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Assessment of Atrial Natriuretic Peptide (ANP) Level and Relationship with Some Physiological Parameters

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Abstract*— The present study investigated the level of atrial natriuretic peptide (ANP) and relationship with some physiological parameters of diabetic male rats induced by streptozotocin. Thirty male rats (*Rattus norvegicus*) were used in this study, and divided into five groups (6 rats for each group). Group1, considered as the negative group, given standard food and water for a period of 30 days. Group 2, served as the positive control (diabetic control), given streptozotocin 60 mg/kg b.w. as a single dose with standard food and water for 15 days. Group 3, received streptozotocin (60 mg/kg) with standard food and water for 15 days, then treated orally with foxiga 1mg/kg every day for a period of 15 days. Group 4, given streptozotocin (60 mg/kg) with standard food and water for 30 days. Group 5, received streptozotocin (60 mg/kg) with standard food and water for 30 day, then treated with foxiga1mg/kg administrated orally every day for a period of 15 day. The results indicated a significant increase (P<0.05) of glucose level in groups (2,3,4,5) compared with group (1). There was a significant decrease of glucose level in group (2) compared with group (3). But there was a significant increase of glucose level in group (3) compared with group (4). There was a significant decrease in group (4) compared with group (5). The results indicated .a significant increase (P<0.05) in atrial natriuretic peptide in groups (2,3,4,5) compared with group (1).There was no significant decrease in level of ANP in group (2) compared with group (3). There was significant increase in level of ANP in group (3) compared with group (4). But there was no significant decrease in group (4) compared with group (5). The result indicated a significant increase (P<0.05) in level of ALP in groups (2,3,4,5). There was no significant decrease in group (2) compared with group (3). ALP level significant increase in group (3) compared with group (4). There was no significant decrease in group (4) compared with group (5). The result indicated significant increase (P<0.05) of level ALT and AST in groups (2,3,4,5) compared with group (1). There was significant decrease in group (2) compared with group (3). ALT and AST levels were significant increase in group (3) compared with group (4). There was significant decrease in group (4) compared with group (5). The result indicated a significant increase (P<0.05) in level of ALP in groups (2,3,4,5). There was no significant decrease in group (2) compared with group (234) compared with group (5).

***Keywords*— Atrial natriuretic peptide, Glucose, liver enzyme, rats**

I. INTRODUCTION

Diabetes mellitus (DM) refers to a group of multifactorial metabolic disorders characterized by elevated blood glucose levels that result from defects in the body's ability to produce and/or insufficiency of insulin action (Kharroubi and Darwish *el at*., 2015; Chala and Ali *el at*., 2016). Without enough insulin, the cells of the body cannot absorb sufficient glucose from the blood; hence blood glucose levels increase, which is termed as hyperglycemia. If the glucose level in the blood remains high over a long period of time, this can result in long term damage to organs, such as the kidneys, liver, eyes, nerves, heart and blood vessels (Ram *et al.*, 2015). Atrial natriuretic peptide (ANP) or atrial natriuretic factor (ANF) or atrial natriuretic hormone (ANH) or atriopeptin; is a circulatory peptide hormone; belongs to the family of endogenous polypeptide mediators of cardiac origin, natruretic peptide having another important members as brain natriuretic peptide (BNP), and Ctype natriuretic peptide (CNP) (Levin *et al*., 1998).

ANP has a characteristic 17 amino acids residue ring structure formed by an intramolecular disulfide bridge between two cysteine residues. The amino- and carboxyl terminal tails vary between the different natriuretic peptides, leading to polypeptides of 28 amino acids (ANP), 32 amino acids (BNP), and 53 amino acids (CNP) (Levin *et al*., 1998). It also exist as a pro-hormone with a relatively high molecular weight that is cleaved before release into the circulation (Magnusson *et al*., 2012). It functions through a natriuretic receptor, which generates cyclic-GMP (Guanosine mono-phosphate) to produce vasodilation and natriuresis (Das *et al*., 2005).

