol levels were observed to be significantly lowered in animals treated with Sitagliptin or Linagliptin (P<0.01) or their concomitant treatments and sertraline (P<0.05) as compared to disease control animals (fig-1d).

2. BEHAVIOURAL PARAMETERS

a. Light Dark Model

i. **No. of Transitions:** The exploratory activity is a direct indicator of the extent of anxiety induced in rats. This can be quantified or analyzed in terms of the number of transitions done by each rat between the light and dark chamber. Results of the study show that the number of transitions were reduced significantly in both normal control and disease control groups, indicating the onset of anxiety in these animals (p<0.05). Furthermore, the number of transitions were observed to be higher in Sitagliptin and Linagliptin treated group as compared to disease control group (p<0.01). Additionally, animals treated with a combination of Sitagliptin or Linagliptin with the standard drug displayed a noticeable decrease in anxiety as shown by the higher number of transitions as compared to Sitagliptin, Linagliptin or sertraline alone (p<0.05) (fig-2a(i)).

ii. **Time spent in Light Chamber:** Studies show that the time spent in light chamber indicates a significantly lower level or absence of anxiety in experimental animals subjected to the light dark box test. The amount of time spent in the light chamber is inversely proportional to the extent of anxiety in experimental animals. Results of the present study show that the animals in the disease control group spent significantly less time in the light chamber as compared to the normal control group (p<0.01). Furthermore, animals treated with Sitagliptin or Linagliptin were observed to spend a significantly higher amount of time in the light chamber as compared to the disease control group (p<0.01). Also, animals treated with a combination of Sitagliptin or Linagliptin with sertraline showed a longer duration of time spent in light chamber as compared to individual treatment (p<0.05). The time spent in dark chamber is one of the important parameters which show a lack of exploratory drive and thus, induction of anxiety. In accordance with the study, results show that the disease control group spent a significantly high amount of time in the dark chamber as compared with other groups (p<0.05). Additionally, the rats treated with Sitagliptin or Linagliptin spent a significantly lesser amount of time in dark chamber as compared to disease control animals (p<0.05). Furthermore, the animals treated with a combination of Sitagliptin or Linagliptin with sertraline showed a significantly lesser time spent in dark chamber as compared to individual treatment groups (p<0.05)(fig-2a(ii)).

b. Porsolt's forced swim test

i. Time of Immobility: The Porsolt's forced swim or despair swim test is a valuable study for evaluating drugs which can modify or alter the onset and intensity of depression in rats. The time of immobility is a direct indicator to evaluate the onset of depression in animals. Results of the study show that the time of immobility is significantly higher in disease control group as compared to normal control group (p<0.01) as well as treatment groups (P<0.01). The time of immobility in animals treated with Sitagliptin or Linagliptin was found to be significantly lesser as compared to disease control animals (p<0.05). Additionally, the time of immobility in animals treated with a combination of Sitagliptin or Linagliptin with sertraline was found to be significantly lesser as compared to individual treatment groups (p<0.05) (fig-2b(i)).

DISCUSSION

Diabetes mellitus is one of the leading metabolic disorders affecting the health care sector in both developed and

