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A review of the use of Streptozotocin (STZ) in the induction of diabetes in rats and subsequent ocular tissue changes

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Abstract

Streptozotocin is widely used in medical research for treating certain cancers of the Islets of Langerhans and to produce an animal model for type 1 diabetes. A study has revealed that when compared to the control group of rats, those injected with STZ exhibited reduced plasma insulin and elevated blood glucose ($p < 0.05$ in all cases). The study also found that diabetic rats weighed significantly less than control animals ($p < 0.05$). In relation to ocular tissues, lacrimal glands from diabetic rats were also found to weigh significantly less ($p < 0.05$) than those from the control group. However, no significant changes in the weights of lens, cornea, sclera and retina were observed between diabetic and control animals. Several other studies found that STZ-induced diabetes can be treated by plant extracts which control the blood sugar level as well as improving the lipid profile and ocular complications such as retinopathy. Experiments are usually performed on male or female rats of a specific body weight, usually between 250 and 300 g. Diabetes is induced in rats by intraperitonial injections of streptozotocin (60 mg/ kg) in citrate buffer, pH 6.3. Animals that exhibit glucosuria after 24 hours, tested by urine test strips are considered diabetic. Plant extracts (6 mg/100g body weight) are orally administered into the stomach of STZ-diabetic rats every third day at a certain consistent time by means of bulbed steel needle for at least a four week period. This is done to determine the efficacy and potency of the plant extracts on diabetes. Histological and transmission electron microscope (TEM) techniques are used to study the changes in the ocular tissues. The dissected ocular tissues should be dehydrated in graded ethyl alcohol series and embedded in Araldite CY212. Ultra thin sections should be contrasted with uranyl acetate and lead citrate for examination by TEM. Streptozotocin induces diabetes in laboratory animal models for scientific studies and breakthroughs in medicine. The use of STZ and plant extracts may prove to be beneficial in the eye health care profession if considered for the studies of hypoglycemic agents that have the potency to prevent the advancement of diabetic retinopathy in diabetic patients.

Key words: streptozotocin, diabetes, rats, ocular tissue, plant extracts.

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Introduction

Eye care practitioners are very conversant with the pathophysiology, disease process and management of diabetes. It is considered of interest to let optometrists know that diabetes can be experimentally induced and that a common method by which the induction of diabetes is done is through the use of a drug known as streptozotocin (STZ). STZ allows experimentalists to test the efficacy and potency of new drugs that are perceived to have some hypoglycemic effects in diabetic sufferers. STZ is a naturally occurring chemical that is particularly toxic to the insulin-producing β-cells of the pancreas in mammals[1]. It is a glucosamine-nitrosourea compound[2]. As with other alkylating agents in the nitrosourea class, it is toxic to cells by causing damage to the DNA[3]. It is similar enough to glucose that it is also transported into the cells by the glucose transport protein GLUT2, but it is not recognized by the other glucose transporters. This explains its relative toxicity to ß-cells, since these cells have relatively high levels[2, 3] of GLUT2. The chemical structure of STZ is shown in Figure 1.

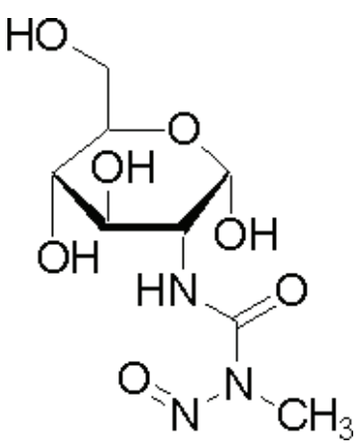

Figure 1: Showing the chemical structure of streptozotocin. STZ has the following systematic chemical IUPAC name: 1-methyl-1-nitroso-3-[2,4,5-trihydroxy-6- (hydroxymethyl) oxan-3-yl]-urea.

STZ was originally identified in the late 1950s as an antibiotic[4]. The drug was discovered in a strain of the soil microbe *Streptomyces achromogenes* by scientists at the drug company Upjohn, which is now part of Pfizer.

In the mid 1960s, STZ was found to be selectively toxic to the β-cells of the pancreatic islets of Langerhans, which normally regulate blood glucose levels by producing the hormone insulin[5]. This suggested the drug's use as an animal model for type 1 diabetes[5], and as a medical treatment for cancers of the β-cells[6]. In the 1960s and 1970s the US National Cancer Institute investigated STZ's use in cancer chemotherapy. Upjohn filed for Food and Drug Administration (FDA) approval of STZ as a treatment for pancreatic islet cell cancer in November 1976, and approval was granted in July 1982. The drug was subsequently marketed as Zanosar[4].

