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Effects of lixisenatide on acute and subacute models of inflammation in male Wistar rats.

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A B S T R A C T

Analogues of Glucagon like peptide-1 authorized for the management of diabetes mellitus type 2 have demonstrated anti-inflammatory effects. However, the effects were not studied in models of inflammation. This study aimed to assess lixisenatide's effect on male Wistar rat's acute and subacute inflammation models. Three groups of six animals each, weighing 180±20 g, were randomly selected. Thirty minutes after per oral administration of gum acacia, aspirin and one hour after subcutaneous lixisenatide administration, 0.05 ml of Carrageenan at 1% was injected into the sub-plantar region of hind paw at left side using normal saline. A digital plethysmograph was used to measure the volume of paw oedema in ml at 0, 0.5, 1, 2, 3, 4, and 5 h, and the increase at each time point in comparison to that at 0 h was calculated. For the subacute study, with similar grouping, a pair of 10 mg sterile cotton pellets and a pair of sterile grass-piths were implanted subcutaneously in the axilla and groin. Blood samples (5 ml) were collected after 10 days for measuring the inflammatory cytokine levels. Rats were sacrificed, grass piths and cotton pellets were collected, the dry weight was calculated using the % inhibition of granuloma; haematoxylin and eosin-stained grass piths were evaluated using ANOVA and Dunnett's test. No significant reduction in rat paw oedema, percentage inhibition of cotton pellets, or levels of inflammatory markers was observed with lixisenatide treatment. The histopathology of grass piths showed abundant fibroblasts, granulation tissue, and collagen. In both acute and subacute inflammation models, lixisenatide did not reduce inflammation significantly. Therefore, GLP-1 analogues exhibit anti-inflammatory effects owing to their hypoglycaemic action in diabetes; however, they do not possess any independent anti-inflammatory effect.

Keywords:
Lixisenatide, aspirin, inflammation, diabetes mellitus, GLP-1 analogue,

INTRODUCTION

Pathogenic events induce inflammation, a local and systemic response in the tissues and microcirculation [1], which helps the body eliminate the prime mediators of cell damage, such as poisons and microbes as well as the resultant necrotic cells and tissues. The inflammatory process is mediated by vascular and cellular events; the former is majorly responsible for the pathogenesis of acute inflammation [2].

The mechanisms employed by the immune system to destroy foreign intruders and necrotic tissues inherently injure normal tissues. When the inflammation is targeted against self-tissues or is poorly controlled, it leads to injury and disease [2].

Chronic inflammation is implicated in type 2 diabetes mellitus (T2DM) and its macrovascular (transient ischaemic attack -TIA, myocardial infarction- MI, peripheral vascular disease) and microvascular consequences (retinopathy, nephropathy, autonomic neuropathy) [3]. The release of chemokines, such as macrophage migration inhibition factor and monocyte chemoattractant protein (MCP-1), from tissues under stress prominently adipose tissue and vascular endothelium, is the underlying cause of this chronic inflammation. These factors induce the release of interstitial and vascular cellular adhesion molecules (ICAM-1 and VCAM-1) as well as E-selectin. In addition, they enable immune cells such as monocytes to penetrate the region of stress, where these cells proliferate in response to chemokine-induced factors such as advanced glycation end products (AGE). These factors activate proinflammatory genes and produce cytokines such as TNF-α, IL-1, IL-6, IL-8, interferon-γ, and C-reactive protein(CRP) [4]. This silent inflammation is dangerous, and it is essential to

develop pharmacological interventions that lower it.

Diabetes outcomes can be improved by insulin sensitizers with anti-inflammatory qualities, such as biguanide (metformin) and thiazolidinediones (agonists of the peroxisome proliferator-activated receptor-gamma, or PPAR-agonists)[4,5]. In terms of anti-inflammatory activity, these drug classes are superior to insulin-secreting agents like glinides or sulphonylureas [3]. Oral hypoglycaemic drugs like α-glucosidase inhibitors moderately influence inflammatory markers. Gliptins, which are inhibitors of dipeptidyl peptidase-4(DPP-4), have remarkable anti-inflammatory potential that could abate the cardiovascular complications of T2DM [3,6]. α-glucosidase inhibitors exhibit moderate effect on inflammatory markers while gliptins and agonists of the glucagon like peptide -1(GLP-1) receptor are more promising [3].

