The Bioscan 5(4) : 655-659, 2010
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

ANTI DIABETIC ACTIVITY BY THE *IN VITRO* ALPHA AMYLASE AND ALPHA-GLUCOSIDASE INHIBITORY ACTIVITY OF *CATHARANTHUS ROSEUS*

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KEY WORDS
Catharanthus roseaus
Anti diabetic activity
α - amylase
α-glucosidase inhibitory activity
Metformin

Received on :
19.06.2010
Accepted on :
27.10.2010

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ABSTRACT
Catharanthus roseus is a plant extensively known for its anti-inflammatory potential. In this study the alcoholic extract of flower and leaf of *Catharanthus roseus* was tested for its α-amylase and α-glucosidase inhibitory activity to understand its anti-diabetic potential.Varying concentration of the extracts of leaf and flower were assayed for their inhibitory action [*in vitro*] on serum amylase, pancreatic amylase, α–amylase from fermented barley and α- glucosidase. Both extracts of the leaf and flower were found to inhibit the enzymes considerably. The leaf extract showed maximum inhibitory action with a concentration of 10 mg/mL [IC_{50}]. IC_{50} for the flower extract was found to be at a concentration of 12.5mg/mL. Further the anti-oxidant property of the herb was also evaluated by its activity to inhibit lipid per oxidation. *Catharanthus roseus* extract of leaf and flower exhibit their anti-diabetic effect by inhibiting the enzymes which has a main role in carbohydrate metabolism like α-amylase and α-glucosidase.

INTRODUCTION

Diabetes mellitus (DM) is the commonest endocrine disorder that affects more than 100 million people worldwide (6% of the population) and in the next 10 years it may affect about five times more people than it does now (WHO/Acadia, 1992; ADA, 1997). In India, the prevalence rate of diabetes is estimated to be 1–5 %. Complications are the major cause of morbidity and mortality in DM. The World Health Organization (WHO) predicts that the number of cases worldwide for diabetes is now 150 billion, which will double by the year 2025.

Diabetes mellitus is a chronic, serious metabolic disorder caused by inherited or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced resulting in impaired function in carbohydrate, lipid and protein metabolism (Quong Luo *et al.,* 2004). Among the two principal form of diabetes, insulin dependent (type ~ 1) and non insulin dependent (type ~ 2) , Non insulin dependent diabetes accounts for 90% of all cases worldwide, due to the body's inability to respond properly to the action of insulin produced by the pancreas (Notkins, 2002). Non insulin dependent diabetes is becoming a pandemic and despite the recent surge in new drugs to treat and present the condition, its prevalence continues to soar. In spite of great strides that have been made in understanding and in the management of this disease, serious problems like diabetic retinopathy, diabetic nephropathy and low extremely amputation continues to confront patients and physicians. The graph of diabetes related mortality is rising and reducing the life expectancy to 5 to 15 years.

Diabetes mellitus in youth is emerging as a serious clinical entity and its incidence has increased over the year. The Indian diabetic population is predicted to rise to > 80.9 million by the year 2030 (Bjork *et al.,* 2003).

In recent years, much interest has been focused on biologically active compounds occurring in natural resources. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethno botanical information reports about 800 plants that may possess anti-diabetic potential (Alarcon-Aguilara *et al.,* 1998). Wide arrays of plant derived active principles representing numerous chemical compounds have demonstrated activity consistent with their possible use in the treatment of Non-insulin dependent diabetes mellitus (NIDDM) (Bailey and Day, 1989). Among these are alkaloids, glycosides, galactomannan, polysaccharides, peptidoglycans, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids and inorganic ions. Even the discovery of widely used hypoglycemic drug, metformin came from the traditional approach of using *Galega officinalis* . Thus, plants are a potential source of anti-diabetic drugs but this fact has not gained enough momentum in the scientific community.

Although, oral hypoglycemic agents such as insulin are the main stay of treatment of diabetes and are effective in

controlling hyperglycemia, they have prominent side effects and fail to significantly alter the course of diabetic complications (Rang and Dale, 1991). As the knowledge of heterogeneity of this disorder increases, there is needed to look for more efficacious agents with lesser side effects. Though development of modern medicine resulted in the advent of modern pharmacotherapeutics including insulin, biguanides, sulfonylureas and thiazolidinediones, there is still a need to look for new drugs as no drug (except strict glycemic control with insulin) has been shown to modify the course of diabetic complications.

This study is hence aimed at identifying safe herbal remedies for treating diabetes and also to identify their possible mechanism of anti diabetic control.

