ORIGINAL RESEARCH ARTICLE

PROTECTIVE ROLE OF *CURCUMA LONGA* EXTRACT SUPPLEMENTATION IN STZ INDUCED DIABETIC RATS

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ABSTRACT:

Diabetes mellitus is a chronic metabolic disorder that prevents the body to utilize glucose completely or partially. It is characterized by raised glucose concentration in the blood and alterations in carbohydrate, protein and fat metabolism. *Curcuma longa* has several biological properties, including antidiabetic and antioxidant activity. The present study was designed to investigate the antidiabetic effect of *Curcuma longa* extract against streptozotocin (STZ) induced perturbations in blood glucose, body weights and hematological alterations in albino rats. Adult male albino Wister rats, weighing 180 ± 20 g was made diabetic by injecting STZ (40mg/kg body weight) intraperitoneally. Diabetic rats were supplemented with ethanolic extract of *Curcuma longa* rhizomes (250 mg/kg body weight) for a period of three weeks. After this period, rats were decapitated and blood was collected from control and experimental rats. Blood glucose levels, WBC, RBC, serum total proteins, albumin, globulin, creatinine, urea and cholesterol were significantly elevated in diabetic rats with decreased hemoglobin and body weight levels when compared with control. The above mentioned parameters were significantly restored to near normal by oral administration of *Curcuma longa extract* once daily for three weeks as compared to untreated rats. The results obtained indicated that *Curcuma longa extract* to be beneficial in preventing diabetes induced alterations in blood glucose, body weights and hematology in rats.

KEYWORDS: Diabetes; Streptozotocin; *Curcuma longa*; Blood glucose; Body weight; hematology; Rats.

INTRODUCTION:

Diabetes mellitus, is a chronic metabolic disorder commonly known as diabetes, is a disorder of carbohydrate metabolism characterized by high blood sugar level (hyperglycemia) and high level of sugar in urine (glycosuria), Insulin lowers the blood glucose level. Insulin is released from the pancreas to normalize the glucose level. In patients with diabetes, the absence or insufficient production of insulin causes hyperglycemia.

This can be due to failure in the formation of insulin or liberation or action [1]. Since insulin is produced by the β-cells of the islets of langerhans, any alterations in the number of functioning cells will decrease the amount of insulin synthesis. Many diabetics can produce sufficient insulin but some stimulus to the islets tissue is needed for its secretion.

This study is a part of the national non-communicable diseases (NCD) risk factor surveillance conducted in different geographical locations in India, This nation-wide NCD risk factor surveillance study showed that the prevalence of self reported Diabetes is higher in urban, intermediate in Peri-urban and lowest in rural areas.

Table 1. Top countries for estimated number of people with Diabetes, 2000-2030

Ranking	2000		2030	
	Country	People with Diabetes (Millions)	Country	People with Diabetes (Millions)
1	India	31.7	India	79.4
2	China	20.8	China	42.3
3	U.S.	17.7	U.S.	30.3
4	Indonesia	8.4	Indonesia	21.3
5	Japan	6.8	Japan	8.9
6	Pakistan	5.2	Pakistan	11.9
7	Brazil	4.6	Brazil	11.3
8	Bangladesh	3.2	Bangladesh	11.1

Ayurvedic Approach in Diabetes treatment:

Ayurvedic physicians have treated diabetes for thousands of years using a combination of regulated lifestyle and herbal formulations. The physicians also prescribed specific herbal formulations for the treatment of diabetes. In recent times, the safety and efficacy of these herbs have been validated by laboratory experiments and clinical trials. A large variety of compounds obtained from several plant families were found to hypoglycemic effect. The glycosides, glycans,

certain triterpenes, various types of sulfide molecules, polysaccharides, oils, vitamins, alkaloids, saponins, glycoproteins, peptides, amino acids and proteins isolated from various plant families showed beneficial effects in reducing the blood sugar. Many Indian medicinal plants are reported to be useful in diabetes [2, 3, 4, 5] and [6]. Medicinal plants used to treat hypoglycemic or hyperglycemic conditions are of considerable interest for ethno-botanical community as they are recognized to contain valuable medicinal properties in different parts of the plant and a number of plants have shown varying degree of hypoglycemic and anti-hyperglycemic activity. The active principles of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents. Several species of medicinal plants are used in the treatment of *Diabetes mellitus*. Traditional plant medicines or herbal formulations might offer a natural key to unlock diabetic complications.

Antioxidants play an important role to protect against damage by reactive oxygen species and their role in diabetes has been evaluated. Many plant extracts and products were shown to possess significant antioxidant activity. In the present study two of such plants were selected for evaluation of their antioxidant potential mediated antidiabetic activity.

***Curcuma longa*:**

In Ayurvedic medicine, turmeric is thought to have many medicinal properties and in India many people use it as a readily available antiseptic for cuts, burns and bruises. Practitioners of Ayurvedic medicine say that it has fluoride which is thought to be essential for teeth. It is also used as an antibacterial agent. It is taken in some Asian countries as a dietary supplement, which allegedly helps with stomach problems and other ailments. It is popular as a tea in Okinawa, Japan. The active ingredient in turmeric is exploding. U.S. National Institutes of Health had four clinical trials to study curcumin treatment for pancreatic cancer, multiple myeloma, Alzheimer's, and colorectal cancer. Curcumin has been used for thousands of years as a safe anti-inflammatory agent in a variety of ailments as part of Indian traditional medicine". A recent study involving mice has shown that turmeric slows the spread of breast cancer into lungs and other body parts. Turmeric also enhances the effect of taxol in reducing metastasis of breast cancer.

Researchers had discovered that turmeric-treated mice were less susceptible to developing type-II diabetes, based on their blood glucose levels, and glucose and insulin tolerance tests. They also discovered that turmeric-fed obese mice showed significantly reduced inflammation in fat tissue and liver compared to controls. They speculated that curcumin in the turmeric lessens insulin resistance and prevents type-II diabetes in these mouse models by dampening the inflammatory response provoked by obesity. Curcumin and its analogues have a variety of physiological and

pharmacological activities such as antiinflammatory, anticarcinogenic and antioxidant properties [4, 5, 6, 7 and 8].

The present research work was carried out to evaluate the beneficial effects and Protective role of Plant Extract of *Curcuma longa* against Streptozotocin induced Diabetes. Other objective of the study was to determine the role of plant extract on hematological and serum biochemical parameters.

