Research Article

Comparative Study of Anxiolytic Effect between DPP-4 Inhibitors in Albino Rats

Dr. Raushan Kumar Ranjan[1], Dr. Adil Ali Shakur[2], Dr. Manish Kumar[3], Dr. Rinky Thakur[4], *Dr. Saajid Hameed[4], Dr. Manoj Kumar[5], Prof. (Dr.) Lalit Mohan[6]

1. Senior Resident, Department of Pharmacology, Indira Gandhi Institute of Medical Sciences, Patna, India.
2. Assistant Professor, Department of Pharmacology, Indira Gandhi Institute of Medical Sciences, Patna, India.
3. Additional Professor, Department of Pharmacology, Indira Gandhi Institute of Medical Sciences, Patna, India.
4. Senior Resident, Department of Pharmacology, Indira Gandhi Institute of Medical Sciences, Patna, India.
5. Veterinary Officer, Central Animal House, Indira Gandhi Institute of Medical Sciences, Patna, India.
6. Professor & Head, Department of Pharmacology, Indira Gandhi Institute of Medical Sciences, Patna, India.

***Corresponding author's E-mail:** saajid36@gmail.com

Received: 11-04-2024; **Revised:** 26-06-2024; **Accepted:** 05-07-2024; **Published on:** 15-07-2024.

ABSTRACT

Background: 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 as well as in neurogenesis. In addition, the potential of GLP-1 receptors in animal models of pain15 and DPP-4 in degradation of substance P and its influence on pain pathway is described. Intrigued with these reports, present study was conducted to compare the anxiolytic effect between different DPP-4 inhibitors in rats.

Materials & Methods: 60 rats were used in this study. They were divided into 2 set comprising five groups of six animals each. The drugs (control gum Accacia, sitagliptin, vildagliptin, teneligliptin and linagliptin) were administered orally, once daily, for ten days. The tests were carried out 45 minutes after the last dose of the drugs on the 10th day. Two models were used in this study to assess anxiety in rats: Elevated plus maze (EPM) & Bright and Dark Arena. One-way ANOVA was used to determine statistical significance of difference between groups of rats given different DPP-4 inhibitors.

Results: Significant difference was noted with respect to time spent in open arm with linagliptin having maximum anxiolytic effect as compared to control group ($p<0.0001$). Similar to findings of elevated plus maze test, linagliptin had maximum anxiolytic activity with time spent in bright arena (29.67 ± 3.06 seconds) as compared to control group (11.50 ± 1.82 seconds) while rats of teneligliptin group demonstrated maximum activity with respect to number of rears (22.00 ± 1.29) as compared to control group (14.67 ± 1.01).

Conclusion: Linagliptin and teneligliptin demonstrated more anxiolytic effect as compared to other DPP-4 inhibitors. They can be preferred in patients with type 2 diabetes and anxiety after generating enough evidence from clinical trials.

Keywords: Anxiety, DPP-4 inhibitors, Elevated plus maze, Bright-dark arena, Albino Rat.

INTRODUCTION

A new therapeutic strategy for type 2 diabetes mellitus (T2DM) has been made possible by the manipulation of the incretin systems. Glucagonlike peptide (GLP)-1, an incretin hormone exhibits diverse actions including insulinotropic effects, neogenesis, differentiation and anti-apoptotic preservation of pancreatic β-cells. [1,2] GLP-1 acts through GLP-1 receptors (R). In individuals with type 2 diabetes, activation of GLP-1R increases the proliferation of pancreatic islet beta-cells, increases insulin production, and reduces blood glucose and food consumption.

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).[3] Because of its short half-life, it is therefore inappropriate for regular clinical use. To achieve or sustain high levels of GLP-1, medications with a significantly longer half-life are created. These medications either work as GLP-1 receptor agonists by stimulating GLP-1 receptors or as endogenous GLP-1 pool restorers by preventing its DPP-4 induced breakdown. [2] There is mounting evidence that the brain 4 also produces GLP-1, specifically from the caudal brain stem, area postrema, and nucleus of the solitary tract 5. It functions as a growth factor in the brain.[4-6] GLP-1 has been shown to enhance neurite outgrowth and to protect against oxidative injury in cultured neuronal cells. [7] Furthermore, GLP-1R is extensively distributed throughout the central nervous system and is crucial in controlling neuronal plasticity and the survival of cells. Mice with elevated hippocampal GLP-1R overexpression exhibited enhanced neurite formation and enhanced learning. [8]

