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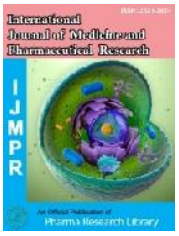

Research Article **Open Access**

Preclinical safety, toxicology and efficacy studies of Recombinant Exendin-4 (Antidaibetic peptide) on wistar rats

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A B S T R A C T

Recombinant Exendin-4 is a 39 amino acid peptide which exhibits sequence identity over 30 amino acids with mammalian glucagon like peptide-1 (GLP-1) and shares many of the gluco-regulatory actions observed with GLP-1. It enhances glucose dependent insulin secretion in the pancreatic beta-cells by suppressing inappropriately elevated glucagon secretion, and slows down the process of gastric emptying in the gut. Unlike GLP-1, Recombinant Exendin-4 exhibit extended kinetics due to its resistance to proteolytic degradation by dipeptidyl peptidase IV (DPP-I). The present study was performed to investigate the efficacy of recombinant Exendin-4 in Albino wister rats. The Efficacy of the Antidiabetic recombinant product was assessed on the high fat diet fed hyperglycemic animals. For this study animals are selected and divided into 4 groups (each group consists of 3 male and 3 female) in which 2 groups of Animals were fed with high fat diet and other 2 groups of animals fed with low fat diet for a period of 4 weeks , only two groups (LFD & HFD) treated with multiple low doses of STZ (30 mg/kg IP at weekly interval for 2 weeks) produced frank hyperglycemia in HFD-fed rats with highly successful rate, after attaining the hyperglycemia the animals (2 groups i.e LFD+ recombinant Exendin-4, HFD+ recombinant Exendin-4, in which one receives the low fat diet and another one receives the high fat diet) were exposed to test drug for a period of 6 weeks along with their diets and assessed their metabolic parameters such as Glucose tolerance test (GTT),Triglyceride levels and blood glucose levels & plasma insulin levels after administering the recombinant Exendin-4 daily subcutaneously twice daily for a period of 6 weeks. The result of this experiment clearly indicates that, recombinant Exendin-4 corrected the hyperglycemia & hyperinsulinemia in high fat fed animals in comparison with low fat fed animals.

Keywords: Exendin-4, Amino acid peptide, GLP-1, DPP-IV, HFD, LFD, STZ, albino wister rats, Haematology.

A R T I C L E I N F O

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1. Introduction

Type 2 diabetes mellitus is a chronic metabolic disorder that results from defects in both insulin secretion and insulin action. An elevated rate of basal hepatic glucose production in the presence of hyper insulinemia is the primary cause of fasting hyperglycaemia; after a meal, impaired suppression of hepatic glucose production by insulin and decreased insulin-mediated glucose uptake by muscle contribute almost equally to postprandial hyperglycaemia (1,2,3). For patients with type 2 diabetes who are no longer achieving good glycaemic control on Oral Anti Diabetic agents (OAD), an effective and safe alternative to insulin could be beneficial which is associated with side effects (4). Recombinant Exendin-4 is a 39 amino acid glucagon like peptide-1 (GLP-1) which improves glycaemic control in people with Type II diabetes mellitus by stimulating insulin production in the pancreas.(5) It enhances glucose-dependent insulin secretion in the pancreatic beta-cells by suppressing inappropriately elevated glucagon secretion, and slows down the process of gastric emptying in the gut and is approved by FDA in 2005(5).

Recombinant-Exendin-4 (VB63) differs in chemical structure and pharmacological action from insulin, sulfonylureas (including D-phenylalanine derivatives and meglitinides), biguanides, thiazolidinediones, and alpha alpha glycosidase inhibitors.(6) Exendin-4 as an incretin mimetic agent binds and activates the known human GLP-1 receptors in the gut. This leads to an increase in both glucose-dependent synthesis of insulin, and in vivo secretion of insulin from pancreatic beta cells, by mechanisms involving cyclic AMP and other intracellular signaling pathways (7,8). Exendin-4 promotes insulin release from pancreatic beta cells in the presence of elevated glucose concentrations and improves glycemic control by reducing fasting and postprandial glucose concentrations in patients with type 2 diabetes (9). The current study was performed to determine the recombinant Exendin-4 internally to male and female albino wiste rats.(10) rats were selected as the test system because one rodent is mandatory as per the regulatory requirement. The dose levels were calculated based on the body surface area of the animals. The duration of the study was 6 weeks the proposed routes of administration used for this study was intravenous and subcutaneous.(11)

2. Materials and Methods

Formulation of test and control compounds:

Vehicle used for both the routes of administration intravenous and subcutaneous was formulated buffer which is stored at 2-80C when not in use. Exend in obtained contained strength of 350 or750µg/vial which is >99% pure in the form of a solution, stored at 2-80C when not in use.Doses were fixed for the test as only two groups (LFD & HFD) treated with multiple low doses of STZ (30 mg/kg IP at weekly interval for 2 weeks) produced frank hyperglycemia in HFD-fed rats with highly successful rate, after attaining the hyperglycemia the animals (2 groups i.e LFD+ recombinant Exendin-4, HFD+ recombinant Exendin-4.