developing countries [IDF 2013] [Donelly et al. 2000]. Despite of many newer drugs emerging for the same, glycemic control remains fundamental to the management of diabetes, especially for preventing complications and improving the quality of life [UKPDS study 1998] [Khuwaja et al.2014]. Recently, Incretins and DPP-IV inhibitors have been an addition to the treatment strategies for diabetes mellitus. Reports of pleiotropic effects of incretins on CNS, GIT, liver, Immune System and Inflammation have also emerged, in addition to their hypoglycemic action [Genugten 2013] [Campbell and Drucker 2013]. One of the important and concerning long term manifestations of diabetes mellitus has been its effect on CNS. Studies conducted in recent years provide evidence for an impaired cerebral glucose metabolism in CNS disorders [Speilman and Klegeris 2014]. Studies also show that chronic unpredictable mild stress (CUMS) increases HPA axis activity which disturbs glucose and lipid metabolism and evokes insulin resistance in peripheral tissues of high fat-fed rats. Hence, diabetes, anxiety and depression have shown a profound correlation according to many reports [Detka et al. 2013] [Park et al. 2008] [Khuwaja et al. 2004] [Khuwaja et al 2010] [Lin et al. 2010]. When these conditions co-exist, the risk of developing co-morbidities, complications, patient suffering and associated costs escalate alarmingly. The role of GLP-1 and DPP-IV has been hypothesized in correlation to the HPA axis and hence stress [Speilman and Klegeris 2014] [Lozano et al. 2010]. However, their role in anxiety and other psychological complications associated with diabetes has not been studied extensively and needs to be evaluated further. Results of our previous studies show that DPP-IV inhibitors, namely Sitagliptin and Linagliptin have a significant role in amelioration of acute anxiety and depression [Shukla and Goswami 2016]. In accordance with the results, the present study was carried out to evaluate the role of DPP-IV inhibitors, Sitagliptin and Linagliptin in CMS model in rats. Results of the present study show that these drugs also have a significant effect on amelioration of chronic stress in the experimental animals as that of our previous findings of acute stress results. This was evident as observed from lowered anhedonia in the treatment rats as compared to disease control group. The increased sucrose preference or sucrose intake clearly depicts the lowering of stress in animals treated with Sitagliptin and Linagliptin. The behavioral parameters also support the observations and lead us to strongly suggest that these drugs have a potential role in lowering stress in experimental animals. Furthermore, the evidence of their stress lowering capacity is strengthened by the decreased cortisol levels in the animals, as the study progressed. All the above observations suggest that DPP-IV inhibitors have a potential role in lowering chronic stress in rats. The possible pathway of action may be the HPA axis [Speilman and Klegeris 2014] [Lozano et al. 2010]. Also, the correlation of cortisol and insulin in the brain is reported and studied [Radahmadi et al. 2006] [Detka et al. 2013] [Chiodini et al. 2007]. This may also be another possible area of DPP-IV action. Hence, further studies would be useful to investigate the mechanisms and markers involved in the DPP-IV mediated amelioration of stress.

CONCLUSION

Results of the present study suggests the role of DPP-IV inhibitors, Sitagliptin and Linagliptin against chronic stress procedure. The possible mechanism of action includes involvement of the modulation of cortisol and the role of GLP-1 in the HPA-axis.

However, further studies need to be done to investigate the exact mechanisms and markers involved in the observed activities of these drugs which may provide new opportunities to explore the pleiotropic action of antidiabetic drugs in improving the conditions of stress and depression in patients of type 2 diabetes.

ACKNOWLEDGEMENTS

The authors would like to acknowledge and thank the management and the Dr. K. Pundarikakshudu, Director of L. J. Institute of Pharmacy for their support during the study.

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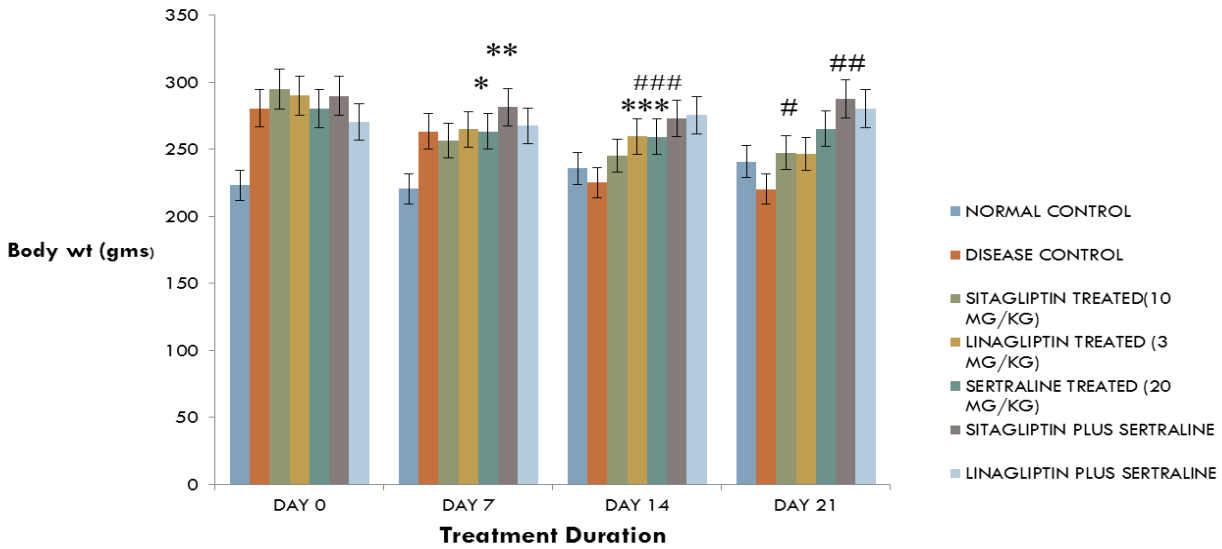
RESULTS