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 as well as in neurogenesis. [9, 10] Hence, in recent years, research involving GLP-1 and its receptors has shifted from T2DM to focus upon various neurodegenerative disorders.[11, 12] Activation of incretin pathway has been shown to stimulate neuronal cell proliferation and prevented cell death.[13] Inhibition of GLP-1 degradation with the DPP-4 inhibitor is also associated with neuroprotection in the diabetic rat, independent of any changes in glycaemia. [14]

In recent studies, anxiety is also characterized by enhanced neurodegeneration and hence GLP-1 could have a role in anxiety. In addition, the potential of GLP-1 receptors in animal models of pain and DPP-4 in degradation of substance P and its influence on pain pathway is described. [15, 16] However, the neurobehavioral effects of DPP-4 inhibitors are not clear.

Various studies have been conducted on anxiolytic effect of DPP-4 inhibitors in animal models but no study has compared anxiolytic effect between DPP-4 inhibitors in our knowledge. Comparative study is needed to find the drug which is safest in terms of anxiolytic effect so that better DPP-4 inhibitor will be prescribed to patient of diabetes mellitus with anxiety disorders.

Intrigued with these reports, present study was conducted to compare the anxiolytic effect between different DPP-4 inhibitorsin rats. The objectives were to compare the efficacy with respect to findings from elevated plus maze & bright and dark arena test between group of rats given teneligliptin, vildagliptin, linagliptin, sitagliptin and normal saline.

MATERIALS AND METHODS

Animals:

An average body weight (150-250 g), male Wistar albino rats have been reared individually in polypropylene cages at 23–25°C with 12 hours light: 12 hours dark cycle with access to standard food pellets and water ad libitum for 15 days. Before the experiment began, all of the experimental animals were given a period of 15 days to acclimate. The ethical clearance for the proposed study was obtained from the IAEC of tertiary care hospital & medical college of eastern India.

Drug:

The test drug in tablet form was obtained commercially and were dissolved in distilled water using gum acacia and administered orally. The drug solutions were made right before the dosage was given.

Experimental Design:

60 rats were used in this study. They were divided into 2 set comprising five groups of six animals each. For 10 days, the medications were taken orally, once a day. The test drugs were administered in doses based on a previous study.[17,18] The test was carried out 45 minutes after the last dose of the drugs on the 10th day.

Table 1: Drug treatment schedule

Group	Drug	Dose	No. of animals	Route of drug administration
Group 1	Control (1% Gum Accacia)	10ml/kg	6	Oral-once daily for 10 days
Group 2	Sitagliptin	10 mg/kg[19]	6	Oral-once daily for 10 days
Group 3	Vildagliptin	10 mg/kg[20]	6	Oral-once daily for 10 days
Group 4	Teneligliptin	4 mg/kg[21]	6	Oral-once daily for 10 days
Group 5	Linagliptin	5 mg/kg[22]	6	Oral-once daily for 10 days

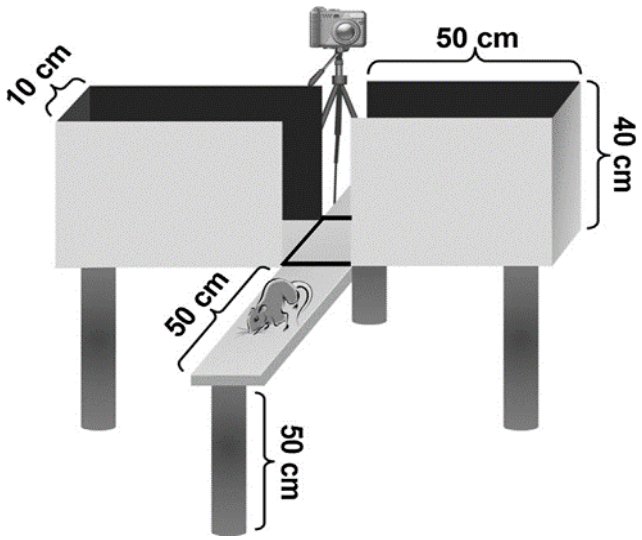

Figure 1: Standard elevated plus maze (EPM) apparatus. *Rats traversed an elevated plus maze with two enclosed and two open arms. Conflict to explore open arms versus remaining in safe enclosed spaces captures anxiety-related behaviors.*[23]

Two models were used in this study to assess anxiety in rats: Elevated plus maze (EPM) & Bright and Dark Arena.

