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METHANOL EXTRACT OF THE LEAVES OF *ICHNOCARPUS FRUCTESCENS* R. BR. (APOCYANACEAE) MODIFIES ANTIDIABETIC AND HYPOLIPIDEMIC CONDITION IN STREPTOZOTOCIN, TRITON AND HIGH FAT DIET INDUCED EXPERIMENTAL ANIMALS

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ABSTRACT

The present study was designed to evaluate the effect of methanol extract of *Ichnocarpus fructescens* on the streptozotocin induced diabetic animals. The extract treated animals were failed to show significant reduction in plasma glucose level. However, the treatment had significantly reduced the plasma triglycerides and total cholesterol levels. Based on these results, another study was conducted to decipher the effect of methanol extract of *I.fructescens* on lipid metabolism. In this study, the plasma total cholesterol and triglyceride levels of *I.fructescens* methanol extract animals had shown a significant 47.17%, 66.36% and 78.27% reduction in triglyceride levels at 100, 200 and 400 mg/Kg respectively. The extract treatment with this extract also reduced the total cholesterol by 40.83%, 49.65% and 53.04% at 100, 200 and 400 mg/Kg respectively in triton (Tyloxapol) induced hyperlipidemic animals. Therefore , a long term study was conducted to determine the efficacy of the extract in High-fat induced obese animals. In this current study, the methanol extract treatment had shown a significant 13.69% reduction in triglyceride levels. In an acute toxicity study, the treatment with the methanol extract of *I.fructescens*, at the doses of 2 and 3g/Kg, the extract showed slight abdominal writhing. But the animals had recovered within 2hrs after treatment and there was no mortality. This indicates the less toxic effect of

this extract. So, the fractionation of this extract will yield some novel prototypes to treat lipid related metabolic disorders.

KEYWORDS: *Ichnocarpus fructescens*, hypolipidemic, antidiabetic, streptozotocin, Tyloxapol, HFD.

INTRODUCTION

Worldwide, over 1200 species of plants have been reported as traditional medicine for diabetes. Some of these plants have been evaluated in laboratories and in a number of cases, their efficacy has been confirmed for insistence. Specific chemical constituents of these plants such as polysaccharides, alkaloids, triterpenoids and xanthones are believed to be responsible for the hypoglycemic effects and they can be related to actions including insulin release and increased glucose metabolism in the body periphery among others. The worldwide epidemic of type 2 diabetes (NIDDM) has been stimulating the search for new concepts and targets for the treatment of this incurable disease. Globally diabetes has shadowed the spread of modern lifestyle and it can be linked to an increase overweight and sedentary population (Vats et al., 2005).

Ichnocarpus fructescens (L) R.Br. is an extensively branched climber of the family Apocynaceae. It is a common evergreen, laticiferous, woody climber with simple opposite leaves. Flowers were greenish white and fruit is a capsule (Matthew,1991). The common name of this plant is Udarkodi, Palvalli (Tamil) (Sivaranjan,1995). It occurs throughout India upto 1200MSL. In Kerala, *I.fructescens* and *H.indicus* are commonly used as the two constituents of saribadvayam. The roots are sweet, refrigerant, febrifuge, aphrodisiac, alterant,diaphoretic, diuretic, depurative, demulant and tonic. They are useful in vitiated conditions of pitta, burning sensation, hyperdisia, fever, seminal weakness, nephrolithiasis, strangury, skin disease, leprosy, diabetes, cephalagio and general weakness. The roots are being used to treat diabetes by many tribes (Chopra *et al*., 1995). The current study investigates the antidiabetic and hypolipidemic effect of the methanol extract of *I.fructescens*.

MATERIALS & METHODS

Plant Material: Leaves of *Ichnocarpus fructescens* were collected and the botanical identity of the plant material was confirmed by the Taxonomists at Department of Botany, Loyola College. The fresh plant materials were washed thoroughly with water and crushed. 1Kg of fresh plant material was extracted with 3L of 3X methanol by cold percolation method. The

combined extracts were concentrated under reduced pressure. The extracts were concentrated under reduced pressure. The extracts were stored at 4^{o}C, till assayed. The preliminary phytochemical tests were carried out for the effective extracts using standard phytochemical methods (Harborne, 1998).