Streptozotocin (STZ)-induced diabetes

The prevalence of diabetes in the South African population is about 18%[7]. The disease is a progressive metabolic disorder and is characterized by hyperglycemia[8, 9]. This is due to lack of insulin or insensitivity of insulin to target cells[10]. Diabetes mellitus (DM) can be divided primarily into two types: type 1 or insulin dependent diabetes mellitus and type 2 or non-insulin dependant diabetes mellitus[8]. Type 1 DM is due to autoimmune destruction of β-cells especially in childhood. On the other hand, type 2 DM is mainly due to hereditary factors, affluent lifestyles and obesity[8]. Both types of DM are associated with common long term complications including cardiomyopathy, nephropathy and digestive insufficiency, especially if the disease is not diagnosed and treated early[11]. Also, both types can damage a variety of ocular tissues including the retina, lens, and the cornea[12].

STZ is widely used in medical research for treating certain cancers of the Islets of Langerhans and to produce an animal model for type 1 diabetes[1, 5]. It has widely been used by many authors[13-17] to induce diabetes in rats. Most importantly, STZ is used for the study of the effectiveness of antidiabetic agents (usually plant extracts) in STZ-induced diabetic rats[18]. It has been used to induce diabetes in rats for various purposes including the investigation of the effects of streptozotocin (STZ)-induced type 1 diabetes on protein concentration and on cation content in ocular tissues of rats[19], reversal of diabetic retinopathy in STZ-induced diabetic rats using a traditional Indian anti-diabetic plant, *Azadirachta Indica*[18] as well as the effects of *Syzygium cordatum* (Hochst.) [Myrtaceae] leaf extract on plasma glucose and hepatic glycogen in STZ-induced diabetic rats[20]. Clearly, the drug is used for various reasons which aim at benefiting mankind. As shown in previous studies[18-20], STZ is widely used to induce diabetes in animals, which subsequently become hyperglycemic. Thereafter, exami-

nations are done to determine the effects of the drug on various parts of the body including the eye. Also, the efficacy and potency of different plant extracts to increase plasma insulin and decrease blood glucose levels may be investigated.

STZ-induced ocular changes and the effects of plant extracts

In a recent study by Roy *et al.*[19], the effects of STZ-induced type 1 DM on protein concentration and on cation contents in ocular tissues of the rat compared to age-matched healthy controls was investigated. Eight weeks after the induction of diabetes, diabetic and control animals were characterized and the results are shown in Table 1.

Table 1: Characteristics of animals and weights of rat ocular tissues. Data were mean ±SEM, N=7, *Significantly different from age-matched control mice ($p < 0.05$).

Parameters measured	Age matched control	Diabetic
Weight of rat (g)	391.83 ± 37.91	190.12 ± 5.41*
Blood glucose level (mg dl^{-1})	92.40 ± 2.42	> 500*
Plasma insulin level (mg dl^{-1})	201.36 ± 572	4.80 ± 1.38*
Weight of cornea (mg)	8.03 ± 2.56	10.1 ± 0.89
Weight of lens (mg)	33.94 ± 5.26	38.44 ± 6.11
Weight of retina and sclera (mg)	33.11 ± 11.15	25.34 ± 11.74
Weight of lacrimal gland (mg)	118.07 ± 4.09	93.77 ± 2.4*

Adapted from Roy *et al.*[19]

The findings by Roy *et al.*[19] reveal that when compared to the control group of rats, those injected with STZ exhibited reduced plasma insulin and elevated blood glucose ($p < 0.05$ in all cases). It was also found that diabetic rats weighed significantly less than control animals ($p < 0.05$). In relation to ocular tissues, lacrimal glands from diabetic rats were also found to weigh significantly less ($p < 0.05$) than those from the control group. However, no significant changes in the weights of lens, cornea, sclera and retina were observed between diabetic and control animals.

In another study by Martin *et al.*[21] a systematic analysis of early changes in the mouse retina as a consequence of diabetes, applying several assessments of apoptosis and morphometric analyses was done. The study showed that the cells in the retinal ganglion cell layer of STZ-induced diabetic mice undergo apoptosis. By ten to twelve weeks, the thickness of retinae of diabetic mice was significantly less than that in age-matched control mice. In Table 2, data adapted from Martin *et al.*[21] shows the average weights and blood glucose levels of control and diabetic mice.