Elevated plus maze (EPM): The apparatus has two open arms (50×10 cm) and two closed arms (50×10×40 cm) with an open roof, around a central square (10×10 cm), such that arms of the similar kind are opposite to each other. The whole maze is elevated 50 centimeters above the floor. The drugs were administered for ten days to four groups of rats. On the 10th day, 45 minutes after drug administration, each rat were placed in the central square of the maze facing one of the closed arms. The number of entries, time spent and the number of rears in each type of arm (open/closed) were recorded for 5 min. An entry was defined as the presence of all four paws in the arm.[23, 24]

Bright and Dark Arena: The apparatus consists of open top wooden box with two distinct chambers viz, black chamber (20 x 30 x 35 cm) painted black and illuminated with dimmed red light and a bright chamber (30 x 30 x 35 cm) painted white and brightly illuminated with 100-watt white light source, located 17cm above the box. The two

chambers are connected with a small open-door way (7.5cm) situated at the floor levels in the center of partition.

The test uses the rodent's natural aversion of bright area as compared to dark area. For the rodents which normally dwell in dark and enclosed environment, exposure to bright lit arena is believed to be a noxious environmental stressor. The reduced number of re-entries to bright arena, decreased time spent in bright arena and decreased exploratory behavior in it are regarded as a marker of anxiety in this paradigm. The apparatus in each model were wiped with 10% ethanol after the test with each rat to eliminate possible bias due to odor of previous animal.[24]

Statistical Analysis

Observed values were noted in a sheet of paper at the time of experiment and then represented in the tabular form using Microsoft excel 365 and then transferred to graph pad version 8.4.3 for further statistical analysis. Continuous data such as time spent, entry and rearing were expressed as mean ± standard error of mean (SEM) and one-way ANOVA was used to determine statistical significance of difference between groups of rats given different DPP-4 inhibitors. We also performed the Tukey HSD ("Honestly Significant Difference") post-hoc test, to indicate which DPP-4 inhibitors were significantly different from which others. Chi-square test was used to evaluate statistical significance of categorical data such as arm of first entry in elevated plus maze test. A p-value of less than 0.05 was taken as measure of significance.

RESULTS

All the rats completed the study with no serious adverse event or death. No abnormal behaviors were noted during the study period.

No significant difference was found between DPP-4 inhibitors and control with respect to percentage of open arm rotation (P>0.05). However, significant difference was noted with respect to time spent in open arm with linagliptin having maximum anxiolytic effect as compared to control group (p<0.0001). However, rats receiving teneligliptin were having a greater number of rears. [Table 2]

Table 2: Effect of various DPP-4 inhibitors on the behavior of rats in the elevated plus-maze test

Parameters (Mean ± SEM)	Control Group (1% Gum Accacia)	Sitagliptin Group	Vildagliptin Group	Teneligliptin Group	Linagliptin Group	P-Value (ANOVA)
% of open arm /total arm ration	30.83 ± 6.13	35.17 ± 5.15	38.67 ± 4.78	36.83 ± 6.08	40.00 ± 5.68	0.80
Time spent in Open Arm in seconds	19.83 ± 3.81	51.33 ± 9.42	59.83 ± 8.29	54.67 ± 9.03	63.33 ± 7.52	**0.005 Group 1 vs Group 5: Diff=43.5000, 95%CI=10.7928 to 76.2072, p=0.0052**
Time Spent in Closed Arm in Seconds	268.00 ± 8.87	237.83 ± 15.64	219.33 ± 14.67	224.50 ± 14.98	216.83 ± 13.14	0.08
Number of Rears in Open Arm	1.50 ± 0.66	2.50 ± 0.93	2.83 ± 0.75	3.00 ± 0.87	2.83 ± 1.01	0.73
Number of Rears in Closed Arm	8.83 ± 1.19	11.33 ± 1.12	12.00 ± 0.97	13.83 ± 1.16	12.17 ± 0.83	**0.04 Group 1 vs Group 4: Diff=5.0000, 95%CI=0.5532 to 9.4468, p=0.0220**