ANP secretion is also modulated by paracrine factors such as endothelin (Endothelial cell derived peptide), hormones as Angiotensin II, prostaglandins (PGF2α and PGE2 but not PGI2 stimulated ANP synthesis) and Vasopressin; Neurohumoral factors like Opiods, Corticotropin releasing factor, but Calcitonin gene-related peptide (CGRP) has inhibitory effect of ANP secretion (Levin *et al*., 1998; Lyssenko *et al*., 2008; Julic *et al*., 2013). Similarly, Nitric oxide (NO) is a potent vasodilator which is produced from L-argenine and increases the production of

cGMP in both cardiac muscle and vascular smooth muscle (Julic *et al*., 2014). aim of study diabetes is a modern disease has recently invaded the world and since lack of studies on the relationship between diabetes and atrial natriuretic peptide (ANP), the present study designed to measurement of ANP level and measurement of some biochemical parameters which included : blood glucose level and liver enzyme.

II. MATERIALS AND METHOD

A. Experimental animals

The present study was carried out at the College of Science, University of Thi-Qar , Iraq ,during the period from July to October. Adult male thirty six male *Rattus norvegicus* were used in the persent study, which divided into 5groups (6 animals for each group),weighted from 190-120g, were maintained on standard rat chow and tap water *ad* libitum with a12-h light/dark cycle in a quiet environment.

B. Induction of Diabetes Mellitus

The male rats intraperitoneally injected by a single dose STZ (Sigma, Chemical Co., St. Louis, MO), 60 mg/kg body wt, dissolved in sodium citrate buffer (0.1 mol/liter, pH 4.5) at a concentration of 20 mg/ml immediately before use. In order to prevent the onset of severe hypoglycemia they have received a solution of 10% glucose instead of normal drinking water over the 24 hours following the treatment. Streptozotocin induces diabetes within 3 days by destroying the beta cells and a mean blood glucose > 250 mg/dL. Diabetic animals and non-diabetic control group were kept in metabolic cages individually and separately and under feeding and metabolism control. Glucose in the blood of diabetic rats exceeded that of the non-diabetic control ones. Diabetes rats were treatment were with Forxiga drug (1mg /1kg/day) orally for 15 day. Animals used as normal control received standard rat pellet with *ad libitum*, distilled water till the end of the experiment.

C. Animals experimental design

Thirty male rats (190-210 gm) were randomly divided into five groups and placed in cages according to the groups, containing 6 rats per group as following.

1-Group1, considered as the negative control group, given standard food and water for a period of 30 days.

2-Group 2, served as the diabetic positive control, given streptozotocin 60 mg/kg b.w. as a single dose with standard food and water for 15 days.

3-Group 3, received streptozotocin (60 mg/kg) with standard food and water for 15 days, then treated with foxiga 1mg/kg administrated orally every day for a period of 15 days.

4- Group 4, given streptozotocin (60 mg/kg) with standard food and Water for 30 days

5- Group 5, received streptozotocin (60 mg/kg) with standard food and water. Then treated with foxiga1mg/kg administrated orally every day for a period of 15 day.

At end of experiment measured body weight of animals and then sacrificed.

D. Blood samples

Blood were drawn from each animal in the experimental groups, by heart puncture method after 12 hours fast. Using 60 gauge syringes, the sample was transferred into clean tube, left at room temperature for 15 minutes for clotting, centrifuged at 3000 rpm for 15 minutes, and then serum was separated and kept in a clean tube in the refrigerator at (-20$_o$C) until the time of assay.

E. Measurement of glucose level (Tietz et al .,2006)

In vitro test for the quantitative determination of glucose in serum, plasma, urine, and cerebrospinal fluid (CSF) on COBAS INTEGRA systems.

F. Measurement of atrial natriuretic peptide level (www.elabscience.com)

This ELISA kit applies to the in vitro quantitative determination of Human ANP concentrations in serum, plasma and other biological fluids.