Table 2: Average weights and blood glucose levels of control and diabetic mice. The normal range (mg/dl) for blood glucose level is between 126.5 ± 12.6 and 140.2 ± 26.6. Data were presented as the mean ±SEM. *Significantly different from age-matched control mice ($p < 0.05$).

Duration of diabetes	Treatment Group	*n*	Weight (g)	Blood Glucose (mg/dl)
2	Control Diabtic	4 6	19.14 ± 0.96 16.74 ± 0.78	142.6 ± 26.8 438.7 ± 34.6*
4	Control Diabtic	4 6	20.93 ± 0.95 17.73 ± 0.80	129.3 ± 21.2 370.6 ± 48.4*
6	Control Diabtic	4 6	22.29 ± 0.96 19.62 ± 0.78	137.0 ± 30.0 370.6 ± 26.8*
8	Control Diabtic	4 6	24.10 ± 1.65 19.90 ± 2.32	119.5 ± 28.6 346.8 ± 21.5*
10	Control Diabtic	4 6	24.62 ± 0.96 21.46 ± 0.78	145.0 ± 30.0 22.81 ± 0.76
12	Control Diabtic	4 6	25.66 ± 0.98 22.81 ± 0.76	152.5 ± 26.8 466.0 ± 24.5*
14	Control Diabtic	4 6	27.33 ± 1.46 22.90 ± 1.02*	125.5 ± 21.8 436.0 ± 23.7*

Adapted from Martin *et al.*[21]

STZ is known to induce not only diabetes but also to stimulate diabetic retinopathy similar to early stage retinopathy in humans[18]. For the study of antidiabetic agents, STZ-induced hyperglycemia in rats is considered to be a good preliminary screening model[18]. STZ is a potent methylating agent for DNA and acts as nitric oxide donor in pancreatic cells. β-cells are particularly sensitive to damage by nitric oxide and free radicals because of their low levels of free radical scavenging enzymes. Major microvascular complications of DM include retinopathy, most common cause of adult blindness in developed countries[22].

A study by Hussain[18] found that the STZ injected rats developed not only diabetes but also showed an external sign of retinopathy after ten days, for example the eyes of diabetic rats looked opaque even from

outside. When the retinae were observed, they had dilated blood vessels and cotton wool spots, clearly indicating the presence of retinopathy.

Subsequent to the rats becoming hyperglycemic, various antidiabetic agents are tested to determine if they have any hypoglycemic and therapeutic effects. For instance, in a study by Brentjens and Saltz[1] it was found that STZ-induced diabetes can be treated by *neem* leaf extracts which control the blood sugar level as well as improving the lipid profile and the retinopathy itself. Also, Teng *et al*.[23] has recently studied the protective effect of puerarin, a major active ingredient extracted from the traditional Chinese medicine Gegen, on diabetic retinopathy in STZ-induced diabetic rats. Retinal histopathological observation and electron microscopic examination were performed. It was found that diabetic retinopathy induced by STZ was significantly reduced by the treatment of puerarin as judged by the reduction of morphological changes of inner nuclear and outer nuclear layer of the retina at any time-point. It was then concluded that puerarin exerts a significant protective effects against diabetic retinopathy in rats, likely regulating the angiogenesis factors expressions, and thus may be an effective and promising medicine for treatment of diabetic retinopathy.

Again, in a recent study by Musabayane *et al*.[20], it has been reported that oral administration of *Syzygium cordatum* leaf extract indicates a short-term hypoglycemic effect in STZ-induced diabetic rats. Furthermore, the results showed that long-term administration of the leaf extract is associated with increases in hepatic glycogen in STZ-diabetic rats. These observations taken together suggest that *Syzygium cordatum* leaf extract has a potential in the management of DM[20]. Whether this plant can exert any significant effects against retinal changes such as neovascularization, cotton wool spots and retinal edema is not known. *Syzygium cordatum* is a native tree of Southern Africa that is commonly found near streams, forest margins or in swampy spots. This evergreen tree has white, fluffy flowers that bloom from around August to November. It bears fruits that are edible berries which turn purple when ripe. In rural areas, the tree is used as a remedy for stomach ache, diarrhea and tuberculosis.