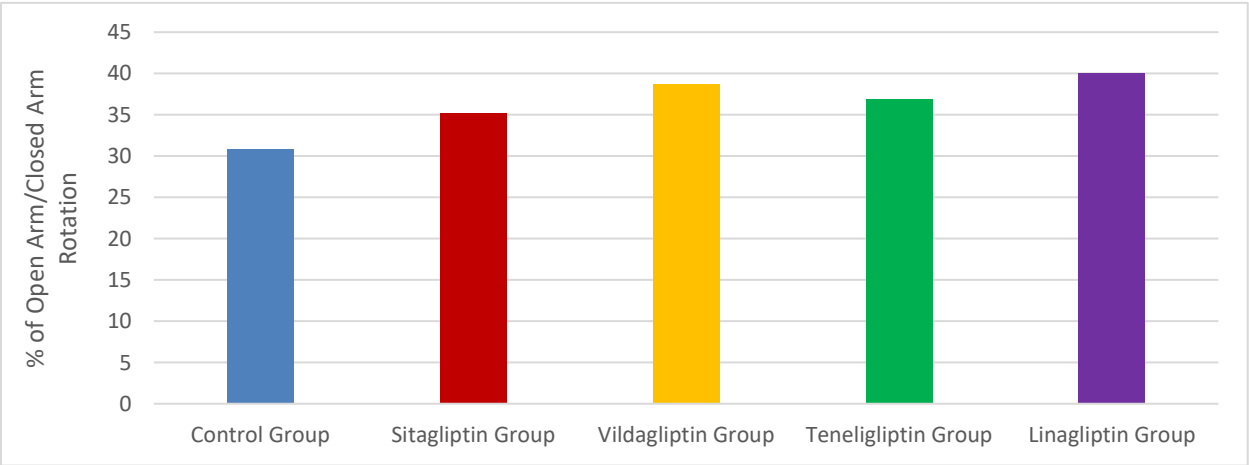

Figure 1: Comparison of open arm/closed arm rotation elevated plus maze between different Groups

Similar to findings of elevated plus maze test, linagliptin had maximum anxiolytic activity with time spent in bright arena (29.67 ± 3.06 seconds) as compared to control group (11.50 ± 1.82 seconds) while rats of teneligliptin group demonstrated maximum activity with respect to number of rears (22.00 ± 1.29) as compared to control group (14.67 ± 1.01). [Table 3]

Table 3: Effect of various DPP-4 inhibitors on the behavior of rats in the bright and dark arena test

Parameters (Mean ± SEM)	Control Group (1% Gum Accacia)	Sitagliptin Group	Vildagliptin Group	Teneligliptin Group	Linagliptin Group	P-Value (ANOVA)
Number of entries into Bright Arena	2.17 ± 0.18	2.33 ± 0.33	2.67 ± 0.25	2.83 ± 0.31	3.00 ± 0.29	0.22
Time spent in Bright Arena in seconds	11.50 ± 1.82	25.17 ± 3.24	27.50 ± 2.97	26.83 ± 3.16	29.67 ± 3.06	**0.001 Group 1 vs Group 5: Diff (95%CI) =6.1352 to 30.2048, p=0.0014**
Number of Rears in Bright Arena	1.17 ± 0.29	1.83 ± 0.56	2.17 ± 0.71	2.67 ± 0.76	2.33 ± 0.48	0.45
Number of Rears in Dark Arena	14.67 ± 1.01	18.17 ± 1.49	21.83 ± 1.31	24.17 ± 1.52	22.00 ± 1.29	**0.0003 Group 1 vs Group 4: Diff (95%CI)=3.9491 to 15.0509, p=0.0003**

