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Original Research Article

# Evaluation of hyperalgesic effect of sitagliptin in albino mice

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ABSTRACT

**Objective:** Various studies have showed the increased incidence of joint pain with the use of DPP-4 inhibitors. There is also some evidence of increase in inflammatory mediators like substance P, SDF-1 and other cytokines with the inhibition of DPP-4 from some experimental studies. But this association is still unclear and DPP-4 inhibitor continue to be prescribed in inflammatory disorders. So, this study was planned to assess the development of hyperalgesia in albino mice with the use of sitagliptin.
**Materials and Methods:** Sitagliptin dissolved in saline was administered in the doses of 10, 20, 30 mg/kg to Albino mice of either sex weighing 25-30 gm. Hyperalgesia was assessed in the mice with hot plate method and acetic acid induced writhing test.
**Results:** We found that reaction time of the mice receiving higher dose of Sitagliptin in hot plate method was lower than that of mice receiving lower doses or distilled water (P-Value <0.05). We also found that after injection of acetic acid, the number of writhing observed in the mice receiving higher dose of Sitagliptin was greater than that of mice receiving lower doses or distilled water (P-Value <0.05).
**Conclusion:** Our findings show that in a cohort of mice receiving Sitagliptin and distilled water at baseline, there was significant association between dose of Sitagliptin and hyperalgesia. However, P-Value was greater than 0.01, but with these finding we can't rule out this association and need for further prospective studies to assess the relationship between DPP-4 inhibitors and hyperalgesia.



## 1. Introduction

Glycemic control is the most important component of diabetes management. The patients of type 2 Diabetes Mellitus who do not achieve good glycemic control with only metformin require addition of other classes of oral anti-diabetic drugs.[1] Dipeptidyl peptidase (DPP)-4 inhibitors are now recommended as a second-line treatment for the management of type 2 diabetes in various guidelines.[2–4] It was found in a systematic review and meta-analysis of 19 randomized controlled trials (RCTs) in which efficacy and safety of DPP-4 inhibitor monotherapy, metformin monotherapy, and combination therapy were compared that there was better improvement of glycemic control in DPP-4 inhibitor treatment with less incidence of weight gain.[5]

In a recent meta-analysis of 16 studies (9 RCTs and 7 observational studies), the safety and efficacy of DPP-4 inhibitors was examined among old age patients (aged ≥65 years). From that meta-analysis, it was concluded that the overall risk to benefit ratio of DPP-4 inhibitors were unclear and inconsistent and some studies also reported increased risks for pain, sepsis, hospitalizations for cardiac disease, chest pain and heart disease related outcomes.[6]

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Possible association of DPP-4 inhibitors with joint pain and inflammatory disorder is also suggested by emerging evidences from some case reports.[7–10] Crickx and colleagues found in their 3 case reports that the use of DPP-4 inhibitors was associated with inflammatory disorders of the joints.[9] Similar finding was seen in other case-control study which showed the association between DPP-4 inhibitors and joint pain.[11] Consequently, a warning was issued by the US Food and Drug Administration regarding risk of severe joint pain with the use of DPP-4 inhibitors for the treatment of type 2 diabetes.[12] In spite of this, DPP-4 inhibitors are regularly prescribed regardless of the history of inflammatory disorders in patients of type 2 Diabetes Mellitus.

Sitagliptin belongs to a group of synthetic drugs that are competitive inhibitors of enzyme dipeptidylpeptidase-4 that cause breakdown of GLP-1. It is a DPP-4 inhibitor widely used in the treatment of diabetes mellitus type 2. Sitagliptin lowers blood glucose by potentiating endogenous incretins (GLP-1 and GIP) by inhibiting DPP-4 and thereby stimulating insulin secretion. It is used for type 2 diabetes mellitus in addition to other oral anti-diabetic drugs.[13]

In addition to endogenous incretin hormones, DPP-4 enzyme also causes degradation of substance P along with other substrates involved in inflammatory pathways. Thus, DPP-4 inhibitors cause increased level of substance P and other cytokines.[14] Substance P and the neurokinin 1 receptor (NK1R) play important roles in the neural processing of a range of painful and stressful stimuli. Substance P play crucial role in nociception in CNS and disruption of the neurokinin 1 receptor generally affect the late phase response to peripheral injury.

