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(Article)

The Potential Protective Effect of Vildagliptin on Paw Edema: An Experimental Study

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ABSTRACT

Paw edema is an inflammatory condition that produces swelling and redness in the paws of animals. In addition, it is a prevalent inflammatory response used as a model to study inflammation and evaluate potential treatment options. Eight rats were assigned to each of three groups: normal control, paw edema, and vildagliptin. The normal control group rats received a vehicle orally for seven days, while the paw edema group received the vehicle for seven days and paw edema induction on the eighth day. Vildagliptin was given orally at 5 mg/kg for seven days, and paw edema was induced on the eighth. Using proper methods, paw weight, edema volume, and inflammatory markers were measured and statistically analyzed. Vildagliptin reduced inflammation caused by paw edema as evidenced by reductions in paw weight, edema volume, and inflammatory markers, suggesting anti-inflammatory effects. Increased SOD and IL-10 levels support its antioxidant and anti-inflammatory capabilities. These findings highlight the therapeutic value of vildagliptin in managing various inflammatory conditions and warrant further investigation to understand the underlying mechanisms and clinical implications.

Keywords: Inflammation; Oxidative stress; Paw edema; Vildagliptin

1. Introduction

Inflammation is a complex biological response that is vital to the body's defense against injury, infection, and tissue damage. It is characterized by a series of physiological processes in which immune cells, blood vessels, and molecular mediators are involved (1). Paw edema is a form of inflammation that causes swelling and inflammation in the paws of animals (2). In addition, it is a common inflammatory response and is commonly used as an experimental model to investigate inflammation and evaluate potential treatment approaches (3). Due to its accessibility and ease of measurement, it functions as a significant model for studying inflammation (4).

Paw edema is caused by a series of events that begin with the production of chemical signals like cytokines and chemokines in response to tissue injury or immunological activation (5). These signals draw immune cells, specifically neutrophils, to the affected area. Neutrophils are the first line of defense and play an essential role in the development of inflammation (6). Upon activation, they release numerous enzymes such as myeloperoxidase (MPO), which contribute to tissue injury and the production of reactive oxygen species (ROS) (7).

The recruitment of immune cells and the production of inflammatory mediators cause increased vascular permeability in the paw tissue. This allows plasma proteins and fluid to leak into the interstitial space, causing swelling and edema (1). Furthermore, the increased blood flow to the affected area also causes the characteristic redness and warmth of inflamed paws (8).

The activation of inflammatory mediators, including cytokines, chemokines, and enzymes, is an essential aspect of the inflammatory process. Myeloperoxidase (MPO), an enzyme found predominantly in neutrophils, has received considerable attention due to its role in neutrophil activation and oxidative stress as it contributes to tissue injury by generating ROS and producing inflammatory mediators (9, 10).

The antioxidant enzyme superoxide dismutase (SOD) plays a crucial function in mitigating the harmful effects of ROS. It converts superoxide radicals into hydrogen peroxide, mitigating oxidative stress and preventing cellular oxidative damage (11). Evaluation of SOD levels can provide valuable information regarding the antioxidant defense mechanisms and oxidative stress in the paw tissue during edema (12).

Furthermore, inflammatory cytokines play a crucial role in the inflammatory response. Interleukin-10 (IL-10) is a powerful immunosuppressant and anti-inflammatory cytokine. IL-10 suppresses the inflammatory response by modulating the synthesis and activity of numerous pro-inflammatory cytokines, chemokines, and enzymes (13). IL-10 levels can be measured to identify the level of anti-inflammatory activity in paw tissue and provide insight into any potential protective effect (5).

Cyclooxygenase-2 (COX-2) is an enzyme involved in the synthesis of prostaglandins, which are lipid mediators that control inflammation, pain, and fever. COX-2 is upregulated by inflammatory stimuli and contributes to the production of pro-inflammatory prostaglandins (8). Increased COX-2 levels are frequently observed in inflammatory tissues and can serve as an indicator of increased inflammation in the paw tissue during edema (14).

The pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) is essential for the initiation and regulation of inflammatory responses. TNF-α is responsible for the recruitment and activation of immune cells, the induction of other pro-inflammatory cytokines, and the regulation of cellular processes (15). Assessing TNF-α levels can provide crucial information about inflammatory activity in the paw tissue and play a role in assessing any potential protective effect (16).

