Preclinical studies of a medicinal plant (coded) based on Traditional Knowledge with special reference to Access and Benefit Sharing – Part 1

N. M Krishnakumar, P.G. Latha, S Rajasekharan, S.R. Suja, Mathew Dan,
B. Sabulal and M Navas
Jawaharlal Nehru Tropical Botanic Garden and Research Institute
Palode, Thiruvananthapuram - 695562, Kerala, India.
plathagopalakrishnan@gmail.com

Abstract

This scientific study was carried out based on traditional knowledge related to a medicinal plant (Code No. 222*) disclosed by a traditional healer after signing Prior Informed Consent (PIC) and Contractual Agreement including non-disclosure agreement with Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI). The claim of the traditional healer was that he is using the particular medicinal plant species for treating diabetes, liver disorders, jaundice and to relieve fatigue. On verification, no scientific study so far has been carried out on this plant species and the therapeutic usage was kept as trade secret by the healer. The main objectives of the preclinical study was to conduct scientific evaluation of the claim disclosed by the traditional healer, to explore the possibilities for developing single/polyherbal formulation and its scientific validation through conducting preclinical studies. In the present study, the authors carried out plant taxonomy, pharmacognosy, phytochemistry, ethnopharmacology and toxicity studies of the plant species (single/polyherbal formulation). The preclinical studies so far carried out by the authors shows that the given coded drug (Single and Polyherbal formulations) possesses significant anti-diabetic, hepatoprotective and antifatigue properties as claimed by the traditional healer. Apart from this, the medicinal plant possesses excellent antioxidant property. The study further shows that the medicinal plant is devoid of any side effects. Based on the study, a patent application was filed with the title 'A novel polyherbal formulation with multiple therapeutic effects as antidiabetic, hepatoprotective, antifatigue and antioxidant' (Application No. 2277/CHE/2011) to Regional Patent Office, Chennai. The traditional healer was also included as one of the inventors in the patent application. This is the first case study on Access and Benefit Sharing (ABS) where a Traditional Healer was included as one of the inventors. The first part of the study highlights the pharmacological study on anti-diabetic effect of the medicinal plant.

Keywords: *Traditional Knowledge, Multiple Therapeutic Effects, Preclinical studies, Antidiabetic, Access and Benefit Sharing*

Introduction

Traditional knowledge (TK) is directly linked with the cultural heritage of the people and has been transferred from generation to generation. In India, more than 80 % of the livelihood needs of the poor directly or indirectly depend upon the use of biological resources and associated TK. It includes both documented and non-documented information. This knowledge was recorded from the non-documented category of information after signing the PIC, Contractual agreement including the non–disclosure agreement with a Traditional healer.

There is an increasing appreciation of the advantages of using science and technology together with TK to

* *Name of the medicinal plant species will be disclosed only after obtaining the Patent*

find mutually beneficial results from development projects. TK is more than a simple compilation of facts drawn from local, often remote environments. It is a complex and sophisticated system of knowledge growing from centuries of wisdom and experience. It also constantly grows and changes with new information. The wisdom derived from philosophy is an advantage when planning for sustainability. Development projects utilizing TK should be implemented through co-operation and mutual understanding, combined with an understanding of the traditional rights of the indigenous people. Therefore, systematically documented TK will provide a platform to the research workers for developing new processes, products, patenting, technology transfer, commercialization, benefit sharing etc.

India is a party to Convention of Biological Diversity (CBD) (1992) recognizing the sovereign rights of the state to use their biological resources. The Convention expects the parties to use their biological resources and to facilitate access to biological resources by other parties subject to national legislation and on mutually agreed upon terms (Article 3 and 15 of Convention of Biological Diversity – CBD). Article 8(j) of the CBD recognizes contributions of local and indigenous communities to the conservation and sustainable utilization of biological resources through TK, practices and innovations and provides equitable sharing of benefits with such people arising from the utilization of such knowledge and innovations.

