"EVALUATION OF ANTIDIABETIC DRUG *ALOGLIPTIN*FOR THE TREATMENT OF INFLAMMATIONIN RATS"

INTRODUCTION

In the present Evaluation, we have selected a *Alogliptin* (Anti diabetic drug) in which variety of pharmacological features are abundant. However, to dateanti-inflammatory activities of this Drug have not beenreported. Its medicinal properties of dipeptidyl peptidase 4 inhibitors (DPP-4) reported by the researchers to opt for the assessment of anti-inflammatory activities in variousexperimental animal models.

Dipeptidyl peptidase-4 (DPP-4) inhibitors are novel oralantihyperglycemic agents for treating type 2 diabetesmellitus patients. Recent studies suggest that several DPP-4inhibitors exert suppressinginflammatory reactions. However, whether or not DPP-4inhibitors suppress arterial inflammation and intimal hyperplasiaafter injury remains undetermined.*Alogliptin*(2-({6-[(3R)-3-aminopiperidinyl-1-yl]-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)yl} methyl)benzonitrilemonobenzoate) (AGP) is a selective DPP-4 inhibitor that has improves glycemic control.However, it remains unknown whether AGP has anti-inflammatoryeffects. [1-7]

DPP4 was first discovered by Hopsu-Havu and Glenner [8] in 1966. This protein is also called CD26 and is a ubiquitously expressed 110-kDa glycoprotein that belongs to the type 2 transmembrane protein family [9]. As a member of the serine peptidase/prolyl oligopeptidase family, DPP4 is often subclassified based on its structure and function as follows: membrane-bound peptidase (fibroblast activation protein (FAP)/seprase), resident cytoplasmic enzyme (DPP8 and DPP9), and nonenzymatic member (DPP6 and DPP10). These proteins share a typical α/β-hydrolase fold. DPP4 comprises four domains: a short cytoplasmic domain, a transmembrane domain, a flexible stalk segment, and the extracellular domain, which is further separated by a glycosylated region, the cysteine-rich region, and the catalytic region [10]. DPP4 can cleave dozens of peptides, including chemokines, neuropeptides, and regulatory peptides, containing a proline or alanine residue at position 2 of the amino-terminal region [11]. Despite the preference for proline at position 2, alternate residues at the penultimate position are also cleaved by DPP4, indicating a required stereochemistry for cleavage. This DPP4 cleavage at post-proline peptide bonds inactivates peptides and/ or generates new bioactive peptides, thereby regulating diverse biological processes.

DM is a low-grade systemic inflammatory disease. Suppressing inflammation slows the progression of DM. In addition to preserving glucose homeostasis, DPP4 inhibitors exert pleiotropic actions, such as anti-inflammatory effects. Alogliptin inhibits Toll-like receptor-4-mediated extracellular matrix signal-regulated kinase (ERK) activation and ERK-dependent matrix metalloproteinase expression in U937 histiocytes [12-13]. DPP4 inhibitors reduce cyclooxygenase-2, IL-1β, macrophage inflammatory protein-2, and TLR-4-mediated IL-6 expression in Zucker Diabetic Fatty rat [14], diabetic apolipoprotein E-deficient mice [15], and C57BL/6J-obese/obese mice [16], which parallels recovery from disease. It is speculated that the anti-inflammatory properties of DPP4 inhibitors may be largely beneficial for DM.

Alogliptin was first approved by the Pharmaceuticals and Medical Devices Agency of Japan in 2010 and by the FDA in 2013 for treating T_2DM. It is a potent and highly selective inhibitor of DPP4 with a mean IC_{50} of 6.9 nM and 1,000-fold increased

selectivity for DPP4 compared with that of the closely related serine proteases DPP2, DPP8, DPP9, FAP/seprase, prolyl endopeptidase, and tryptase [17]. Alogliptin exhibits favorable pharmacokinetic, pharmacodynamic, and pharmacologic safety profiles. Therefore, alogliptin as a monotherapy or add-on to metformin, pioglitazone, glipizide, glibenclamide, voglibose, or insulin significantly improves glycemic control compared with placebo or active comparators in adult and elderly patients with inadequately controlled T_2DM [18,19]. Because the kidney is the main excretion route for alogliptin, accounting for 60% to 71% [18] of excretion, the oral dose should be reduced or withdrawn in patients with renal impairment.