MATERIALS AND METHODS

Materials

The leaf and flower [pink coloured flower species] of the plant *Catharanthus roseus* were collected from the coastal region of Kalpakkam, (located on north of Chennai and south of Pondycherry) Tamil Nadu, India. The chemicals used for the study were from Sigma Chem. (St. Louis, MO, USA).

Preparation of extract

The Alcoholic extract of leaves and flowers of *Catharanthus roseus* was prepared by Soxhlet extraction using 50g of the dry powder in 200mL of ethanol.

Chemical characterization of the extract

The extract was tested for the presence of alkaloids, flavanoids, phenol, tannins, saponins etc. Total alkaloids were determined by Dragendroff's test. Briefly, 1mL of extract, few drops of Dragendroff's reagent was added orange colour were noticed indicating presence of alkaloids and Mayer's test was also done to confirm the presence of alkaloids. To the extract 2mL of Mayer's reagent was added. The formation of yellow colour precipitate confirmed the presence of alkaloids. Total flavones and flavanones in the extract were determined by using Shinoda test. To the extract, a few magnesium turnings and 1-2 drops of concentrated HCl were added, formation of red colour showed the presence of flavones. To the extract, 10%NaOH was added, yellow to orange color showed the presence of flavanones. (Harborne, 1998; Sadasivam and Manickam, 1992; Ogbonnia *et al.,* 2008; Nooman *et al.,* 2008; Mohd. Nawagish and Ahmad, 2007).

Total phenols were determined by ferric chloride test, to the extract, few drops of 10% aqueous ferric chloride was added, appearance of blue colour indicated the presence of phenols.

Effect of *Catharanthus roseus* extract on serum amylase, pancreatic amylase and α-amylase from fermented barley

The alcoholic extract of leaves and flowers was tested for its serum amylase inhibitory activity by the Yukihiko hara method, (1990). Three Test tubes were taken and labeled as blank, test (T) and control (C). To each test tube 2.5mL of phosphate buffer of pH 6.8 was added. 1mL of starch substrate and sodium chloride was added to all the three test tubes. The test tubes were incubated at 37ºC for 10 min. After incubation, 0.5mL of extract was added and 0.2 mL of enzyme [serum amylase, pancreatic amylase, α-amylase from fermented barley] was added to the test tube T. The contents of the test tube were mixed well and incubated at 37ºC for 10 min. After incubation 0.5mL of 2N NaOH was added to the test tube T and C.0.2mL of enzymes was added to the control C.5.7mL of distilled water alone serves as blank.0.2mL of dinitrosalicylic acid was added to all the test tubes.

The contents were mixed well and kept in a boiling water bath for 15minutes.The intensity of reddish orange colour was read at 540nm. The percentage of inhibitory action of serum amylase was calculated from the following formula.

O.D = optical density.

$$\text{Percentage inhibition} = \frac{\text{O.D of control-O.D of test}}{\text{O.D of control}} \text{ X } 100$$

Effect of Catharanthus roseus on α -glucosidase

The α – glycosidase inhibitory activity of *Catharanthus roseus* was tested by the method of Li *et al.,* 2004. Three Test tubes were taken and labeled as blank, test (T) and control (C) .200μL of substrate, starch and 200μL of enzyme [α-glucosidase] to the test tube T and C were added. This was followed by the addition of 0.5mL of extract to T. The contents were mixed well and incubated at 37ºC for 30 min. Trichloroacetic acid was added to all the test tube after incubation. The test tubes were then centrifuged for 10 min. The liberated glucose from the substrate by the action of enzyme was reacted with 1.5mL of anthrone reagent. The contents were boiled for 15 min. The intensity of color developed was read at 640nm. The percentage of inhibitory action of α-glucosidase was calculated from the following formula.

$$\text{Percentage inhibition} = \frac{\text{O.D of control-O.D of test}}{\text{O.D of control}} \text{ X } 100$$

Effect of *Catharanthus roseus* extract on lipid per oxidation

The anti-oxidant potential of the extract of *Catharanthus roseus* leaves and flowers were tested by the ability to inhibit Lipid peroxidation. The lipids in the cell membranes are highly susceptible to per oxidative damage and are broken down into number of small units to form malonialdehyde. In Blood cells, lipid per oxidation was induced by the addition of 0.1mL of 25μM ferrous sulphate, 10mL of ascorbic acid, 10 mM KH_2PO_4. Three Test tubes were taken and labeled as blank, test (T) and control (C). The (T) tubes were incubated at 37ºC for one hr with different concentration of extracts. To the test tubes 1mL of 15% TCA and 0.5mL of 0.375% TBA [thio barbituric acid] were added and test tubes were placed in the boiling water bath for 30min. The tubes were centrifuged and the supernatant was taken and read at 532 nm.