GLP-1 belongs to the family of incretin peptides, which have a variety of effects on blood sugar regulation.[7,8]. GLP-1 receptor agonists activate the GLP-1 receptor, facilitating the release of insulin that is reliant on glucose, in the pancreas, slow gastric emptying, suppress glucagon output from the pancreas, and decrease appetite, which is beneficial especially in obese diabetic patients [7,8]. GLP-1 does not decrease glucose below fasting levels; therefore, the risk of hypoglycemia is low with GLP-1 receptor agonists [9].

The GLP-1 analogue, exenatide, considerably lowers oxidative damage and inflammatory indicators in patients with T2DM [10]. Liraglutide, another GLP-1 analogue, minimises the levels of reactive oxygen species and the inflammatory processes induced by TNF-α, in endothelial cells [11].

The European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) approved lixisenatide, a synthetic GLP-1 receptor agonist, for the treatment of type 2 diabetes. In animal models, lixisenatide and ligarglutide penetrate the blood-brain barrier and reduce oxidative stress and the brain's response to chronic inflammation in Alzheimer's disease [12]. Lixisenatide protects ß-cells from apoptosis induced by lipids and cytokines in vitro in a rat pancreatic ß-cell line [13]. Long-term administration of lixisenatide in diabetic rodent models inhibits the decline in glucose tolerance over time.

In db/db mice, intraperitoneal administration of lixisenatide twice a day, effectively reduced disease progression and enhanced glycemic control [14]. Lixisenatide reduced inflammation and atheroma plaque size and instability in Apoe−/−Irs2+/−mice, a model of insulin resistance, atherosclerosis, and metabolic syndrome, by reorganizing macrophages in accordance with the M2 phenotype [15].

Distinguishing between the anti-inflammatory benefits linked to the intrinsic activities of glucose-lowering medication and those stemming from improved glucose regulation is critical. Determining the possible contributions of the anti-inflammatory qualities of these glucose-lowering drugs in preventing, delaying, and ameliorating macro- and micro-vascular diabetic complications is of great clinical interest [3].

This research aimed to determine the anti-inflammatory effects of lixisenatide in male Wistar rats.

OBJECTIVES

1) To assess the effect of lixisenatide in carrageenan generated rat paw oedema (acute model) as well as foreign body-induced granuloma (subacute model)

2) To evaluate the effect of lixisenatide on inflammatory markers, namely TNF-α, IL-1β, and CRP

MATERIALS AND METHODS

Healthy male Wistar albino rats weighing 180±20 g were provided by J. N. Medical College's central animal house in Belagavi. For 10 days before the experiments, these animals were housed under standard conditions and were acclimatized to a 12 h cycle of light and dark. They were fed a regular diet of Amrut brand rat chow pellets along with an unlimited access to water. Institutional animal ethics committee (IAEC) approval was granted for the study.

Acute model: carrageenan-induced rat paw oedema

Three groups of six rats each were given water ad libitum and were allowed to fast throughout the night before the experiment day. The control group received an oral 0.5 ml suspension of gum acacia at 1% while other groups received clinically equivalent doses of either aspirin in 1% gum acacia orally or lixisenatide subcutaneously. Aspirin served as the standard anti-inflammatory drug.

Carrageenan (1%; 0.05 ml) was injected into the sub-plantar region of the left hind paw using normal saline [16] at 30 min following oral administration of gum acacia or aspirin and 1 h after subcutaneous administration of lixisenatide [12].

To facilitate uniform dipping during subsequent measurements, a mark was applied to the leg at the malleolus. With the aid of a digital plethysmograph, the volume of paw oedema was measured in milliliters (ml) using the water displacement method at 0 hours (shortly after injecting carrageenan). The same process was performed again at 0.5, 1, 2, 3, 4, and 5 h. The actual oedema was defined as the difference between that at zero and at subsequent readings.