The transcription factor, nuclear factor-kappa B (NFκB) regulates multiple aspects of innate and adaptive immune functions and is a key mediator of inflammatory responses. NFκB induces the expression of numerous pro-inflammatory genes, such as those encoding cytokines and chemokines, and participates in inflammasome regulation (17). NFκB activation is a crucial stage in the inflammatory process and can contribute to the sustained production of inflammatory mediators. Evaluating NFκB levels can provide insights into the activation of inflammatory pathways in the paw tissue during edema (18).

Prostaglandin F2-alpha (PGF_2-α) is a pro-inflammatory prostaglandin that contributes to the inflammatory response. It is produced by COX-2 and functions in a variety of physiological and pathological processes, including inflammation, vasoconstriction, and pain. Measuring PGF_2-α levels can reveal the extent of inflammation in the paw tissue and provide insight into any potential protective effect (8).

Vildagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, has drawn interest for its possible anti-inflammatory properties as well as its ability to modulate inflammatory responses (19). DPP-4 is an enzyme that degrades incretin hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). By inhibiting DPP-4, Vildagliptin increases the levels of GLP-1 and GIP, which play crucial functions in glucose metabolism and insulin secretion (20).

In addition to its effects on glucose regulation, vildagliptin has been shown to have anti-inflammatory properties. Several investigations have demonstrated its anti-inflammatory properties by inhibiting the production and activity of pro-inflammatory cytokines and chemokines (21, 22, 23). By modulating the inflammatory response, vildagliptin may have a protective effect in inflammatory conditions, including paw edema (24, 25).

One of the primary mechanisms by which Vildagliptin exerts its anti-inflammatory effects is by inhibiting NFκB activation (26, 27). Moreover, vildagliptin has been shown to modulate the release of cytokines involved in inflammation, such as TNF-α (28, 29). In addition, it has been discovered that vildagliptin enhances IL-10, which may shift the balance toward an anti-inflammatory environment and protect against paw edema-induced inflammation (27, 30, 31).

This study was conducted to investigate the potential protective effect of Vildagliptin on paw edema by evaluating the modulation of inflammatory and oxidative stress parameters, including MPO, SOD, IL-10, COX-2, TNF-α, NFκB, and PGF_2-α in an experimental rat model.

2. Material and methods

2.1. Animal

Twenty-four male Wistar rats (170–200 g, 8 weeks old) were provided by the animal facility at the Faculty of Pharmacy, Egyptian Russian University, and were housed in the animal house for one week before the experiment to acclimatize. The experiment protocol was approved by the research ethics committee at the Faculty of Pharmacy, Egyptian Russian University. The protocol approval code is ERUFP-PO-23-003.

2.2. Chemicals

Vildagliptin was purchased as tablets under the brand name Galvus® (Novartis Company). It was ground in a porcelain mortar and suspended in 1% carboxymethyl cellulose (CMC) directly

before administration by oral gavage. Carrageenan was purchased from Sigma Aldrich Company (St. Louis, MO, USA) and dissolved in 0.9% normal saline before induction of paw edema.

2.3. Experimental design

The animals used in the study were divided into three groups, each consisting of eight rats. The groups were as follows:

1) **Normal control group:** Rats in this group received a vehicle (10 ml/kg P.O) for seven consecutive days.

In this group, paw edema induction was not performed. Instead, a subplantar injection of 0.9% normal saline, equivalent in volume to the carrageenan injection, was administered.

2) **Paw edema group:** Rats in this group received vehicle (10 ml/kg P.O) for seven consecutive days.

On the eighth day, paw edema was induced in the subplantar region of the left hind paw of these rats by carrageenan, as described by previous studies (32, 33).

3) **Vildagliptin group:** Rats in this group received vildagliptin (5 mg/kg P.O) for seven consecutive days (27).

On the eighth day, paw edema was induced in these rats as well.

Five hours after inducing paw edema, rats from the three groups were sacrificed under pentobarbital sodium (100 mg/kg)-induced deep anesthesia, and their left and right paws were cut and weighed. Then, paw exudates were extracted for further evaluation of inflammatory and anti-inflammatory biomarkers.