MATERIAL AND METHODS:

Procurement and Maintenance of Animals:

Healthy female albino wistar rats (180±20g) were procured from Sri Venkateswara Enterprises, Bangalore, Karnataka, India (Reg. No: 237/99/CPCSEA), Animals were maintained in the animal house of Sri Venkateswara University, Dept of Zoology, Tirupati. Rats were kept in sterilized polypropylene cages lined with paddy husk (18"x10"x8"), The animals were maintained under a regulated 12 h light: 12 h dark scheduled at 24±1^{o}C and relative humidity of 55±15%. Rats were provided standard rat chow (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*.

Procurement of chemicals:

All the chemicals used in the present study were Analar Grade (AR) and were obtained from Sigma (St. Louis, MO, USA), Fisher (Pitrsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (NEW Delhi, India), Qualigens (Mumbai, India) scientific companies.

For the present work Barnstead Thermoline water purification plant was used for Nano pure water, Kubota KR 200000T centrifuge for centrifugation of the homogenates and Hitachi UV -2000 Spectrophotometer for measuring the optical density values were used for high –quality results.

Streptozotocin:

Systematic (IUPAC) name: - 2-deoxy-2-({[methyl (nitroso) amino] carbonyl} amino)-β-Dglucopyranose

Streptozotocin is a mixture of α- and β-stereoisomers. It occurs as pale yellow or off-white crystals, powder, or platelets, while the research grade may be off-white to tan solid. It is very soluble in water, ketones, and lower alcohols, slightly soluble in polar organic solvents, and insoluble in monopolar organic solvents. The pure compound is sensitive to humidity and light. Streptozotocin decomposes to diazomethane in alkaline solutions at 0°C. When heated to decomposition, it emits toxic fumes of nitrogen oxides (IARC 1978, HSDB 2001),

Preparation of *Curcuma longa* extract:

The fine powder of *Curcuma longa* rhizome powder were purchased (AGMARK symbol) in Tirupati. The power is extracted by cold percolation with 95% ethanol for 24h. The extract was

recovered and 95% ethanol was further added to the plant material and the extraction was continued. The process was repeated three times. The three extractions were pooled together, combined, filtered and the filtrate was concentrated to dryness under reduced pressure in rotary evaporator. The resulting ethanol extract was air-dried. Finally light yellow powdery, crude ethanol extract of *Curcuma longa* was obtained. Without any further purification the plant crude ethanol extract was used in the study. Dose equivalent to 250mg kg/body was calculated and suspended in 2% v/v Tween 80 solution for the experiment [4].

Induction of Diabetes:

Streptozotocin (STZ, 2-deoxy-2-({[methyl (nitroso) amino] carbonyl} amino)-β-Dglucopyranose) frequently used dosage is 40mg/kg BW [9] Single injection of STZ given intravenously or intraperitonially to the adult rats to induce diabetes. After fasting for 18hrs, rats were injected intraperitonially with a single dose of 40mg STZ (Sigma, St. Louis, Mo., USA) freshly dissolved in 0.1 M cold sodium citrate buffer, (pH 4.5), After injection, they had a free access to food and water was given 5% glucose solution to drink overnight to counter hypoglycemic shock. Diabetes in rats was identified by moderate polydipsia and marked polyuria. From the second day onwards fasting blood samples were collected from the rats by tail vein and blood glucose was measured by Accu chek Sensor comfort glucometer (Manufacture-Johnson and Johnson) to know the induction of diabetes. If the blood glucose levels were more than 300mg/dL, insulin (IIU Protamine Zinc Insulin) is given to the diabetic rats for diabetic condition for one week. After one week the rats with hyperglycemia (blood glucose level 250mg/dL) were selected and used for the experiment [10].

Grouping of animals:

Group -1 : Normal Control rats.

Group- 2 : Diabetic rats (Streptozotocin)

Group -3 : Diabetic rats treated with 250 mg/Kg b.w. of *Curcuma longa*.

The blood samples were collected after completion of treatment i.e. on 22nd day of the treatment. The blood was used for the hematological parameters and separated serum was used for the serum biochemical parameters.

Estimation of Blood glucose:

Estimation of Blood glucose levels was carried out by using Accu Chek glucometer (Sensor Comfort),

Body Weight Changes:

Body weights of all groups of (eight) rats were recorded before and after treatments. The body weights of all groups were recorded at an interval of one week till the completion of the experiential period (21 days),

Hematology:

Blood samples were collected at the end of experimentation period immediately after sacrifice the blood was collected from the jugular vein and the blood was allowed into a graduated centrifuge tubes containing 10% EDTA, a common anticoagulant used for routine hematological work. The blood parameters like total erythrocytic count, total leucocyte count, hemoglobin and hematocrit (PCV) were estimated by using standard procedures.

Biochemical Parameters:

With out adding anticoagulant, the blood was collected into separate tubes and subject for centrifugation and serum collected was used for biochemical analysis. The parameters such as of glucose, total proteins, albumin, globulin, total cholesterol, creatinine, blood urea nitrogen, and bilirubin were estimated by using diagnostic kits supplied by SD fine, Ranbaxy, span diagnostics Ltd., India, and the procedures mentioned in the kit.

RESULTS AND DISCUSSION:

Blood Glucose:

Blood glucose levels were measured using glucometer (Accu Chek) in control, diabetic, diabetic treated with *Curcuma longa* extract groups before and after treatment. In group II, the blood glucose levels were significantly increased after induction with STZ when compared with control.

Table1. Showing Blood glucose levels in the control and experimental animals:

Days	Group-I	Group-II	Group-III
1st Day	89.33 ±8.38	282.16 ±37.92	280.16 ±34.62
10th Day	106.50 ±9.35	306.66 ±30.32	208.67 ±24.41
21st Day	119.52 ±12.56	271.33 ±42.78	137.16 ±23.92

Values are mean ± S.D. of 6 individual rats

Blood glucose levels were significantly decreased in the group III, where the rats were subjected to *Curcuma longa* extract. The various blood glucose values of alterations are as shown in Table-1 and Figure-1.

Most of the body cells use the sugar called glucose as their major source of energy. Glucose molecules are broken down within cells in order to produce adenosine triphosphate (ATP) molecules, energy-rich molecules that power numerous cellular processes. Glucose molecules are delivered to cells by the circulating blood and therefore, to ensure a constant supply of glucose to cells, it is essential that blood glucose levels be maintained at relatively constant levels. Level

constancy is accomplished primarily through negative feedback systems, which ensure that blood glucose concentration is maintained within the normal range of 70-110 mg/dl.

