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# Acute Toxicity Study of Recombinant Exendin-4 in Swiss Albino Mice

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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-IV) and has higher potency for in vivo glucose lowering effect. The present study was performed to investigate the single-dose toxicity of recombinant Exendin-4 in Swiss Albino Mice. The test compound was injected at 10 times the intended therapeutic dose for once in 10 (5M+5F) mice intravenously (IV) and in another group of 10 (5M+5F) mice it was injected subcutaneously. Animals were observed for a period of 14 days for mortality and morbidity. In overall study, we found that the single high dose administration of recombinant Exendin-4 did not exert any toxic effects in Swiss Albino Mice until the end of the observation period. No treatment related changes were detected for body weight, feed intake, haematology, clinical chemistry, and organ weight. There were no adverse clinical signs indicative of an anaphylactic response and no mortality and morbidity were observed during the experimental period.

**Keywords:** Exendin-4, Amino acid peptide, GLP-1, DPP-IV, Swiss Albino Mice, 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. 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. 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 [6,7]. 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 [8]. The current study was performed to determine the single-dose toxicity of recombinant Exendin-4 internally to male and female Swiss albino mice. Mice 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 14 days and the proposed routes of administration used for this study was intravenous and subcutaneous.

## 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 2-8$^0$C 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 2-8$^0$C when not in use. Doses were fixed for the test as Therapeutic dose (TD)-0.9 µg/Kg, Byetta TD-0.9 µg/Kg, Average dose (AD)-4.5 µg/Kg, High dose (HD) (10XTD)-9.0/µg/Kg, same volume formulation ion buffer was administered to control group as the test group.

**Test facilities and test sites:**

All the studies were performed at Virchow Biotech Private Limited (CPSCEA No: 546/02/a/ CPSCEA), Hyderabad, India.

**Animals and Husbandry:**

8-10 weeks aged Swiss Albino Mice obtained from National Centre for Laboratory Animal Sciences, National Institute of Nutrition (Hyderabad, India) 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 ± 3 °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 [9].

**Experimental design:**

**Table 1**

| S.No | Test group | Test species | No. of animals | Route of administration | Dose µg /Kg | Observations |
|---|---|---|---|---|---|---|
| 1 | **Group-I** HD (10XTD) | Mice | 5M+5F | IV | 13.0 | Morbidity and mortality |
| 2 | **Group-II** HD (10XTD) | Mice | 5M+5F | SC | 13.0 | |

**Experimental procedure:**

Before test compound administration, mice were placed in a restrainer, and the tail was warmed to dilate the veins in order to make the procedure easier. Ten animals of Group I (males: females) received a single intravenous injection of high dose (HD) and other group animal received a single subcutaneous injection of high dose (HD). Volume of administration was according to the body weight and surface area of the animals, which were observed for morbidity, mortality over the period of 14 days [10]. The vehicle control and test compounds were administered to animals. Ten animals of Group I (males: females) were given a single intravenous injection of high dose (HD) and ten animals of Group II (males: females) were given a single subcutaneous injection of high dose (HD). After 14 days, all the animals were sacrificed; tissues and blood samples were collected and stored for further investigations. The two additional satellite groups i.e. Group 2 and Group 7 (Satellite VC and HD) were observed for reversibility,

persistence, or delayed occurrence of toxic effects for a post treatment of 14 days. Later these animals were also sacrificed and various investigations were conducted (10).

**Examinations:**

**Physical Examination:** Safety assessment of the test compound was conducted using standard International guidelines which include monitoring every day the cage side observations such as lying on side, resting alertness, faeces excretion, urine output. Physical examinations were also made periodically such as hair coat, lacrimation (eye secretions), salivation (slight or excess), respiration rate (increased, decreased, shallow, deep. gasping), eyelid closure, biting and convulsions (after the exposure to test compound). Neurological activity was evaluated by abnormal gait (spastic, waddling, dragging hind limbs), Ataxic gait (falling frequently; walking inability), static limb position (abnormal limb positions), and locomotor activity. Bodytemperatures were recorded for all the animals before and after exposing totest compound. Allergic profile of the test compound was observed by congestion of eyes, hair loss and skin reaction at the site of injection.