1. PHYSIOLOGICAL PARAMETERS

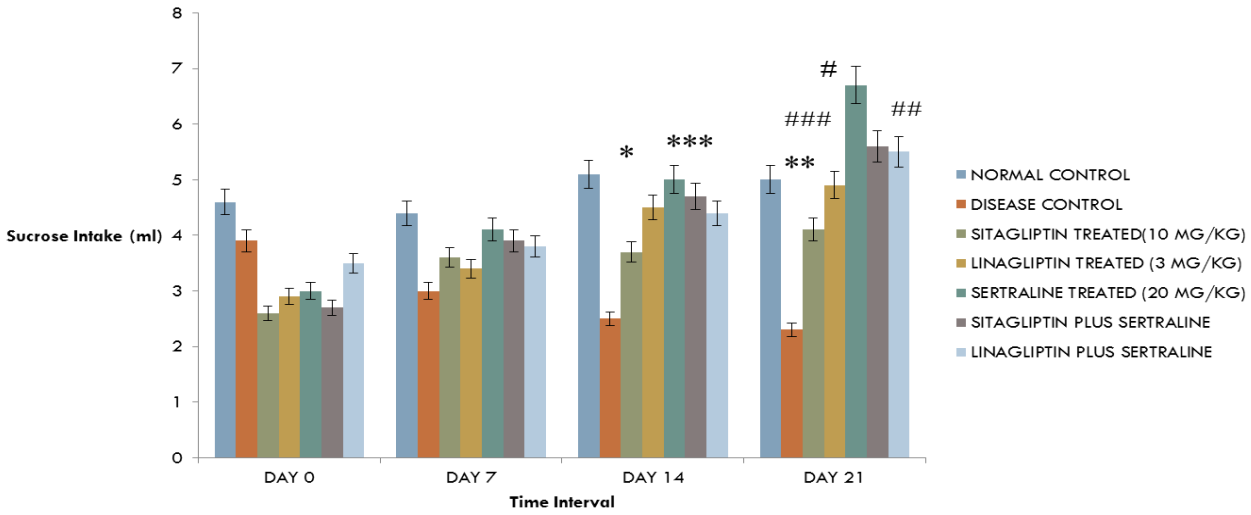
a. Body Weight (g)



Data showing body weight in each group
* Shows significant difference between disease control and treatment groups (P<0.05)
** Shows significant difference between sitagliptin and sertraline (P<0.05)
*** Shows non-significant difference between linagliptin and sertraline
# Shows significant difference between sitagliptin and combination (P<0.05)
Shows significant difference linagliptin and combination (P<0.01)
shows non-significant difference between sitagliptin alone and linagliptin

Fig.1a: Effect of drug treatments on body weight of rats

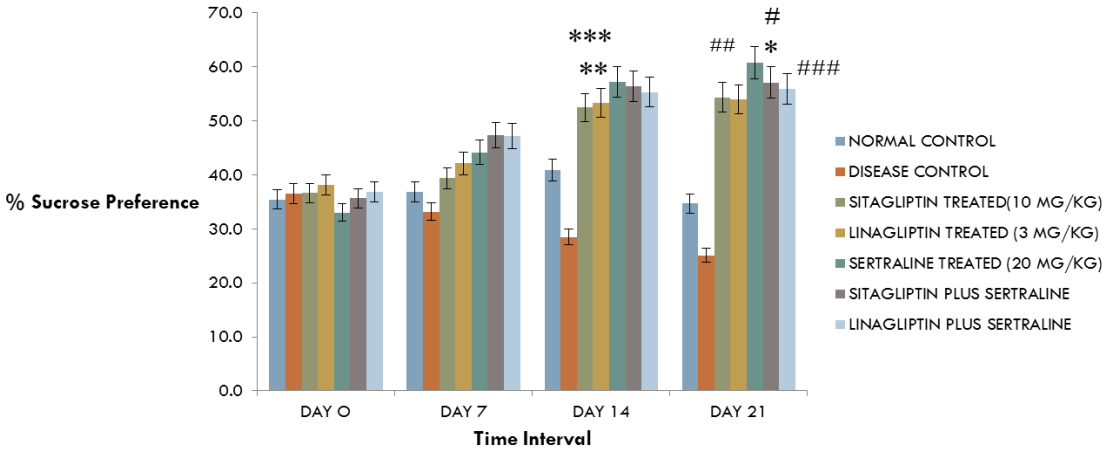
b. Sucrose Intake (ml)