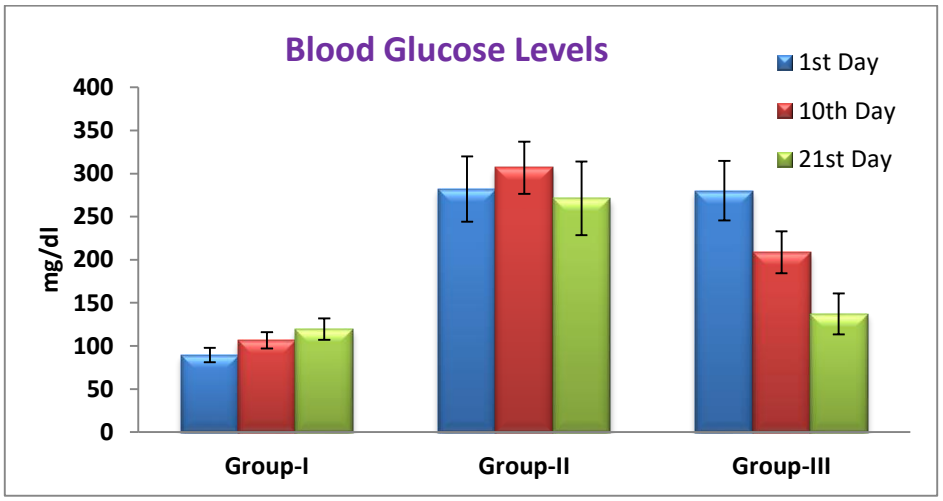

Figure 1. Blood glucose levels in control and experimental animals

The levels of glucose in the blood are monitored by the cells in the pancreas. If the blood glucose level falls to dangerous levels (as in very heavy exercise or lack of food for extended periods), the Alpha cells of the pancreas release glucagon, a hormone which alerts the liver to increase blood glucose levels and converts stored glycogen into glucose (Glycogenesis), Thus glucose is released into the blood stream, increasing blood sugar levels. There are several other causes for an increase in blood sugar levels. Among them diabetic stress due to the accumulation of reactive oxygen spices is a major cause.

In the present study blood glucose levels were maintained at normal levels in control rats. A significant increase in glucose levels found in STZ treated rats could be due to the distruction of pancreatic beta-cells by STZ induced oxidative stress. The elevation of glucose in STZ treated rats was due to an oxidative stress produced in the pancreas, due to a single strand break in pancreatic islets DNA [11]. In experimental diabetes, enzymes of glucose and fatty acid metabolism are markedly altered; hence blood glucose levels were increased [12]. An increased hyperglycemia has been reported to induce oxidative stress due to glycation of proteins and accumulation of polyols [13]. One of the consequences of hyperglycemia is increased metabolism of glucose by sorbitol pathway. Besides this, other path ways, such as fatty acid and cholesterol biosynthesis favor hyperglycemia [14]. Hyperglycemia is currently considered to be primarily responsible for the auto-oxidative glycosylation, formation of hydro peroxides and free radicals, in particular the hydroxyl radical and low density lipoprotein oxidation [15].

The action of STZ in the pancreas is preceded by its rapid uptake by the B cells [16]. Rapid uptake by insulin-secreting cells has been proposed to be one of the important features determining

STZ diabetogenicity. Another aspect concerns the formation of reactive oxygen species [17]. A similar uptake of STZ also takes place in the liver. However, the liver and other tissues are more resistant to reactive oxygen species in comparison to pancreatic β cells and this resistance protects them against STZ toxicity [18, 19]. The formation of reactive oxygen species is preceded by STZ reduction. In beta cells of the pancreas its reduction occurs in the presence of different reducing agents. Since STZ exhibits a high affinity to the SH-containing cellular compounds, reduced glutathione (GSH), cysteine and protein-bound sulfhydryl groups (including SH containing enzymes) are very susceptible to its action [20]. However, other reducing agents such as ascorbate may also participate in this reduction [21, 22] proposed that one of the SH-containing compounds essential for proper glucose-induced insulin secretion is glucokinase (EC 2.7.1.2), being very vulnerable to STZ. STZ reacts with two -SH groups in the sugarbinding side of glucokinase resulting in the formation of the disulfide bond and inactivation of the enzyme. Glucose can protect glucokinase against the inactivation hindering the access of alloxan to the -SH groups of the enzyme [22].

In case of rats which were subjected to both STZ and plant extracts, the decrease in blood glucose was due to the hypoglycemic activity of the extracts. Changes of blood glucose levels in the group III where diabetic rats were treated with plant extract is due to the flavonoid and triterpenoid compounds in them. A number of investigations had reported that 6-gingerol, tannins, polyphenolic compound, flavonoids, triterpenoids posses analgesic, hypoglycemic and other pharmacological actions in various experimental animal models [23, 24].The plant favorably affected glycolytic, gluoneogenic, and liogenic enzymes to restore glucose homeostasis in STZ-induced diabetic rats [25]. The administration of *Curcuma longa* powder to diabetic animals has been shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrate and lipid metabolism close to normal values in animal model systems [25, 26]. Oxidant induced alterations in the glucose utilizing system during diabetic manifestation is partially reversed by the administration of herbal extracts (Methanol extracts (75%) of *Aegle marmelos, Momordica charantia, Trigonella foenum-graecum, curcuma longa, Eclipta prostrata, Salacia oblonga, Coriandrum sativum, Vernonia anthelmintica* and *Murraya koenigii*) having antioxidant activity. Various reports demonstrated that the *Curcuma longa* have hypoglycemic, hypocholserolemic and hyperinsulinomic effects on type 1 and type 2 *Diabetes mellitus* patients and experimental diabetic animals [27, 28]. Oral administration of extract from *Curcuma longa* lowers blood glucose and attenuates STZ-induced hyperlipidemia in diabetic rabbits. [29]. *Curcuma longa* rhizomes he been reported to possesses active constituents showing blood glucose lowering activity in STZ induced diabetic rats [30]. Curumin has been shown to lower blood glucose levels in typ-2 diabetic KK-ay mice [31] and STZ treated rats [32]. The

administration of an aqueous extract of turmeric and abromine powder resulted in a significant reduction in blood glucose.