Animals: Male albino rats weighing 140-170g were kept in polypropylene cage under controlled temperature, humidity and 12/12 hour light/dark cycles. The animals were fed *ad libitum* with normal laboratory chow diet containing 74% carbohydrates, 22% protein and 4% fat. This study got clearance from the Institutional Animal Ethical Committee (IAEC-ERI-LC-11).

Fixation of Doses: The doses for the study were based on Irwin test for the extracts at 1, 2 and 3g/Kg (Roux *et al*., 2004). The extracts were dissolved in a vehicle containing 0.2% polysorbate-80, 0.5% Sodium carboxy methyl cellulose, 0.9% Sodium chloride, 0.9% benzyl alcohol and 97.2% distilled water (Lee, 2001). Non-diabetic male rats weighing 150±5g were used in this study. The experimental animals were appropriately grouped and placed in Laboratory for acclimatization. Three animals were used for each group. On the morning of the experiment day, food and water were removed from the cages. The the animals were treated orally with the vehicle or the extracts. At 0, 15, 30, 60, 120, 180 minutes and 24hours after treatment of the extracts behavioral alterations were observed.

Assessment of antidiabetic effect of the methanol extract *I.fructescens* in Streptozotocin induced diabetic rats

Induction of Diabetes: Six week old male albino rats 145±5g were used in the study. Diabetes was induced according to Wu and Huan (2008), but the amount of streptozotocin used to induce diabetes was 40mg/Kg. On 10th day, after the induction , blood samples were collected for the estimation of plasma glucose levels. Animals having plasma glucose levels=200mg/dL were included in the study.

Treatment with extracts

Based on the body weight and plasma glucose levels, the animals were assigned randomly into five groups with six animals in each group. Group-I was Normal rats treated with vehicle; Group-II was Diabetic rats treated with vehicle; Group-III was diabetic rats which received Glibenclamide (5mg/Kg) (Habibuddin *et al*., 2008); Group-IV, V & VI was Diabetic rats which received *I.fructescens* methanol extract of 50mg/Kg, 100mg/Kg & 200mg/Kg

respectively. Treatment were given orally between 12.00 p.m. to 2.00 p.m. daily once regularly for four weeks.

At the end of experiment, the animals were sacrificed and the blood was collected on EDTA containing tubes. Liver samples were sliced and snap-freezed in liquid N_2, stored at -70^{o}C for the analysis of glycogen (Carroll *et al*.,1956). Plasma glucose levels were measured by GOD-POD method. Total cholesterol levels were measured by CHOD-POD method. Triglyceride levels were measured by GPO-POD method. Urea and Nitrogen levels were measured by DAM method (Wybenga *et al*., 1972). Creatinine levels were measured by Jaffe's Kinetic method.

Assessment of hypolipidemic effect of methanol extract *I.fructescens*

Effect of methanol extract of *I.fructescens* in Triton WR-1339 induced hyperlipidemic animals: This study was performed according to the method of Vogel (Vogel, 1997). Male wistar rats weighing 175-200g were used in this study. Triton WR-1339 dissolved in phosphate buffered saline at pH 7.4 and injected intraperitonealy to the animals at the concentration of 200mg/Kg. Then the animals were treated with different concentration of extract (100, 200 & 400mg/Kg) or with fenofibrate (65mg/Kg). The animals in control group have received vehicle alone. After seven hours, plasma samples were collected from orbital sinus and Total cholesterol and Triglyceride levels were measured by CHOD-POD method and GPO-POD method.

Effect of methanol extract of *I.fructescens* in High fat diet induced hyperlipidemic animals: This study was performed according to the method of Guido and Joseph (1992). Male wistar rats weighing 170-200g were used in this study. The animals were fed with a powdered pellet diet containing 20% saturated fat for 2 weeks. 'Dalda' available from local market was used as a source of saturated fat. The animals randomly divided into four groups of animals containing four animals per group. Group I was normal animals fed with commercial pellet feed, Group II was disease control animals fed with high fat diet, Group III was positive control animals fed with high fat diet and treated with Fenofibrate (65mg/Kg) and Group IV was animals fed with high fat diet and treated with the methanol extract of *I. fructescens*. The treatment was given orally for 15 consecutive days. Total cholesterol and Triglyceride levels were measured for every 5 days using enzymatic, spectrophotometric methods.

STATISTICAL ANALYSIS

One way ANOVA and Student's *t*-test (SPSS program; Version 12.0) were used to compare the data with the level of significance set at $P = 0.05$.