G. Measurement of liver enzyme

1) Alanine aminotransferases level(ALT) (Greiling et al.,1995)

In vitro test for the quantitative determination of the catalytic activity of ALT (EC 2.6.1.2; L-alanine: 2-oxoglutarate aminotransferase) in human serum and plasma on COBAS INTEGRA systems. This method sheet describes the application for ALT without pyridoxal phosphate activation (test ALTL, 0-495). The application for ALT activated with pyridoxal phosphate is described in the method sheet Alanine Aminotransferases Pyridoxal Phosphate Activated.

2) Aspartate aminotransferase level(AST) (Bergmeyer et al .,1985 ; Eccls et al .,1989)

In vitro test for the quantitative determination of the catalytic activity of AST (EC 2.6.1.1; L-aspartate: 2-oxoglutarate aminotransferase) in human serum and plasma on COBAS INTEGRA systems.This method sheet describes the application for AST without pyridoxal phosphate activation (test ASTL,0-494). The application for AST activated with pyridoxal phosphate is described in the method sheet Aspartate Aminotransferase - Pyridoxal phosphate activated.

3) Alkaline phosphatase level(ALP) (Schumann et al .,2011)

In vitro test for the quantitative determination of the catalytic activity of alkaline phosphatase (EC 3.1.3.1; or tho-phosphoric monoester phosphohydrolase, alkaline optimum) in serum and plasma on COBAS INTEG systems.

III. RESULTS AND DISCUSSION .

A. Glucose level

The results indicated significant increase ($P<0.05$) of glucose level in groups (2,3,4,5) compared with group (1). There was significant decrease of glucose level in group (2) compared with group (3). But there was significant increase

of glucose level in group (3) compared with group (4). There was a significant decrease in group(4) compared with group(5).

TABLE1. Effect of forxiga on Serum Glucose of diabetic Male rats.

Parameters / Groups	**Glucose (mg/dl) Mean ± S.D**
Group 1	88.16 ± 8.75^{d}
Group 2	329.33 ± 62.25^{b}
Group 3	155.50 ± 14.23^{d}
Group 4	427.33 ± 230.02^{a}
Group 5	277.0 ± 42.70^{bc}
L.S.D	146.57

Uncontrolled blood glucose levels cause conditions of high (hyperglycaemia) or low (hypoglycaemia) blood sugar (Sacher *et al*., 2001). Diabetes symptoms identified by raised blood glucose, changed lipids, carbohydrate, and enhanced opportunity for diabetic difficulties and oxidative stress (Davis *et al*., 2006; Al-Assaf *et al*., 2012).

Low dose streptozotocin is known to induce rapid destruction of pancreatic*β*-cells leading to impaired glucose-stimulated insulin release and insulin resistance, both of which are marked features of type 2 diabetes (Sundaram *et al*. 2013). The elevated blood glucose is a result of reduced glucose uptake in muscle and adipose tissue and increased gluconeogenesis, hepatic glucose production and glycogen breakdown (Guignot and Mithieux 1999; Sundaram *et al*. 2013).The HFD/STZ-induced diabetic rats revealed signs of polyphagia, polyuria and polydipsia. At 1 mg/kg, dapagliflozin stimulated significant excretion of glucose in the urine in rats that were already glucosuric because of diabetes. Notably, 1 mg/kg, dapagliflozin stimulated a significant increase in urine glucose excretion in rats within 6 h post-dose, whereas in normal rats, glucose excretion was not significantly increased at this dose over 24 h. The data are suggestive of a greater impact of dapagliflozin in the diabetic kidney to reduce the renal glucose threshold (Katsuno *et al* .,2007). Over the first 6 h after ZDF rats were treated with dapagliflozin, significant dose-dependent plasma glucose lowering was observed, simultaneous with enhancement of glucose excretion in the urine. Rats show significant loss of insulin secretory function; previous studies have shown little acute effect on insulin level in this model with compounds acting by this mechanism (S.H., J.R.T., L.X., W.G. Humphreys, W.N.W., J.R.T., unpublished data). Significant correction of ambient hyperglycemia was achieved acutely by dapagliflozin in these experiments, with no evidence of hypoglycemia observed. Based on our observations that dapagliflozin administration resulted in acute glycosuria accompanied by plasma glucose lowering in fed diabetic rats, expected that hyperglycemic fasting glucose levels would be lowered in rats as well. While we anticipated that elevated FPG could be acutely lowered by this mechanism, it was measured only in the 2-week studies and only on days 15 of treatment. The data confirm that dapagliflozin reduced FPG levels in rats over the treatment period.