2.4. Measurements of paw edema

On the malleolus of the hind limb, measurement points have been marked for use in subsequent measurements. Edema was measured in milliliters using a plethysmograph at 0 hours, i.e., immediately after carrageenan injection, and repeated at 1, 2, 3, 4, and 5 hours after carrageenan injection. The percentage of weight increase between the left and right paws of each animal was determined using the following equation:

$$Percentage\ of\ weight\ increase = \frac{Weight\ of\ the\ left\ paw - Weight\ of\ the\ right\ paw}{Weight\ of\ the\ right\ paw} \times 100$$

2.5. Measurement of inflammatory mediators

The level of MPO level was measured using a colorimetric assay kit provided by Abcam Company (Cambridge, UK), Cat# K744-100, according to manufacturer instructions. TNF-α and NFκB levels were measured by enzyme-linked immunosorbent assay (ELISA) using kits provided by Wuhan USCN Business Company (Houston, Texas, USA), Cat# SEA133Ra, and SEB824Mu, respectively, according to manufacturer instructions. The COX-2 level was measured by ELISA using a kit provided by My BioSource (San Diego, CA, USA), Cat# MBS725633, according to manufacturer instructions. PGF2-α level was measured by ELISA using a kit provided by Assay Genie Company (Dublin 2, Ireland), Cat# RTES00662, according to manufacturer instructions. The exudate protein content was calculated using the Bradford method (34).

2.6. Measurement of SOD as an antioxidant marker

The level of SOD level was measured in exudates of paw edema by colorimetric method using a kit provided by Biodiagnostic Company (Giza, Egypt), Cat# SD 25-21, according to manufacturer instructions.

2.7. Measurement of IL-10 as an anti-inflammatory marker

The IL-10 level was measured in exudates of paw edema by ELISA using a kit provided by USCN Business Company (Houston, Texas, USA), Cat# SEA056RA, according to manufacturer instructions.

2.8. Statistical analysis

The statistical analysis of the data was conducted using GraphPad Prism 9.5.1 Demo (GraphPad Software, San Diego, CA). One-way analysis of variance (ANOVA), except for paw edema volume, which was analyzed using two-way ANOVA, was employed to evaluate the differences between the various groups. Post-hoc analysis was performed using Tukey's multiple comparisons test. The results are presented as mean ± standard deviation (SD). The significance level was set at $P < 0.05$ to determine statistical significance.

3. Results

3.1. Impact of Vildagliptin Pretreatment on paw weight in a paw edema inflammation model

As shown in **Figure 1**, the rats in the paw edema group exhibited a significant increase in the average percentage of weight gain in the left paw (7087.13%) when compared to the normal control group. Conversely, rats pretreated with vildagliptin demonstrated a significant reduction in the average percentage of weight gain in the left paw (44.63%) compared to the paw edema group, **Figure 1**.

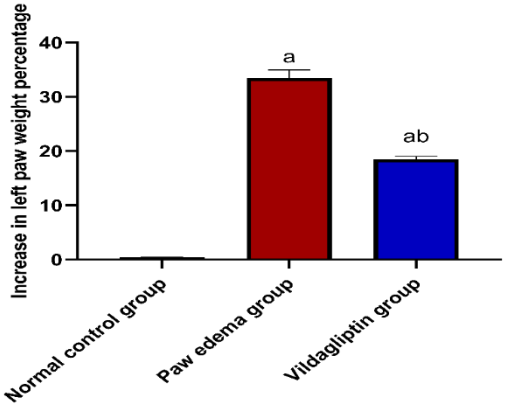

Figure 1: Impact of Vildagliptin Pretreatment on paw weight gain in a paw edema inflammation mode. Rats were treated with 1% CMC (10 ml/kg, P.O.; normal control group), 1% CMC (10 ml/kg, P.O.; paw edema group), or vildagliptin (10 mg/kg, P.O.; vildagliptin group), for seven days and on the eighth day, paw edema was induced in both the paw edema group and the vildagliptin group. Data are presented as mean ± SD; one-way ANOVA followed by Tukey's multiple comparisons test; n=8. [a] Significantly different from normal control group at $p < 0.05$, [b] Significantly different from paw edema group at $p < 0.05$.