Data showing sucrose intake in each group
* Shows significant difference between disease control and treatment groups (P<0.001)
** Shows significant difference between sitagliptin and sertraline (P<0.01)
*** Shows non-significant difference between linagliptin and sertraline (P<0.01)
# Shows significant difference between sitagliptin and combination (P<0.01)
Shows significant difference linagliptin and combination (P<0.01)
shows significant difference between sitagliptin alone and linagliptin (P<0.05)

Fig.1b: Effect of drug treatments on sucrose intake of rats

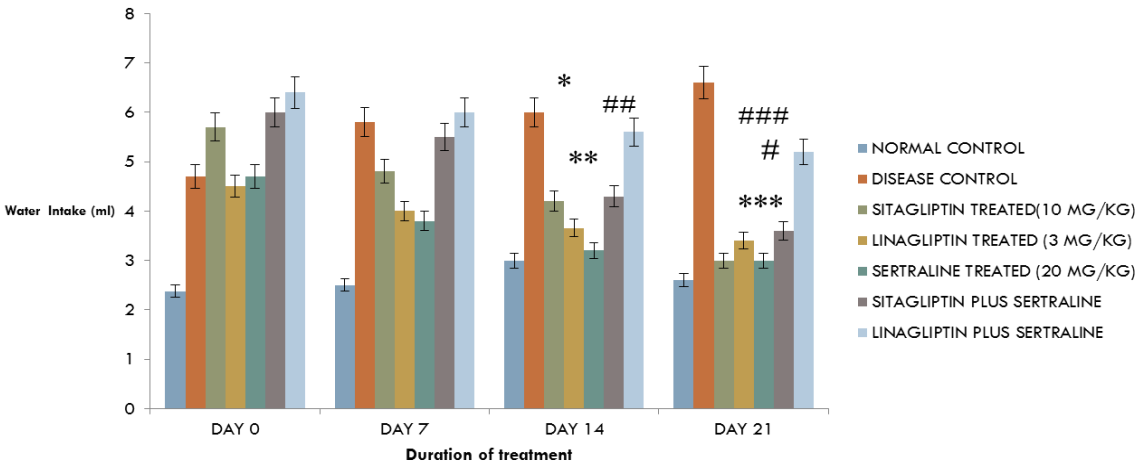
c. Anhedonia (% Preference for sucrose)



Data showing % preference for sucrose in each group
* Shows significant difference between disease control and treatment groups (P<0.01)
** Shows significant difference between sitagliptin and sertraline (P<0.05)
*** Shows non-significant difference between linagliptin and sertraline (P<0.05)
# Shows significant difference between sitagliptin and combination (P<0.05)
Shows significant difference linagliptin and combination (P<0.05)
shows no significant difference between sitagliptin alone and linagliptin