Subacute model: foreign body-induced granuloma method

Three groups of rats with six in each group, were anaesthetised with thiopentone sodium, and the axillae and groin were clipped; a tiny incision was made and pairs of sterile cotton pellets (10 mg) and pairs of sterile grass-piths (25x2 mm) were randomly implanted subcutaneously. The wounds were sutured and following the recovery from anaesthesia, each rat was

housed in a separate cage. Throughout the experiment aseptic measures were observed. The treatment course initiated from the implantation day and was followed regularly for total of 10 days, with administration of treatment repeated for every 24 h in the control, aspirin, and lixisenatide groups.

On the 11th day, heart puncture was used to extract 5 ml of blood for measuring inflammatory cytokines. Cotton pellets and grass piths have been obtained from rats that had been sacrificed under an overdose of thiopentone anesthesia. To determine their dry weight, the pellets—devoid of excessive tissue—were dried in an incubator for an entire night at 60 °C. The initial weight (10 mg) was subtracted from the measured weights to determine the net granuloma formation. The average dry weight of granulomas for each group was determined and reported as mg/100 g of body weight.

The granuloma dry weight percentage inhibition was calculated with the help of following formula

$$\text{Percentage inhibition of granuloma dry weight} = \left[1 - \left(\frac{\text{dry weight of granuloma in treated group}}{\text{Dry weight of granuloma in control group}}\right)\right] X100$$

The grass piths maintained in 10 % formalin were processed in Laboratory of the Department of Pathology, J.N. Medical College, Belagavi. The granulation tissue in each group was examined under a microscope after the sections were dyed with hematoxylin and eosin.

Blood samples were collected before sacrificing the rats. These were centrifuged and the serum concentrations of inflammatory markers, namely TNF-α, IL-1β and CRP was measured using ELISA.

Statistical analysis

The data for each group is mentioned as Mean ± SEM. The data was analysed using one-way analysis of variance (ANOVA) and Dunnett's test, in Graph Pad Prism, to compare outcomes to those in the control group. Statistical significance was established at p-value less than 0.05.

The aspirin and lixisenatide treatment groups were compared using a one-way ANOVA and Bonferroni's test. Statistical significance was defined at p-value less than 0.05.

RESULTS

This research explored the possible anti-inflammatory properties of the diabetes drug, lixisenatide in acute as well as subacute inflammation models in male Wistar rats at therapeutically equivalent doses.

Carrageenan-induced acute inflammation

No discernible decrease in the volume of paw oedema was elicited in the lixisenatide treatment group as compared to the control group, as per the results of the post-hoc analysis using Dunnett's test (Table 1). In comparison to the control group, the paw oedema volume in the aspirin treatment group decreased significantly ($p<0.05$) at 2, 4, and 5 h (Table 1). These findings imply that lixisenatide does not exhibit statistically significant anti-inflammatory effect in the acute model of inflammation.

Subacute inflammation (granuloma induced by a foreign body)

When calculated as mg per 100 g body weight, the mean dry weight of the 10-day-old granulomas (aspirin 200 mg/kg) in the standard control group was significantly ($p < 0.001$) lower than that of the control group. However, in comparison to the standard control (Aspirin-200 mg/kg) group, the lixisenatide (1.8 mg/kg) treated group did not show a statistically significant decrease in granuloma weight (Table 2).

The effect of lixisenatide on serum levels of inflammatory indicators namely TNF–α, IL-1β and CRP was studied. There was no significant difference in the levels between control and treatment groups (Table 3). The histopathology results provided additional insights into the mode of action of lixisenatide in inflammation. Haematoxylin and eosin staining of grass pith sections revealed an abundance of granulation tissue in the control animals (Fig 1) but a marked reduction in fibroblasts, granulation tissue and collagen in the aspirin group (Fig 2). While in the drug-treated group, fibroblasts, granulation tissue and collagen were seen to be abundant with no marked reduction (Fig 3). The findings show that in the subacute form of inflammation, lixisenatide did not show any anti-inflammatory effects.