Body Weights:

Body weights of rats were measured using a digital balance at an interval of 10 days during the experimental period. The initial average weight of animals was in the range of 180 ± 200g. In group II, the body weights were significantly decreased after induction with STZ when compared with the control rats. In the group III, the body weights were significant increased when compared with the diabetic (group II) rats. The changes of body weights are as shown in Table-2 and figure-2.

Table 2. Body Weight levels in the control and experimental animals:

Days	Group-I	Group-II	Group-III
1st Day	242.53±28.41	229.66±31.38	227.64±15.73
10th Day	260.43±24.31	188.42±13.76	220.88±25.57
21st Day	276.17±19.28	140.19±14.71	237.33±19.02

Values are mean ± S.D. of 6 individual rats

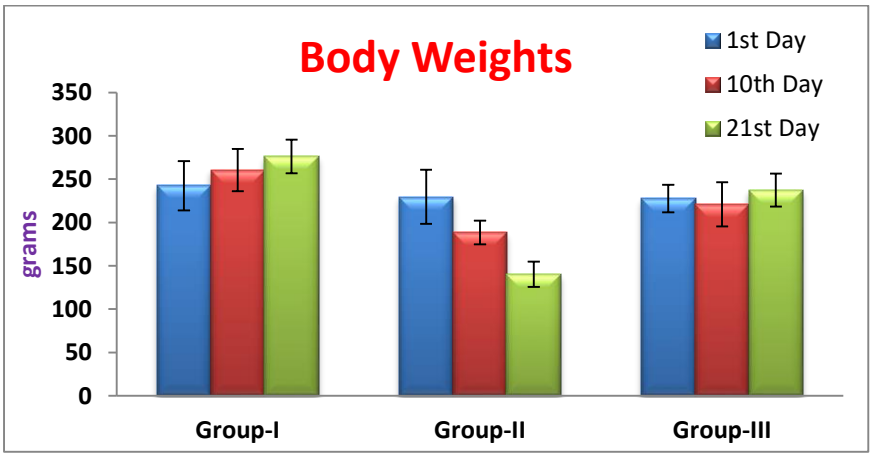

Figure 2. Body weights levels in control and experimental animals

Body weight is determined by energy intake on one hand and energy expenditure on the other. Imbalance between energy intake and expenditure results in a change in body weight. Organisms expend energy to perform daily work required for survival, such as finding food or evading predators. Metabolic efficiency refers to the amount of energy an organism has to exert to perform a given amount of work.

Metabolic efficiency varies among different species of organisms and among different individuals within a species. An individual with high metabolic efficiency will expend less energy to perform a specific task, such as climbing a set of stairs, than an individual with low metabolic efficiency. Compared with an individual with low metabolic efficiency, an individual with high metabolic efficiency is better able to preserve body weight during negative daily energy balance (expenditure exceeding intake), but likely to gain more weight during positive energy balance (intake exceeding expenditure), The ability of an organism to minimize reduction in body weight during long periods of starvation is likely associated with its survival. As a result, millions of years of evolution may have favored organisms with high metabolic efficiency [33, 34, 35, 36].

A constellation of clinical studies has established the close link between obesity and type 2 diabetes[37, 38]. This correlation, however, is not perfect; many diabetic patients are not obese, and many obese individuals are perfectly responsive to insulin. Regardless of whether a causal relationship exists between obesity and the body's response to insulin, beneficial effects of weight loss on the metabolic parameters of many diabetic patients are well documented [39, 40]. Thus, it is not surprising that a combination of weight loss and exercise is an effective treatment for many diabetic patients [41].

In the present study, STZ induced diabetic rats showed decreased level of body weights. The decrease in body weight in diabetic rats clearly shows a loss or degradation of structural proteins. Weight loss which is one of the clinical features of *Diabetes mellitus* may be due to the degeneration of the adipocytes and muscle tissues to make up for the energy lost from the body due to frequent urination and over conversion of glycogen to glucose. Weight loss is a very serious issue in the management of *Diabetes mellitus* [42].

Due to diabetes the structural proteins are known to contribute for the body weight [43]. STZ induced diabetes is characterized by a severe loss in body weight. The control diabetic animals showed a significant decrease in body weight compared with normal rats [44]. Changes in body weight in adult and non adult diabetic rats varied. Since the non adult diabetic rats are in the growing age, diabetic loss of weight is not seen in them and they even show a slight weight gain. In adult rats, however diabetes is an accompanied by loss of weight [45]. Weight loss during diabetes is mainly related to urinary glucose excretion because cells become to use glucose. Another factor could be also the osmotic diuresis resulting in hyper osmotic dehydration.

In the case of diabetic rats treated with *Curcuma long* extract (group III) increased levels in body weights were observed. They showed almost same response as that of control rats. This shows that *Curcuma long* plant extract apposes degeneration of the adipocytes and muscle tissues which

occurs during diabetic stress in order to make up for the energy lost from the body due to frequent urination and over conversion of glycogen to glucose.

Hematological Parameters:

Significant decreased levels of hemoglobin, during diabetes when compared with corresponding control group. But WBC and RBC was increased in diabetes rats.

Table 3. Blood Parameters levels in the control and experimental animals:

Parameters	Group I	Group II	Group III
Haemoglobin (gm/dl)	12.8± 0.7	9.2± 0.86	12.4± 0.98
RBC (millions/µl)	4.5± 0.32	3.8± 0.37	4.8± 0.43
WBC (cells/µl)	5600± 348.23	5800± 316.23	6400± 361.24

Values are mean ± S.D. of 6 individual rats

Administration of *Curcuma longa* extract tended to bring the values to near to normal range and the effect was more pronounced in the group of rats treated with plant extract.