Thus for its medicinal properties reported in the texts prompted us to select Evaluation of *Alogliptin*for the Treatment of inflammation in different experimental animal models.

MATERIALS AND METHODS

Determination of anti-inflammatory activity:

Carrageenan induced paw edema [20,21,22,23]:

Group A: Toxicant control (0.1 ml of 1% w/v Carrageenan, hind paw)

Group B: Standard (Ibuprofen 40 mg/ kg, p.o)

Group C: *Alogliptin* (1 mg/kg/day p.o)

Group D: *Alogliptin* (2 mg/kg/day p.o)

Group E: *Alogliptin* (3 mg/kg/day p.o)

Experimental Procedure:

5 groups ofWister albino rats of either sex weighing 180- 220 g, selected for the study were kept in colony cages at ambient temperature of 28±2°C and relative humidity of 45 to 55% with a 12:12 h light/dark cycle.The animals were fasted for 12 h before commencing the experiment with water *ad libitum*. The fasting was continued till completion of the experiment. Group A was served as normal toxicant control treated with toxicant carrageenan, group B with Ibuprofen (40 mg/kg p.o.) served as standard, groups C, D and E administered with *Alogliptin*(low, medium and high doses p.o) respectively. The rats in Groups B, C, D and E were administered with 0.1 ml of 1% w/v of carrageenan into sub plantar region of right hind paw of rats 1 h after the administration of Ibuprofen/Alogliptin. Immediately thereafter the oedema volumes of the injected paws were measured plethysmographically at prefixed time intervals.

2. Histamine induced paw edema[20,21,22,23]:

Group A: Toxicant control (0.1 ml of 1% w/v histamine, hind paw)

Group B: Standard (Ibuprofen 40 mg/ kg)

Group C: *Alogliptin* (1 mg/kg/day p.o)

Group D: *Alogliptin* (2 mg/kg/day p.o)

Group E: *Alogliptin* (3 mg/kg/day p.o)

Experimental Procedure:

5 groups ofWister albino rats of either sex weighing 180- 220 g, selected for the study were kept in colony cages at ambient temperature of 28±2°C and relative humidity of 45 to 55% with a 12:12 h light/dark cycle.The animals were fasted for 12 h before commencing the experiment with water *ad libitum*. The fasting was continued till completion of the experiment. Group A was served as normal toxicant control treated with toxicant Histamine, group B with Ibuprofen (40 mg/kg p.o.) served as standard, groups C, D and E administered with *Alogliptin*(low, medium and high doses p.o) respectively. The rats in Groups B, C, D and E were administered with 0.1 ml of 1% w/v of Histamine into sub plantar region of right hind paw of rats 1 h after the administration of Ibuprofen/Alogliptin. Immediately thereafter the oedema volumes of the injected paws were measured plethysmographically at prefixed time intervals.

For comparison purpose, the volume of oedema was measured at prefixed time intervals. The difference between paw volumes of the treated animals was measured and the mean oedema volume was calculated. Percentage reduction in oedema volume was calculated by using the formula,

$$\text{Percentage reduction} = \frac{Vo - Vt}{Vo} \times 100$$

Where, Vo = Volume of the paw of control at time‘t’.

Vt = Volume of the paw of drug treated at time‘t’.

Statistical analysis:

All results will be expressed as mean ± SEM from 6 animals. Statistical difference in mean will be analyzed using one-way ANOVA (analysis of variance) followed by Post hoc test (Dunnett‘s‘t’ test). $P< 0.05^{*}$, 0.01^{**} and 0.001^{***} will be considered as statistically significant.

RESULTS

1. Anti-inflammatory activity of *Alogliptin* in Carrageenan induced paw oedema model in rats:

The *Alogliptin* with three selected doses i.e. 1, 2 and 3 mg/kg/day have exhibited a significant reduction in paw oedema volume in carrageenan induced paw oedema in rats at different time intervals. Results are tabulated in Table No. 1. Ibuprofen (40 mg/kg) was used as standard reference and it has significantly reduced paw oedema volume by 32.97% at 1st h, 57.48% at 2nd h, 70.94% at 3rd h and 82.03% at 4th h, thus standard drug has exhibited time dependent reduction in oedema volume.

During 1st h of study *Alogliptin* with low, medium and high doses have significantly reduced oedema volume by 14.05%, 26.75%, 45.67% respectively, which was found to be a time dependent effect.