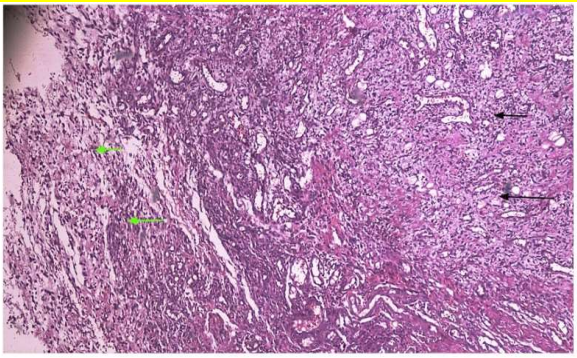

Figure 1 - Photomicrographs of grass pith with granulation tissue.
The control group showing abundant fibrous tissue, granulation tissue and inflammatory cells.

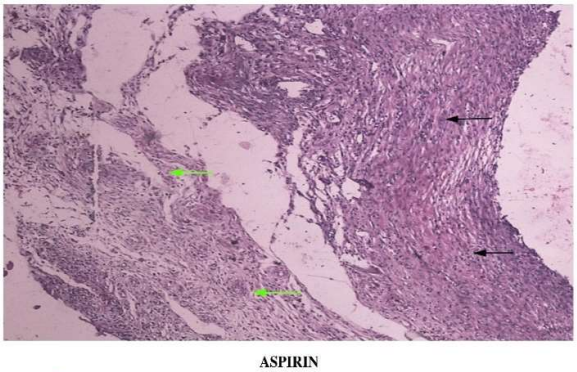

Figure 2 - Photomicrographs of grass pith with granulation tissue.
The aspirin-treated group showing a significantly lower amount of fibrous tissue, granulation tissue and inflammatory cells in comparison to the control group.

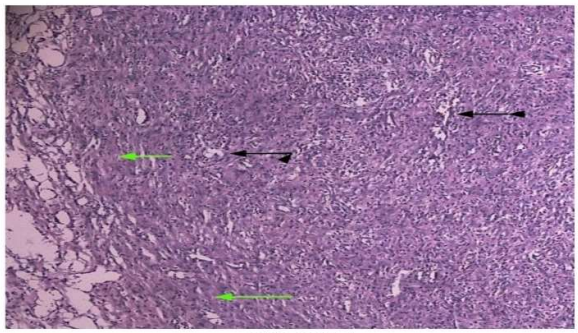

Figure 3 - Photomicrographs of grass pith with granulation tissue.
The lixisenatide-treated group showing abundant granulation tissue, inflammatory cells, and fibrous tissue

Figure 1, 2 & 3: Photo micrographs of grass pith with granulation tissue (H&E Stain-10x).

Abundant granulation tissue, inflammatory cells, and fibrous tissue were observed in the control and lixisenatide-treated groups. The amount of fibrous tissue, tissue with granulation, and inflammatory cells were significantly low in the aspirin-treated group compared that in the control.

Table 1: Effect of various treatments on carrageenan induced paw edema

Time post carrageenan injection	**Control** Paw oedema volume (ml) (Mean±SEM)	**Aspirin** Paw oedema volume (ml) (Mean ±SEM)	**Lixsenatide** Paw oedema volume (ml) (Mean ±SEM)	**ANOVA Result** F5,10 value
0.5 h	0.001 ± 0.046	0.033 ± 0.029	-0.03± 0.126	1.687
1 h	0.015 ± 0.043	-0.121±0.035	-0.033±0.114	2.439
2 h	0.053 ± 0.041	-0.183±0.049*	-0.095± 0.086	2.367
3 h	0 ± 0.101	-0.165±0.039	0.016 ± 0.170	1.081
4 h	0.028 ± 0.081	-0.175±0.056*	-0.148± 0.056	3.720
5 h	0.025 ± 0.082	-0.156±0.059*	-0.195± 0.080	2.701