This has been translated into action by Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI) formerly known as Tropical Botanic Garden and Research Institute (TBGRI), Palode, Thiruvananthapuram, during the year 1996 when it developed an unique benefit sharing model, popularly known as 'ABS- Kani Model' or 'TBGRI-Model of Benefit Sharing'. The model relates to the development of scientifically validated herbal drug, 'Jeevani' based on a lead from the Kani tribe of Kerala. This unique benefit sharing model won the 'UN-Equator Initiative Award' during the last Earth Summit, in 2002 held at Johannesburg, South Africa. This is the second case study (Phase I) on Access and Benefit Sharing (ABS) carried out by JNTBGRI based on a medicinal plant (coded drug). In a classic revamp to the existing ABS model, the traditional healer is included as an inventor and not just an informant. He will be eligible for all the rights even later when the technology transfer gets complete and for a share of the royalty when the drug gets commercialized.

Materials and Methods

1. Preparation of the coded drug extracts

100 g of the coded drug 222 was extracted with 1000 ml of ethanol overnight, at room temperature with constant stirring. The extract was filtered, concentrated and the solvent evaporated completely on a rotary evaporator and dried in a desiccator and they were reconstituted in 0.5 % Tween-80 to required concentrations and used for the experiments.

2. Animals

Wistar albino rats, male and female (200–250 g) and Swiss albino mice, male and female (25–30 g), obtained from the Institute's Animal house were used for the present study. They were housed under standard conditions and fed commercial rat feed (Lipton India Ltd., Mumbai, India) and boiled water *ad libitum*. All experiments involving animals were carried out according to National Institute of Health (NIH) guidelines, after getting the approval of the Institute's Animal Ethics Committee.

3. Glucose Tolerance Test (GTT).

Wistar albino male rats were divided into five or six groups and fasted for 15 hr. Distilled water was used as normal control, a reference drug Glibenclamide (5 mg/kg) and coded drugs at different concentrations were orally administered. Thirty min later, glucose (1.25 g/kg) was orally administered to each rat. Blood samples were taken from tail vein at 0 min (just before

glucose administration), 30, 90 and 150 min for the assay of glucose (Peungvicha *et al*., 1998).

4. Alloxan-induced diabetic study

The alloxan-induced diabetic studies of the leaf ethanolic extract of coded drug 222 was carried out on Wistar albino male rats. Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg). Two days after alloxan injection, rats having plasma glucose level greater than 140 mg/dl (alloxan–induced) were included in the study. Treatment with coded drug extract was started 48 h after alloxan injection. Blood samples were drawn at weekly intervals till the end of the study (i.e. 3 weeks). Fasting blood glucose estimation was done on day 1, 7, 14 and 21 of the study. On day 21, blood was collected by cardiac puncture under carbon dioxide inhalation anaesthesia from overnight fasted rats and fasting blood sugar estimated. Serum was separated and analyzed for biochemical parameters. Liver, pancreas and kidney tissue samples were collected for histopathological studies (Nagappa *et al*., 2003).

4.1. Biochemical Estimations

The collected blood was allowed to coagulate for 1h at room temperature and centrifuged at 1500 rpm for 15 min at 37ºC to separate the serum. . The serum was then used for the assay of marker enzymes, namely Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Serum Bilirubin (SB) and Serum Alkaline phosphatase (ALP) according to standard methods. Other serum parameters such as serum cholesterol, triglycerides and total protein levels were estimated and compared with the standard modern drug, glibenclamide.

4.2. Histopathological Studies

Liver, pancreas and kidney specimens obtained from the control and treated groups of the experiment were trimmed to small pieces and preserved in formalin (10% solution) for 24h. They were subjected to dehydration with acetone of strength 70, 80 & 100% respectively, each for 1h. The infiltration and impregnation was done by treatment with paraffin wax, twice each time for 1h. Specimens were cut into sections of 3-7 µm thickness and stained with haematoxylin and eosin. Mounting of the specimens was done by the use of Distrene Phthalate Xylene (DPX).

5. Statistical analysis

All the analyses were carried out in triplicate. The statistical significance between the samples were determined by the analysis of variance (ANOVA) and the data were recorded as mean ± Standard Deviation (SD) , $P \leq 0.05$ was considered to be statistically significant. Significant differences between means were determined by Duncan's Multiple Range tests (Raghava, 1987). The computer software employed for the statistical analysis was IBM SPSS Statistics, version 20 (USA).