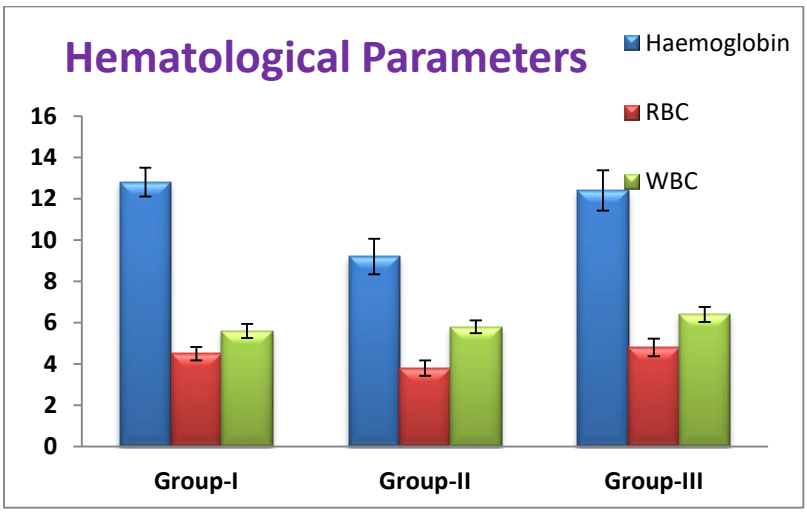

Figure 3. Blood Parameters levels in the control and experimental animals

In diabetic rat haemoglobin (Hb) levels were found to be low when compared to normal rats, as the Hb synthesis might also be depressed. Thus *Curcuma longa* treated rats showed improved levels of Hb because of its glucose lowering effect. The various proteins including hemoglobin undergo an enzymatic glycation in diabetes. Glycosylated hemoglobin was found to be increased in diabetes condition and the amount of increase is directly proportional to that of fasting blood

glucose level [46]. Lowered levels of hemoglobin were observed in diabetic rats which might be due to the increased formation of HbA1c. Hyperglycemia is the clinical hallmark of poorly controlled diabetes, which is known to cause glycation, and also known as nonenzymatic glycosylation. HbA1c was found to increase in patients with *Diabetes mellitus* and the increase was directly proportional to the fasting blood glucose levels. Previous studies reported that the active components present in *Curcuma longa* were effective in raising the hemoglobin levels in rats.

The link between chronic diseases and anemia is well characterized [47]. The occurrence of anaemia in *Diabetes mellitus* has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia. Oxidation of these proteins and hyperglycaemia in *Diabetes mellitus* causes an increase in the production of lipid peroxides that lead to haemolysis of RBC. The major pathological consequences of free radical induced membrane lipid peroxidation include increased membrane rigidity, decreased cellular deformability, reduced erythrocyte survival, and lipid fluidity [48]. In this study, the RBC membrane lipid peroxide levels in diabetic rats were not measured. The reversal effect shown by the *Curcuma longa* were effective in reduce the RBC levels in rats.

Peripheral WBC count has been shown to be associated with insulin resistance, type 2 diabetes, coronary artery disease, stroke, and diabetes micro- and macrovascular complications [49, 50]. Peripheral blood leukocytes are composed of polymorphonuclear cells, including monocytes as well as lymphocytes. Polymorpho- and mononuclear leukocytes can be activated by advanced glycation end products, oxidative stress, angiotensin II [51, 52], and cytokines in a state of hyperglycemia. Leukocytes may be activated through the release of cytokines, such as TNF-α, transforming growth factor-1, superoxide, nuclear factor κB (NF-κB), monocyte chemoattractant protein 1, interleukin-1β, and others [53] to participate in the pathogenesis of diabetic micro- and macrovascular complications. The profile of the WBC count reflects the balance between the rate of granulocyte production and that of WBC. [54] reported that diabetes in mice was accompanied by moderate neutrophilic leukocytosis and prolonged circulation times of neutrophils and monocytes, and a shortened circulation time of lymphocytes, which increases the susceptibility to infection. The active components present in *Curcuma longa* decreases the WBC count.

Serum Biochemical Parameters:

A significant increase in serum total proteins (5.42), albumin (2.52) and globulin (3.86) was recorded in diabetic untreated rats when compared to the normal control rtes (Group-I), *Curcuma longa* treated diabetic rats showed significant decrease in serum total proteins (4.72), albumin (2.24) and globulin (2.68) levels compared to the diabetic rats and also nearly levels of the control rats.

Table 4. Serum Biochemical Parameters levels in the control and experimental animals:

Parameters	Group I	Group II	Group III
Total Proteins (g/dl)	4.68± 0.53	5.42± 0.92	4.72± 0.34
Albumin (g/dl)	2.12± 0.32	2.52± 0.48	2.24± 0.36
Globulin (g/dl)	2.44± 0.38	3.86± 0.97	2.68± 0.82

Values are mean ± S.D. of 6 individual rats

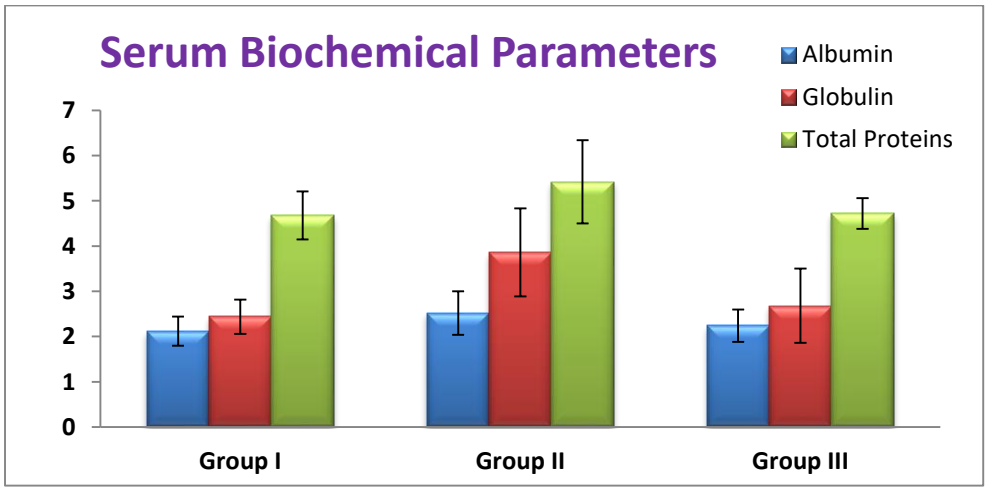

Figure 4. Serum Biochemical Parameters levels in the control and experimental animals

Hyperlipidemia is a known complication of *Diabetes mellitus* and coexists with it and is characterized by increased levels of cholesterol and also changes in lipoprotein patterns. Interest in the study of plasma lipids in diabetes arises from the widely acknowledged higher incidence of atherosclerotic disease which is a major cause of premature death in diabetic patients whether it is type-I or type-II [55].