n=6 in each group. Post hoc analysis using Dunnett'sTest, *p≤0.05

Table 2: Effects of various treatments on granuloma dry weight

Drug Treatment	**Mean granuloma dry weight mg/100 g body weight (Mean±SEM)**	**Percentage inhibition**
Control	37.25 ± 2.954	-
Aspirin	21.58 ± 2.509*	42.07%
Lixisenatide	36.833 ± 1.866	9.06%

n=6 in each group. ANOVA:F3,20=9.980, p<0.001. Post hoc analysis using Dunnett's test:
*p<0.001, SEM: Standard error of mean

Table 3: Effects of various treatments on serum inflammatory markers

Drug Treatment	Serum levels (Mean±SEM)		
	TNF–α (pg/ml)	IL-1β (pg/ml)	CRP (ng/ml)
Control	4.74 ± 2.81	3634 ± 1133.9	11.45 ± 1.57
Aspirin	24.71 ± 5.57	1622.41 ± 706.31	14.99 ± 1.75
Lixisenatide	46.73 ± 12.83	1782.35±1130.60	13.34 ± 1.78

n=6 in each group. Post hoc analysis using Dunnett's test.
ANOVA: F3,17=2.573 for TNF-α; F3,20=0.9248 for IL-1β; F3,20=0.7725 for CRP

DISCUSSION

The GLP-1 analogue, lixisenatide was evaluated for its impact on male Wistar rat models of acute and subacute inflammation. Lixisenatide, primarily used for the management of diabetes mellitus (DM) through modulation of insulin secretion and satiety, exerts pleiotropic effects, including anti-inflammatory, neuroprotective, antioxidative, and protective cardiovascular effects [16, 17]. Lixisenatide's anti-inflammatory effects are attributed to its ability to modulate the immune response and reduce cytokine production [18, 19], making it a potential therapeutic option for a range of inflammatory disorders, such as rheumatoid arthritis and atherosclerosis. However, these findings are based on in vitro studies evaluating the effect of lixisenatide on inflammation and not on animal models of inflammation. Purportedly, this is the first research to evaluate the anti-inflammatory potential of lixisenatide in acute (carrageenan-induced rat paw oedema) and subacute (cotton pellet-induced granuloma) inflammatory models. These specific models were chosen because they are simple, basic, and time-tested anti-inflammatory screening methods.

In the acute model, carrageenan was injected during the peak plasma concentration of the single administration of lixisenatide (1 h); there was no reduction in oedema suggesting that lixisenatide did not impart any anti-inflammatory effect. However, only a single dose of lixisenatide was studied; this could have resulted in the lack of discernible variations in inflammation owing to its pharmacokinetic properties. Lixisenatide did not cause a significant reduction in granuloma dry weight, levels of serum inflammatory markers–TNF-α, IL-1β and CRP, and granuloma dry weight percentageConsequently, in both acute and subacute inflammatory models, the chosen incretin analog has no anti-inflammatory impact. This is in line with a few earlier reports [3], which are in contrast to those that suggest that incretins have independent anti-inflammatory properties [4].

Exenatide, a GLP-1 analogue exhibits anti-inflammatory effect in rats with streptozotocin-induced diabetes and fed on a high-fat diet; this was observed as a significant decrease in serum levels of the inflammatory markers, TNF -α as well as IL-6. This discrepancy in the effects of GLP-1 analogues on inflammation could be attributed to the differences in the effect of incretins on glucose levels.

GLP-1 analogues promotes the production of insulin in the beta cells of pancreas in a glucose-dependent way, decrease inflammatory markers, glucagon secretion, and slow gastric emptying. These actions are associated with a decrease in post prandial blood glucose [5]. In addition, incretins decrease HbA1c and body weight [13Numerous pro-inflammatory mediators are produced as a result of oxidative stress and endoplasmic reticulum stress induced by hyperglycemia. These pro-inflammatory mediators cause peripheral tissues and the pancreatic islets inflammation, and they additionally lead peripheral tissues develop insulin resistance[14]. Considering the role of hyperglycemia in inflammation, the anti-inflammatory effect of incretins could be attributable to their blood glucose-lowering properties ather than to direct anti-inflammatory effects.