Fed plasma glucose levels were measured only on day 15 in the first chronic study, and dose-dependent reductions were observed; however, the acute experiments in rats demonstrate that this reduction can occur within hours after a single oral dose. Despite the fact that these rats excreted large amounts of glucose over the course of the 2-week study (Devenny *et al* .,2007).

B. Atrial natriuretic peptide

The results indicated a significant increase ($P<0.05$) in atrial natriuretic peptide in groups (2,3,4,5) compared with group (1).There was no significant decrease in level of ANP in group (2) compared with group (3). There was significant increase in level of ANP in group (3) compared with group (4). But there was no significant decrease in group(4)compared with group(5).

TABLE2. Effect of forxiga on Serum Glucose of diabetic Male rats.

Parameters / Groups	**ANP(pg/ml) Mean ± S.D**
Group 1	62.16 ± 6.49^{c}
Group 2	131.33 ± 21.77^{ab}
Group 3	118.16 ± 28.73^{b}
Group 4	173.0 ± 85.32^{a}
Group 5	141.50 ± 39.03^{ab}
L.S.D	44.43

Al-though an increase of blood glucose may increase plasma volume and thus also ANP secretion and plasma ANP concentrations. The increases over the control at both time intervals were 100%, similar to that reported in 10-week STZ-diabetic rats (Choi *et al* ., 1994). Such increases in plasma ANP concentrations may down-regulate receptors in both the kidney and the endothelium (Valentin *et al*.,1994). Although the significance of an increase in plasma ANP concentration in response to hyperglycaemic volume expansion is easy to understand, the underlying mechanism is by no means clear.

Plasma ANP levels are also increased in patients with insulin-dependent diabetes mellitus and diabetic nephropathy(Shinoda *et al*.,1990;Lieberman *et al* .,1999). This increase in ANP levels could be a consequence of the plasma volume expansion present in both STZ-diabetic rats (Allen *et al* .,1990;Benigni *el at* .,1990) and patients with diabetes mellitus.

Despite increased plasma levels of ANP, the response to maneuvers known to stimulate the release of the peptide, such as saline infusion, is impaired (Hebden *et al* .,1989;Patel *et al* .,1989). This has been interpreted as a consequence of partial exhaustion of the storage pool of the hormone as also demonstrated by morphologic studies in which the abundance of secretory granules is decreased in atrial cardiomyocytes obtained from diabetic rats (Hebden *et al* .,1989 ; Chua *et al* .,1988). Blunted glomerular and tubular responses to volume expansion(Patel *et al* .,1990;Patel *et al* .,1989) and exogenous ANP infusion (Benigni *et al* .,1990) have been reported in rats with STZ-induced diabetes. Similarly, a decreased natriuretic response

to endogenously secreted ANP has been shown in type 1 diabetic subjects after head-out water immersion (Lieberman *et al* .,1991).

C. Level of liver enzyme

The result indicated significant increase ($P<0.05$) of level ALT and AST in groups (2,3,4,5) compared with group (1) .There was significant decrease in group (2) compared with group (3) . ALT and AST levels were significant increase in group (3) compared with group (4). There was a significant decrease in group (4) compared with group (5).

In same table (3) the result indicated a significant increase ($P<0.05$) in level of ALP in groups (2,3,4,5). There was no significant decrease in group (2) compared with group (3). ALP level significant increase in group (3) compared with group (4). There was no significant decrease in group (4) compared with group (5).