The percentage of LPO was inhibition was calculated from the following formula.

$$\text{Percentage inhibition} = \frac{\text{O.D of control-O.D of test}}{\text{O.D of control}} \text{ X } 100$$

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

C.*roseus* was found to contain flavones, sugar, phenol, tannins, glycosides and alkaloids. Saponin, steroids, quinones were

found to be absent (Table 1). The investigation of the Phytochemical constituent show that ethanol extract of *Catharanthus roseus* contains Flavonoids. Flavones and iso-flavonoid, glycosides form the ingredient of many Ayurvedic formulation used for the treatment of diabetes and also it stimulates insulin secretion by pancreatic islet cells. It also controls blood glucose and modulates the metabolism of glucose and blood lipid and decrease outputs of lipid peroxidation and scavenge the free radicals in non-insulin dependent diabetic rats.The flavonoids also act as anti-inflammatory agents. The Phytochemical analysis of *Catharanthus roseus* shows a positive result for tannins. The Tannoids inhibit aldose reductase in-vitro and prevent hyperglycemia induced lens opacification on organ culture. It also inhibit sorbitol formation in the lens and might counter the polyol pathway induced oxidative stress. Thus tannoids are effective in delaying development of diabetic cataract in rats (Suryanarayana, 2007).

The high Phenolic content of the extract support the anti-amylase activity. The phenolic substances have ability to interact with and / or inhibit proteins / enzymes (Rohan *et al.,* 2002)**.**

The Phytochemical analysis of *Cathranthus roseus* shows the positive result for alkaloids. The Alkaloids present in this extract was found to lower the blood sugar level and act on hemostatics. It also lowers the number of white cells in blood. So, it is also used as an anti-cancer drug. They work by preventing mitosis in metaphase, these alkaloids binds to tubulin, thus preventing the cell from making the spindles it needs to divide. This is different from the action of taxol which interferes with cell division by keeping the spindles from being broken down.

Analysis of the Phytochemical constituents support the strong anti-diabetic activity of the herb *Cathranthus roseus*.

Antioxidant property of *Cathranthus roseus*

The alcoholic extract of leaf and flower *of Catharanthus roseus* of different concentrations such as 10mg, 12.5mg, 25mg and 50 mg/mL were tested for their ability to inhibit Lipid peroxidation. The leaf extract was found to show 26% inhibition at a concentration range of 50 mg/mL. The flower extract was found to show a significant inhibition of 47% at a concentration of 50 mg/mL. IC_{50} of the flower extract was found to be 50 mg/mL.

From the results, it is clear that the ethanolic extract of leaves and flowers of *Catharanthus roseus* possesses significant antioxidant properties. The flower extract was found to possess much more significant antioxidant properties than the leaf extract.

Antioxidant property of Catharanthus roseus assessed by the ability to inhibit lipid per oxidation

The antioxidant property of the extract could be explained by its rich content of Flavonoids (Table 1), which has the ability to decrease the output of lipid peroxidation and scavenge free radicals**.**

Earlier studies were also found to confirm this property of *Cathranthus roseus*(Fig.1).Alcoholic stem extract of *Catharanthus roseus* was found to regulates carbohydrate metabolism and improves antioxidant status in streptozotcin nicotinamide induced Diabetic rats. *Catharanthus roseus* stem extract in graded doses caused a significant increase in enzymatic antioxidants such as catalase, superoxide dismutase, glutathione synthetase peroxidase and enzymatic antioxidants such as ascorbic acid, Ceruloplasmin and tocopherol. **(**Punitha *et al.,* 2005).

Table 1: Qualitative phytochemical analysis of *Catharanthus roseus*

S. N.	Phyto-chemicals tested	Inference
1	Flavones	P
2	Steroids	A
3	Anthraquinones	A
4	Phenol	P
5	Tannins	P
6	Saponins	A
7	Glycosides	P
8	Reducing sugar	P
9	Alkaloids	P
10	Quinones	A

P = Present ; A = Absent

The α-amylase inhibitory activity of *Catharanthus roseus*

In-vitro, α-amylase inhibitory activity of *Catharanthus roseus* was tested. α–amylases from three different sources was used namely pancreatic α-amylase, serum α-amylase and α-amylase from fermented barley (supposed to be rich source of α-amylase).