In the present investigation, results show a significant in plasma albumin, globulin, total protein levels in diabetic rats which are in agreement with many earlier reports. These alterations in diabetes are due to enhanced catabolism of proteins [46, 56]. It is well known that in insulin deficiency (diabetes) decreased protein synthesis and increased protein degradation lead to release of amino acids which are directed for gluconeogenesis. Lowered albumin and globulin in diabetic rats might be due to increased degradation and/or decreased production and/or increased urinary excretion of these substrates. Microalbuminuria in STZ-diabetic rats and humans is well documented with increased albumin excretion range (AER) [57] and formation of advanced glycation and products (AGEs) leading to kidney damage and diabetic glomerulopathy [58]. *Curcuma longa* supplementation appears to have rectified this abnormality in diabetic rats as evidenced by significantly elevated serum albumin levels in rats receiving *Curcuma longa* observed that the risk of

progression to over proteinuria can be reduced by improved glycemic control. In the present study also, the glycemic control exerted by *Curcuma longa* might have contributed to the restored plasma albumin levels. Moreover, supplementation of *Curcuma longa* might have induced protein synthesis by effective utilization of the available amino acids and also by reducing protein catabolism and/or by regulating certain signal transduction mechanisms and enzymes. Phytochemicals of *Curcuma longa* extract appear to have mitigated the metabolic abnormalities and restored the urea and creatinine levels.

Insulin is the principal regulatory hormone involved in the tight regulation of fuel metabolism. In response to blood glucose levels, it is secreted by the β-cell of the pancreas and exerts its effects by binding to cell surface receptors that are present on virtually all cell types and tissues [59]. In the present study, normal rats treated with *Curcuma longa* extract showed normal levels of insulin while diabetic rats had shown very low levels of insulin as a consequence of pancreatic β-cell damage indicating low pancreatic β-cell activity followed by Streptozotocin.

The serum biochemical parameter of control rats are Creatinine (2.16), Urea (26.945) and (95.74) was tabulated in Table-1. Increased levels of the creatinine (2.83), urea (35.36) and cholesterol (135.38) was recorded in the diabetic untreated rats (group-II), *Curcuma longa* treated rats shows significantly decreased levels of creatinine (2.34), urea (28.28) and cholesterol (102.68) when campared to the diabetic rats and also near to the normal rats (Table-5 and Figure-5),

Table 5. Serum Biochemical Parameters levels in the control and experimental animals

Parameters	**Group I**	**Group II**	**Group III**
Creatinine (mg/dl)	2.16± 0.16	2.83± 0.42	2.34± 0.68
Urea (mg/dl)	26.94± 3.94	35.36± 6.46	28.28± 5.98
Cholesterol (mg/dl)	95.74± 10.92	135.38± 23.6	102.68± 18.74

Values are mean ± S.D. of 6 individual rats

Hyperlipidemia is a known complication of *Diabetes mellitus* and coexists with it and is characterized by increased levels of cholesterol and also changes in lipoprotein patterns [60]. Interest in the study of plasma lipids in diabetes arises from the widely acknowledged higher incidence of atherosclerotic disease which is a major cause of premature death in diabetic patients whether it is type-I or type-II [51, 55].

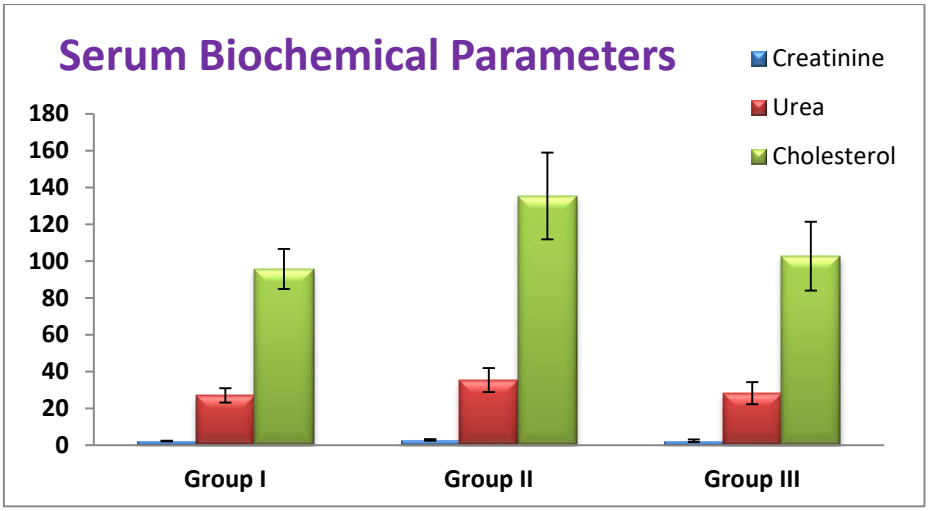

Figure 5. Serum Biochemical Parameters levels in the control and experimental animals

In the present investigation, results show a significant in urea and creatinine levels in diabetic rats which are in agreement with many earlier reports. These alterations in diabetes are due to enhanced catabolism of proteins [56]. It is well known that in insulin deficiency (diabetes) decreased protein synthesis and increased protein degradation lead to release of amino acids which are directed for gluconeogenesis. Due to increased catabolism of proteins and amino acids, hepatic ureagenesis and creatinine production are elevated in diabetic rats [46]. As a consequence, increments in urea and creatinine levels occur in plasma. Microalbuminuria in STZ-diabetic rats and humans is well documented with increased albumin excretion range (AER) [57] and formation of advanced glycation and products (AGEs) leading to kidney damage and diabetic glomerulopathy [58]. *Curcuma longa* supplementation appears to have rectified this abnormality in diabetic rats as evidenced by significantly elevated serum albumin levels in rats receiving *Curcuma longa* observed that the risk of progression to over proteinuria can be reduced by improved glycemic control. In the present study also, the glycemic control exerted by *Curcuma longa* might have contributed to the restored plasma albumin levels. Moreover, supplementation of *Curcuma longa* might have induced protein synthesis by effective utilization of the available amino acids and also by reducing protein catabolism and/or by regulating certain signal transduction mechanisms and enzymes. Phytochemicals of *Curcuma longa* extract appear to have mitigated the metabolic abnormalities and restored the urea and creatinine levels.

Insulin is the principal regulatory hormone involved in the tight regulation of fuel metabolism. In response to blood glucose levels, it is secreted by the β-cell of the pancreas and exerts its effects by binding to cell surface receptors that are present on virtually all cell types and tissues [59]. In the present study, normal rats treated with *Curcuma longa* extract showed normal

levels of insulin while diabetic rats had shown very low levels of insulin as a consequence of pancreatic β-cell damage indicating low pancreatic β-cell activity followed by Streptozotocin.