Table (3): Effect of forxiga on Serum Glucose of diabetic Male rats.

Parameters / Groups	**ALT(U/L) Mean ± S.D**	**AST(U/L) Mean ± S.D**	**ALP(U/L) Mean ± S.D**
Goup 1	46.83±6.24^{d}	31.83±2.13^{e}	45.88±4.56^{c}
Group 2	123.33±5.53^{b}	62.16±7.62^{c}	70.33±4.41^{b}
Group 3	102.83±13.10^{c}	52.16 ±6.96^{d}	68.83±0.87^{b}
Group 4	139.83±6.21^{a}	89.66±10.57^{a}	92.16±9.90^{a}
Group 5	126.16 ±4.79^{b}	74.0 ±12.31^{b}	88.33±1.14^{a}
L.S.D	7.67	8.53	6.87

The aminotransferases (AST and ALT) levels were significantly increased in the liver of STZ-treated animals and ALP levels were determined to evaluate the hepatic functions (Degirmenchi *et al.* 2002). The increase in aminotransferases levels may be due to the cellular damage in the liver caused by STZ-induced diabetes. Although ALT is also present in mitochondria and cytosol, the mitochondrial form is low in activity and is very unstable. The detailed mechanism by which enzymes are released from the cytosol and mitochondria of hepatocytes is not completely known. Experimental studies have shown that subtle membrane changes are sufficient to allow passage of intracellular enzymes to the extracellular space (Garella,1997).Very large concentration gradient between the hepatocytes and the sinusoidal space usually exists for enzymes.

Cell damage increases permeability causing cytosolic isoenzymes to spill into the sinusoids and from there into the peripheral blood (Garella). Baxter & Schofield reported an increase in AST and ALT in diabetics. It had been shown by Rogers *et al.*(1986), that mitochondrial activity was decreased 53 % per gram of diabetic liver and cytoplasmic AST activity was increased 3-4 fold in STZ diabetic rats. (Voss *et al.,* 1988) proposed that STZ in hyperglycemic animals caused a time dependent rise in AST, ALT, and ALP levels. The work of (Barneo *et al*1990) showed that STZ induced diabetes in rats produced alterations in hepatic functions as described in poorly controlled diabetics. This alteration in hepatic function may be because of increase activity and mRNA levels of araginase as reported by (Salimuddin *et al.*,2008) in their study. Hepatocellular dysfunction was evaluated by the AST and ALT activities in plasma. The results of our study showed that STZ diabetes in rats produced alterations in the hepatic functions as well as structure of hepatocytes. The increase in the levels of AST and ALT in diabetic rats after 15 and30 day treatment was also reported by many other workers (Zhang *et al*., 1995; Isogai *et al*., 1997). Okada *et al*. (1997) reported that AST activity was lower than the amount of enzyme in diabetic rat tissues. It is suggested that this may be due to the inactivation of cytosolic AST in the diabetic rat tissues by a glycation reaction, accompanied by impairment in glucose utilization in STZ induced diabetes. In our study, the levels of ALP were significantly increased in the liver of treated animals.

The mechanism of release of membrane bound enzyme is less well understood. Alkaline phosphatase is a membrane bound glycoprotein enzyme. It is present in highest concentrations in the sinusoids and in the endothelium of the central and periportal veins; smaller concentrations occur in the biliary canaliculi. Barneo *et al.*evaluated cholestasis by plasma ALP activity in STZ induced diabetes and their results showed that ALP levels were raised. Leibovitch *et al.* (1991) observed increased levels of serum ALP in pathological conditions involving the kidneys and liver. Increase in the levels of ALP in diabetic rats was also reported by Ramesh & Pugalendi. Pseudocholinesterase levels in serum are useful as test of liver functions.