Also, in the study by Hussain[18], treatment of diabetic rats with water extract of *Azadiracta indica (neem),* following the induction of diabetes through the injection of STZ, showed considerable improvement in the glucose tolerance (see Table 3). The results in diabetic rats showed abnormal tolerance during Glucose Tolerance Tests (GTT). After treatment with water *A. indica* the glucose tolerance pattern was normal. Nowadays the level of glycosylated hemoglobin (HbA_{1C}) is considered as a more reliable index of glycemic control than fasting blood values. These values were therefore estimated in Table 4. In the untreated diabetic animals, the initial HbA_{1C} value increased to 9.4 ± 2.4% and to 6.4 ± 0.9 in the neem treated group, when compared with the normal value of 3.4 ± 0.2%. After treatment the HbA_{1C} level did not come down but remained more or less the same. In the *neem* treated group the HbA_{1C} value came down to 2.8 ± 0.1% which is in the lower range of normal value. Thus, water extract of *neem* leaves brought about very good glycemic control. Also, Table 5 shows that the gain in body weight in the untreated diabetic rats from 150 ± 6 g to 190 ± 6.09 (26.6%) was much less than that in normal rats from 160 ± 9.0 to 230 ± 9.0 (43.7%). But after treatment for four weeks with water extract of *neem* leaves, gain in weight in the diabetic treated animals was almost equal to that in normal (41.8%). Thus this study indicated how effective STZ is in in-

Table 3: Effect of treatment with water extract of *Azadirachta indica* leaves on glucose tolerance in diabetic rats after 16 weeks treatment. N=5, *$p < 0.05$ when compared with untreated diabetic group.

Plasma glucose mg/dl, mean ±SD					
Group	0 hr	0.5 hr	1 hr	1.5 hr	2 hr
Normal	95.5 ± 26.0	143.5 ± 13.9	137.2 ± 32.0	118.4 ± 11.3	106.0 ± 13.9
Diabetic untreated	170.6 ± 43.0	250.6 ± 79.0	271.0 ± 91.0	285.6 ± 82.0	274.0 ± 84.8
Diabetic treated	88.2 ± 25.6	114.0 ± 20.1	117.5 ± 18.0	102.7 ± 0.3	94.9 ± 26.0*

Adapted from Ali Hussain[18]

ducing diabetes as well as proving that extracts of plant of *Azadiracta* indica have some antidiabetic agents which can be used in the reversal of physical complications of diabetes including ocular conditions such as diabetic retinopathy.

Table 4: Effect of treatment with water extract of leaves *A. indica* on glycoslated hemoglobin (HbA_{1C}). The normal value for HbA_{1C} is 3.4 ± 0.2.

Group	**Initial**	**After 4 weeks treatment HbA_{1C} (%)**
Normal	3.4 ± 0.2	4.0 ± 0.1
Diabetic untreated	9.5 ± 2.4	9.3 ± 2.0
Diabetic + *neem*	6.4 ± 0.9	2.8 ± 0.1

Table 5: Effect of water extract of *Azadirachta indica* on the body weight of rats. *$p <$ 0.05 when compared with untreated diabetic group.

Group	**Initial body weight (g)**	**After 4 weeks treatment HbA_{1C} (%)**
Normal	160 ± 9.0	230 ± 9.0
Diabetic untreated	150 ± 6.0	190 ± 6.0
Diabetic + *neem*	158 ± 5.0	224 ± 9.0*

Adapted from Ali Hussain[18]

Method used to induce diabetes in rats

Experiments are usually performed on male or female rats of a specific body weight, usually between 250-300 g body weight. However, male rats are mostly preferred because they are significantly larger than their female counterparts[24]. The rats are then maintained under laboratory conditions of 12-hour light or 12-hour dark regime, and kept at biomedical resource centres. The rats receive daily both food (Epol-diet 4700, Epol, South Africa) and water *ad libitum*[20].

Induction of Diabetes Mellitus

Diabetes is induced in rats by intraperitonial injections of streptozotocin (60 mg/kg) in citrate buffer, pH 6.3. Animals that exhibit glucosuria after 24 hours, tested by urine test strips (Rapidmed Diagnostics, South Africa), are considered diabetic. Plasma glucose is then measured after one week to confirm the diabetic state. This can be done until such time when the diabetic state of the animal is confirmed. Vehicle (citrate buffer) injected animals are then used as controls[20].