RESULT & DISCUSSION

The present study reports for the first time, the effect of *I.fructescens* methanol extract as a hypolipidemic and antihyperglycemic agent, thus scientifically validating the traditional claim. For the selection of doses, an acute toxicity evaluation was carried out according to the method of Roux *et al.,* 2004. At the doses of 2 and 3g/Kg, the extract showed abdominal writhing. But the animals had recovered within two hours after treatment and there was no mortality.

The methanol extract of *I.fructescens* gave positive results for Lieberman Burchard test, Nollers test and Anthrone test. This indicated the presence of Steroids, Terpenoids and Carbohydrates in the extract. Further it gave positive results for Tannins, Carboxylic acids, Saponins and Alkaloids.

The effect of methanol extract of *I.fructescens* on the body weight of streptozotocin induced diabetic animals shown in the table 1. The diabetic control animals had shown a 10.60% reduction in body weight from zero week values. The Metformin treated animal had shown 1.98% increase in body weight. The extract treated animals had shown only 4.43%, 4.489% and 1.17% decrease in the body weight when compared with the zeroth day values. This can be correlated with the reduction proteolysis by the reduction in serum urea nitrogen levels (Oliveira *et al.,* 2008).

The treatment with the extract of *I.fructescens* had reduced magnitude of the loss in body weight, the extracts were failed to reduce the plasma glucose levels, in a dose dependent manner (Table 1). Kumarappan and Mandal (2007) had reported that the polyphenol rich extract of the roots of *I.fructescens* had reduced the plasma glucose levels in a significant manner. Babu *et al.,* (2007) had reported that the antidiabetic effect of the methanol extract of *I.fructescens* might be due to the modulation of insulin secretion. In this present study, there is no reduction in plasma glucose levels was observed. This may be due change in the plant part used for extraction or some other unknown factors. It was already reported that decotion prepared from *Eugenia jambolana* of Indian region had reduced the plasma glucose levels (Achrekar *et al*., 1991; Grover *et al*., 2000, 2002).

Table-2 & Figure-1 shows the effect of treatment on the total cholesterol and triglycerides level of streptozotocin induced diabetic animals. In this study, the treatment with the extract of *I.fructescens* had significantly reduced the total cholesterol and triglycerides levels. The extract treated animals had shown 23.91%, 28.02% and 19.08% of reduction in plasma cholesterol levels compared to the diabetic control at 50, 100 and 200mg/Kg doses respectively. Likewise, the treatment with the extract had results 8.87%, 6.31% and 10.65% reduction in triglyceride levels at 50, 100 and 200mg/Kg doses respectively. Further, the treatment with this extract had significantly reduced the lipid peroxidase levels. In 2012, Saravanan & Ignacimuthu had reported the hexane extract of *I. frutescens* reveals medicinal property for antiobesity effect. Based on these results, another study was conducted to investigate the effect of methanol extract of *I.fructescens* on lipid metabolism. Tyloxapol (Triton WR-1339) is a nonionic surfactant which induces a transient hyperlipidemic condition and used as inducer for screening of products with hypolipidemic effect in laboratory animals (Vogel and Vogel, 1997). Tyloxapol increases the plasma lipid levels by increasing the hepatic LDL synthesis.

In this study, the plasma total cholesterol and triglyceride levels of *I.fructescens* methanol extract on animals had shown a significant 47.17%, 66.36% and 78.27% reduction in triglyceride levels at 100, 200 and 400 mg/Kg respectively. The extract treatment with this extract also reduced the total cholesterol by 40.83%, 49.65% and 53.04% at 100, 200 and 400 mg/Kg respectively (Table-3 & Figure-2). Therefore, a long term study was conducted to determine the efficacy of the extract in High-fat induced obese animals. In this current study, the methanol extract treatment had shown a significant 13.69% reduction in triglyceride levels (Table-4) and also reduced the hepatic lipid content and cholesterol levels in a significant manner. Diabetes mellitus is a chronic metabolic disorder affecting approximately 4% population world wide and the rate is expected to increase by 5.4% in 2025 (Kim *et al*., 2006). The world wide prevalence of diabetes was expected to increase by 42% from 51-72 million in the developed countries and by 170% from 84-228 million in the developing countries by the year 2025 (King *et al*., 1998). Diabetes is a heterogenous clinical entity, characterized by high blood glucose levels or hyperglycemia arising from a deteriorated tissue response to the biological effects of insulin (Salitiel and Kahn, 2001) and impaired glucose induced insulin secretion (Bell and Polonsky, 2001). The major risk determinants of coronary heart disease (CHD) are cigarette smoking, hypertension, diabetes and hypercholesterolemia. Of these hypercholesterolemia appears to be the most important in