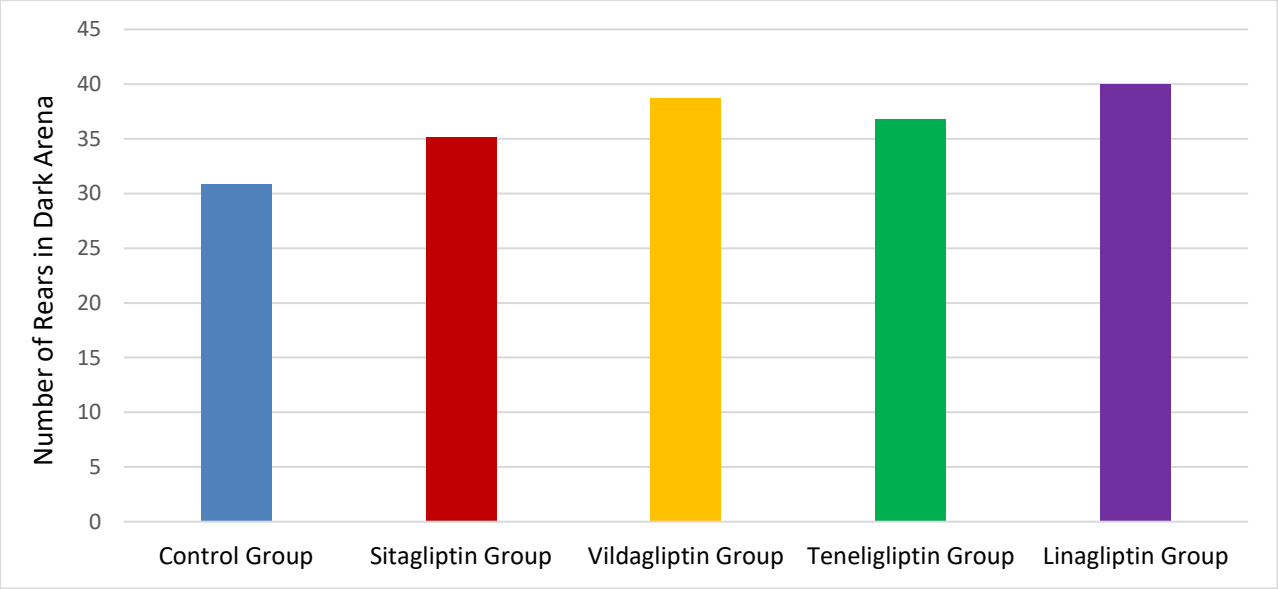

Figure 2: Comparison of number of rears in dark arena between two groups

DISCUSSION

In this study, we have compared the anxiolytic effect of commonly utilized DPP-4 inhibitors between various groups via elevated plus maze and bright and dark arena test. No significant difference was found between DPP-4 inhibitors and control with respect to percentage of open arm rotation (P>0.05). However, significant difference was noted with respect to time spent in open arm with linagliptin having maximum anxiolytic effect as compared to control group (p<0.0001). However, rats receiving teneligliptin were having a greater number of rears.

Similar to findings of elevated plus maze test, linagliptin had maximum anxiolytic activity with time spent in bright arena (29.67 ± 3.06 seconds) as compared to control group (11.50 ± 1.82 seconds) while rats of teneligliptin group demonstrated maximum activity with respect to number of rears (22.00 ± 1.29) as compared to control group (14.67 ± 1.01).

In the study conducted by Sharma A.N. et al., In the elevated plus maze (EPM) test in rats, sitagliptin exhibited anxiolytic effect, according to a dose-response study. After consuming a meal high in ethanol for seven days, tolerance to the anxiolytic action of ethanol was seen. Rats given a daily dose of sitagliptin showed resistance to the anxiolytic effect of ethanol, however, with tolerance becoming apparent on day 13 after the start of ethanol consumption. When rats were taken off of an ethanol diet after 15 days of consumption, they experienced withdrawal symptoms 8 to 12 hours after stopping the diet. On the other hand, rats on a 15-day ethanol-diet and concurrent sitagliptin therapy showed a 24-hour delay in the onset of withdrawal anxiety.[25]

In the study conducted by Mahalmani VM et al., compared to the control group, sitagliptin demonstrated a decrease in depression symptoms and hippocampus cytokine gene expression. With sitagliptin, there was a statistically significant decrease in nitric oxide levels in relation to serum oxidative stress indicators. The glutathione levels increased, and the catalase levels decreased in both the standard and test groups, but the differences in outcomes were not statistically significant.[26]