During 2nd h of study *Alogliptin* with low, medium and high doses have significantly reduced oedema volume by 26.68%, 38.17%, 53.14% respectively a time dependent effect.

During 3rd h of study *Alogliptin* with low, medium and high doses have significantly reduced oedema volume by 28.42%, 42.10%, 60.84% respectively a time dependent effect was noted.

During 4th h of study *Alogliptin* with low, medium and high doses have significantly reduced oedema volume by 36.92%, 51.49%, 65.46% respectively a time dependent effect was noted and result are graphically represented in Fig No.1.

2. Anti-inflammatory activity of *Alogliptin* in Histamine induced paw oedema model in rats:

The *Alogliptin* with three selected doses i.e. 1, 2 and 3 mg/kg have exhibited a significant reduction in paw oedema volume in histamine induced paw oedema in rats at different time intervals. Results are tabulated in Table No. 2. Ibuprofen (40 mg/kg) was used as standard reference and it has significantly reduced paw oedema volume by 58.73% at 1st h, 70.90% at 2nd h, 84.72% at 3rd h and 91.22% at 4th h, thus exhibited a time dependent reduction in oedema volume.

During 1st h of study *Alogliptin* with low, medium and high doses have significantly reduced oedema volume by 9.25%, 20.63%, 39.68% respectively, which was found to be a time dependent effect.

During 2nd h of study *Alogliptin* with low, medium and high doses have significantly reduced oedema volume by 14.77%, 26.59%, 45.90% respectively noted a time dependent effect.

During 3rd h of study *Alogliptin* with low, medium and high doses have significantly reduced oedema volume by 19.95%, 34.62%, 54.58% respectively a time dependent effect was noted.

During 4th h of study *Alogliptin* with low, medium and high doses have significantly reduced oedema volume by 27.41%, 48.24%, 69.07% respectively a time dependent effect was noted and result are graphically represented in Fig No.2.

DISCUSSION

The present study is the first providing evidence that DPP-IV inhibitionwith *Alogliptin* has protective effects of diabeticanimals by a mechanism independent of enhanced insulin secretion. In the system of medicine a very good numbers of anti diabetic's medicine are reported to produce anti-inflammatory activities. Hence in the present study a plant by name *Alogliptin*has considered to evaluate its anti-inflammatory activities scientifically. For this *Alogliptin* were tested against different inflammatory models in rats.

Carrageenan induced paw oedema model is used for screening of NSAIDs and inflammation produced by its biphasic in nature with the release of serotonin, bradykinin and histamine at I Phase followed by release of prostaglandins in II Phase which is shown in Table No.1 and Fig. No.1.

Histamine being an important mediator of inflammation and also a potent vasodilator that causes increase in vascular permeability. In both phases due to release of these mediators cause pain and fever and *Alogliptin* significantly reduced paw oedema in II Phase of the inflammation indicating there effect on prostaglandins which is shown in Table No.2 and Fig. No.2.

The present study evaluation of Anti diabetic drug *Alogliptin*confirms a positive anti inflammatory effect, hence these might have contributed for the anti-inflammatory activity.

CONCLUSION

The results of recent studies suggest that dipeptidyl-peptidase-4 inhibitors (*Alogliptin*) have anti inflammatory effect on experimental models in rats.

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Table No: 1. Anti-inflammatory effects of *Alogliptin* in Carrageenan induced paw oedema model in rats at different time intervals

S.No.	Groups	Treatment	1 h	% ROV	2 h	% ROV	3 h	% ROV	4 h	% ROV
A	Toxicant	Carrageenan (1% w/v)	**0.370 ±0.018**	--	**0.461 ±0.017**	--	**0.475 ±0.020**	--	**0.501 ±0.017**	--
B	Standard	Ibuprofen 40 mg/kg	**0.248 ±0.025*****	32.97	**0.196 ±0.024*****	57.48	**0.138 ±0.008*****	70.94	**0.090 ±0.017*****	82.03
C	*Alogliptin*	1 mg/kg	**0.318 ±0.020**^{ns}	14.05	**0.338 ±0.027**^{ns}	26.68	**0.340 ±0.017***	28.42	**0.316 ±0.015****	36.92
D	*Alogliptin*	2 mg/kg	**0.271 ±0.021**^{ns}	26.75	**0.285 ±0.024****	38.17	**0.275 ±0.013*****	42.10	**0.243 ±0.014*****	51.49
E	*Alogliptin*	3 mg/kg	**0.201 ±0.013***	45.67	**0.216 ±0.010*****	53.14	**0.186 ±0.016*****	60.84	**0.173 ±0.017*****	65.46

n = 6, Significant at P < 0.05*, 0.01** and 0.001***, ns = not significant. ROV- Reduction of Oedema Volume.

