https://doi.org/10.33472/AFJBS.6.10.2024.4584-4590

African Journal of Biological Sciences

Evaluation Of Safety Profile And Pre-Clinical Toxicity Testing Of Exenatide On Wistar Rats

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Article History
Volume 6,Issue 10, 2024
Received:29 Apr 2024
Accepted : 27 May 2024
doi: 10.33472/AFJBS.6.10.2024.4584-4590

ABSTRACT
The peptide exenatide, which is present in the lizard Heloderma suspectum, is quite similar to the one found in GLP-1. This GLP-1 receptor agonist has a substantially longer biological halflife than GLP-1 and is administered sub-cutaneously twice daily for therapeutic purposes. There are two parts to this experimental investigation. We monitored acute toxicity for 14 days and sub-acute toxicity for 28 days. Under the skin, participants in an acute toxicity trial took 200-800 mg/kg of exenatide in a single dosage. Researchers looked at subacute toxicity by administering 150 mg/kg/day, 250 mg/kg/day, and 500 mg/kg/day dosages for 28 days. Tests on rats at doses up to 800 mg/kg revealed no harmful effects, alterations in behaviour, or mortality. Thus, a subcutaneous hazardous dosage with an LD50 greater than 800 mg/ml is required. Also, sub-acute toxicity experiments validated the drug's safety, and rats given doses of 1 mg/kg, 10 mg/kg, and 100 mg/kg did not exhibit any clinical signs, variations in biochemistry, or histology when compared to the control group ($p<0.05$). It follows that exenatide is safe for use in future research.

Keywords: Exenatide, toxicity, safety, diabetes, GLP-1

Introduction

The prevalence of the metabolic disorder diabetes mellitus (DM) is skyrocketing around the globe. Demir et al. (2021) found that various difficulties can arise from disturbances in the metabolism of carbohydrates, proteins, and lipids. Awad et al. (2020) postulate that by 2025, DM will have surpassed all other non-communicable diseases in the globe. Another theory is that DM will be more common in the developing countries. In undeveloped nations, there is no organised healthcare system. Exenatide, the first incretin mimetic, has increased the therapeutic choices for treating type 2 diabetes (Padhi et al., 2020).

Insulin secretion is stimulated after a carbohydrate-rich meal by incretins, which are hormones produced in the gut. According to Fisman et al. (2021) and Müller et al. (2019), the incretin hormone glucagon-like peptide-1 (GLP-1) has multiple functions in type 2 diabetes, including suppressing

glucagon release, slowing stomach emptying, inducing satiety, and improving beta cell function. It also promotes insulin secretion under hyperglycemic settings. Because of these positive effects, GLP-1 is being considered as a potential therapy for type 2 diabetes. It is not feasible to use GLP-1 as a treatment for type 2 diabetes due to its short biological half-life (Marx et al., 2022). Kamruzzaman et al. (2021) and Abdul-Ghani et al. (2019) cite clinical research and anecdotal evidence that exenatide significantly lowers HbA1c, fasting-and postprandial glucose, and significantly reduces body weight in type 2 diabetic individuals. Exenatide treatment improves beta cell activity and increases beta cell mass, according to animal research.

In 2005, the United States authorised the first GLP-1 RA for clinical use, and its name was exenatide. Two daily injections of the original exenatide formulation were necessary for the medication's short-acting effects (Nauck and Meier, 2019). Patients with type 2 diabetes have a number of essential and well-established treatment options, including GLP-1 receptor agonists, which can be taken alone, in combination with insulin or oral antihyperglycemic drugs, or in a coformulation with long-acting insulins (Romera et al., 2019). These medications have a low risk of hypoglycemia, are very successful in lowering HbA1c, suppressing appetite, and reducing glycemic variability. The homology to either GLP-1 or exendin-4 determines whether a GLP-1 receptor agonist has a short or long half-life (Llewellyn et al., 2023; Nauck et al., 2022).