terms of causation, because in some countries where the average level of cholesterol in the middle aged men is remaining low, the risk of CHD is also low in people with diabetes mellitus (Jarrett, 1978). The CARE trial (Warnica, 2004) had demonstrated that the reduction in 28% of reduction in cholesterol levels assosciated with the 15% reduction in the risk of myocardial infarction, even in diabetic condition. The detailed investigation of this present study supports the use of this plant in traditional medicine and the fractionation of this extract yields some novel prototypes to treat lipid related metabolic disorders.

Table-1 Effect of *Ichnocarpus fructescens* methanol extract on Body weight and Plasma glucose of the STZ diabetic animals after four weeks treatment

Groups	Body Weight		Plasma glucose	
	0th Week	4th Week	0th Week	4th Week
Diabetic control	165.00±4.00	147.50±3.22	268.73±22.80	416.62±56.47
Metformin HCl (120mg/Kg)	185.00±10.20	188.75±13.90*	254.58±32.14	229.76±57.11
***I.fructescens* methanol extract (50mg/Kg)**	197.50±10.30	188.75±8.26*	272.28±23.18	371.68±66.61
I.fructescens methanol extract (100mg/Kg)	173.75±6.88	165.00±7.35	254.16±1.59	483.75±34.80
I.fructescens methanol extract (200mg/Kg)	170.00±6.45	168.00±5.00*	252.70±1.93	453.33±19.95

Values are (in gms) Mean±SEM for four animals; *- indicates values vary significantly from corresponding diabetic control values

Table-2 Effect of *Ichnocarpus fructescens* methanol extract on total cholesrerol, triglycerides, lipoperoxidase and glycogen levels of the STZ diabetic animals

Groups	Diabetic control	Metformin HCl (120mg/Kg)	*I.fructescens* methanol extract (50mg/Kg)	I.fructescens methanol extract (100mg/Kg)	I.fructescens methanol extract (200mg/Kg)
Total Cholesterol (mg/dL)	131.01±4.80	102.84±4.36**	99.68±4.24**	94.30±1.50**	106.01±0.94**
Triglycerides (mg/dL)	160.44±3.40	137.97±6.75*	146.20±6.54*	150.31±4.36	143.35±4.51*
Lipoperoxidase (mM/L)	65.21±5.88	43.47±2.85*	37.17±5.27*	31.70±5.59**	20.77±3.82**
Liver glycogen (mg/g))	17.51±2.55	36.10±5.22*	12.65±3.86	14.10±1.92	20.01±2.00

Values are (in gms) Mean±SEM for four animals; *- indicates values vary significantly from corresponding diabetic control values

Table-3 Effect of *Ichnocarpus fructescens* methanol extract on total cholesrerol and triglycerides levels of Triton WR-1339 induced hyperlipidemic animals after seven hours

Groups	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)
Triton Control	161.10±15.06	710.47±33.50
Fenofibrate (65mg/Kg)	60.88±5.53*	244.02±24.60*
***I.fructescens* methanol extract (100mg/Kg)**	95.32±12.32*	375.32±25.32*
I.fructescens methanol extract (200mg/Kg)	81.11±4.00*	238.99±26.30*
I.fructescens methanol extract (400mg/Kg)	75.65±5.32**	154.32±15.32**

All values (mg/dL) Mean±SEM for four animals; * - indicates values deviated significantly from control values

Table-4 Effect of *Ichnocarpus fructescens* methanol extract on serum total cholesrerol and triglycerides levels in High fat diet (HFD) induced hyperlipidemic animals

Groups	Total Cholesterol (mg/dL)		Triglycerides (mg/dL)	
	5th Day	15th Day	5th Day	15th Day
HFD control	121.39±4.33	127.16±8.64	193.65±8.10	202.77±21.47
I.fructescens methanol extract (200mg/Kg)	117.28±4.45	132.92±9.26	182.93±18.83	175.00±5.45*
Fenofibrate (65mg/Kg)	135.49±6.07	139.81±7.89	189.28±42.32	152.97±17.23**

All values (mg/dL) Mean±SEM for four animals; * - indicates values deviated significantly from control values