Results and Discussion

1. Glucose tolerance test (GTT):

The Glucose Tolerance Test is a standard test used to determine whether a mammal produces sufficient insulin to promote the uptake of glucose from the blood after it is given a high glucose feeding or not. This test must be performed after a significant period of fasting to ensure that the level of glucose in the blood is low enough so that it does not trigger the release of more than trivial amounts of insulin. As glucose enters the blood from the intestine, the blood glucose level of the mammal begins to rise. This elevation in blood glucose level triggers the release of insulin from the ß-cells of the Islets of Langerhans in the pancreas. The insulin is released into the blood and is transported to all the tissues of the body. In these tissues, insulin stimulates the cellular uptake of glucose present in the blood. As the body tissues take up glucose, the level of glucose in the blood will

decrease. Thus we can assume that, these coded drugs have the ability to stimulate insulin secretion from the ß-cells of the Islets of Langerhans in the pancreas of the normal fasted rats after glucose loading. Glucose tolerance test (GTT) of 222 ethanolic leaf extract was carried out in overnight fasted Wistar albino male rats (Tsble 1). The blood glucose levels were significantly reduced by the ethanolic extract of 222 (125 mg/kg) at 90 min (47.62 %) and 150 min (32.74%) after glucose loading.

2. Alloxan-induced diabetic study

Alloxan induced diabetes model is almost comparable to type I diabetes with near complete β-cell destruction (Chattopadhyay *et al*., 1997). Alloxan administration results in cellular damage which causes increased serum levels of enzymes and other biochemical parameters like cholesterol, triglycerides etc. In alloxan-induced diabetic rats, the blood glucose level was in the range of 307 – 329 mg/100ml, which can be considered as severe diabetes. When treated with 222 ethanolic extract at 125 mg/kg dose treated group, the blood glucose level was decreased to 203 mg/dl from the 21st day of the treatment (Table 2).

The body weight of animals treated with 222 ethanolic extract was significantly increased compared to the diabetic control (Table 3). Serum parameters like ALT and AST levels were significantly reduced compared to diabetic control (Table 4). The total protein level was significantly increased in the extract treated group compared to the standard drug glibenclamide. Histopathological studies also supported the biochemical findings. The liver of alloxan-treated rats showed degenerated parenchymatous cells with severe necrosis and dilated sinusoids. The histopathological observations of the liver pretreated with 222 (125 mg/kg) dose exhibited near normal appearance and minimal necrosis of liver cells (Fig 1). Kidney sections of diabetic animals showed thickening on the walls of nephrons filling their lumen and expanded glomerulus. The thickening of the walls was reversed and the glomerular architecture was restored by the extract treatment (Fig 2). Diabetic animals showed atrophy of β-cells and vascular degenerative changes in the islets of pancreas (Fig 3). But the treatment with the coded drug extracts 222 (125 mg/kg) dose increased the number of islets compared to the diabetic control. The blood glucose lowering effect of the extracts of coded drug 222 was very significant at 125 mg/kg.

Table 1: Effect of crude ethanolic extract of the dried leaves of the plant 222 on blood sugar level (mg/dl) in oral Glucose Tolerance Test

Groups	Initial reading (before drug administration)	'0' min (before glucose loading)	30 min	90 min	150 min
Normal control	64.00±0.89	92.00±0.89	145.00±0.89	111.00±0.89	109.00±0.89
222 leaf ethanolic extract (125 mg/kg)	84.00 ±0.89	79.50±0.89	107.00±0.89	44.00 ±0.89**	56.50 ±0.89
222 leaf ethanolic extract (250 mg/kg)	71.00±0.89	66.00±0.89**	96.00±0.45	71.00±0.89	73.00±0.89
222 leaf ethanolic extract (500 mg/kg)	53.00±0.89	68.50±0.89	107.00±0.45	70.50±0.89	64.50±0.89
Standard control Glibenclamide (5 mg/kg)	61.00±0.89	66.00±0.89**	87.50±0.45	43.00±0.89**	34.00±0.89

*Values are the mean ± SD, n=6 in each group, analysis of variance (ANOVA) followed by Duncan's multiple range test. **Significance P≤0.05 compared to the standard control.*

Table 2: The effect of 222 ethanolic extract on rat serum glucose levels (mg/dl) on Alloxan-induced diabetic study.