Oral glucose tolerance tests (OGTT)

The rats are then divided into groups for Oral Glucose Tolerance Test (OGTT): non-diabetic control, treated non-diabetic, control STZ-diabetic, and treated STZ-diabetic rats. Animals in all groups are then orally administered glucose (1.4 g/100 kg) by means of a bulbed steel tube. In those animal groups in which the effect of the plant extracts is to be examined, oral glucose load is followed by administration of the plant extract (6 mg/100g). The median dose (6 mg/100g body weight) is generally used for this type of study. The plant extracts should be freshly dissolved in dimethyl sulfoxide (DMSO, 2ml) and normal saline (19 ml) before use[20].

Blood samples are then collected from the tail vein of the rat every 30 minutes over 2.5 hours for glucose determination using Elite glucometer. To establish whether the plant extract has activities comparable to drugs already in use, OGTT studies are conducted in separate groups of non-diabetic and streptozotocin (STZ)-diabetic rats that have been subcutaneously injected with metformin (50 mg/100g)[20].

Administration of plant extracts in STZ-induced diabetic rats

Plant extracts (6 mg/100g body weight) are orally administered into the stomach of STZ-diabetic rats every third day at a certain consistent time by means of bulbed steel needle for a ±4-week period. The groups of control non-diabetic and STZ-diabetic animals are then given an equal volume of DSMO-saline dissolution medium free of the crude extract. All animals should preferably be housed individually

in separate Makrolon polycarbonate metabolic cages to facilitate the measurement of amount of food and water intake daily. The weights of the animals should also be recorded once every week. However, an animal's life may be terminated by an overdose of halofane gas if it exhibit signs of pain before the actual time of sacrifice[20].

Evaluation of ocular tissue changes and the effects of plant extracts on the tissue changes

If the study focuses on the effects of STZ and the plant extracts on the ocular tissues, after at least four weeks, the eyes will be removed from each of the untreated and treated STZ-diabetic rats. The rats will be anaesthetized through halofane gas inhalation before the removal of the eyes. This will be done an hour before dissection takes place. The eyes will then be dissected into cornea, lens and retina. Fluorescein angiography may be used to examine the abnormalities in the retinal blood vessels of the rats. The fluorescent dye may be deposited into the blood system of the rat through a cardiac puncture using a 24 G 1" needle. The dye travels through the body including the eyes. A special camera may be used to photograph the retina as the dye passes through it. The photographs will show the changes that may occur in the retina and the location of those changes[25].

Histological and transmission electron microscope (TEM) techniques are then used to study the changes in the ocular tissues. The dissected ocular tissues should be dehydrated in graded ethyl alcohol series and embedded in Araldite CY212. Ultra thin sections should be contrasted with uranyl acetate and lead citrate for examination by TEM. Immunohistochemical staining method combined with light microscopy and Transmission electron microscopy may also be used to study proteins in the retinal tissues[25].

Conclusion and recommendation

Streptozotocin induces diabetes in laboratory animal models for scientific studies and breakthroughs in medicine. STZ use may also prove to be beneficial in the eye health care profession if considered for the studies of the effects of different plant extracts on their hypoglycemic effects on STZ-induced diabetic rats. However, collaborative studies with experts in physiological chemistry, pharmacology and those from biomedical professions may be vital in the success of such studies. In the light of the above background, it is recommended that a study on the efficacy and potency of some of the known South African indigenous plant extracts be conducted in STZ-induced diabetic rats. This will help to determine if they have any therapeutic effects on both DM and associated ocular complications and thereby improve the quantity of choices and cheaper medical treatment for both DM and its ocular complications.