These disturbing findings highlight the need of raising knowledge about the importance of safe medication dosage, especially among healthcare providers and patients. Acute and subacute toxicological studies contribute to drug pharmacology, guarantee safe use, and may prevent the occurrence of adverse effects—a big problem with synthetic drugs—according to Mirza and Panchal (2019). Typically, initial information on acute toxicity tests is obtained before doing subacute toxicity studies, according to the OECD 407 (OECD, 2008a, OECD, 2008b). Data on potential health risks from short-term, repetitive exposure to medications or chemicals can be found there, and it also aids in establishing doses for longer-term, subchronic investigations (Eaton et al., 2018). To back up the move to clinical trials and, eventually, drug molecule marketing, subacute toxicity studies in rats are also seen as a necessary step.

Material and methods Animals and Ethical statement

The institute's animal house provided us with healthy male and female Wistar rats weighing 100-150 g. Results from the toxicity test were analysed in accordance with OECD Guidelines No. 425 and 407. All animals were housed in plastic cages and kept separately by sex at 22 ± 2°C, 55 ± 10% relative humidity, and a 12-hour light/dark cycle. No restrictions were placed on the rats' food and water. The lab was the site of the experiment. The animals were given a week to adjust to their new surroundings and undergo examinations before the actual experiment.

Acute toxicity study test

Animals were subjected to acute toxicity studies following the OECD guideline 425. Male Wistar rats were starved for 24 hours for this research. Two pairs of male and female rats were pre-experimented with in 24 total rats to prepare for the acute investigation. The drug's physicochemical characteristics were assessed by mixing exenatide with a sodium carboxymethyl cellulose solution with a concentration of 0.2%. For every rat, we gave them a cumulative dose of 200, 350, 450, 600, and 800 mg/kg of medicine. For the first half an hour after administration, they were closely monitored. After that, they were monitored for the first 24 hours and then again for the next 72 hours. Fatality, lethargy, lacrimation, nasal haemorrhage, paralysis, piloerection, salivation, skin, water use, and drowsiness were the criteria examined (OECD, 2008).

Sub-acute toxicity study

High dose daily treatment for 28 days ranging from one-tenth to one-fourth of LD50 is used in the subacute phase. Ten percent are selected during the 28-day trial. In the group receiving a medium dose, half of the high dose is medium. The low-dose group receives half the recommended medium dose at a lower level. There were a total of eight animals divided into four groups. A control group was established. A solution of 0.2% sodium carboxymethyl cellulose was administered to the control group. The medicine was administered subcutaneously to the other three groups at doses of 1 mg/kg b.w/day, 10 mg/kg b.w/day, and 100 mg/kg b.w/day, respectively. On a weekly basis, we tracked the animals' weight, as well as any changes in their behaviour or physical appearance. An intraperitoneal injection of 5 millilitres per kilogramme of chloralose dissolved in 25% urethane (w/v) was used to put the animals to sleep on the 28th day of treatment. Cardiac puncture was used for blood sample collection. Following collection of blood samples in EDTA and heparinized tubes, they were sent for haematological and biochemical investigation.

Hematological Index

In order to conduct the haematological assays, blood samples were drawn and deposited into tubes that contained EDTA-K2. The rats' haemoglobin index was examined once the medication administration period came to a close. Enzymes, substrates, and metabolic products were identified using standard haematological and biochemical testing. These indicators were measured: red blood cell count (RBC), white blood cell count (WBC), granulocyte count (GRAN), lymphocyte count (LYM), monocyte count (MON), thrombocytocrit (PCT), hematocrit (HCT), platelet distribution width (PDW), mean corpuscular haemoglobin (MCH), haemoglobin (HGB), red blood cell distribution width (RDW), mean corpuscular volume (MCV), mean platelet volume (MPV), mean corpuscular haemoglobin concentration (MCHC), and platelet count (PLT).