Groups	DAY 1	DAY 7	DAY 14	DAY 21
Normal control	76.50 ± 1.87	72.67 ± 4.72	73.33 ± 5.42	73.33 ± 3.98
Toxin control Alloxan (150 mg/kg)	321.33 ± 4.03	317.67 ± 10.93	313.33 ± 11.33	307.67 ± 10.29
Ethanolic extract 222 (125 mg/kg)	271.00 ± 6.26	233.33 ± 7.60	209.33 ± 5.09	203.67 ± 4.93
Standard control Glibenclamide (5 mg/kg)	232.67 ± 3.72	163.00 ± 3.58	120.33 ± 3.61	109.00 ± 7.10

*Values are the mean ± SD, n=6 in each group, analysis of variance (ANOVA) followed by Duncan's multiple range test. **Significance P≤0.05 compared to the standard control.*

Table 3: Alloxan-induced diabetic study of coded drug 222 ethanolic extract: Influence on body weight (in grams).

Groups	Day 1	Day 7	Day 14	Day 21
Normal control	125.00 ± 0.01	127.50 ± 1.89	130.00 ± 1.32	130.67 ± 1.08
Toxin control (alloxan 150 mg/kg)	125.00 ± 0.01	92.50 ± 1.10	92.50 ± 1.10	90.83 ± 1.34
222 ethanolic extract (125 mg/kg)	125.00 ± 0.01	111.67 ± 1.38	110.00 ±1.04	125.00 ± 0.01**
Standard control (Glibenclamide 5 mg/kg)	150.00 ± 0.01	150.00 ± 1.16	125.00 ± 0.01	109.17 ± 1.34**

*Values are the mean ± SD, n=6 in each group, analysis of variance (ANOVA) followed by Duncan's multiple range test. **Significance P≤0.05 compared to the standard control.*

Table 4: The effect of 222 ethanolic extract on rat serum parameters on Alloxan-induced diabetic study.

Groups	Glucose (mg/dl)	AST (IU/L)	ALT (IU/L)	Cholesterol (mg/dl)	Total protein (g/dl)	Triglycerides (mg/dl)
Normal Control	73.60±2.41	108.00±2.37	43.87±1.48	180.33±1.54	11.00±3.35	102.67±1.37
Toxin Control Alloxan (150mg/kg)	203.10±1.78	312.67±1.14	236.00±2.22	212.82±2.07	3.30±2.80	221.00±5.37
222 ethanolic extract 125 mg/kg	84.07±1.39	58.67±2.53**	59.00±1.07**	182.00±2.11	6.33±2.16**	191.67±4.03
Standard Control Gliben-clamide (5 mg/kg)	69.37±2.84	164.00±2.68**	47.33±1.39**	178.47±0.81	5.67±1.21**	150.33±2.25

*Values are the mean ± SD, n=6 in each group, analysis of variance (ANOVA) followed by Duncan's multiple range test. **Significance P≤0.05 compared to the standard control.*