**Biochemical parameters:**

Urine samples were collected before test compound administration and after 14 days exposure to the test compound (Urine collection was done for 12 hours before testing) and tested using Ames Multistix reagent strips to evaluate Urobilinogen, Protein, pH, RBC, Specific gravity, Ketone, Bilirubin and Glucose. Serum samples were analyzed for glucose, creatinine, total protein, ALP, SGOT, SGPT, Bilirubin, Potassium by using analytical kits purchased from Wipro Biomed and protocols were followed as suggested along with the kits. Animals were starved for approximately 22 hours; blood samples were drawn from orbital plexus and collected in commercially available heparinized vacuette tubes, and centrifuged at 3000 rpm for 10 minutes to separate the plasma for clinical chemistry analyses. Quality control samples were supplied by Transasia Bio-Medicals Ltd (ERBA controls for biochemistry) was used to establish the precision and accuracy of the analyses. Quality control samples at two levels (level 1 and level 2) supplied by Wipro Biomed were used to establish the precision and accuracy of the analyses.

**Haematology:**

Whole blood samples were investigated for Total white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (Plt), and mean platelet volume (MPV) and differential leucocytes count. These investigations were carried out using the pre exposure and post exposure blood samples. All the blood samples were drawn into tubes containing EDTA K2 from the orbital plexus using heparin zedmicrohaematocrit tubes and analyzed on an automated blood cell counter (Medonic-CA 620).

**Genotoxicity and Cytotoxicity:**

The genotoxicity and cytotoxicity of VB 17 upon rat wasfound through bone marrow micronucleus assay (9, 10). The bone marrow was flushed from the femur using fetal calfserum and made fine colloid using the syringe and centrifuged in 800 rpm/5 minutes. The supernatant was poured out and the sediment is over layered with one or two drops of calf serum. This drop is dropped into the slides and the smear is air-dried. The staining was done using May-Grunwald stain and followed by giesma stain. The polychromatic cells(PCEs) were bluish; the monochromatic cells were pink and are smaller than PCEs. The micronuclei appeared as deep purple. Scoring was done for 2000 polychromatic erythrocytes (PCEs, reticulocytes, immature erythrocytes) and the presence of NCEs (mature erythrocytes) and micro nucleated PCEs (11).

**Necropsy:**

After the experimental period, Mice were fasted overnight (water was provided) later euthanized by cervical dislocation method and subjected to detailed gross necropsy and observations were recorded, the following organs were collected from each animal and the weights were recorded using a top loading electronic weighing machine: Liver, Spleen, Kidney, Lung, Heart, Brain and Ovaries or Testes. After opening the chest and abdominal cavities, an in-situ examination of organs was done. The individual organs were again examined for gross morphological changes (if any) after removal.

**Histopathology:**

The organs and tissue samples ( brain, thymus, spleen, bone marrow, kidney, wound site/ site of application, heart, lung, trachea, thyroid, sternum, liver, gastro intestinal tract, testes and ovaries) collected from all the animals were preserved in 10% buffered neutral formalin.

**Statistical Analysis:**

Sample size required for the study was determined as per the protocol of the regulatory authorities. The study design takes care of 'a priori' rationale for the target difference between the treatments and the control. Proper measures were always taken to avoid bias, particularly by applying randomization methods, local control methods and blinding of the study. Randomization ensured the allocation of treatments to animals/groups was independent of their characteristics and were similar in all the groups. It was also taken care while randomization, base variables were homogenized and were allotted to different groups. Treatment groups were compared with vehicle control by Fisher's exact or Chi-square test.

The group comparisons were analysed 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. After confirming homogeneity, TWO-way ANOVA was done for between group significant F-ratio. Post-hoc tests by means of Dunnett's were carried out for comparison of treatment groups with vehicle control. In case of heterogeneity of variance, the data was subjected to Log transformation. Even after transformation, if the heterogeneity persisted, the distribution free methods (Nonparametric methods) were used for analysis of data. Two tailed probability level of $<0.05$ was set in these experiments for rejecting the null hypothesis.