Biochemical Analysis

We collected blood samples, rotated them at 4,000 rpm for 10 minutes at 4°C, and then put them in the freezer. Blood biochemical indices of rats in each group were examined at the conclusion of the medication administration period. The primary biochemical markers included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL), total protein (TP),urea nitrogen (BUN), serum albumin (Alb), creatinine (Cr), blood sugar (Glu), total protein (TP), globulin (GLO), triglyceride (TG), total cholesterol (TCH), total bile acid (TBA), lactate dehydrogenase (LDH), creatine kinase (CK), total cholesterol (TC), glucose (GLU), and uric acid (UA).

Statistical Analysis

The average plus or minus standard error of the mean was shown. SPSS 20 was used for statistical analysis. 1-Way ANOVA with LSD post-hoc testing. When $P < 0.05$, significance was established.

Result Acute toxicity study

Acute toxicity research results showed that the medications were safe at doses between 200 and 800 mg/kg. The treated groups did not exhibit any changes in behaviour or mortality at the levels that were examined. This means that the medications may have an LD50 larger than 800 mg/kg body weight. Table 1 shows the changes in rat weight that occur during medication acute toxicity trials. The weights of the control and experimental groups did not differ statistically.

Table 1 Acute toxicity testing of exenatide suspension

Group	Dosage	Mortality rates (%)	Survival (n=4)
1	200	0	4
2	350	0	4
3	450	0	4
4	600	0	4
5	800	0	4

Sub-acute toxicity study

The organs examined in the sub-acute toxicity study—the heart, liver, pancreas, adipose tissues, and kidneys—did not exhibit any noticeable pathological alterations in either the control or experimental groups. Experiment rats were monitored throughout the sub-acute trial and no mortality or toxic effects were noted at doses of 1 mg/kg, 10 mg/kg, and 100 mg/kg. There was no statistically significant change in food consumption despite recording it, suggesting that exenatide does not appear to have any discernible impact on food consumption (Figure 1). Figure 2 shows that the mean body weights of the treatment and control groups of rats did not differ significantly ($p > 0.05$). In addition, exenatide has not altered the appearance of the fur, motor functions, skin, eyes, secretions, mucous membranes, excretions, or autonomic nervous system.