SUMMARY AND CONCLUSION:

In present investigation, anti-diabetic properties of *Curcuma longa* in STZ induced diabetic rat hematological parameters and serum biochemical parameters were studied with the blood glucose and body weight levels. Wistar stain male albino rats of 3 months age were used in the present study. They were maintained in the animal house at 24±2° C, humidity of 45-64% with photoperiod of 12 hours light and 12 hours darkness. Regarding selection of age and grouping of animals as mentioned in "Material and methods" was taken in to consideration to select 3 months old rats as adult age in this experimental design for expected results. They were maintained in clean poly propylene cages and fed with standard rat pellet diet (Hindustan lever Ltd., Mumbai) and water *ad libitum.* The animals of same age group were divided in to 3 groups, each group consists of six animals and the division of groups is as follows.

Group -1 : Normal Control rats.

Group- 2 : Diabetic rats (Streptozotocin)

Group -3 : Diabetic rats treated with 250 mg/Kg b.w. of *Curcuma longa*.

The blood samples were collected after completion of treatment i.e. on 22nd day of the treatment. The blood was used for the hematological parameters and separated serum was used for the serum biochemical parameters.

The summary of the results from this study is presented as follows:

1. No significant blood glucose level changes were observed in control rats. In diabetic rats blood glucose levels were increased. *Curcuma longa* rats, which were subjected to *Curcuma longa* extract supplementation showed decreased levels of blood glucose. This may be due to the antidiabetic compounds present in *Curcuma longa*.

2. We observed body weight changes in the current investigation in all experimental rats. In diabetic rats, the body weights were significantly decreased after induction of STZ. The decrease in body weight in diabetic rats clearly showed a loss or degradation of structural proteins. Weight loss which is one of the clinical features of *Diabetes mellitus* may be due to the degeneration of the adipocytes and muscle tissues to make up for the energy loss from the body due to frequent urination and over conversion of glycogen to glucose. In the *Curcuma longa* treated rats, body weights were gained near to control levels after treatment with *Curcuma longa* plant extract.

3. The blood parameters revealed significant alterations in all experimental groups. In group-II (Diabetic rats) the blood parameters such as Hemoglobin, RBC, WBC counts were highly decreased

which suggest the anemic condition in the body, and increased count was observed in group-III (Diabetic + *Curcuma lona*) treated rats.

4. Increased levels were observed in Albumin, Globulin, Total proteins, Creatinine, Urea and Cholesterol in group-II (Diabetic) rats indicating its impact on Soluble proteins, disturbance on immune mechanism etc, whereas the same were decreased in group-III treated with *Curcuma longa* plant extract of diabetic rats.

To conclude the present findings reveals that treatment with selected dosage of *Curcuma longa* extract is beneficial in countering the alterations in various blood and serum biochemical parameters. This study drawn a conclusion, stating that *Curcuma longa* treatment to diabetic rats may be beneficial to improve the metabolic efficiency and thereby improve the health status. Thus *Curcuma longa* may be useful in the formulation of herbal drugs which can be used in the treatment of diabetes.

REFERENCES:

1. Papadakis KA, Tabibzadeh S. 2002. Gastrointest Endosc Clin N Am. 12(3):433-49.
2. Kirtikar, K.R., Basu, B.D., (1975), Indian -Medicinal Plants, Vol 9, 2nd ed. 2435-2439.
3. Nadkarni, K. M. (1997), Popular Prakashan, pp, 1308-1315.
4. Sadak Basha S, Guru Sekhar M, Mannur Ismail S, Sree Vani P, Pushpa Latha B, Radha Madhavi YR and M. Bhaskar (2010a), Asian J. Exp. Biol. Sci. 1 (3): 627-632.
5. Sadak Basha S, Guru Sekhar M, Radha Madhavi Y.R, Mannur Ismail S and M. Bhaskar (2010b), Int. J. Pharmocol. Biol. Sci. 4 (1): 93-102.
6. Guru Sekhar M, Sadak Basha S, Radha Madhavi YR, Rama Krishna S, Mannur Ismail S and Bhaskar M. (2010), Adv. Phrmacol. Toxicol. 11(1): 95-105.
7. Osawa T. (2002), Nippon Rinsho. 60 Suppl 10:53-9.
8. Sreejayan, Rao MN. (1997), J Pharm Pharmacl. 49(1):105-7.
9. Ganada, OP., Simonson, DS. (1993), Diabetes Rev. 1,286-302.
10. Radha Madhavi Yeggnisetty Ramachaiahgari, Swapna Rekha Somesula, Pradeepkiran Jangampalli Adi, Ismail Shaik Mannur, Madhuri Enamalaa, Bhaskar Matcha. (2012), Digest J Nano. Biostru. 7(1): 175-184.
11. Omamto, H., Uchigata, Y. (1981), Nature. 294:284-286.
12. Gottried, S. P., Rosenberg, B. (1973), Clin, Chem. 19:107-108.
13. Low, P, A., Nickander, K. K., Tritscher. H. 1997. Diabetes. 46, S38-S42.
14. Vijay Kumar, M., Govindarajan, R., Rao, G. M. M., Rao, Ch. V., Shirwaikar, A., Mehrotra, S.,

Oushpangadan, P. (2006), J. Ethnopharma. 104:356-361.