## 3. Results and Discussion

There was no mortality during the 14days of experimental period. No toxic signs and abnormal behaviour in both the groups which were exposed to the test compound at 10 times of intended therapeutic dose. Test compound was well tolerated upon acute administration to mice at the highest dosage level. (9.0ug/kg BW) and no untoward effects or mortality occurred following acute intravenous and subcutaneous administration in mice (shown in table 2).

**Table 2**

| S.No | Days of observation | Group-I | | Group-II | |
|---|---|---|---|---|---|
| | | M | F | M | F |
| 1 | Base line | 0/5 | 0/5 | 0/5 | 0/5 |
| 2 | 1 | 0/5 | 0/5 | 0/5 | 0/5 |
| 3 | 2 | 0/5 | 0/5 | 0/5 | 0/5 |
| 4 | 3 | 0/5 | 0/5 | 0/5 | 0/5 |
| 5 | 4 | 0/5 | 0/5 | 0/5 | 0/5 |
| 6 | 5 | 0/5 | 0/5 | 0/5 | 0/5 |
| 7 | 6 | 0/5 | 0/5 | 0/5 | 0/5 |
| 8 | 7 | 0/5 | 0/5 | 0/5 | 0/5 |
| 9 | 8 | 0/5 | 0/5 | 0/5 | 0/5 |
| 10 | 9 | 0/5 | 0/5 | 0/5 | 0/5 |
| 11 | 10 | 0/5 | 0/5 | 0/5 | 0/5 |
| 12 | 11 | 0/5 | 0/5 | 0/5 | 0/5 |
| 13 | 12 | 0/5 | 0/5 | 0/5 | 0/5 |
| 14 | 13 | 0/5 | 0/5 | 0/5 | 0/5 |
| 15 | 14 | 0/5 | 0/5 | 0/5 | 0/5 |

Group-I: Intravenous
Group-II: Sub-cutaneous
Test compound exposed once.

**Functional Observation Battery:**

**In-Life Observations:**

Findings of general behaviour indicated no activity changes in any test compound induced group. 80% of the animals from all the groups were walking around in the open field during the observation. We found statistically significant (<0.05) differences in the body weight between the groups on. May be the difference in body weights was due to the initial body weight variation between the groups.

The weight gain in all the groups was uniform and no significant treatment change was observed in any group. No dose related and statistically significant decrease in food consumption was recorded in all treated groups during the dosing period. Following the recovery period, food intake was similar in all groups. The water intake was found to be adequate in all groups of animals. The body temperature was found to be normal in all the animals before and after exposure of the test compound. There was no recorded dose related significant increase in blood pressure in all the animals before and after exposure of the test compound.

**Physical Examination**

Physical examination was conducted twice in a week. 70-80% of the animals from all the groups were found to be alert. Remaining animals were found with high and low body movements and some were showed exploratory movements at the time of observation. There were no abnormalities in the faecal colour, consistency & amount during the experimental period in all the groups. There were no abnormalities in the urine output and colour during the experimental period in all the groups. The clean groomed hair coat was observed in all the experimental animals during the experimental period. No excessive lacrimation was observed during the experimental period in all the groups. There was no excess salivation observed during the experimental period both in vehicle control group and test compound groups. There were no significant abnormalities in respiration character & rate in all groups of animals during the experiment.

The eye prominence was found to be normal in all the groups of animals during the experimental period .There was no aggressiveness and biting character in animals exposed to test compound or vehicle (results were not shown). Neurological Examination Static limb position was normal without tilt and Wadding. There was no significant ataxic gait in any group of animals. There were no convulsions recorded after the administration of test compound and during the post exposure in any group of animals.

**Clinical Laboratory Investigations:**

**Clinical chemistry:**

There was no difference in all clinical chemistry parameters at any time of point in any of the treated animals (results shown in table 3, 4, 5).