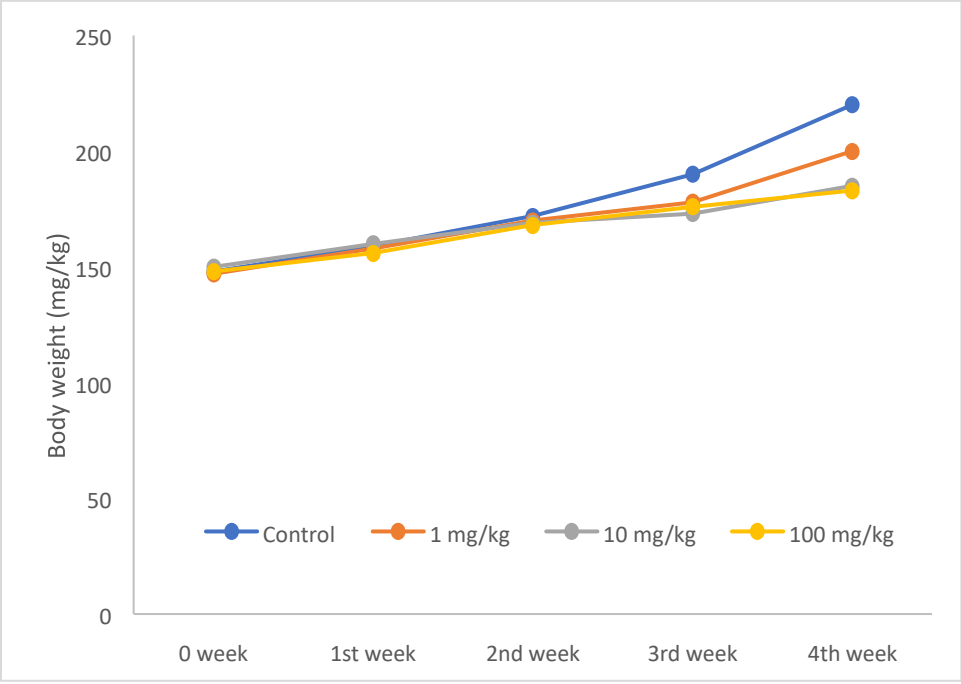

Figure 1 Change in body weight of animals after treatment with the drug

Hematology Index

With the exception of MCH levels in both male and female rats, there was no statistically significant change in any of the relative blood parameters (Mon, Lym, HBC, WBC, and PCT) between the exenatide-treated group and the control group (Table 2). The results were most pronounced in the group that received the lowest dosage. There was no significant departure from the range of control variables in these statistical analyses. We need to go further into the reasons behind discrepancies.

Table 2 Hematological data of animals after treatment with exenatide on animals

Parameter	Control	1 mg/kg drug	10 mg/kg drug	100 mg/kg drug
WBCs (10^3/ul)	5.14 ± 1.24	4.73 ± 1.06	5.03 ± 0.94	5.11 ± 1.46
RBCs (10^6/ul)	7.19 ± 0.34	6.85 ± 0.94	6.95 ± 0.46	7.22 ± 0.46
PLT (10^3/ul)	840.13 ± 12.33	976.13 ± 15.14	857.24 ± 13.26	879.56 ± 10.45
HGB (g/dl)	123.45 ± 4.39	116.55 ± 3.23	125.45 ± 5.34	130.45 ± 7.54
MCH (pg)	15.55 ± 0.58	17.34 ± 0.46	14.73 ± 0.45	14.46 ± 0.45
MCV (fL)	57.49 ± 1.55	54.67 ± 2.78	56.56 ± 1.23	55.53 ± 1.24
MCHC (g/L)	364.34 ± 3.20	357.77 ± 4.23	369.45 ± 8.45	375.34 ± 6.45
PCT (ng/L)	0.54 ± 0.032	0.49 ± 0.023	0.52 ± 0.077	0.46 ± 0.056
Monocytes (10^9/uL)	0.04 ± 0.01	0.05 ± 0.02	0.05 ± 0.01	0.04 ± 0.01
Lymphocytes (10^9/uL)	2.85 ± 0.94	2.43 ± 0.47	2.36 ± 0.57	2.96 ± 0.94

Biochemical analyses

Biochemical studies revealed statistical differences ($p < 0.05$) between the control group and the exenatide treatment group, as shown in Table 3, as compared to the control group. As an example, in male rats, the levels of UA, ALB, LDH, and CK were significantly higher and GLU was significantly lower in the medium-and/or high-dose groups compared to their respective controls ($p < 0.01$ and $p < 0.05$, respectively). On the other hand, only in female rats were the levels of GLO, TBIL, and TG statistically reduced ($p < 0.01$) in the groups given medium and/or high doses compared to their respective control groups. All of the biochemical markers measured in the sub-acute toxicity study—including urea, uric acid, creatinine, ALT, AST, alkaline phosphatase, , acid phosphatase, triglycerides, HDL, and LDL—were within the rat reference range. There was no statistically significant difference between the medication and control groups with respect to the biochemical results (Table 3).