Concentrations of the range 10mg, 12.5mg, 16 and 80 mg/mL of the ethanol extract of leaf and flower was tested on the amylases from the sources mentioned above.

The leaf extract showed a significant pancreatic amylase inhibitory activity of 63% at a concentration of 80mg/mL. For a similar concentration the flower extract showed 15% inhibition compared with the commercial drug (Glycomet - Metformin tablet - α-amylase inhibitor) which showed 85% inhibition. IC_{50} of the flower extract was found to be 16mg/mL (Fig. 2).

The extract of leaf and flower showed significant serum amylase inhibitory activity when compared to pancreatic amylase. The leaf extract showed a significant serum amylase inhibitory activity of 80% at a concentration of 10mg/mL. While the flower extract showed a 50% inhibitory activity at a concentration of 12.5mg/mL and 16mg/mL (Fig.3).The percentage inhibition showed by the commercial drug for similar concentration were 90%, 80% and 60% respectively.

The α-amylase from fermented barley seeds were also tested with varying concentration of the leaf and flower extract of *Catharanthus roseus*. The leaf extract showed an inhibitory activity of 61% at a concentration of 80mg/mL. While for a similar concentration, the flower extract showed 66% inhibition (Fig. 4).

From the results, it is evident that the ethanolic extract of leaves and flowers had potent á-amylase inhibitory activity comparable to the commercial drug Metformin.

α-amylase begins the process of starch digestion. It takes starch chains and break them into small pieces with two or three glucose units. Some phenolic compounds in sweet potato, strawberry, Raspbery, Olive oil, pears, coca and Lentils are reported to be effective human α-amylase inhibitors. (Matsui

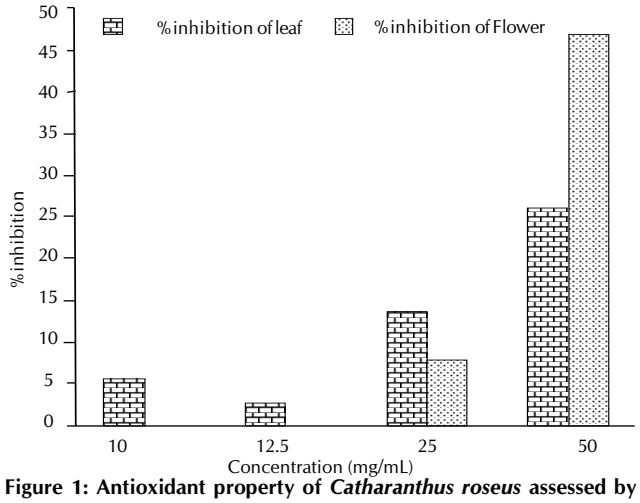

Figure 1: Antioxidant property of *Catharanthus roseus* assessed by the ability to inhibit lipid per oxidation

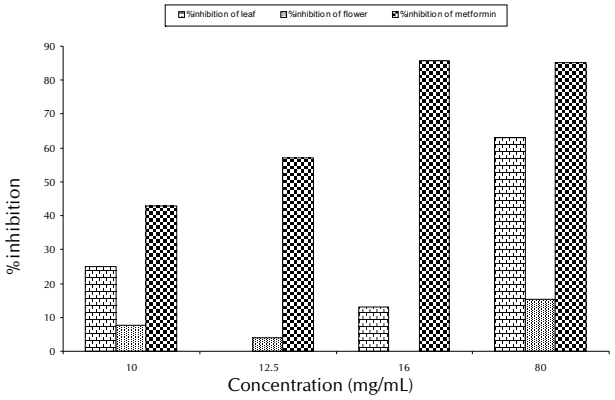

Figure 2: α-Amylase inhibitory activity (pancreas)

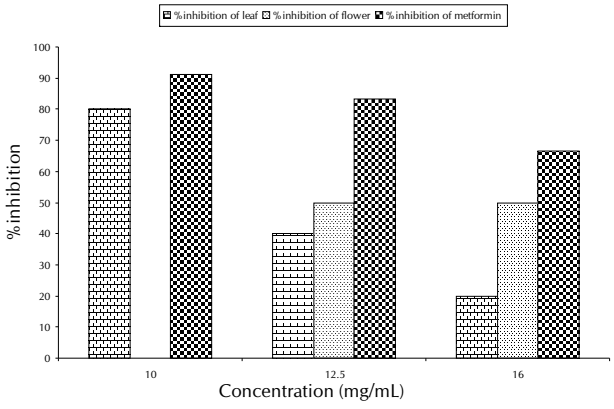

Figure 3: α-Amylase inhibitory activity (Serum)

et al., 2001). Flavonoids and anthocyacin are also reported to have inhibitory activity against α-amylase (Matsui *et al.,* 2001**).**