3.2. Impact of vildagliptin pretreatment on paw volume in a paw edema inflammation model

As shown in **Table 1**, rats in the paw edema group have shown a significant increase in the paw edema volume 0, 1, 2, 3, 4, and 5 hours after paw edema induction (5.53%, 35.93%, 56.02%, 45.44%, 34.69%, and 31.44%, respectively) compared to the normal control group. On the other hand, rats pretreated with vildagliptin have shown a significant decrease in paw edema volume 1,

2, 3, 4, and 5 hours after paw edema induction (16.87%, 18.37%, 22.59%, 19.55%, and 16.90%, respectively) in comparison to the paw edema group **(Table 1)**.

Table 1: Impact of vildagliptin pretreatment on paw volume in a paw edema inflammation model.

Time after paw edema induction	Volume displacement in ml (mean ± SD)		
	Normal control group	Paw edema group	Vildagliptin group
0 hours	0.918 ± 0.025	0.969 ± 0.026 [a]	0.960 ± 0.044 [a]
1 hour	1.007 ± 0.014	1.369 ± 0.039 [a]	1.138 ± 0.045 [ab]
2 hours	0.989 ± 0.012	1.542 ± 0.027 [a]	1.259 ± 0.045 [ab]
3 hours	0.971 ± 0.013	1.412 ± 0.027 [a]	1.093 ± 0.014 [ab]
4 hours	0.949 ± 0.012	1.278 ± 0.020 [a]	1.028 ± 0.005 [ab]
5 hours	0.931 ± 0.010	1.224 ± 0.047 [a]	1.017 ± 0.009 [ab]

Rats were treated with 1% CMC (10 ml/kg, P.O.; normal control group), 1% CMC (10 ml/kg, P.O.; paw edema group), or vildagliptin (10 mg/kg, P.O.; vildagliptin group), for seven days and on the eighth day, paw edema was induced in both the paw edema group and the vildagliptin group. Data are presented as mean ± SD; two-way ANOVA followed by Tukey's multiple comparisons test; n=8

[a] Significantly different from the normal control group at $p < 0.05$.

[b] Significantly different from the paw edema group at $p < 0.05$.

3.3. Impact of vildagliptin pretreatment on inflammatory mediators in a paw edema inflammation model

As shown in **Figure 2**, rats in the paw edema group have shown a significant increase in exudate levels of MPO, TNF-α, NFκB, COX-2, and PGF2-α (451.27%, 240.12%, 384.70%, 360.99%, and 373.79%, respectively) compared to the normal control group. However, rats pretreated with vildagliptin have shown a significant decrease in exudate levels of MPO, TNF-α, NFκB, COX-2, and PGF2-α (50.96%, 52.82%, 48.04%, 33.48%, and 50.02%, respectively) compared to the paw edema group, **Figure 2**.