**Table 3:** biochemical parameters of the control mice and test mice expressed asmean ±s.d

| **Parameters/ Group** | **Glucose mg/dl** | | **Creatinine mg/dl** | | **SGPT U/L** | | **SGOT U/L** | |
|---|---|---|---|---|---|---|---|---|
| Days (Post exposure) | 0 | 14 | 0 | 14 | 0 | 14 | 0 | 14 |
| Vehicle Control (5 Males ) | 84.6± 10.52 (5) | 92±11 (5) | 1.06± 0.45 (5) | 1.06± 1.23 (5) | 35.3 ±3.4 (5) | 29.5±9. 32 (5) | 27.83 ±6.57 (5) | 35.5 ±4.65 (5) |
| Vehicle Control (5 Females ) | 82±14.6 (5) | 82.1±20 (5) | 1.06±1.44 (5) | 1.12±1.26 (5) | 42 ± 12.32 (5) | 44.6 ± 10.1 (5) | 33.45 ±9.2 (5) | 42 ±7.32 (5) |
| Group-I HD (10XTD) Males IV | 78.4±9.3 (5) | 86.5±14.4 (5) | 0.76±0.23 (5) | 0.71±1.19 (5) | 29.12±5.4 (5) | 29.8 ±8.3 (5) | 34.56 ±7.2 (5) | 34.8 ±5.7 (5) |
| Group-I HD (10XTD) Females IV | 91.8±12.8 (5) | 74.6±9.6 (5) | 0.85±1.56 (5) | 1.1±1.27 (5) | 25.8±9.26 (5) | 22.8±4. 6 (5) | 33.8 ±7.3 (5) | 41.1 ±6.6 (5) |
| Group-I1 HD (10XTD) Males SC | 76.6±9.3 (5) | 83.52±14.4 (5) | 0.76±0.23 (5) | 0.71±0.19 (5) | 31.1±5.4 (5) | 29.8±6. 31 (5) | 35.5 ±7.2 (5) | 34.8 ±5.75 (5) |
| Group-I1 HD (10XTD) Females SC | 91.8±14.8 (5) | 84.6±8.62 (5) | 0.85±0.22 (5) | 1.1±0.17 (5) | 22.8±8.26 (5) | 22.8±5. 6 (5) | 33.8 ± 8.32 (5) | 41.1 ±6.6 (5) |

**Table 4:** biochemical parameters of the control mice and test mice expressed asmean ±s.d

| **Parameters/ Group** | **Total protein gm/l** | | **Total bilurubin mg/dl** | | **BUN mg/dl** | | **Cholestrol m/dl** | | **ALP IU/L** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Days (Post exposure)** | 0 | 14 | 0 | 14 | 0 | 14 | 0 | 14 | 0 | 14 |
| **Vehicle Control (Males )** | 6.22± 1.43 (5) | 5.86 ± 1.54 (5) | 0.21 ± 1.03 (5) | 0.21 ± 1.02 (5) | 29.1 ± 1.7 (5) | 25.3 ± 1.1 (5) | 63 ± 5.7 (5) | 67.45 ± 11.4 (5) | 231 ± 12.1 (5) | 213 ± 18.6 (5) |
| Vehicle Control (Females ) | 6.53 ± 1.77 (5) | 6.2 ± 1.2 (5) | 0.25 ± 1.07 (5) | 0.20 ± 1.03 (5) | 28 ± 4.6 (5) | 26.3 ± 6.2 (5) | 55 ± 9.5 (5) | 69.8 ± 19.2 (5) | 172 ± 37.5 (5) | 153 ± 24.9 (5) |
| Group-I HD (10XTD) Males IV | 5.91 ± 0.6 (5) | 6.4 ± 1.03 (5) | 0.21 ± 0.02 (5) | 0.21 ± 0.02 (5) | 24.5 ± .9 (5) | 22.3 ± 3.61 (5) | 72.6 ± 14.1 (5) | 84.1 ± 22 (5) | 158 ± 56.6 (5) | 149 ± 23.3 (5) |
| Group-I HD (10XTD) Females IV | 5.78 ± 1.1 (5) | 6.7 ± 1.6 (5) | 0.18 ± 0.13 (5) | 0.17 ± 0.02 (5) | 24.6 ± 6.7 (5) | 24.1 ± 2.3 (5) | 59 ± 8.04 (5) | 59.5 ± 11.8 (5) | 163 ± 27.1 (5) | 201 ± 32.1 (5) |
| Group-I1 HD (10XTD) Males SC | 7.1 ± 0.46 (5) | 6.85 ± 0.75 (5) | 0.28 ± 0.07 (5) | 0.22 ± 0.08 (5) | 26.6 ± 4.1 (5) | 23.8 ± 3.1 (5) | 62.5 ± 11.8 (5) | 64 ± 18.3 (5) | 127 ± 24.9 (5) | 143.6 ± 29.8 (5) |
| Group-I1 HD (10XTD) Females SC | 7.1 ± 0.43 (5) | 7.1 ± 0.46 (5) | 0.18 ± 0.04 (6) | 0.24 ± 0.07 (5) | 23.8 ± 3.3 (5) | 24 ± 3.1 (5) | 62.5 ± 11.8 (5) | 65 ± 5.7 (5) | 151 ± 26.3 (5) | 127 ± 24.9 (5) |