Table No: 2. Anti-inflammatory effects of *Alogliptin* in Histamine induced paw oedema model in rats at different time intervals

S.No.	Groups	Treatment	1 h	% ROV	2 h	% ROV	3 h	% ROV	4 h	% ROV
A	Toxicant	Histamine (1% w/v)	**0.378 ±0.011**	--	**0.440 ±0.019**	--	**0.491 ±0.008**	--	**0.456 ±0.015**	--
B	Standard	Ibuprofen 40 mg/kg	**0.156 ±0.015*****	58.73	**0.128 ±0.013*****	70.90	**0.075 ±0.021*****	84.72	**0.040 ±0.019*****	91.22
C	*Alogliptin*	1 mg/kg	**0.343 ±0.024^{ns}**	9.25	**0.375 ±0.023^{ns}**	14.77	**0.393 ±0.010****	19.95	**0.331 ±0.015*****	27.41
D	*Alogliptin*	2 mg/kg	**0.300 ±0.018***	20.63	**0.323 ±0.029*****	26.59	**0.321 ±0.010*****	34.62	**0.236 ±0.015*****	48.24
E	*Alogliptin*	3 mg/kg	**0.228 ±0.016*****	39.68	**0.238 ±0.015*****	45.90	**0.223 ±0.010*****	54.58	**0.141 ±0.015*****	69.07

n = 6, Significant at P < 0.05*, 0.01** and 0.001***, ns = not significant. ROV- Reduction of Oedema Volume.

Fig No. 1

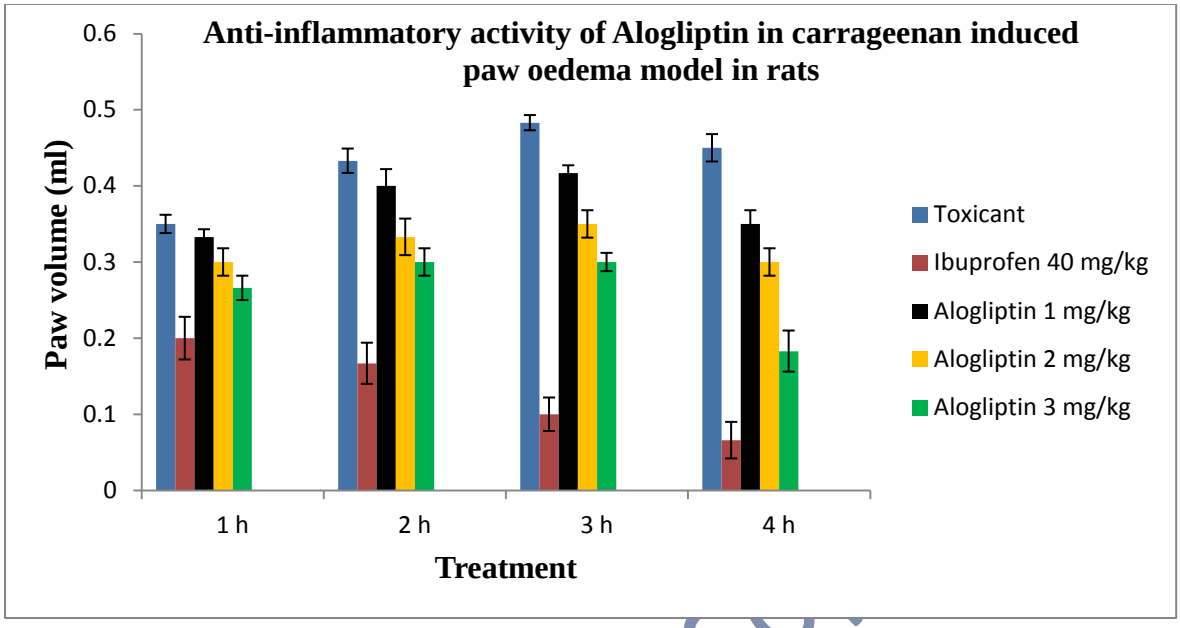

Fig No. 2