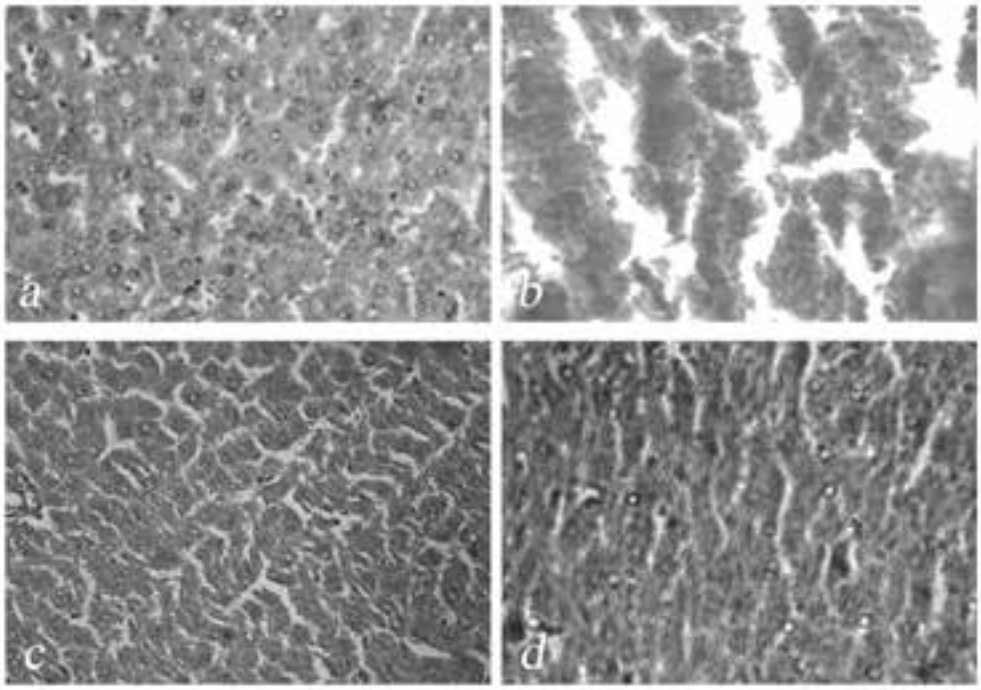

Figure 1

Histological evidence of the protective effect of the ethanolic extract of the coded drug 222/glibenclamide on the liver of Alloxan-induced diabetic rats. a)[f] Normal control rat liver showing normal hepatocytes with well brought out nuclei and cytoplasm (x 400). b) Liver of diabetic rat showing degenerated parenchymatous cells with severe necrosis and dialation of sinusoids (x 400). c) Liver of diabetic rat after the treatment with ethanolic extract of coded drug 222, showing hepatocytes with near normal appearance and minimal necrosis (x 400). d) Liver of diabetic rat after the treatment with glibenclamide showing normalcy of hepatocytes arrangement (x 400).