**Table 5:** Biochemical parameters of the control mice and test mice expressed asmean ±s.d

| **Parameters/ Group** | **Sodium mEq/l** | | **Potassium mmol/L** | | **Chloride mEq/l** | | **HbA1C** | |
|---|---|---|---|---|---|---|---|---|
| **Days (Post exposure)** | 0 | 14 | 0 | 14 | 0 | 14 | 0 | 14 |
| **Vehicle Control (5 Males )** | 139 ± 4.1 (5) | 140.5 ± 4.5 (5) | 4.1 ± 0.8 (5) | 4.7 ± 0.5 (5) | 94.1 ± 4.7 (5) | 93.8 ± 11.1 (5) | 6.41 ± 0.39 (5) | 6.46 ± 0.33 (5) |
| **Vehicle Control (5 Females )** | 143.5 ± 2.6 (5) | 140 ± 2.06 (5) | 3.9 ± 0.82 (5) | 4.51 ± 0.36 (5) | 101 ± 5.3 (5) | 101 ± 3.2 (5) | 6.51 ± 0.42 (5) | 6.38 ± 0.31 (5) |
| **Group-I HD (10XTD) Males IV** | 138 ± 2.8 (5) | 139.6 ± 3.3 (5) | 4.2 ± 0.80 (5) | 5.1 ± 0.55 (5) | 100 ± 3.3 (5) | 102 ± 5.3 (5) | 6.6 ± 0.37 (5) | 6.38 ± 0.19 (5) |
| **Group-I HD (10XTD) Females IV** | 138 ± 2.4 (5) | 141 ± 3.3 (5) | 4.1 ± 0.6 (5) | 4.1 ± 0.8 (5) | 97.4 ± 5.1 (5) | 101 ± 3.2 (5) | 5.6 ± 0.25 (5) | 6.46 ± 0.30 (5) |
| **Group-I1 HD (10XTD) Males SC** | 137.6 ± 3.3 (5) | 138 ± 3.3 (5) | 4.6 ± 0.2 (5) | 4.2 ± 0.82 (5) | 100.5 ± 5.7 (5) | 98 ± 5.09 (5) | 6.28 ± 0.34 (5) | 5.4 ± 0.25 (5) |
| **Group-I1 HD (10XTD) Females SC** | 138 ± 1.3 (5) | 139 ± 4.1 (5) | 3.9 ± 0.43 (5) | 4.51 ± 0.44 (5) | 101 ± 7.2 (5) | 101.5 ± 4.5 (5) | 6.41 ± 0.33 (5) | 6.46 ± 0.34 (5) |

**Genotoxicity and Cytotoxicity:**

Scoring was done for 2000 polychromatic erythrocytes (PCEs, reticulocytes, immature erythrocytes) and the presence of NCEs (mature erythrocytes) and micro nucleated PCEs. The frequency of micronuclei has shown no significance which indicates lack of genotoxicity.

**Hematology:**

All hematology values were normal and no statistically significant differences observed between test groups and vehicle control. It may be concluded that under the given experimental conditions and based on these results there were no hematological changes attributable to the administration of test material (results shown in table 6, 7).