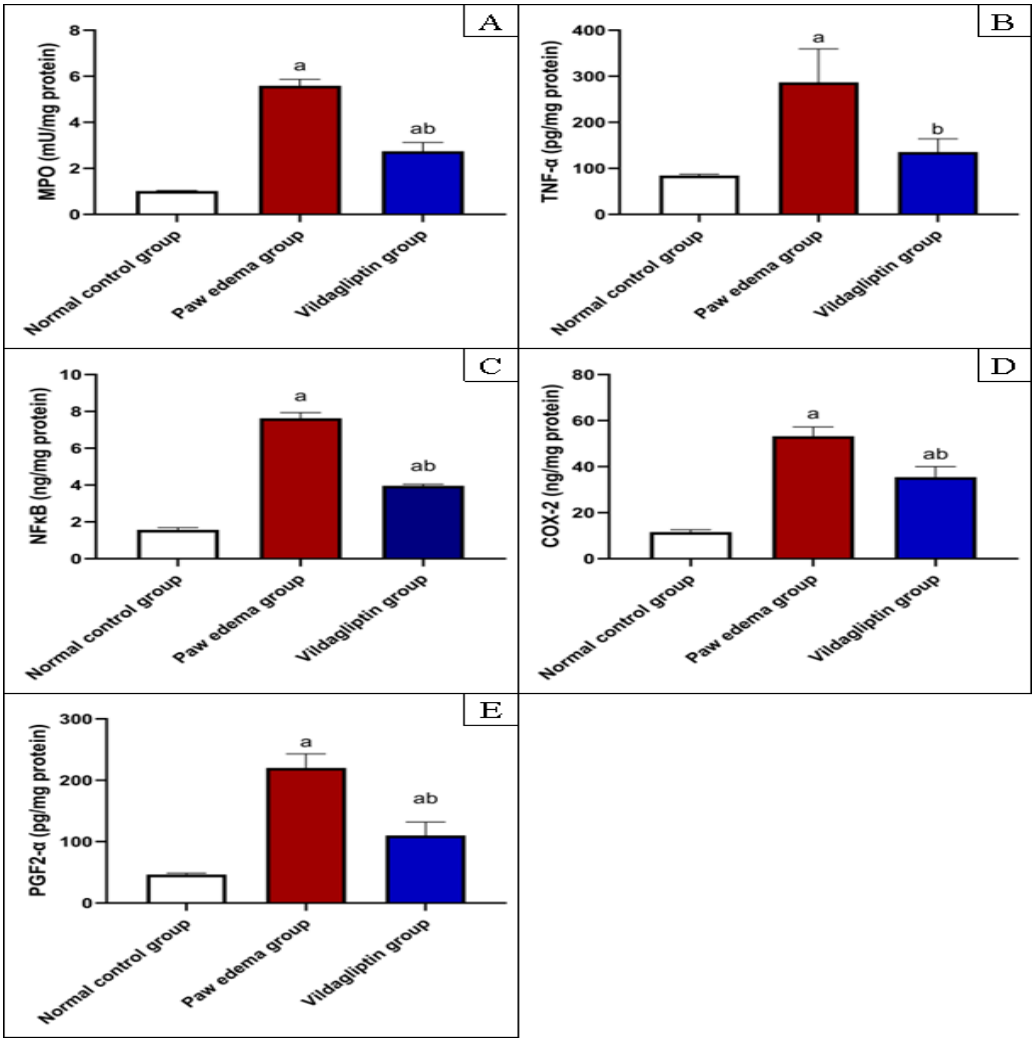

Figure 2: Impact of vildagliptin pretreatment on inflammatory mediators in a paw edema inflammation model. A: MPO, B: TNF-α, C: NFκB, D: COX-2, E: PGF2-α. Rats were treated with 1% CMC (10 ml/kg, P.O.; normal control group), 1% CMC (10 ml/kg, P.O.; paw edema group), or vildagliptin (10 mg/kg, P.O.; vildagliptin group), for seven days and on the eighth day, paw edema was induced in both the paw edema group and the vildagliptin group. Data are presented as mean ± SD; one-way ANOVA followed by Tukey's multiple comparisons test; n=8. a Significantly different from normal control group at $p < 0.05$, b Significantly different from paw edema group at $p < 0.05$.

3.4. Impact of vildagliptin pretreatment on antioxidant activity in a paw edema inflammation model

As shown in **Figure 3**, rats in the paw edema group have shown a significant decrease in the exudate level of SOD (73.85%) compared to the normal control group. Conversely, rats pretreated with vildagliptin have shown a significant increase in the exudate level of SOD (146.82%) in comparison to the paw edema group, **Figure 3**.

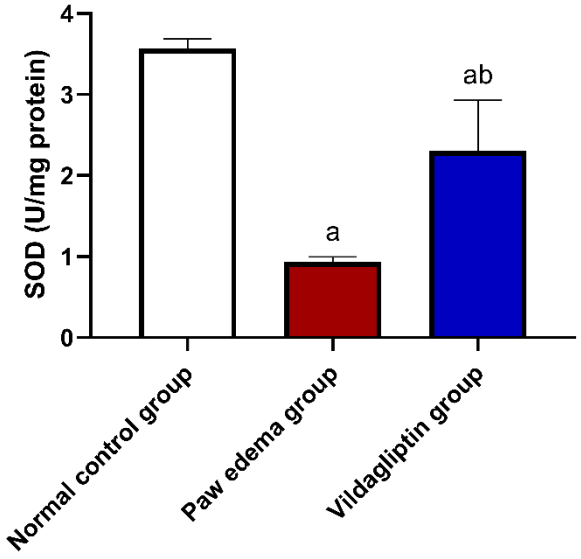

Figure 3: Impact of vildagliptin pretreatment on SOD level in a paw edema inflammation model. Rats were treated with 1% CMC (10 ml/kg, P.O.; normal control group), 1% CMC (10 ml/kg, P.O.; paw edema group), or vildagliptin (10 mg/kg, P.O.; vildagliptin group), for seven days and on the eighth day, paw edema was induced in both the paw edema group and the vildagliptin group. Data are presented as mean ± SD; one-way ANOVA followed by Tukey's multiple comparisons test; n=8. [a] Significantly different from normal control group at $p < 0.05$, [b] Significantly different from paw edema group at $p < 0.05$.