In the present study, AST and ALT decreased in both groups after treatment with dapagliflozin. This is because the mechanism of the SGLT2i on theimprovement of liver function is believed to be due to the recovery of metabolic imbalance by controlling body weight and lipid composition while ALT is a parameter associated more with NAFLD and metabolic diseases than with AST (Sorbi *et al* .,1999). Furthermore, after multivariate analysis to correct for other metabolic parameters such as body weight change, ALT reduction was still significant in the dapagliflozin group. This result suggests that in addition to controlling body weight, SGLT2i also have other own effects on reducing ALT in NAFLD. Although ALT doesn't correlate well with severity of NAFLD, ALT is associated with metabolic disease and cardiovascular risk factors in NAFLD. Therefore reduction and normalization of ALT can be used to assess the effect of SGLT2i on NAFLD (Schindhelm *el at* .,2006 ; Bea *et al* .,2015).

IV. Conclusion

1- The study indicated increase of atrial natriuretic peptide in DM patients, it is means elevate of may atrial natriuretic peptide be a risk factors.

2- The current study recorded the effect of diabetes on renal function, oxidant – antioxidant markers and lipid profile.

3-There was negative correlation between atrial natriuretic peptide and HDL, creatinine and albumin.

4-There was weak correlation between atrial natriuretic peptide and glucose, TG and ALP.

5-There was moderate correlation between atrial natriuretic peptide and cholesterol, VLDL, ALT, AST, MDA and urea.

5-Patients with severe renal impairment, end-stage renal disease, on dialysis, or who have a history of serious hypersensitivity reaction to dapagliflozin should not use this medication.

REFERENCES

Al-Assaf A.H., 2012. Antihyperglycemic and antioxidant effect of ginger extract on streptozotocin-diabetic rats, Paki, J. Nutriti., 11: 1107–1112.

Allen, T. J., M. E. Cooper, R. C. O'Brien, L. A. Bach, B. Jackson, and G. Jerums. 1990. Glomerular filtration rate in streptozotocin-induced diabetic rats. Role of exchangeable sodium, vasoactive hormones, and insulin therapy. Diabetes. 39:1182-1190.

Benigni, A., N. Perico, J. Dadan, M. Gabanelli, M. Galbusera, P. Boccardo, T. Mennini, and G. Remuzzi. 1990. Functional implications of decreased cortical atrial natriuretic peptide binding in experimental diabetes. Circ. Res. 66:1453-1460.

Bergmeyer HU, Hørder M, Rej R. Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentrationof enzymes. Part 2. IFCC Method for aspartate aminotransferase. J Clin Chem Clin Biochem 1986;24:497-510.

Chala, T. and Ali, G. (2016). Recent advance in diabetes therapy: pancreatic beta cell regeneration approaches Diabetes Manag 6(6) 108–118.

CHOI, K.C., H.C. PARK & J. LEE. 1994. Attenuated release of atrial natriuretic peptide and vasorelaxation in streptozotocin-induced diabetic rats. J. Korean Med. Sci. **9:** 101–116.

Das SR, M. H. Drazner, D. L. Dries, G. L. Vega, H. G. Stanek,S. M. Abdullah, R. M. Canham, A. K.Chung, D. Leonard, F. H. Wians and J. A. De-Lemos, 2005. Impact of body mass and body composition on circulating levels of natriuretic peptides: results from the Dallas Heart Study. *Circulation*, 112: 2163-68.

Davis S., 2006. Insulin, oral hypoglycemic agents and the pharmacology of endocrine pancreas, McGraw-Hill, Medical Publishing Division. 1037–1058.

Degirmenchi, I.; Kalender, S.; Ustuner, M. C.; Kalender, Y.; Gunes, H. V.; Unal, N. & Basaran, A.The effects of acarbose and Rumex patientia on liver ultrastructure in streptozotocin-induced diabetic (type-II) rats. *Drugs Exp. Clin. Res., 28*:229-34, 2002.

ECCLS. Determination of the catalytic activity concentration in serum of L-aspartate aminotransferase (EC 2.6.1.1,ASAT). Klin Chem Mitt 1989;20:198-204.

Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer Verlag 1995.

Hebden, R. A., M. E. Todd, and J. H. McNeill. 1989. Relationship between atrial granularity and release of atrial natriuretic factor in rats with diabetes mellitus. Am. J. Physiol. 257:R932-R938.

Isogai, M.; Kurota, H. & Yamaguchi, M. Hepatic calcium binding protein regucalcin concentration is decreased by Streptozotocin diabetic state and ethanol injection in rats. *Mol. Cell Biochem., 168(1-2)*:67-72, 1997.

Jujic, A., P. Nilsson, G. Engstrom, B. Hedblad, O. Melander and M. Magnusson, 2013. Atrial natriuretic peptide and type 2 Diabetes development, evidence of causal association from the prospective Malmo diet and cancer study. Europian Heart Journal, 310: P5048.

Jujic A , P. M. Nilsson, G. Engström, B. Hedblad, O. Melander and M. Magnusson, 2014. Atrial natriuretic peptide and type 2 Diabetes development –Biomarker and genotype association study. PLoS One. 9(2): e89201.

Kharroubi, A. and Darwish, H. (2015). Diabetes mellitus: The epidemic of the century. World Journal of Diabetes. 6 (6):850-867.

Lieberman, J. S., L. Parra, L. Newton, J. D. Scandling, N. Loon, and B. D. Myers. 1991. Atrial natriuretic peptide and response to changing plasma volume in diabetic nephropathy. Diabetes. 40:893-901.

Levin ER, D. J. Gardner and W. K. Samson, 1998. Natriuretic peptides. N Engl J Med., 339: 321–28. Patel, K. P., and P. L. Zhang. 1989. Reduced renal responses to volume expansion in streptozotocin-induced diabetic rats. Am. J. Physiol. 257:R672-R679.

Ram, M.; Singh, V.; Kumawat, S. Kumar, D. Lingaraju, M.C. Uttam, S. Rahal, A. Tandan, S.K. (2015). Deferoxamine modulates cytokines and growth factors to accelerate cutaneous wound healing in diabetic rats. Eur J Pharmacol 764:9–21.

Sacher, R.A., Mcpherson R.A., 2001. Widmann's Clinical Interpretation of Laboratory Tests. 11th Edn. F.A. Davis Company. 13–79.

Schwab, K.O., Doerfer, J., Hecker, W., Kordonouri, O., Peter, B., Holl, R.W., (2006). Spectrum and prevalence of atherogenic risk factors in 27,358 children, adolescents, and young adults with type l diabetes: cross-sectional data from the German diabetes docum entation and quality management system (DPV). Diabetes Care 218-225.

Schumann G, Klauke R, Canalias F, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37° C. - Part

9. Reference procedure for the measurement of catalytic concentration of alkaline phosphatase. Clin Chem Lab Med 2011 Sep;49 (9):1439-46.

Shinoda, T., M. Ishihara, F. Kurimoto, T. Aizawa, K. Hiramatsu, T. Shirota, N. Takasu, and T. Yamada. 1990. Elevated plasma atrial natriuretic peptide level in the early phase of microalbuminuria in patients with non-insulindependentdiabetes mellitus. Clin. Nephrol. 34:202-207.

Tietz NW, ed. Clinical Guide to Laboratory Tests, 4th ed. Philadelphia: WB Saunders Co 2006;444-451.

VALENTIN, J.P., L.A. SECHI & M.H. HUMPHREYS. 1994. Blunted effect of ANP on hematocrit and plasma volume in streptozotocin-induced diabetes mellitus in rats. Am. J. Physiol. **266** (2 Pt 2): R584–R591.

Zhang, A.; Vetromenn, J.; Van Gaal, L. & De Leeuw, I. Effects of pravastatin on lipid levels in vitro oxidizability of non-HDL lipoproteins and microalbuminuria in IDDM patients. *Diabetes Res. Clin. Pract., 29(3)*:189-94, 1995.