15. Hunt JV, Dean RT and Wolf SP (1988), Biochem 256: 205-212.
16. Weaver DC, McDaniel ML, Naber SP, Barry CD, Lacy PE. 1978. Diabetes. 27(12):1205-14.
17. Boquist, L., Ericsson, I., Lorentzon, R. and Nelson, L. (1985), *FEES Lett.* 183, P. 173 – 176.
18. Malaisse, W. J., Lebrun, P. and Herchuelz, A. (1982), Pflugers Arch. 395(3), 201-203.
19. Tiedge, M., Lortz, S., Drinkgern, J. and Lenzen, S. (1997), Diabetes. 46(11), 1733-1742.
20. Lenzen, S. and Munday, R. (1991), Biochem Pharmacol. 42(7), 1385-1391.
21. Zhang, C. F., Zhu, X. H., Dong, F. T., Ye, J. J., Fei, P. F., Zhang, Q. N. and Du, H. (1992), Chin Med J Eng. 105(3), P. 234-236.
22. Lenzen, S., Tiedge, M. and Panten, U. (1987), Acta Endocrinol (Copenh), 115(1), P. 21-29.
23. Jiang, H., Xie, Z., Koo, H. J., McLaughlin, S. P., Timmermann, B. N. and Gang, D. R. (2006), Phytochemistry. 67, P. 232-244.
24. Ojewole, A. (2006), Phytother. Res. 20, P. 764-772.
25. Raju, J., Gupta, D., Rao, A. R., Yadava, P. K. and Baqucr, N. Z. (2001), Mol Cell Biochem. 224(1-2), P. 45-51.
26. Vats, V., Yadav, S. P. and Grover, J. K. (2003), J. Ethnopharmacol. 85(2-3), P. 237-242.
27. Khosla, P., Gupla, D. D., and Nagpal, R. K. (1995), Int.J. Pharmaco. 27, P. 89 93.
28. Puri, D., Prabhu, K. M. and Murlhy, P. S. (1995), Indian J Clin Bioehem. 9, 13-16.
29. Sarah, N., Oluwatosin, A. and Edith, A. (2009), Pak. J. Nutr. 8 (5), P. 625-628.
30. Shnkar, T. N. B., Shanta, N. V., Ramesh, H. P., Murthy, I. A. S. and Murthy, V. S. (1980), Ind. J. Exp. Bio. 18(1), P. 73-75.
31. Nishiyama, T., Mae, T., Kishida, H., Tsukagawa, M., Mimaki, Y., Kuroda, M., Sashida, Y., Takahashi, K., Kawada, T., Nakagawa, K. and Kitahara, M. (2005), J. Agri. Food and Chem. 53, P. 959-963.
32. Mahesh, T., Balasubashini, M. and Menon, V. (2005), J. Medicinal Food. 8, P. 251-255.
33. Neel, J. V. (1962), Am J Hum Genet. 14, P. 353–362.
34. Knowler, W. C., Pettitt, D. J., Bennett, P. H. and Williams, R.C. (1983), Am J Phys Anthropol. 62, P. 107 –114.
35. Ravussin, E. and Bogardus, C. (1990), Infusionstherapie. 17, 108 –112.
36. Sharma, A. M. (1998), J Mol Med. 76, P. 568-571.
37. Mokdad, A. H., Ford, E. S., Bowman, B. A., Dietz, W. H., Vinicor, F., Bales, V. S. and Marks, J. S. (2001), JAMA. 289, P. 76 –79.
38. Hu, F. B., Manson, J. E., Stampfer, M. J., Colditz, G., Liu, S., Solomon, C. G. and Willett, W. C. (2001), N Engl J Med. 345, P. 790 –797.

39. Olefsky, J., Reaven, G. M. and Farquhar, J. W. (1974), J Clin Invest. 53, P. 64 –76.
40. Pi-Sunyer, F. X. (1993), Ann Intern Med. 119, P. 722 –726.
41. Klein, S., Sheard, N. F., Pi-Sunyer, X., Daly, A., Wylie-Rosett, J., Kulkarni, K. and Clark, N. G. (2004), Am J Clin Nutr. 80, P. 257 –263.
42. Reno, J. and Leland, J. (1999), Newsweek. 134, P. 56-57.
43. Rajkumar, L. and Govindarajulu, P. (1991), Indian JExp Biol. 29, P. 1081-1083.
44. Al-Amin., Martha Thomson Khaled, K., Al-Qattan., Ritta Peltonen, S. and Muslim, A. (2006), British Journal of Nutrition. 96, P. 660-666.
45. Akbarzadeh, A., Norouzian, D., Mehrabi, M. R., Jamshidi,S. H., Farhangi, A., Allah Verdi, A., Mofidian, S. M. A. and Lame Rad, B. (2007), Ind. J. Cli. Biochem. 22, P. 60-64.
46. Mude Ravi Naik., Jangampalli Adi Pradeepkiran., Somesula Swapna Rekha. and Match Bhaskar. (2012), Digest Journal of Nanomaterials and Biostructures. 7 (2), P. 649 – 655.
47. Weiss, G. and Goodnough, L.T. (2005), Eng. J. Med. 352, P. 1011–1023.
48. Kolanjiappan, K., Manoharan, S. and Kayalvizhi, M. (2002), Clin Chim Acta. 326, P. 143–149.
49. Lee, C. D., Folsom, A. R., Nieto, F. J., Chambless, L. E., Shahar, E. and Wolfe, D. A. (2001), Am J Epidemiol. 154, P. 758–764.
50. Tong, P. C., Lee, K. F., So, W. Y., Ng, M. H., Chan, W. B. and Lo, M. K. (2004), Diabetes Care. 27, P. 216–222.
51. Radha Madhavi, Y. R., Swapna Rekha, S., Pradeepkiran, J. A., Ismail S. M., Maduri, E. Bhaskar, M. (2010), Digest Journal of Nanomaterials and Biostructures. 7(1), P. 175-184.
52. Lee, F. T., Cao, Z., Long, D. M., Panagiotopoulos, S., Jerums, G. and Cooper, M. E. (2004), J Am Soc Nephrol. 15, P. 2139–2151.
53. Shanmugam, N., Reddy, M. A., Guha, M. and Natarajan, R. (2003), Diabetes. 52, P. 1256–1260.
54. Kozlov, I. A., Novitski, V. V. and Baìkov, A. N. (1995), Biull Eksp Biol Med. 120, P. 33–35.
55. Betteridge, D. J. (1989), Brit Med Bull. 45 (1), P. 285-311.
56. Willatgamuwa, S. A., Platel, K., Saraswathi, G. and Srinivasan, K. (1998), *Nutr Res.* 18, P. 131-124.
57. Berg, T. J., Bangstad, H. J., Torjesen, P. A., Osterby, R., Bucala, R. and Hanssen, K. F. (1997), Metabolism. 46(6), P. 661-665.
58. Bangstad, H. J., Osterby, R. and Dal- Jorgensen, K. (1993), Diabetoiogia. 36, P. 523-529.
59. Cheng, A., Dube, N., Gu, F. and Tremblay, M. C. (2002), Eur J Biochem. 269, P. 1050-1059.
60. Bagdade, J. D., Root, R. K. and Bulger, R. J. (1974), *Diabetes*. 23, P. 9-15.