Lixisenatide transforms macrophages into an M2 phenotype, alleviating inflammation by reducing atheroma plaque size and instability in a mouse model of insulin resistance, metabolic syndrome, and atherosclerosis [11]. Liraglutide and lixisenatide crossed the blood-brain barrier, lower oxidative stress, and reduce the brain's chronic inflammatory response in animal models of Alzheimer's disease [5]. These differences could be attributed to variation with species, experimental design, and disease model.

The effects of incretin (liraglutide) based therapy on acute paw oedema induced by carrageenan and subacute inflammation induced by cotton pellet implantation in rats were studied; however, no anti-inflammatory effects of incretin-based therapy were observed in these models of inflammation [15]. In the model of rat paw edema generated by carrageenan, liraglutide did not show a significant anti-edema effect. This suggests that liraglutide does not inhibit the inflammatory mediators involved in the early and late phases of edema, such as histamine, serotonin, prostaglandins, lysosomes, and proteases. In

addition, liraglutide's anti-inflammatory effects were variable and context-dependent. In the acute model it did not show significant anti-inflammatory effect but had a potentiating effect in the subacute model when combined with ibuprofen. Despite its limited standalone anti-inflammatory effects, liraglutide may still have potential uses in chronic inflammatory conditions, especially when used in combination with other anti-inflammatory drugs like ibuprofen [20].

Based on these findings and literature, we conclude that incretin-based therapy does not exhibit anti-inflammatory effects independent of their glucose-lowering potential. Incretin-based therapy might offer the advantage of reducing inflammation associated with diabetes; however, it does not possess the potential to be used as an independent anti-inflammatory agent. We speculate that the similarity in the observations is due to the induction of inflammation in normal non-diabetics and by extension in non-hyperglycemic rats.

This speculation needs to be confirmed through evaluating the effects of the drugs on inflammation in diabetic rats. To further explore this hypothesis, future studies should investigate the underlying mechanisms of inflammation and the potential impact of different inflammatory pathways on glucose homeostasis. In addition, evaluation in a chronic model of inflammation with regular follow up will provide more robust data.

CONCLUSION

Lixisenatide had no discernible anti-inflammatory impact in either acute or subacute models of inflammation. Therefore, the anti-inflammatory effect observed in earlier studies could be attributed to its hypoglycemic effect and not to independent anti-inflammatory effect. Future studies should focus on validating these effects in inflammatory models in diabetic animals. However, this study provides a key perspective for understanding the anti-inflammatory potential of hypoglycaemic drugs employed in the treatment of type 2 diabetes mellitus.