3.5. Impact of vildagliptin pretreatment on anti-inflammatory activity in a paw edema inflammation model

As shown in **Figure 4**, rats in the paw edema group have shown a significant decrease in the exudate level of IL-10 (75.36%) compared to the normal control group. On the contrary, rats

pretreated with vildagliptin have shown a significant increase in the exudate level of IL-10 (91.55%) compared to the paw edema group **(Figure 4)**.

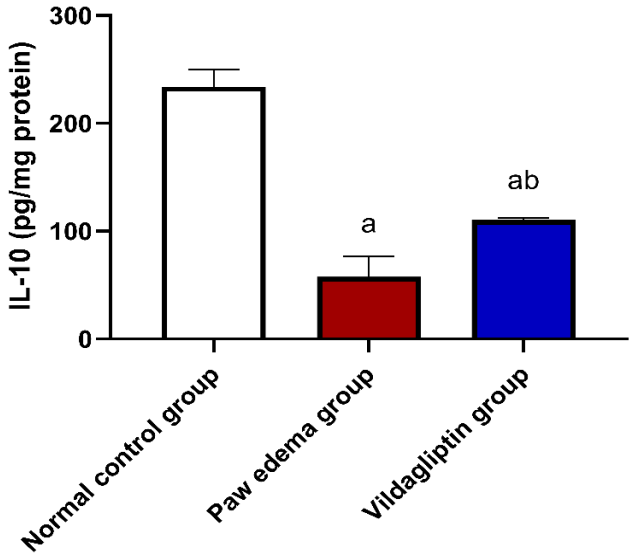

Figure 4: Impact of vildagliptin pretreatment on IL-10 in a paw edema inflammation model. Rats were treated with 1% CMC (10 ml/kg, P.O.; normal control group), 1% CMC (10 ml/kg, P.O.; paw edema group), or vildagliptin (10 mg/kg, P.O.; vildagliptin group), for seven days and on the eighth day, paw edema was induced in both the paw edema group and the vildagliptin group. Data are presented as mean ± SD; one-way ANOVA followed by Tukey's multiple comparisons test; n=8. [a] Significantly different from normal control group at $p < 0.05$, [b] Significantly different from paw edema group at $p < 0.05$.

4. Discussion

Paw edema is a common inflammatory response that is characterized by swelling and increased permeability in the affected tissues (35). It is often used as a model to study acute inflammation and evaluate potential anti-inflammatory interventions (2, 36). The present study aimed to investigate the potential protective effect of vildagliptin, an anti-diabetic agent, on the paw edema inflammation model.

In this study, a paw edema model was induced in rats using carrageenan, a well-established method for inducing localized inflammation. The effects of vildagliptin were evaluated by assessing various parameters related to inflammation, including paw weight, paw edema volume,

and levels of inflammatory markers such as MPO, TNF-α, NFκB, COX-2, PGF_2-α, SOD, and IL-10.

The results obtained from the study revealed significant differences among the groups in various parameters, indicating the potential anti-inflammatory effects of vildagliptin. The paw edema group showed a significant increase in paw weight compared to the normal control group, suggesting the development of edema in the inflamed paw. However, rats pretreated with vildagliptin exhibited a significant reduction in paw weight gain compared to the paw edema group, indicating a potential protective effect against edema formation.

Furthermore, the paw edema group demonstrated a significant increase in paw edema volume at multiple time points compared to the normal control group. This finding is consistent with the characteristic swelling observed in paw edema models. Interestingly, rats pretreated with vildagliptin showed a significant decrease in paw edema volume at these time points compared to the paw edema group, suggesting a potential inhibitory effect on edema development.

The previous findings are consistent with previous studies that have established a correlation between the induction of acute inflammation by carrageenan and an increase in paw weight percentage and paw volume. These effects have been successfully reversed using standard anti-inflammatory agents as well as experimental compounds (1, 36, 37, 38).