Table 2 Biochemical profile of animals after treatment with exenatide on animals

Parameter	Control	1 mg/kg drug	10 mg/kg drug	100 mg/kg drug
ALT (ul/mL)	35.14 ± 2.24	34.73 ± 1.06	35.03 ± 0.94	32.11 ± 1.46
AST (ul/mL)	67.19 ± 1.34	66.85 ± 3.94	63.95 ± 2.46	67.22 ± 2.46
HDL (mg/dL)	30.13 ± 2.33	29.13 ± 1.14	27.24 ± 1.26	29.56 ± 1.45
LDL (mg/dL)	12.45 ± 1.39	11.55 ± 1.23	13.31 ± 1.34	13.45 ± 1.54
Triglycerides (mg/dL)	25.55 ± 1.58	27.34 ± 1.46	24.73 ± 1.45	24.46 ± 1.45
Alkaline phosphatase (U/L)	67.49 ± 4.55	64.67 ± 3.78	66.56 ± 3.23	65.53 ± 2.24
Acid phosphatase (U/L)	26.34 ± 1.20	27.77 ± 2.23	29.45 ± 2.45	25.34 ± 2.45
Uric acid (mg/dL)	3.54 ± 0.32	3.49 ± 0.23	3.02 ± 0.77	2.86 ± 0.56
Creatinine (mg/dL)	0.45 ± 0.01	0.55 ± 0.02	0.48 ± 0.01	0.44 ± 0.01
Blood urea (mg/dL)	42.85 ± 3.94	40.43 ± 2.47	38.35 ± 2.57	36.96 ± 2.94
TG (mmol/L)	0.75 ± 0.04	0.72 ± 0.04	0.68 ± 0.02	0.69 ± 0.03
TC (mmol/L)	1.64 ± 0.45	1.55 ± 0.64	1.74 ± 0.77	1.73 ± 0.73

Discussion

There are two parts to this study that assess the drug's toxicity profile in diabetic rats. Phase 2 evaluates sub-acute toxicity, while Phase 1 identifies acute toxicity. Acute exposure to the medicine did not cause any harmful effects or alterations in the rats' bodies, suggesting that it may be generally safe. Exenatide has an acute subcutaneous LD50 of 800 mg/kg in rats. To find the dose at which rats did not experience any negative side effects, our present sub-chronic sub-cutaneous toxicity study used the LD50 as its foundation. The doses tested were 1, 10, and 100 mg/kg, with 1-10 and 100 mg/kg representing high, medium, and low doses, respectively. Clinic drug safety data about exenatide's subacute toxicity is currently lacking, as far as we are aware. So, to find out how safe and poisonous exenatide is, we ran a battery of tests on Wistar rats in this study.

The sub-acute toxicity research examines the effects on rats of a substance's toxicity after 28 days of repeated subcutaneous doses. This research provides proof of any shift in the organism's metabolic characteristics, morphology, or hemology. The toxicity consequences could be determined using this work as a foundation. The haematological parameters did not show any statistically significant differences between the control group and the group that was treated with exenatide. The therapy groups showed statistically significant changes in the following biochemistry assays: AST, ALP, GLU, GLO, TBIL, TG, BUN, LDH, UA, and CK. According to these findings, the half-life of exenatide was around 2000 mg/kg BW, and the dose at which the drug had the least amount of negative effect in rats was 50 mg/kg.

If any foreign substances, such as those in poly herbal formulation, have a negative impact on blood, it can be discovered through haematological parameter testing. Important biochemical markers include creatinine, urea, uric acid, AST, ALT, acid phosphatase, triglycerides, alkaline phosphatase, HDL, LDL. There was a difference in AST between the oxyclozanide-treated and untreated control groups, according to the biochemical studies. It is well-known that damage to related cells and tissues triggers the release of AST and ALT into the blood. As a practical and specific enzyme biomarker of hepatocyte injury, most toxicological studies commonly evaluate an increase in ALT. An increase in AST concentration is typically associated with a higher number of damaged hepatocytes rather than a reflection of the pathological lesion's severity or reversibility. Over the course of the 28 days, there was no statistically significant change in the ALT, a marker of liver damage. Consequently, it is possible that many tissues contributed to or were linked with the AST alterations found in this study.

Conclusion

The drugs were determined to be safe at doses up to 800 mg/kg in the acute toxicity trial. At the levels that were examined, no serious adverse effects were noted. No statistical differences were observed between the control group and the group that was treated with exenatide based on the haematological parameters. Changes in AST, ALP, GLU, GLO, TBIL, TG, UA, BUN, LDH, and CK were found statistically between the therapy groups in biochemistry testing.

According to these findings, the half-life of exenatide was around 800 mg/kg BW, and the dose of 200 mg/kg in rats was the one with the least amount of side effects.

Declaration

All authors have stated that they have no competing interests.

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