Animals and Husbandry:

Albino wister rats obtained from Sri venkateswara enterprizes, Banglore, were used for the study following one week acclimatization and also they were observed daily for clinical signs of any existing disease. Animals were maintained in an environment controlled room at a temperature of 20 ± 30 °C and relative humidity of 30 to 70 per cent which was monitored daily and were fed ad libitum with standard pellet feed. They were housed 2 or 3 per cage with slatted floor and automatic photoperiod of 12 h light and 12 h darkness was set. Body weight, food and water consumption measurements were collected. (12,13)

Experimental procedure:

The test compound was injected the intended therapeutic dose for acute study in 10 (5M+5F) rats intravenously (IV) and Efficacy of the Antidiabetic recombinant product was assessed on the high fat diet fed hyperglycemic animals. For this study animals are selected and divided into 4 groups (each group consists of 3 male and 3 female) in which 2 groups of Animals were fed with high fat diet and other 2 groups of animals fed with low fat diet for a period of 4 weeks , only two groups (LFD & HFD) treated with multiple low doses of STZ (30 mg/kg IP at weekly interval for 2 weeks) produced frank hyperglycemia in HFD-fed rats with highly successful rate, after attaining the hyperglycemia the animals (2 groups i.e LFD+ recombinant Exendin-4, HFD+ recombinant Exendin-4, in which one receives the low fat diet and another one receives the high fat diet **)** were exposed to test drug for a period of 6 weeks along with their diets and assessed their metabolic parameters such as Glucose tolerance test (GTT), Triglyceride levels and blood glucose levels & plasma insulin levels after administering the recombinant Exendin-4 daily subcutaneously twice daily for a period of 6 weeks.

Examinations

Efficacy:

Efficacy of the Antidiabetic recombinant product was assessed on the high fat diet fed hyperglycemic animals. The hyperglycemia in HFD-fed rats with highly successful rate, after attaining the hyperglycemia the animals (2 groups i.e LFD+ recombinant Exendin-4, HFD+ recombinant Exendin-4, in which one receives the low fat diet and another one receives the high fat diet **)** were exposed to test drug for a period of 6 weeks along with their diets and assessed their metabolic parameters such as Glucose tolerance test (GTT),Triglyceride levels and blood glucose levels & plasma insulin levels after administering the recombinant Exendin-4 daily subcutaneously twice daily for a period of 6 weeks.[14,15] **Statistical Analysis:** The group comparisons were analyzed by means of Kruskal-Wallis one-way ANOVA and individual group comparisons by Mann- Whitney U test (treatment groups with vehicle

control. Heterogeneity of variance was tested by Levene's statistic.p<0001.

3. Results and Discussion

There was efficacy during the 6weeks of experimental period. No toxic signs and abnormal behaviour in both the groups which were exposed to the test compound intended therapeutic dose.(17) Test compound was well tolerated upon acute administration to rats at the highest dosage level.(30mg/kg LFD+HFD,+r-Exendin-4), no untoward effects. [18]

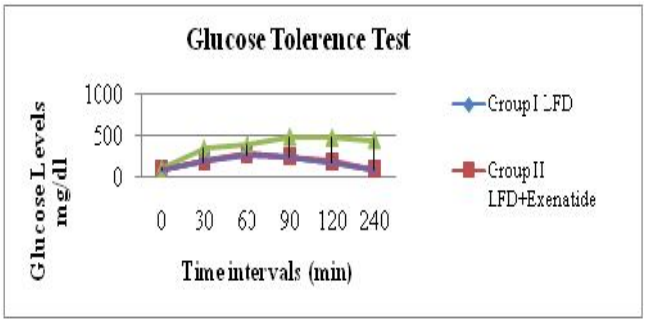

Figure 1: Glucose Tolerance Test

Table 1: Body weight data in grams

Weeks	Group I LFD	Group II LFD+r-Exendin-4	Group III HFD	Group IV HFD+r-Exendin-4
1	200	225	215	220
2	220	235	240	235
3	235	240	270	260
4	260	260	310	300
5	280	270	330	310
6	310	290	350	320
7	330	300	365	335
8	340	325	395	350
9	360	340	420	360
10	370	360	450	375
Mean ± SD	**290.5±60.39**	**284.5±46.51**	**334±77.08**	**306±52.970**

The values are expressed in as Mean ± SD (n=6). ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05 when compare to (LFD vs. LFD+EXENATIDE) and (HFD vs. HFD+EXENATIDE), NA- Not Applicable. Statistical comparison was performed by using one-way ANOVA followed by Bonferroni multiple comparison test. Body weights were monitored throughout the study period, there was no significant changes occurred in body weights.