REFERENCES

1. Murphy H. S. Inflammation. In: Rubin E, Strayer D. S, editors. *Rubin's Pathology. Clinicopathologic Foundations of Medicine.* Philadelphia: Wolters and Kluwers Health. Lippincott Williams & Wilkins; 2015.
2. Kumar V, Abbas A. K, Fausto N and Aster J. C. *Robbins and Cotran. Pathologic Basis of Disease.* 8th ed. Philadelphia: Saunders, An imprint of Elsevier; 2010.
3. Scheen A. J, Esser N and Paquot N. Antidiabetic agents: Potential anti-inflammatory activity beyond glucose control. Diabetes Metab., 2015; 41: 183-194.
4. Hattori Y, Suzuki K, Hattori S and Kasai K. Metformin inhibits cytokine-induced nuclear factor kappa B activation via AMP-activated protein kinase activation in vascular endothelial cells. Hypertension, 2006; 47: 1183-1188.
5. Ceriello A. Thiazolidinediones as anti-inflammatory and anti-atherogenic agents. Diabetes Metab. Res. Rev., 2008; 24: 14-26.
6. Kagal U. A, Angadi N. B and Matule S. M. Effect of dipeptidyl peptidase 4 inhibitors on acute and subacute models of inflammation in male Wistar rats: An experimental study. Int. J. Appl. Basic Med. Res., 2017; 7(1): 26-31.
7. Anderson S. L and Trujillo J. M. Lixisenatide in type 2 diabetes: Latest evidence and clinical usefulness. Ther. Adv. Chronic Dis., 2016; 7(1): 4-17.
8. Inzucchi S. E, Bergenstal R. M, Buse J. B, Diamant M, Ferrannini E, Nauck M, et al. Management of hyperglycemia in type 2 diabetes: A patient-centered approach: Position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care, 2012; 35(6): 1364-1379.
9. Sposito A. C, Berwanger O, de Carvalho L. S and Saraiva J. F. GLP-1RAs in type 2 diabetes: Mechanisms that underlie cardiovascular effects and overview of cardiovascular outcome data. Cardiovasc. Diabetol., 2018; 17(1): 157.
10. Wu J, Xu X, Zhu J, Ding B, Du T, Gao G, et al. Effect of exenatide on inflammatory and oxidative stress markers in patients with type 2 diabetes mellitus. Diabetes Technol. Ther., 2011; 13: 143-148.
11. Shiraki A, Oyama J, Komoda H, Asaka M, Komatsu A, Sakuma M, et al. The glucagon-like peptide 1 analogue liraglutide reduces TNF-α induced oxidative stress and inflammation in endothelial cells. Atherosclerosis, 2012; 221: 375-382.
12. Holscher C. The incretin hormones glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide are neuroprotective in mouse models of Alzheimer's disease. Alzheimers Dement., 2014; 10: 47-54.
13. Tews D, Werner U and Eckel J. Enhanced protection against cytokine and fatty acid-induced apoptosis in pancreatic beta cells by combined treatment with glucagon-like peptide-1 receptor agonist and insulin analogues. Horm. Metab. Res. 2008; 40: 172-180.
14. Werner U, Haschke G, Herling A. W and Kramer W. Pharmacological profile of lixisenatide: A new GLP-1 receptor agonist for treatment of type 2 diabetes. Regul. Pept., 2010; 164: 58-64.
15. Vinue A, Navarro J, Cervera A. H, Cubas M. G, Blasco I. A, Hervas S. M, et al. The GLP-1 analogue lixisenatide decreases atherosclerosis in insulin-resistant mice by modulating macrophage phenotype. Diabetologia, 2017; 60(9): 1801-1812.
16. Oezer K, Kolibabka M, Gassenhuber J, Dietrich N, Fleming T, Schlotterer A, et al. The effect of GLP-1 receptor agonist lixisenatide on experimental diabetic retinopathy. Acta Diabetol., 2023; 60(11): 1551-1565.

17. Xiao M, Lu D, Tian J, Yu Y, Zhang Q, Zhang L, et al. The protective effects of GLP-1 receptor agonist lixisenatide on oxygen-glucose deprivation/reperfusion (OGD/R)-induced deregulation of endothelial tube formation. RSC Adv. 2020; 10(17): 10245-10253.
18. Chung Y. W, Lee J. H, Lee J. Y, Ju H. H, Lee Y. J, Jee D. H, et al. The anti-inflammatory effects of glucagon-like peptide receptor agonist lixisenatide on the retinal nuclear and nerve fiber layers in an animal model of early type 2 diabetes. Am. J. Pathol. 2020; 190(5): 1080-1094.
19. Itoh A, Irie J, Tagawa H, Kusumoto Y, Kato M, Kobayashi N, et al. GLP-1 receptor agonist, liraglutide, ameliorates hepatosteatosis induced by anti-CD3 antibody in female mice. J. Diabetes Complications., 2017; 31(9): 1370-1375.
20. Rajashekar Y. R, Padmanabha T. S and Chandrakantha T. Variable anti-edema and anti-granuloma effects of liraglutide and teneligliptin on experimental Wistar albino rat inflammatory models. Natl. J. Physiol. Pharm. Pharmacol., 2018; 8(1): 51-55.