A transmembrane glycoprotein that is widely distributed and whose soluble form is found in plasma, CD26/DPP-4 is crucial for the immune system, especially for T cell activation.[27] DPP-4 is a type of serine protease that cleaves amino-terminal dipeptides containing either L-proline or L-alanine at the penultimate position. It also regulates the bioactivity of a number of chemokines, peptide hormones, and neuropeptides, such as substance P, glucagon-like peptide 1 (GLP-1), and NPY, primarily by encouraging a faster degradation of these molecules.[28, 29]

While NPY-truncation by DPP-4 to NPY3-36 is thought to have an anxiogenic effect due to the greater binding affinity of NPY3-36 to the receptor Y2 and diminished Y1-receptor stimulation, the anxiety-reducing properties of NPY have been shown to be mainly caused by the interaction of complete NPY with Y1-receptors.[30] According to recent research on animals, DPP-4 regulates the activity of the hypothalamic-pituitary-adrenal (HPA) axis and the response to stress.[31, 32] Different psychiatric diseases have been linked to changes in soluble DPP-4 levels.[33, 34]

Our study findings clearly support the findings of above in-vitro studies. These concepts should be considered in development of new DPP-4 inhibitors with additional pleotropic activities.

CONCLUSION

Linagliptin and teneligliptin demonstrated more anxiolytic effect as compared to other DPP-4 inhibitors. Very few in-vivo and clinical trial have been conducted that has evaluated and compared anxiolytic effect of different DPP-4 inhibitors. The evidence generated in this study should persuade researchers to conduct further researches to generate more evidence for endocrinologists to select appropriate DPP-4 inhibitor in patients with type 2 diabetes mellitus and anxiety disorders.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Baggio LL, Drucker DJ. Therapeutic approaches to preserve islet mass in type 2 diabetes. Annu Rev Med. 2006;57:265-81. doi: 10.1146/annurev.med.57.110104.115624. PMID: 16409149.
2. Drucker DJ. Enhancing incretin action for the treatment of type 2 diabetes. Diabetes Care. 2003 Oct;26(10):2929-40. doi: 10.2337/diacare.26.10.2929. PMID: 14514604.
3. Vilsbøll T, Agersø H, Krarup T, Holst JJ. Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects. J Clin Endocrinol Metab. 2003 Jan;88(1):220-4. doi: 10.1210/jc.2002-021053. PMID: 12519856.
4. Alvarez E, Roncero I, Chowen JA, Thorens B, Blázquez E. Expression of the glucagon-like peptide-1 receptor gene in rat brain. J Neurochem. 1996 Mar;66(3):920-7. doi: 10.1046/j.1471-4159.1996.66030920.x. PMID: 8769850.
5. Larsen PJ, Tang-Christensen M, Holst JJ, Orskov C. Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. Neuroscience. 1997 Mar;77(1):257-70. doi: 10.1016/s0306-4522(96)00434-4. PMID: 9044391.
6. Hamilton A, Hölscher C. Receptors for the incretin glucagon-like peptide-1 are expressed on neurons in the central nervous system. Neuroreport. 2009 Aug 26;20(13):1161-6. doi: 10.1097/WNR.0b013e32832fbf14. PMID: 19617854.
7. Perry T.,HaugheyN.J.,Mattson M.P., EganJ.M.,Greig N.H. Protection and reversal of excitotoxic neuronal damage by glucagon-like peptide-1 and exendin-4. J.Pharmacol.Exp.Ther. 2002;302:881–888.
8. During MJ, Cao L, Zuzga DS, Francis JS, Fitzsimons HL, Jiao X, Bland RJ, Klugmann M, Banks WA, Drucker DJ, Haile CN. Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. Nat Med. 2003 Sep;9(9):1173-9. doi: 10.1038/nm919. Epub 2003 Aug 17. PMID: 12925848.
9. Hölscher C. Potential role of glucagon-like peptide-1 (GLP-1) in neuroprotection. CNS Drugs. 2012 Oct 1;26(10):871-82. doi: 10.2165/11635890-000000000-00000. PMID: 22938097.
10. Salcedo I, Tweedie D, Li Y, Greig NH. Neuroprotective and neurotrophic actions of glucagon-like peptide-1: an emerging opportunity to treat neurodegenerative and cerebrovascular disorders. Br J Pharmacol. 2012 Jul;166(5):1586-99. doi: 10.1111/j.1476-5381.2012.01971.x. PMID: 22519295; PMCID: PMC3419902.
11. Bertilsson G, Patrone C, Zachrisson O, Andersson A, Dannaeus K, Heidrich J, Kortesmaa J, Mercer A, Nielsen E, Rönnholm H, Wikström L. Peptide hormone exendin-4 stimulates subventricular zone neurogenesis in the adult rodent brain and induces recovery in an animal model of Parkinson's disease. J Neurosci Res. 2008 Feb 1;86(2):326-38. doi: 10.1002/jnr.21483. PMID: 17803225.
12. Martin B, Golden E, Carlson OD, Pistell P, Zhou J, Kim W, Frank BP, Thomas S, Chadwick WA, Greig NH, Bates GP, Sathasivam K, Bernier M, Maudsley S, Mattson MP, Egan JM. Exendin-4 improves glycemic control, ameliorates brain and pancreatic pathologies, and extends survival in a mouse model of Huntington's disease. Diabetes. 2009 Feb;58(2):318-28. doi: 10.2337/db08-0799. Epub 2008 Nov 4. PMID: 18984744; PMCID: PMC2628604.
13. Li Y, Tweedie D, Mattson MP, Holloway HW, Greig NH. Enhancing the GLP-1 receptor signaling pathway leads to proliferation and neuroprotection in human neuroblastoma cells. J Neurochem. 2010 Jun;113(6):1621-31. doi: 10.1111/j.1471-4159.2010.06731.x.Epub 2010 Apr 2. PMID: 20374430; PMCID: PMC2912144.
14. Jin HY, Liu WJ, Park JH, Baek HS, Park TS. Effect of dipeptidyl peptidase-IV (DPP-IV) inhibitor (Vildagliptin) on peripheral nerves in streptozotocin-induced diabetic rats. Arch Med Res. 2009 Oct;40(7):536-44. doi: 10.1016/j.arcmed.2009.09.005. PMID: 20082866.