Fig.1c: Effect of drug treatments on anhedonia of rats

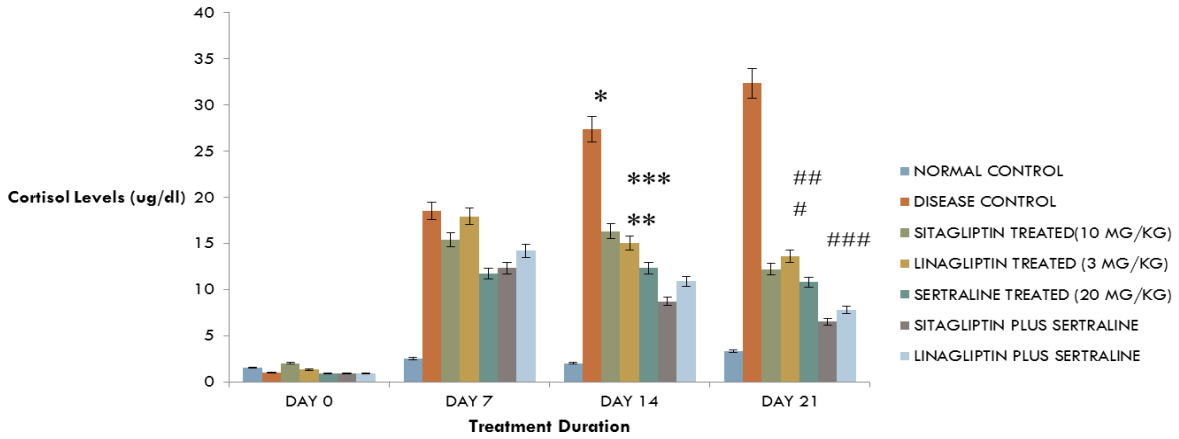
d. Water Intake (ml)



Data showing water intake in each group
* Shows significant difference between disease control and treatment groups (P<0.01)
** Shows significant difference between sitagliptin and sertraline (P<0.01)
*** Shows non-significant difference between linagliptin and sertraline
# Shows significant difference between sitagliptin and combination (P<0.05)
Shows significant difference linagliptin and combination (P<0.01)
shows non-significant difference between sitagliptin alone and linagliptin

Fig.1d: Effect of drug treatments on water intake of rats

e. Cortisol Levels



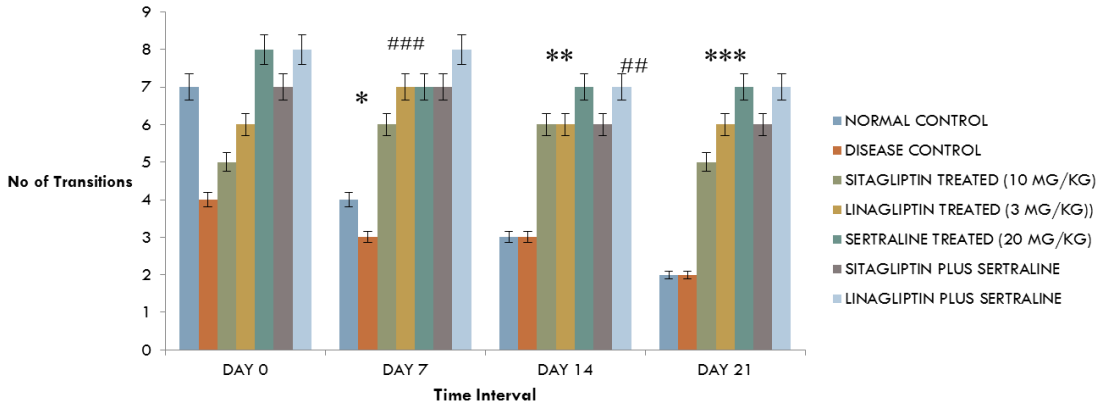
Data showing cortisol levels in each group
* Shows significant difference between disease control and treatment groups (P<0.001)
** Shows significant difference between sitagliptin and sertraline (P<0.01)
*** Shows non-significant difference between linagliptin and sertraline (P<0.01)
# Shows significant difference between sitagliptin and combination (P<0.01)
Shows significant difference linagliptin and combination (P<0.01)
shows non-significant difference between sitagliptin alone and linagliptin