**Table 6:** Blood cells count of the control mice and test mice expressed asmean ±s.d

| **Parameters/ Group** | **WBC X $10^6$/µL** | | **RBC X $10^3$/µL** | | **Hgb g/dL** | | **Hct %** | |
|---|---|---|---|---|---|---|---|---|
| **Days (Post exposure)** | 0 | 14 | 0 | 14 | 0 | 14 | 0 | 14 |
| **Vehicle Control (5 Males )** | 7.48±1.53 (5) | 7.48±0.85 (5) | 6.53±0.73 (5) | 8.32±0.48 (5) | 17.25±1.47 (5) | 17.4±11.97 (5) | 57.35±2.99 (5) | 62.3±4.1 (5) |
| **Vehicle Control (5 Females )** | 7.51±1.15 (5) | 9.36±1.86 (5) | 8.32±0.41 (5) | 7.64±0.63 (5) | 18.5±1.18 (5) | 18.6±0.64 (5) | 52.2±6.7 (5) | 63.2±5.69 (5) |
| **Group-I HD (10XTD) Males IV** | 7.5±1.21 (5) | 8.55±1.19 (5) | 7.64±0.63 (5) | 7.47±1.00 (5) | 18.6±0.64 (5) | 16.25±0.93 (5) | 61.1±3.0 (5) | 61.1±9.3 (5) |
| **Group-I HD (10XTD) Females IV** | 8.1±1.26 (5) | 7.8±0.45 (5) | 7.06±0.89 (5) | 9.71±0.74 (5) | 17.25±1.20 (5) | 18.7±0.72 (5) | 51.8±4.97 (5) | 61.2±3.0 (5) |
| **Group-I1 HD (10XTD) Males SC** | 6.0±1.2 (5) | 6.5±1.21 (5) | 5.18±1.0 (5) | 5.71±1.39 (5) | 14.7±1.20 (5) | 15.3±1.23 (5) | 43.9±3.65 (5) | 46.2±5.33 (5) |
| **Group-I1 HD (10XTD) Females SC** | 8.0±0.81 (5) | 8.7±1.24 (5) | 7.18±1.17 (5) | 6.91±0.59 (5) | 18.5±0.82 (5) | 15.3±1.26 (5) | 60.85±1.42 (5) | 52.1±2.93 (5) |

**Table 7:** Blood cells analysis of the control mice and test mice expressed as mean ±s.d

| **Parameters/ Group** | **MCV fL** | | **MCH Pg** | | **MCHC g/dL** | | **Plt X103/µL** | |
|---|---|---|---|---|---|---|---|---|
| **Days (Post exposure)** | 0 | 14 | 0 | 14 | 0 | 14 | 0 | 14 |
| **Vehicle Control (5 Males )** | 71.12±1.55 (5) | 72.5±1.14 (5) | 22.26±1.06 (5) | 22.3±1.25 (5) | 29.4±1.44 (5) | 27.9±1.52 (5) | 8.2±1.68 (5) | 9.6±1.42 (5) |
| **Vehicle Control (5 Females )** | 70.45±2.82 (5) | 80.32±3.76 (5) | 21.92±1.67 (5) | 25.62±1.23 (5) | 27.35±1.32 (5) | 32.32±2.1 (5) | 8.63±1.12 (5) | 7.42±0.56 (5) |
| **Group-I HD (10XTD) Males IV** | 79.75±1.84 (5) | 78.3±3.42 (5) | 22.0±1.70 (5) | 24.5±1.07 (5) | 28.25±1.19 (5) | 28.7±2.0 (5) | 7.2±0.96 (5) | 8.2±0.46 (5) |
| **Group-I HD (10XTD) Females IV** | 75.15±3.17 (5) | 74±1.41 (5) | 20.5±1.45 (5) | 21.85±1.19 (5) | 20.85±0.97 (5) | 23.3±0.91 (5) | 8.3±1.71 (5) | 8.8±1.71 (5) |
| **Group-I1 HD (10XTD) Males SC** | 74.3±4.3(5) | 79.4±2.2 (5) | 28.25±1.19 (5) | 28.7±2.0 (5) | 29.4±1.95 (5) | 29.4±1.65 (5) | 10.0±1.0 (5) | 6.8±1.43 (5) |
| **Group-I1 HD (10XTD) Females SC** | 72.6±1.24 (5) | 72.7±1.28 (5) | 22.0±0.78 (5) | 21.5±0.78 (5) | 29.15±1.5 (5) | 30.3±1.93 (5) | 9.1±1.68 (5) | 9.4±0.87 (5) |