In addition to evaluating paw weight and edema volume, this study aimed to investigate the levels of various inflammatory markers to gain further insight into the potential anti-inflammatory mechanisms of vildagliptin. One such marker is MPO, an enzyme released by neutrophils during inflammation, serving as an indicator of neutrophil infiltration (10). Additionally, pro-inflammatory mediators including TNF-α, NFκB, COX-2, and PGF_2-α were examined, as they play key roles in the inflammatory response (8, 27).

The results demonstrated that the paw edema group exhibited significantly elevated levels of MPO, TNF-α, NFκB, COX-2, and PGF_2-α compared to the normal control group, indicating the presence of inflammation. These findings are consistent with previous studies that have reported similar increases in these inflammatory markers under inflammatory conditions (39, 40, 41).

Interestingly, rats pretreated with vildagliptin demonstrated significant reductions in the levels of these inflammatory markers compared to the paw edema group. These findings suggest

that vildagliptin administration may attenuate the inflammatory response by modulating the production or release of these pro-inflammatory mediators. The observed decrease in MPO levels suggests a potential inhibitory effect on neutrophil infiltration, while the reductions in TNF-α, NFκB, COX-2, and PGF_2-α levels indicate a potential downregulation of the inflammatory cascade. Previous studies have consistently demonstrated that Vildagliptin possesses the ability to modulate these parameters and effectively attenuate inflammation (21, 26, 27, 30, 42).

Moreover, the study evaluated the levels of SOD and IL-10, markers associated with antioxidant and anti-inflammatory responses, respectively (43, 44). The paw edema group exhibited a significant decrease in SOD levels compared to the normal control group, indicating impaired antioxidant defense mechanisms in the inflamed paw. These findings align with previous studies that have reported similar reductions in SOD levels under inflammatory conditions (45, 46). In contrast, rats pretreated with vildagliptin showed a significant increase in SOD levels compared to the paw edema group, suggesting a potential enhancement of antioxidant activity. This observation is consistent with previous studies demonstrating the ability of vildagliptin to elevate SOD levels in various experimental models (31, 47).

Furthermore, the paw edema group exhibited a significant decrease in IL-10 levels compared to the normal control group whereas, the vildagliptin group demonstrated significant increases in IL-10 levels compared to the paw edema group. IL-10 is an anti-inflammatory cytokine known to regulate the immune response and suppress pro-inflammatory mediators (13, 48). The elevated levels of IL-10 in the vildagliptin group suggest a potential shift towards an anti-inflammatory state, indicating the ability of vildagliptin to modulate the immune response and promote anti-inflammatory processes.

The findings of this study support the notion that vildagliptin has a potential protective effect against paw edema-induced inflammation. The observed reductions in paw weight, paw edema volume, and levels of inflammatory markers indicate that vildagliptin administration may attenuate the inflammatory response associated with paw edema. These findings contribute to the understanding of the anti-inflammatory properties of vildagliptin and suggest its potential therapeutic utility in the management of edema-related inflammatory conditions.

The mechanisms underlying the observed anti-inflammatory effects of vildagliptin are likely multifactorial. Vildagliptin is known to inhibit DPP-4, an enzyme involved in the

degradation of incretin hormones such as GLP-1 (20). GLP-1 has been shown to possess anti-inflammatory properties, including the modulation of immune cell function and the suppression of pro-inflammatory cytokines (49, 50). Therefore, it is possible that vildagliptin's effects on inflammation are mediated, at least in part, by GLP-1-dependent mechanisms.

Additionally, vildagliptin has been reported to exert antioxidant effects by increasing the activity of antioxidant enzymes and reducing oxidative stress markers in various tissues (21, 26, 51). The observed increase in SOD levels in the vildagliptin group supports the notion that vildagliptin may enhance antioxidant defenses, thereby counteracting oxidative stress and inflammation.

5. Conclusion

The findings of this study suggest that vildagliptin pretreatment exerts a protective effect against paw edema-induced inflammation. The observed reductions in paw weight, paw edema volume, and levels of inflammatory markers, along with the increase in SOD levels and IL-10 levels, indicate the potential anti-inflammatory and antioxidant properties of vildagliptin. These findings contribute to the growing body of evidence supporting the therapeutic potential of vildagliptin in the management of inflammatory conditions. Further investigations are warranted to elucidate the underlying mechanisms and evaluate the long-term effects of Vildagliptin treatment in various inflammatory models and clinical settings.

Conflict of Interest

All authors declare that they have no competing interests.

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