Fig.1e: Effect of drug treatments on cortisol levels

2. PHYSIOLOGICAL PARAMETERS

a. Light Dark Model

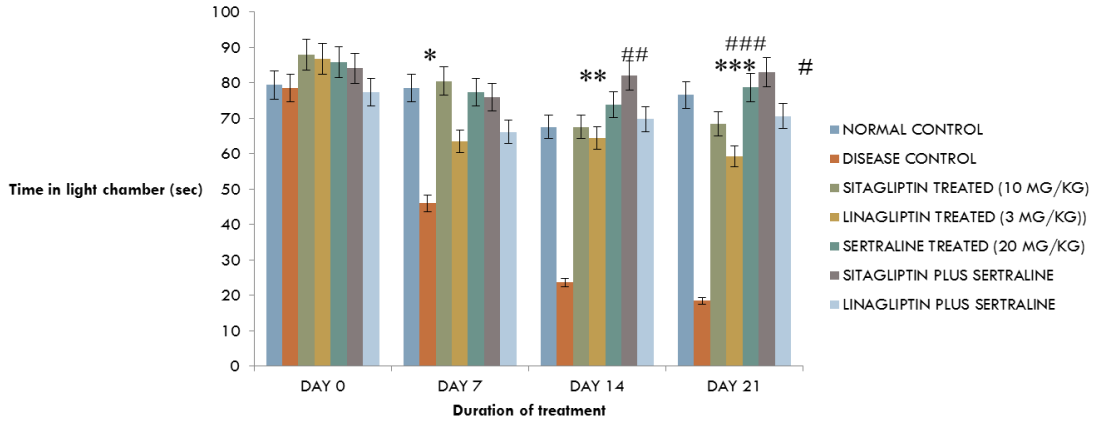
i. No. of Transitions



Data showing no of transitions in each group
* Shows significant difference between disease control and treatment groups (P<0.001)
** Shows significant difference between sitagliptin and sertraline (P<0.01)
*** Shows non-significant difference between linagliptin and sertraline (P<0.01)
# Shows non-significant difference between sitagliptin and combination
Shows significant difference linagliptin and combination (P<0.01)
shows significant difference between sitagliptin alone and linagliptin(P<0.05)

Fig.2a (i): Effect of drug treatments no. of transitions in light dark model

ii. Time spent in Light Chamber

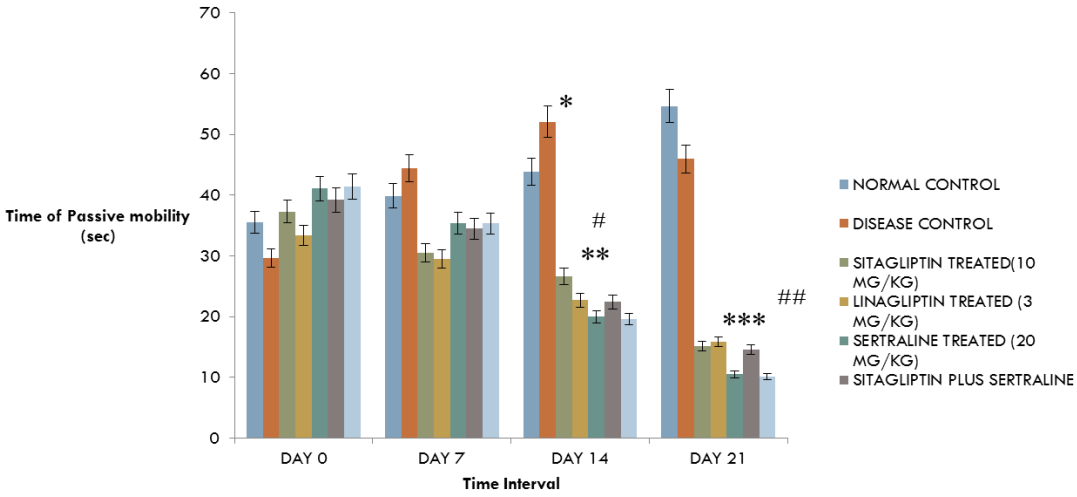


Data showing time spent in light chamber in each group
* Shows significant difference between disease control and treatment groups (P<0.001)
** Shows significant difference between sitagliptin and sertraline (P<0.01)
*** Shows non-significant difference between linagliptin and sertraline (P<0.01)
# Shows significant difference between sitagliptin and combination (P<0.01)
Shows significant difference linagliptin and combination (P<0.01)
shows significant difference between sitagliptin alone and linagliptin(P<0.05)

Fig.2a (ii): Effect of drug treatments on time spent in light dark model

b. Porsolts's Forced Swim Test

i. Time of Immobility



Data showing time of immobilty in each group
* Shows significant difference between disease control and treatment groups (P<0.01)
** Shows significant difference between sitagliptin and sertraline (P<0.05)
*** Shows non-significant difference between linagliptin and sertraline (P<0.05)
# Shows significant difference between sitagliptin and combination (P<0.05)
Shows significant difference linagliptin and combination (P<0.05)
Shows non-significant difference between sitagliptin alone and linagliptin

Fig.2b (i): Effect of drug treatments on time of immobility using FST