15. Gong N, Xiao Q, Zhu B, Zhang CY, Wang YC, Fan H, Ma AN, Wang YX. Activation of spinal glucagon-like peptide-1 receptors specifically suppresses pain hypersensitivity. J Neurosci. 2014 Apr 9;34(15):5322-34. doi: 10.1523/JNEUROSCI.4703-13.2014. PMID: 24719110; PMCID: PMC6608999.

16. Grouzmann E, Bigliardi P, Appenzeller M, Pannatier A, Buclin T. Substance P-induced skin inflammation is not modulated by a single dose of sitagliptin in human volunteers. Biological Chemistry. 2011 Mar;392(3):217-221.

17. Osman AS, Gad MH, Hareedy AH, Mishriki AA, Rasheed EAMA. Sitagliptin attenuates cognitive impairment in the rat model of Aluminum-induced Alzheimer's disease. J Adv Pharm Edu Res 2019;9(3):53-61.

18. Sayed NH, Fathy N, Kortam MA, Rabie MA, Mohamed AF, Kamel AS. Vildagliptin Attenuates Huntington's Disease through Activation of GLP-1 Receptor/PI3K/Akt/BDNF Pathway in 3-Nitropropionic Acid Rat Model. Neurotherapeutics. 2020 Jan;17(1):252-268. doi: 10.1007/s13311-019-00805-5. PMID: 31728850; PMCID: PMC7007456.

19. Ferreira L, Teixeira-de-Lemos E, Pinto F, Parada B, Mega C, Vala H, et al. Effects of sitagliptin treatment on dysmetabolism, inflammation, and oxidative stress in an animal model of type 2 diabetes (ZDF rat). Mediators Inflamm. 2010;2010:592760. Available from: http://dx.doi.org/10.1155/2010/592760

20. Kosaraju J, Murthy V, Khatwal RB, Dubala A, Chinni S, Muthureddy Nataraj SK, Basavan D. Vildagliptin: an anti-diabetes agent ameliorates cognitive deficits and pathology observed in streptozotocin-induced Alzheimer's disease. J Pharm Pharmacol. 2013 Dec;65(12):1773-84. doi: 10.1111/jphp.12148. Epub 2013 Oct 10. PMID: 24117480.