**Necropsy:** No gross changes were observed in all the organs of all groups (results shown in the table 8).

**Table 8:** Organ weights of control mice and the mice exposed to test compound expressed asmean ±s.d

| **Group** | **Heart** | **Lungs** | **liver** | **kidney** | **spleen** | **brain** | **Reproductive organs** |
|---|---|---|---|---|---|---|---|
| **Vehicle Control (5 Males)** | 180.2±3.14 | 239.4± 6.2249 | 1320 ± 11.17634 | 388 ± 12 | 123.8 ± 10.178935 | 376.6 ± 14.863059 | 199.8 ± 5.6499833 |
| **Vehicle Control (5 Females)** | 179.23 ± 5.789032 | 168.4 ± 4.678902 | 1245 ± 19.465789 | 344.4 ± 19.450345 | 123.2 ± 6.983056 | 348.4 ± 12.67498 | 493.2 ± 10.456789 |
| **Group-I HD (10XTD) MALES IV** | 174.2 ± 4.14728827 | 174.8 ± 3.2710854 | 1104 ± 16.201851 | 405 ± 13.472193 | 111.4 ± 8.5615419 | 360.4 ± 18.80957203 | 208.8 ± 5.449770637 |
| **Group-I HD (10XTD) Females IV** | 176.4 ± 3.84707681 | 249.4 ± 5.2249401 | 1414 ± 21.412613 | 344.4 ± 24.945941 | 145.2 ± 12.557866 | 395.6 ± 16.87601849 | 515.2± 22.17430946 |
| **GROUP-I1 HD (10XTD) Males SC** | 181.2 ± 3.19374388 | 213.8 ± 4.6043457 | 1133 ± 26.168683 | 460±18 | 117.6 ± 9.5812316 | 335.6 ± 22.08619478 | 254.4 ± 19.320973.6 |
| **GROUP-I1 HD (10XTD) Females SC** | 126.6 ± 5.45893762 | 222.4 ± 8.2036577 | 1170 ± 18.525657 | 266.4 ± 18.187908 | 125.8 ± 13.179529 | 346.2 ± 16.11521021 | 455.6 ± 16.63730747 |

**Histopathology**

Recombinant Exenedin-4 did not produce any compound related organ toxicity / microscopic findings. Microscopic evaluation of following organs indicating that the heart, liver, spleen, sternum, brain, stomach thymus, urinary bladder, trachea, ovaries, cervix and testes were found to be normal after 14 days of administration in all groups. As a parameter to evaluate the local tolerance skin/site ofinjection was examined, there was no evidence of skin reactions/ lesions/ erythema/oedema in any group. It was observed that the various histological changes seen were mostly found in control group also, the changes were common to colony bred animals and hence not considered as significant.

**Discussion:**

Secretory β-cell dysfunction is a stronger contributing factor than insulin resistance in the development and aggravation of type 2 diabetes (14). GLP-1 receptor agonist therapy has been reported to be more effective in patients with relatively high levels of insulin secretion (15) and one such drug in this line is Recombinant exendin-4. Although control of hyperglycemia remains the primary goal, effectiveness and efficiency of the drug without any side effects is also considered to be important. We tried to find

out the toxicity levels of one of the known effective drug approved by FDA in 2005, Recombinant exendin-4. The drug was found to be non-toxic even at the 10 times of the therapeutic dose and thus could be known as a safer drug in mice. The in house test drug was from Byetta.