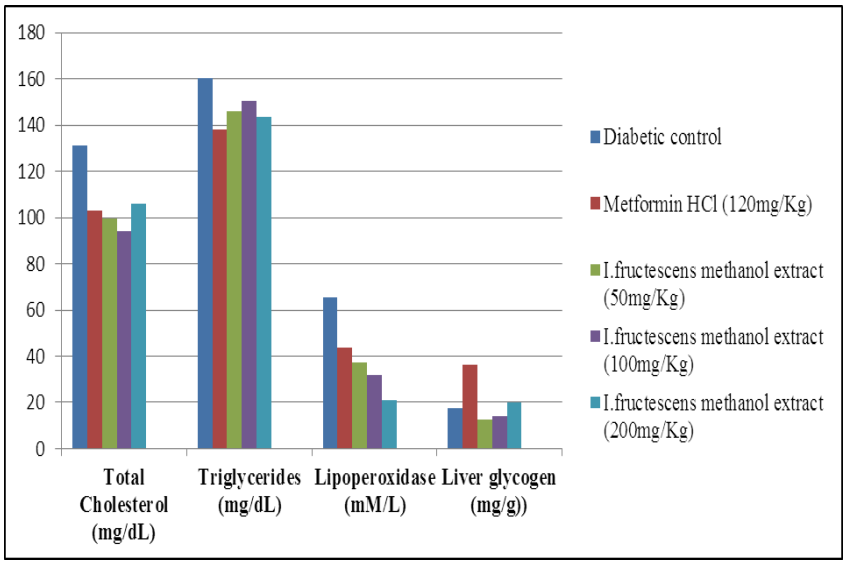

Figure-1 Effect of *Ichnocarpus fructescens* methanol extract on total cholesrerol, triglycerides, lipoperoxidase and glycogen levels of the STZ diabetic animals

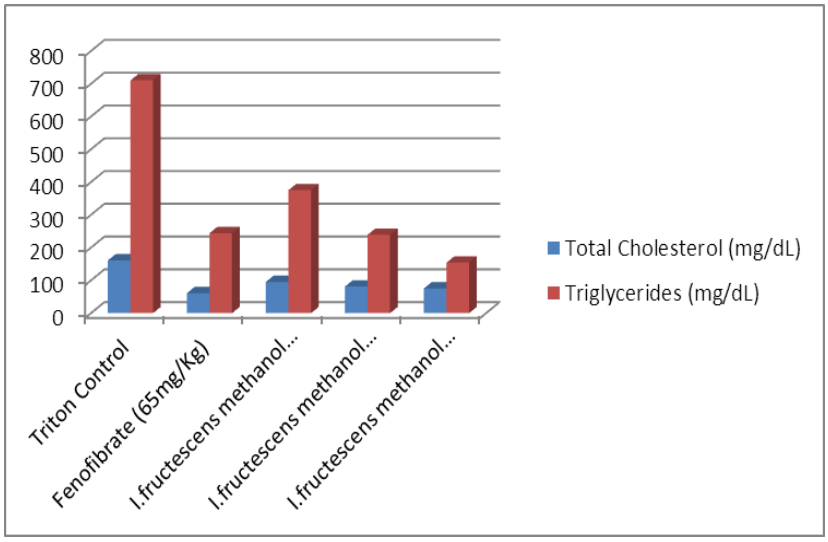

Figure-2 Effect of *Ichnocarpus fructescens* methanol extract on total cholesrerol and triglycerides levels of Triton WR-1339 induced hyperlipidemic animals after seven hours

REFERENCE

1. Achrekar, S., Kaklij, G. S., Pote, M. S., & Kelkar, S. M. (1991). Hypoglycemic activity of *Eugenia jambolana* and *Ficus benghalensis*: mechanism of action in vivo. *Journal of Ethnopharmacology*, 5: 143-148.
2. Babu, P. S., Ignacimuthu, S., Agastine, P. 2008. Insulin secreatogogue effect of *Ichnocarpus fructescens*, leaf extract in experimental diabetes: A dose dependent study. *Chemico Biological Interactions*, 172: 159-171.
3. Bell, G. I., & Polonsky, K. S. (2001). Diabetes mellitus and genetically programmed defects in beta cell function. *Nature*, 414: 788-791.
4. Carroll, N. V., Longley, R. W., & Roe, J. H. (1956). The determination of glycogen in liver and muscles by use of Anthrone reagent. *The Journal of Biological Chemistry*, 220: 583-593.
5. Chopra, R. N., Nayar, S. L., & Chopra, I. C. (1995). Glossary of Indian medicinal plants, National Institute of Science Communication and Information resources, CSIR, New Delhi.
6. Grover, J. K., Vats, V., & Rathi, S. S. (2000). Antihyperglycemic effect of *Eugenia jambolana* and *Tinospora cordifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. *Journal of Ethnopharmacology*, 73: 461-470.