So, this study was planned to assess the development of hyperalgesia in albino mice with the use of sitagliptin.

## 2. Materials and Methods

This study was conducted in the Department of Pharmacology and Central Animal House of IGIMS, Patna. This study was approved from Institutional Animal Ethics Committee of IGIMS, Patna.

### *2.1. Materials*

#### *2.1.1. Animals*

Albino mice of either sex weighing 25-30gm, 15-20 weeks old were used for this study. They were housed in clean polypropylene cage in groups of four at room temperature maintained at 27-31 $^0$C with standard laboratory feed and water. They were divided into 2 sets. Each set had 4 groups and each group had 6 mice. Group-1 animal were administered normal saline and the rest groups were administered Sitagliptin 10 mg, 20 mg, 30 mg respectively to mice of group-2, group-3 and group-4. The ethical clearance for use of animals was obtained from the committee constituted for this purpose.

#### *2.1.2. Drug*

Sitagliptin dissolved in saline was administered in the doses of 10, 20, 30 mg/kg. The test drug in tablet form was dissolved in distilled water and administered orally. Drug solution were prepared just before administration. Drugs or vehicle were administered once daily for ten days.

### *2.2. Methods*

#### *2.2.1. Hot plate method*

In this method heat is used as a source of pain. All animals were individually placed on the Eddy's hot plate (Techno instruments, India) maintained at constant temperature, 55$^0$C and the time taken by the animal for the reaction either by licking the paw or jumping or raising the limbs which ever were observed first was taken as the end point. Animals having basal reaction time not exceeding 15 seconds were included in the study. Reaction time was noted before and 15, 30, 60 and 120 minutes after the drug or vehicle administration in each animal.

#### *2.2.2. Writhing test*

Freshly prepared 0.6% acetic acid solution in the volume of 10ml/kg was administered intra-peritoneally to each animal which received either the vehicle (Group 1) or the test drug Sitagliptin (Groups 2-4) one hour before the challenge. The time of onset of writhing and the number of abdominal contractions or writhing in the following 15 minutes was recorded.

#### *2.2.3. Statistical analysis*

Results obtained from this study were presented in tabular form and data were interpreted by using Microsoft Excel 2007 software. P- Value was calculated using Analysis of variance (ANOVA).

## 3. Observations & Result (Tables 1 and 2 )

## 4. Discussion

To our knowledge, this is the first study to examine the association between DPP-4 inhibitor use and hyperalgesia.

We found that reaction time of the mice receiving higher dose of Sitagliptin in hot plate method was lower than that of mice receiving lower doses or distilled water (P-Value <0.05). We also found that after injection of acetic acid, the number of writhing observed in the mice receiving higher dose of Sitagliptin was greater than that of mice receiving lower doses or distilled water (P-Value <0.05). These results showed that Sitagliptin caused hyperalgesia in mice and this hyperalgesia increased with dose.

Although many mechanisms have been proposed for the development of hyperalgesia with the use of DPP-

**Table 1:** Effect of Sitagliptin on basal reaction time of mice in hot plate method (Set 1)

| Drug (Dose) | Weight (in grams) | Reaction Time after 15 minutes (in seconds) | Reaction Time after 30 minutes (in seconds) | Reaction Time after 60 minutes (in seconds) | Reaction Time after 120 minutes (in seconds) |
|---|---|---|---|---|---|
| | | | Mean +/- SEM | | |
| Group 1 Control Distilled Water (10ml/kg) | 38.83 ± 1.40 | 6.54 ± 0.36 | 6.19 ± 0.36 | 5.94 ± 0.35 | 5.76 ± 0.33 |
| Group 2 Sitagliptin (10 mg/kg) | 44.17 ± 1.72 | 6.02 ± 0.34 | 5.66 ± 0.34 | 5.44 ± 0.33 | 5.28 ± 0.32 |
| Group 3 Sitagliptin (20 mg/kg) | 36.33 ± 2.23 | 5.57 ± 0.32 | 5.21 ± 0.32 | 5.00 ± 0.31 | 4.85 ± 0.30 |
| Group 4 Sitagliptin (30 mg/kg) | 35.5 ± 2.77 | 5.08 ± 0.30 | 4.78 ± 0.29 | 4.61 ± 0.28 | 4.47 ± 0.28 |
| P- Value | | 0.033951 | 0.038414 | 0.04311 | 0.042849 |