## 4. Conclusion

Our short term results indicated that recombinant Exenedin-4 administered 10X of therapeutic dose, once intravenously and subcutaneously to mice at 13.0µg/Kg as per the regulatory guidelines, in comparison with the control (vehicle) to thetest compound did not show any significant changes in physical, physiological and clinical chemistry parameters which were assessed throughout the experimental period. Local tolerance of Exendin-4 was evaluated as a clinical observation and morphologic pathology of injection site. No adverse effect such as irritation, inflammation and edema was noticed at the site of injection. Histopathological observations of major organs revealed some changes in liver and lungs. However similar observations were made in the vehicle control group and hence not considered as significant. No specific outcome of toxicological significance was noted with the administration of Exendin-4 as compared to the control. The latter point is crucial, at the light of many recent acquisitions on the so-called vital organs [16, 17].

An increasingly accepted concept in terms of anatomy, metabolism, and physiological regulationwas well tolerated upon administration to male and female Swiss Albino mice at a high dose level. No treatment related changes were detected for body weight, feed intake, hematology, clinical chemistry, and organ weight. In conclusion, no mortality or morbidity was observed during 14 days of experimental period. Hence, recombinant Exendin-4 is safe to administer at the dose level of 13ug/kg body weight to the Swiss albino mice through different routes of administration.

## 5. References


1. Ralph A., De Fronzo MD. Pharmacologic therapy for type 2 diabetes mellitus. *Annals of Internal medicine*, **1999**, 131(4): 281-303.
2. Kahn S.E., Hull R.L., Utzschneider K.M. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature. **2006**, 444: 840–846.
3. Cavaghan, M.K., Ehrmann, D.A. & Polonsky, K.S. Interactions between insulin resistance and insulin secretion in the development of glucose intolerance**.** Journal of Clinical Investigation. **2000**, 106: 329–333.
4. Rand JS, Fleeman LM, Farrow HA, Appleton DJ, Lederer R. Canine and feline diabetes mellitus: nature or nurture, Nutrition. **2004**, 134: 2072S–80S.
5. Ranganath LR. Incretins: pathophysiological and therapeutic implications of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1. Journal of Clinical Pathology. **2008**, 61: 401-9.
6. Gallwitz B. Glucagon-like peptide-1 analogues for Type 2 diabetes mellitus: current and emerging agents. Drugs. **2011**, 71: 1675-88.
7. Dhanesha N, Joharapurkar A, Shah G, Dhote V, Kshirsagar S, Bahekar R, et al. Exendin-4 reduces glycemia by increasing liver glucokinase activity: an insulin independent effect. Pharmacological Reports. **2012**, 64:140-9.
8. *Nielsen LL*, Young AA, Parkes DG. Pharmacology of exenatide (synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes. *Regulatory Peptides.* **2004**, 117: 77-*88.*
9. OECD/ OCDE, OECD Guideline for Testing of Chemicals, Acute Oral Toxicity- Acute Toxicity Class Method, 423. Adopted 17th December, **2001**.
10. EFPIA/ECVAM paper on good practice in administration of substances and removal of blood, Journal of Applied Toxicology. **2001,** 21: 15-23.
11. Lipnick RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA, Walker AP, Chu I, Goddard M, Segal L, Springer JA and Myers RC. Rationale for the up-and-down procedure as submitted to the OECD Food and Chemical Toxicology. **1995,** 33: 223-231.
12. Schmid W. The micronucleus test Mutation Research. **1975**, 31(1):9–15.
13. Adler, I.D. (**1984**) Cytogenetic Tests in Mammals. In: Mutagenecity Testing, A Practical Approach, Venitt, S. and J.M. Parry (Eds.). IRL Press, Oxford, pp: 275-306.
14. Rhee SY, Woo JT. Theprediabetic period: review of clinical aspects. Diabetes Metabolism Journal. **1984,** 35: 107–116.
15. Takabe M, Matsuda T, Hirota Y, Hashimoto N, Nakamura T, Sakaguchi K, Ogawa W (**2012**) Seino S: C-peptide response to glucagon challenge is correlated with improvement of early insulin secretion by liraglutide treatment. Diabetes Research and Clinical Practice. 98: e32–e35.
16. Tchernof A, Despres JP. Pathophysiology of human visceral obesity: an update, Physiological Reviews. **2013**, 93: 359–404.
17. Bartelt A, Heeren J. Adipose tissue browning and metabolic health, Nature Reviews Endocrinology. **2014**, 10: 24–36.