7. Grover, J. K., Yadav, S., & Vats, V. (2002). Medicinal plants of India with anti-diabetic potential. *Journal of Ethnopharmacology*, 81: 81-100.
8. Habibuddin, M., Daghriri, H. A., Humaira, T., Al Qahtani, M. S., Hefzi, A. A. (2008). Antidiabetic effect of alcoholic extract of *Caralluma sinaica* L. on Streptozotocin induced diabetic rats. *Journal of Ethnopharmacology*, 117: 215-220.
9. Harborne, J. B.(1998). Phytochemical methods, Chapman and Hall, London.
10. Jarrette, J. (1978). Diabetes and the heart: Coronary heart disease. *Clinical Endocrinology and Metabolism*, 6: 389-402.
11. Kim, S. H., Hynn, S. H., & Choung, S. Y. (2006). Antidiabetic effect of cinnamon extract on blood glucose in db/db mice. *Journal of Ethnopharmacology*, 104: 119-123.
12. King, H., Aubert, R. E., & Herman, W. H. (1998). Global burden of diabetes, 1995-2005: prevalence, numerical estimates and projections. *Diabetes Care*, 22: 1414-1431.
13. Kumarappan, C. T., & Mandal, S. C. (2008). Polyphenolic extract of *Ichnocarpus fructescens* attenuates diabetic complications in streptozotocin treated diabetic rats. *Indian Journal of Pharmacology*, 307-322.
14. Lee, K. M. (2001). Overview of drug product development. *Current protocols in pharmacology*, 7(3): 1-10
15. Matthew, K. M. (1991). An excursion flora of Central Tamilnadu, India. Oxford & IBH Publishing co. New Delhi, India.
16. Oliveira, H. C., Dos Santos, M. P., Grigulo, R., Lima, L. L., Martins, D. T. O., Lima, J. C. S., Stoppiglia, L. F., Lopes, C. F., Kawashita, N. F. (2008). Antidiabetic activity of *Vatairea macrocarpa* extract in rats. *Journal of Ethnopharmacology*, 115: 515-519.
17. Roux, S., Sable, E., & Porsolt, R. D. (2004). Primary observation (Irwin) test in Rodents for assessing acute toxicity of a test agent and its effect on behavior and physiological function. *Current protocols in pharmacology*, 10(10): 1-23.
18. Salitiel, A. R., & Kahn, C. R. (2001). Insulin signaling and the regulation of glucose and lipid metabolism. *Nature*, 414: 799-806.
19. Saravanan, M., & Ignacimuthu, S. (2012). Effect of *Ichnocarpus frutescens* (L.) R. Br. hexane extract on preadipocytes viability and lipid accumulation in 3T3-L1 cells. *Asian Pacific Journal of Tropical Medicine*, 6(5): 360-365.
20. Sivaranjan, V. V. (1995). Ayurvedic drugs and their plant sources, Oxford & IBH Publishing co. New Delhi, India.
21. Vats, R. K., Kumar, V., Kothari, A., Mital, A., & Ramachandran, U. (2005). Emerging targets for diabetes. *Current Science*, 88: 241-249.

22. Vogel, H. G., & Vogel, W. H. (1997). Drug discovery and evaluation-Pharmacological assays. *Springer-Verlag, Berlin*, 598-610.
23. Warnica, J. W. (2004). The CARE trial: the effect of prevastatin on coronary events after myocardial infarction in patients with average cholesterol levels, *In: Statins- The HMG-CoA reductase inhibitors in perspective (2nd edition)*, Martin and Duntitz, London, pp123-143.
24. Wu, K. K., Huan, Y. (2008). Streptozotocin induced diabetic models in mice and rats. *Current protocols in pharmacy*, 47(5): 1-14
25. Wybenga, D. R., Di Giorgio, J., & Pileggi, V. J. (1971). Manual and automated methods for urea nitrogen measurement in whole serum. *Clinical Chemistry*, 17(9): 891-895.