**Table 2:** Effect of Sitagliptin on acetic acid induced abdominal writhing in mice (Set 2)

| Drug (Dose) | Weight (in grams) | Time for onset of writhing (in Minutes) | Total number of writhing in 15 minutes |
|---|---|---|---|
| Group 1- Control Distilled Water (10ml/kg) | 38.83 ± 1.40 | 3.44 ± 0.20 | 25 ± 1.32 |
| Group 2- Sitagliptin (10 mg/kg) | 44.17 ± 1.72 | 3.01 ± 0.17 | 28.5 ± 1.41 |
| Group 3- Sitagliptin (20 mg/kg) | 36.33 ± 2.23 | 2.95 ± 0.26 | 30.83 ± 2.61 |
| Group 4- Sitagliptin (30 mg/kg) | 35.5 ± 2.77 | 2.54 ± 0.15 | 34 ± 2.13 |
| P- Value | | 0.039173 | 0.025767 |

4 inhibitors,[10] the exact mechanism is still uncleared. One possible mechanism is increase in levels of certain cytokine by inhibition of DPP-4 enzyme.[10,15] Levels of these inflammatory mediators are higher in old age.[16,17] Therefore, use of DPP-4 inhibitors in the for type 2 diabetes in elderly patients may adversely increase cytokine levels and contribute to joint pain.

In addition to GLP-1, DPP-4 enzyme also degrades many cytokines, including stromal cell-derived factor-1 (SDF-1) which is a pro-inflammatory mediator involved in induction of rheumatoid arthritis.[18] Many other substrates like the super family of glucagon, some hormones and chemokines are also degraded by DPP-4. How-ever, it must be considered that DPP-4 is not the only enzyme that causes breakdown of chemokines. In addition to the increase in the expression of SDF-1, inhibition of DPP-4 also increases MMP-1 and MMP-3 in synovial fibroblasts 19. All these mediators have similarity in X-Ala/X-Pro sequence at the N-terminus, which is the target recognized by DPP-4 for the degradation and inhibition.[19] These findings could suggest us the reason for induction of increase in SDF-1 levels, and MMP-1 and MMP-3 activity and worsening of joint pain by inhibition of DPP-4 activity in patients with RA.

In an experimental study it was found that there was increased severity of antigen-induced arthritis in the mice which were genetically deficient for DPP-4.[20]

This hypothesis is also supported by the expression of DPP-4 in cells involved in acute and chronic inflammation such as fibroblasts, T lymphocytes, and macro-phages. So, the inhibition of DPP-4 has impact on the inflammatory homeostasis of the bones and joints.[20,21] It was also found in some studies that the peripheral T lymphocytes in patients of rheumatoid arthritis have increased expression of DPP-4 on their cell membranes.[22]

In a systematic review and meta-analysis of 67 randomized controlled trial (that included 79,110 adults with type 2 diabetes) there was increased risk for any joint pain with the use of DPP-4 inhibitors in the treatment regimen.[23] However, no association of severe joint inflammation with the use of DPP-4 inhibitors was found in a population-based cohort study done on Taiwanese patients suffering with type 2 diabetes.[24] Despite the increased prevalence of joint pain in patients of type 2 Diabetes Mellitus and the associated morbidity,[25] the association of hyperalgesia with the use of DPP-4 inhibitors is still uncleared.

## 5. Conclusion

Our findings show that in a cohort of mice receiving Sitagliptin and distilled water at baseline, there was significant association between dose of Sitagliptin and hyperalgesia. However, P-Value was greater than 0.01,

but with these finding we can't rule out this association and need for further prospective studies to assess the relationship between DPP-4 inhibitors and hyperalgesia. Further prospective studies are needed to explore the causal relationship between DPP-4 inhibitor use and hyperalgesia. If hyperalgesic effect is fully proved, use of sitagliptin will not be recommended to patient of inflammatory disease like osteoarthritis.

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## 7. Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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None.

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