21. Kuthati Y, Rao VN, Busa P, Wong CS. Teneligliptin Exerts Antinociceptive Effects in Rat Model of Partial Sciatic Nerve Transection Induced Neuropathic Pain. Antioxidants (Basel). 2021 Sep 9;10(9):1438. doi: 10.3390/antiox10091438. PMID: 34573072; PMCID: PMC8465046.

22. ElGamal RZ, Tadros MG, Menze ET. Linagliptin counteracts rotenone's toxicity in non-diabetic rat model of Parkinson's disease: Insights into the neuroprotective roles of DJ-1, SIRT-1/Nrf-2 and implications of HIF1-α. Eur J Pharmacol. 2023 Feb 15;941:175498. doi: 10.1016/j.ejphar.2023.175498. Epub 2023 Jan 6. PMID: 36623635.

23. Sweis BM, Bachour SP, Brekke JA, Gewirtz JC, Sadeghi-Bazargani H, Hevesi M, et al. A modified beam-walking apparatus for assessment of anxiety in a rodent model of blast traumatic brain injury. Behav Brain Res. 2016;296:149–56. Available from: http://dx.doi.org/10.1016/j.bbr.2015.09.015

24. Riebe CJ, Wotjak CT. A Practical Guide to Evaluating Anxiety-Related Behavior in Rodents. Methods in Pharmacology and Toxicology. 2012;167–85.

25. Sharma A.N., Pise A., Sharma J.N. et al. Dipeptidyl-peptidase IV (DPP-IV) inhibitor delays tolerance to anxiolytic effect of ethanol and withdrawal-induced anxiety in rats. Metab Brain Dis.2015; 30: 659–667. https://doi.org/10.1007/s11011-014-9603-7

26. Mahalmani VM, Hogade AP, Mishra SK. Effect of sitagliptin on depression in male wistarrats.IntJBasic Clin Pharmacol. 2020;9:200-6.

27. Klemann C, Wagner L, Stephan M, von Hörsten S. Cut to the chase: a review of CD26/dipeptidyl peptidase-4's (DPP4) entanglement in the immune system. Clin Exp Immunol. 2016;185:1–21. doi: 10.1111/cei.12781

28. Wagner L, Klemann C, Stephan M, von Hörsten S. Unravelling the immunological roles of dipeptidyl peptidase 4 (DPP4) activity and/or structure homologue (DASH) proteins. Clin Exp Immunol. 2016;184:265–83. doi: 10.1111/cei.12757

29. Wagner L, Kaestner F, Wolf R, Stiller H, Heiser U, Manhart S, et al. Identifying neuropeptide Y (NPY) as the main stress-related substrate of dipeptidyl peptidase 4 (DPP4) in blood circulation. Neuropeptides. 2016;57:21–34. doi: 10.1016/j.npep.2016.02.007

30. Sajdyk TJ, Schober DA, Smiley DL, Gehlert DR. Neuropeptide Y-Y2 receptors mediate anxiety in the amygdala. PharmacolBiochemBehav. 2002;71:419–23. doi: 10.1016/S0091-3057(01)00679-7

31. Canneva F, Golub Y, Distler J, Dobner J, Meyer S, von Hörsten S. DPP4-deficient congenic rats display blunted stress, improved fear extinction and increased central NPY. Psychoneuroendocrinology. 2015;53:195–206. doi: 10.1016/j.psyneuen.2015.01.007

32. Golub Y, Schildbach EM, Touma C, Kratz O, Moll GH, von Horsten S, et al. Role of hypothalamus-pituitary-adrenal axis modulation in the stress-resilient phenotype of DPP4-deficient rats. Behav Brain Res. 2019;356:243–9. doi: 10.1016/j.bbr.2018.08.029

33. Maes M, Meester D, Scharpé S, Desnyder R, Ranjan R, Meltzer HY. Alterations in plasma dipeptidyl peptidase IV enzyme activity in depression and schizophrenia: effects of antidepressants and antipsychotic drugs. Acta Psychiatr Scand. 1996;93:1–8. doi: 10.1111/j.1600-0447.1996.tb10612.x

34. Deng J, Lamb JR, McKeown AP, Miller S, Muglia P, Guest PC, et al. Identification of altered dipeptidyl-peptidase activities as potential biomarkers for unipolar depression. J Affect Disord. 2013;151:667–72. doi: 10.1016/j.jad.2013.07.015