Metformin is one such commercial drug which exerts glycemic control by its α-amylase inhibitory activity. Thus metformin improves glucose tolerance in patients with type 2 diabetes. The most common side effect of Metformin are upper respiratory tract infection, diarrhea, edema and headache. By the α-amylase inhibitory activity the rate of digestion of carbohydrate and the consequent absorption of glucose in

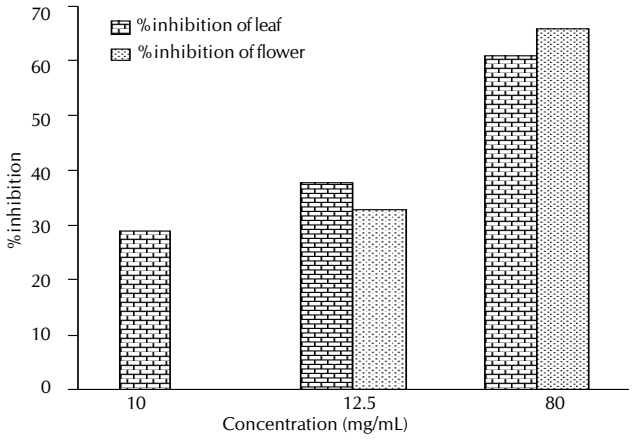

Figure 4: α-Amylase inhibitory activity (Fermented Barley)

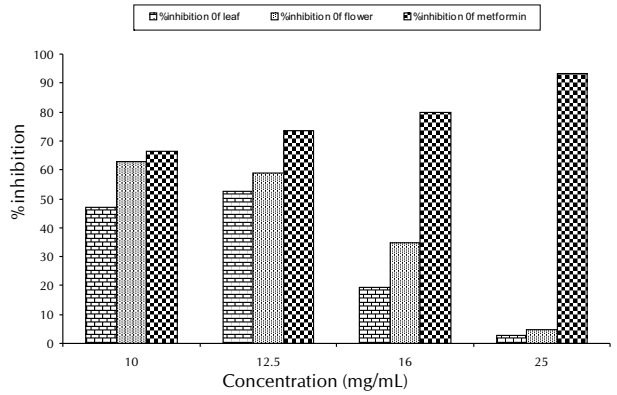

Figure 5: α-Glucosidase inhibitory activity

reduced. The α-amylase inhibitory action of *Catharanthus roseus* might play a role in diabetic treatment.

α-glucosidase inhibitory activity

The in-vitro α-glucosidase inhibitory activity of ethanolic extract of leaf and flower of *Catharanthus roseus* was tested. Varying concentration of the extract 10, 12.5, 16 and 25mg/mL were taken and assayed for the α-glucosidase inhibitory activity.

The leaf extract showed a significant α-glucosidase inhibitory activity of 53% at a concentration of 12.5mg/mL. For a similar concentration, the flower extract also showed significant inhibitory activity of 59% in comparison with the commercial drug metformin which showed about 73% inhibition for similar concentration (Fig. 5).

From the result, it is evident that ethanolic extract of leaf and flower of *Catharanthus roseus* has significant á-glucosidase inhibitory activity comparable to the commercial drug Metformin.

α-glucosidase is one of the numbers of glucosidases located in the brush border surface membrane of intestinal cells and is a key enzyme of carbohydrate metabolism (Caspary, 1978**).** α-glucosidase inhibitory activity block the actions of á-glucosidase enzyme in the small intestine which is the rate limiting step in the conversion of Oligosaccharide and disaccharide to monosaccharide, necessary for gastro intestinal absorption.

From the results, it can be concluded that ethanolic extract of leaf and flower of *Canthranthus roseus* can be excellent choice of drug with α-glucosidase inhibitory activity and can thus reduce the rate of digestion and absorption of carbohydrates.