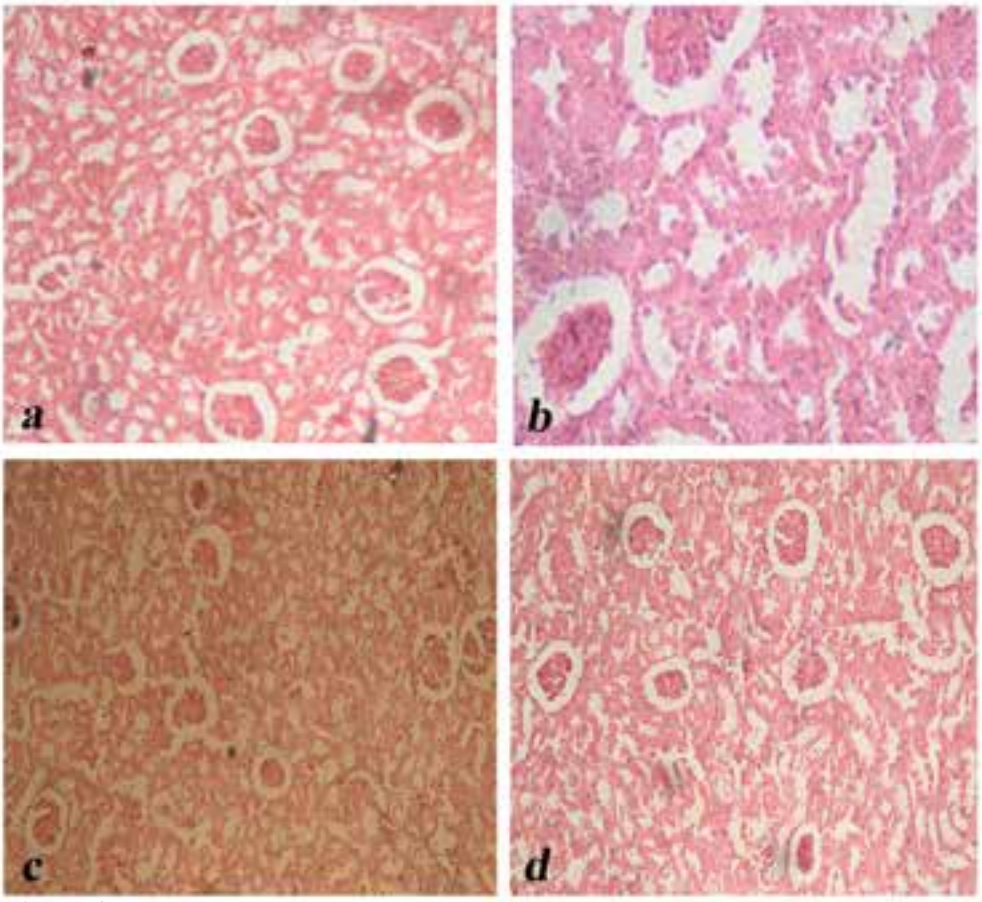

Figure 2

Histological evidence of the protective effect of ethanolic extract of 222/glibenclamide on kidney of alloxan- induced diabetic Wistar rats. a) Normal control rat kidney showing normal Bowman's capsule and renal tubules (x 300). b) Kidney of diabetic rat showing degenerative changes, expanded glomerulus and thickening of the walls of renal tubules (x 300). c) Kidney of diabetic rat after treatment with 222 ethanolic extract (125 mg/kg) showing glomerulus with reduction in thickening of walls of renal tubules (x 300). d) Kidney of diabetic rat after treatment with glibenclamide showing normalcy of Bowman's capsule, almost similar to normal rat kidney (x 300).

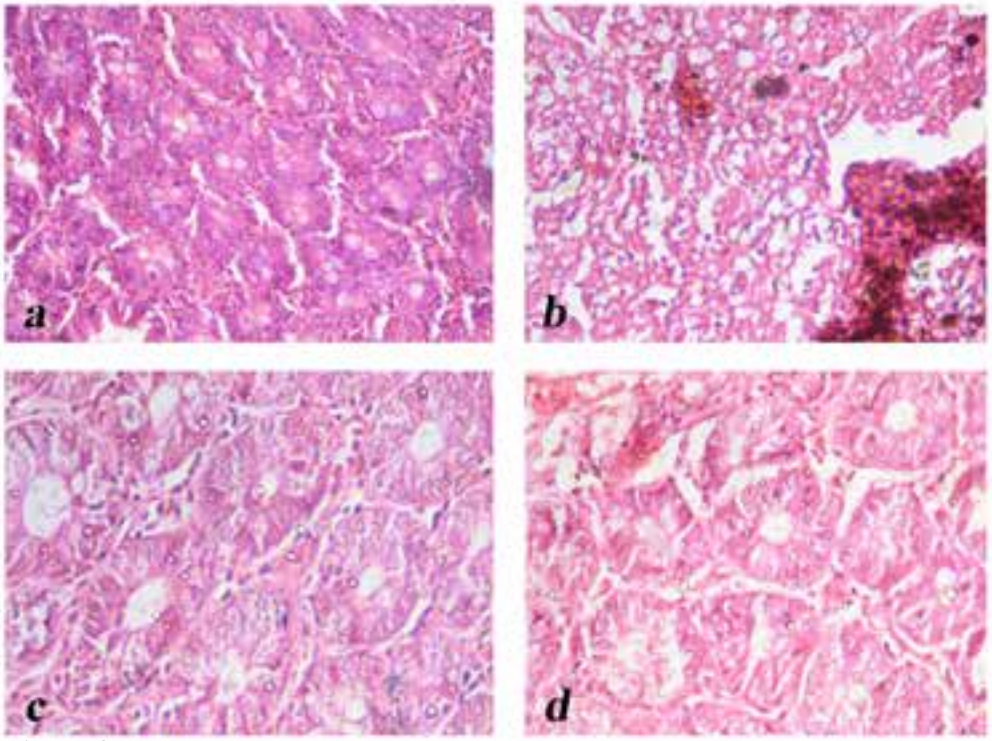

Figure 3

Histological evidence of the protective effect of ethanolic extract of coded drug 222/glibenclamide on pancreas of Alloxan-induced diabetic rats. a) Normal control rat pancreas showing patches of abundant β-cells (x 400). b) Pancreas of diabetic rat showing minimal number of pancreatic β-cells (x 400). c) Pancreas of diabetic rat after treatment with 222 (125 mg/kg) extract showing pancreatic β-cells almost similar to that of normal control (x 400). d) Pancreas of diabetic rat after treatment with glibenclamide (5 mg/kg), showing pancreatic β-cells almost similar to that of normal control (x 400).

Conclusion

Alloxan induces diabetes by destroying β-cells and this model is almost comparable to type I diabetes with near complete β-cell destruction. The blood glucose lowering effect of the extract 222 at 125 mg/kg could possibly be due to increased peripheral glucose utilization and inhibition of tubular reabsorption mechanism for glucose in kidney. The drug may be mimicking one or more actions of insulin at the insulin receptor level or it may be influencing one or more post receptor events. The second part of this paper will highlight streptozotocin-induced diabetic study.

Literature cited

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