References

1. Brentjens R, Saltz L. Islet cell tumors of the pancreas: the medical oncologist's perspective. *Surg Clin North Am* 2001 **81** 527-542.
2. Wang Z, Gleichmann H. GLUT2 in pancreatic islets: crucial target molecule in diabetes induced with multiple low doses of streptozotocin in mice. *Diabetes* 1998 **47** 50–56.
3. Schnedl WJ, Ferber S, Johnson JH, Newgard CB. STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells. *Diabetes* 1994 **43** 1326-1333.
4. Vavra JJ, Deboer C, Dietz A, Hanka LJ, Sokolski WT. Streptozotocin, a new antibacterial antibiotic. *Antibiot Annu* 1959 **7** 230–5.
5. Mansford KR, Opie L. Comparison of metabolic abnormalities in diabetes mellitus induced by streptozotocin or by alloxan. *Lancet* 1968 **1** 670-671.
6. Murray-Lyon IM, Eddleston AL, Williams R, Brown M, Hogbin BM, Bennett A, Edwards JC, Taylor KW (1968). Treatment of multiple-hormone-producing malignant islet-cell tumour with streptozotocin. *Lancet* 1968 **2** 895-898.
7. South African National Department of Health Directorate: chronic diseases, disabilities and geriatrics. National guideline: Prevention of Blindness in South Africa. December 2002.
8. Kumar PJ, Clark M. Diabetes mellitus and other disorders of metabolism. In: *Clinical Medicine*, Saunders (London) 2002 1069-1121.
9. Duane MJ, Cosgrove KE, Shepard RM, Aynsley-Green A, Lindley KJ. Hyperinsulinism in infancy: From basic science to clinical disease. *Physiol Rev* 2004 **84** 239-275.
10. Saltiel AR, Kahn CR. Insulin signaling and the regulation of glucose and lipid metabolism. *Nature* 2001 **414** 799-806.
11. Jullien J. Cardiac complications in Non-insulin Dependent Diabetes Mellitus. *J Diabetes Complicat* 1999 **11** 123-130.
12. Aiello LM. Perspectives on diabetic retinopathy. *Am J Ophthalmol* 2003 **136** 122-135.
13. Koevary SB, Nussey J, Kern TS. Long-term, topical administration increases the severity of retinal vascular pathology in streptozotocin-induced diabetic rats. *Optometry* 2007 **78** 574-581.
14. Zhu B, Wang W, Gu Q, Xu X. Erythropoietin protects retinal neurons and glial cells in early-stage streptozotocin-induced diabetic rats. *Exp Eye Res* 2008 **86** 375-382.

15. Abdouh M, Talbot S, Couture R, Hasséssian HM. Retinal plasma extravasation in streptozotocin-diabetic rats mediated by kinin B(1) and B(2) receptors. *Br J Pharmacol* 2008 **154** 136-143.
16. Sánchez-Chávez G, Hernández-Berrones J, Luna-Ulloa LB, Coffe V, Salceda R. Effects of diabetes on glycogen metabolism in rat retina. *Neurochem Res* 2008 **33** 1301-1308.
17. Lee S, Morgan GA, Harris NR. Ozagrel reverses streptozotocin-induced constriction of arterioles in rat retina. *Microvasc Res* 2008 **76** 217-223.
18. Ali Hussain HEM. Reversal of diabetic retinopathy in streptozotocin induced diabetic rats using traditional Indian antidiabetic plant, Azadirachta Indica (L.). *Indian J Clin Biochem* 2002 **17** 115-123.
19. Roy K, Harris F, Dennison SR, Phoenix DA, Singh J. Effects of streptozotocin-induced type 1 diabetes mellitus on protein and ion concentrations in ocular tissues of the rat. *Int J Diabetes Metabolism* 2005 **13** 154-158.
20. Musabayane CT, Mahlalela N, Shode FO, Ojewole JAO. Effects of Syzigium cordatum (Hochst.) [Myrtaceae] leaf extract on plasma glucose and hepatic glycogen in streptozotocin-induced diabetic rats. *J Ethnopharmacol* 2005 **97** 485-490.
21. Martin PM, Roon P, Van Ells TK, Ganapathy V, Smith SB. Death of retinal neurons in streptozotocin-incuded diabetic mice. *Invest Ophthalmol Vis Sci* 2002 **45** 3330-3336.
22. Kelvin R, Moss S. Visual impairment and diabetes, In Albert KR, De Fronzo Keen HP, Zimmet Eds. *International Textbook of Diabetes Mellitus.* Chi Chester; Willey 1992 1373-1384.
23. Teng Y, Cui H, Yang M, Song H, Zhang Q, Su Y, Zheng J. Protective effect of puerarin on diabetic retinopathy in rats. *Mol Biol Rep* 2008.
24. Ma H, Yang R, Thomas S, Kinnamon JC. Qualitative and quantitative differences between taste buds of the rat and mouse. *Neuroscience* 2007 **8** 5.
Ramos-Vara, JA. Techical Aspects of Immunohistochemistry. *Vet Pathol* 2005 **42** 405-426.