In summary, this work shows that the alcoholic extract of leaf and flower of *Catharanthus roseus* has adequate antidiabetic potential by its ability to inhibit α-amylase and α-glucosidase. When compared to the flower extract the leaf extract was found to significantly inhibit α-amylases. While, the α-glucosidase inhibitory activity of the leaf and flower extract was almost the same. Our result provide a more detailed view of the anti diabetic properties of canthranthus roseus and it is first of its kind to reveal the α-amylase and α-glucosidase inhibitory activity. The significant α-amylase and α-glucosidase inhibitory activity of the leaf and flower extract of *Catharanthus roseus* together with its potent antioxidant potential can make it the future safe drug of choice in diabetes treatment. Future studies will address the molecular mechanisms by which the plant and its active compounds regulate glucose homeostasis.

REFERENCES

Alarcon-Aguilara, F. J., Roman-Ramos, R., Perez-Gutierrez, S., Aguilar-Contreras, A., Contreras-Weber, C. C. and Flores-Saenz, J. L. 1998. Study of the anti-hyperglycaemic effect of plants used as ant diabetics. *J. Ethno Pharmacology*. **61(2):** 101-110.

Bailey, C. J. and Day, C. 1989. Traditional plant medicines as treatmentfor diabetes. *Diabetes Care*. **12:** 553-564.

Bjorks Kapur, A., King, H., Nair, J. and Ramachandran, 2003. A. Global policy:aspects of Diabetes in India. *Health Policy*. **66(1):** 61-72.

Caspary, W. F. 1978. Source malabsorption in Man after ingestion of α- glycosidase hydrolase inhibitor. *Lancet*. **(1):** 1231-1233

Harborne, J. B. 1998. Phytochemical Methods. *A guide to modern techniques of plant analysis,* 3rd Edn., Chapman and Hall, London. p. 235.

Li, Y., Peng, G., Li, Q., Wen, S., Huang, T. H., Roufogalis, B. D. and Yama-HARA, J. 2004. Salacia oblonga improves cardiac fibrosis and inhibits post prandial hyper glycemic in obese zucker rats. *Life Scis*. **75:** 1735-1746.

Matsui, T., Ueda, T., Oki, T., Sugita, K., Terahara, N. and Matsumoto, K., 2001. Glucosidase inhibitory action of natural acylated anthocyanins.1. survey of natural of pigments with potent inhibitory activity. *J. Agri. Foof Chem*. **49:** 1948-1951.

Mohd. Nawagish, Ansar, S. H. and Ahmad, S.2007. Preliminary Pharmacognostical Standardisation of *Lawsonia* inermis Linn. Seeds. *Research J. Botany*. **2(3):** 161-164.

Notkins, A. L. 2002. Immunolgiz and genetic factors in typical Diabetes. *J. Biochem*. **227(46):** 43545-43548.

Nooman, A., Khalaf, Ashok, K., Shakya, Atif Al-Othman., Zaha El-Agbar. and Husni Farah. 2008. Anttioxidant activity of some common plants. *Turk. J. Biol*. **32:** 51-55.

Ogbonnia, S., Adekunle, A. A., Bosa, M. K. and Envuru, V. N. 2008. Evaluation of acute and subacute toxicity of *Alstonia congensis* Engler (Apocynaceae) bark and *Xylopia aethiopica* (Dunal)A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. *African J. Biotechnology*. **7(6):** 701-705.

Punitha, I. S. R., Rajendaran, K., Shrwaikar, A. and Shrwaikar, A.2005. Alocholic stem extracts of *V.roseus* regulates Carbohydrate Metabolism and Improves Anti-oxidant status in streptozotocin-nicotinamide Induced Diabetic Rats. Department of pharmacognosy, Manipal college of pharmaceutical sciences, Manipal 576 104, India.

Quong Luo., Yizhong Cai and Jun Yan. 2004. Hypoglycemic and Hypolipedimic effects and anti-oxidant activity of first extracts from lyceum barbarcum. *Life Sci*. **76:** 137-49.

Rang, H. P. and Dale, M. M. 1991. The Endocrine System Pharmacology,Second Ed. Longman Group Ltd, UK. pp. 504-508.

Rohan, S., Rawel, H. M. and Kroll, J. 2002. Inhibitory effects of plants phenol on the activity of selected enzymes. *J. Agric Food. Chem*. **50:** 3566-3571.

Sadasivam, S. and Manickam, A. 1992. Biochemical methods for Agricultural Sciences, Wiley Eastern Limited. Ansari Road, Daryaganj, New Delhi. pp. 1-20.

Suryanarayana, P., Saraswat, M., Petrash, J. M. and Reddy, G. B. 2007. Emblica officinalis and its enriched tannoids delay streptozotocin-induced diabetic cataract in rats. *Mol VIS*. **13:** 1291-7.