Table 2: Blood glucose levels: Initial and Final Blood Glucose Levels

Sex	Animal No.	Blood Glucose Levels (mg/dl) of normal and final diet feeding animals							
		Group I		Group II		Group III		Group IV	
		Initial	final	Initial	final	Initial	final	Initial	final
Males	1	87	95	95	135	85	149	83	134
	2	83	94	93	113	81	158	95	124
	3	90	98	87	105	98	169	97	115
Females	4	92	92	84	112	90	162	78	120
	5	97	90	92	100	87	194	89	105
	6	95	93	81	100	95	170	90	101

Table 3: Fat diet blood Glucose Levels

Sex	Animal No.	Blood Glucose Levels (mg/dl) after 4weeks fat diet			
		Group I LFD	Group II LFD+ STZ (30 mg/kg IP at weekly interval for 2 weeks)	Group III HFD	Group IV HFD+ STZ (30 mg/kg IP at weekly interval for 2 weeks)
Males	1	98	267	136	367
	2	97	340	141	430
	3	98	254	154	450
Females	4	95	345	167	473
	5	95	289	180	490
	6	93	256	148	445

Table 4: Insulin levels ng/ml

Animal No:	Group I LFD	Group II LFD + Exenatide	Group III HFD	Group IV HFD+Exenatide
1	0.15	0.17	0.35	0.16
2	0.17	0.18	0.4	0.19
3	0.13	0.14	0.45	0.18
4	0.14	0.16	0.38	0.14
5	0.15	0.17	0.5	0.11
6	0.13	0.17	0.45	0.16
Mean ± SD	**0.145±0.015**	**0.165±0.013**	**0.421±0.054**	**0.156±0.028*****

The values are expressed in as Mean ± SD (n=6). ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05 when compare to (LFD vs. LFD+EXENATIDE) and (HFD vs. HFD+EXENATIDE), NA-Not Applicable. Statistical comparison was performed by using one-way ANOVA followed by Bonferroni multiple comparison test. From the above table it was depicted that decrease insulin levels in HFD+EXENATIDE group when compared with HFD group.

Table 5: Glucose tolerance test

Time(min)	Group I LFD	Group II LFD+r-Exendin-4	Group III HFD	Group IV HFD+r-Exendin-4
0	100	105	110	105
30	205	190	350	200
60	290	279	400	270
90	250	248	490	250
120	180	190	480	190
240	105	99	450	108

Table 6: Plasma triglyceride levels mg/dl

Animal No:	Group I LFD	Group II LFD+r-Exendin-4	Group III HFD	Group IV HFD+r-Exendin-4
1	50	45	80	50
2	55	40	78	55
3	48	40	89	48
4	45	41	90	47
5	50	45	75	51
6	47	40	80	48
Mean ± SD	**49.16±3.430**	**41.83±2.483***	**82±6.000**	**49.83±2.920*****

The values are expressed in as Mean ± SD (n=6). ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05 when compare to (LFD vs. LFD+EXENATIDE) and (HFD vs. HFD+EXENATIDE), NA-Not Applicable. Statistical comparison was performed by using one-way ANOVA followed by Bonferroni multiple comparison test. From the above table it was depicted that slightly decrease Triglyceride levels in LFD+EXENATIDE group when compared with LFD group and more significantly decrease the Triglyceride levels in HFD+EXENATIDE group when compared with HFD group.

Discussion

Type-II diabetes has a complex pathophysiology characterized by deficient insulin activity arising from decreased insulin secretion and compromised insulin action in peripheral target tissues or a combination of these two abnormalities.(19) For patients with type 2 diabetes who are no longer achieving good glycemic control on Oral Anti Diabetic agents (OAD), an effective and safe alternative to insulin could be beneficial. Recombinant Exendin-4 is a 39 amino acid glucagon like peptide-1 (GLP-1) which improves glycemic control in people with Type II diabetes mellitus by stimulating insulin production in the pancreas. Exendin-4 promotes insulin release from pancreatic beta cells in the presence of elevated glucose concentrations and improves glycemic control by reducing fasting and postprandial glucose concentrations in patients with type 2 diabetes. [20]

4. Conclusion

Efficacy of the Antidiabetic recombinant product was assessed on the high fat diet fed hyperglycemic animals. For this study animals are selected and divided into 4 groups (each group consists of 3 male and 3 female) in which 2 groups of Animals were fed with high fat diet and other 2 groups of animals fed with low fat diet for a period of 4 weeks , only two groups (LFD & HFD) treated with multiple low doses of STZ (30 mg/kg IP at weekly interval for 2 weeks) produced frank hyperglycemia in HFD-fed rats with highly successful rate, after attaining the hyperglycemia the animals (2 groups i.e LFD+ recombinant

Exendin-4, HFD+ recombinant Exendin-4, in which one receives the low fat diet and another one receives the high fat diet **)** were exposed to test drug for a period of 6 weeks along with their diets and assessed their metabolic parameters such as Glucose tolerance test (GTT), Triglyceride levels and blood glucose levels & plasma insulin levels after administering the recombinant Exendin-4 daily subcutaneously twice daily for a period of 6 weeks. The result of this experiment clearly indicates that, recombinant Exendin-4 corrected the hyperglycemia & hyperinsulinemia in high fat fed animals in comparison with low fat fed animals.

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