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HYPOGLYCAEMIC ACTIVITY OF *Psidium guajava* LINN. FLOWERS AGAINST ALLOXAN INDUCED DIABETES IN MALE ALBINO RATS

Priyadharshini,G. and Anuradha,R.

PG and Research Department of Biochemistry, Sengamala Thayaar Educational Trust Women's College, Sundarakkottai Mannargudi

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ABSTRACT

Over the past decade, herbal medicine have been accepted universally and they have an impact on both world health and international trade. Diabetes mellitus is a metabolic disorder in the endocrine system. Plants provide a potential source of hypoglycemic drugs and are widely used in several traditional systems of medicine to prevent diabetes.Qualitative phytochemical analysis of aqueous extract of flower of *psidium guajava* was carried out with a view of developing leads for a new therapeutic products. The study was designed to investigate the hypoglycaemic effects of oral administration of aqueous extracts of *psidium guajava* flower in alloxan induced diabetic rats. After oral administration of the flower extract to diabetic rats, the blood glucose level significantly reduced, which is much faster and more than that of metformin. The aqueous extracts of flower of *psidium guajava* significantly reduced plasma glucose, creatinine, urea, AST, ALT, ACP, ALP, LPO, glucose-6-phosphatase and fructose1,6 bisphosphatase but increased level of serum insulin, protein, CAT, SOD, glycogen synthase and hexokinase. For all the above biochemical parameters observed and it was reverted toits normal level after flower extract treatment. The present investigation suggests that flowers of *psidium guajava* exhibit hypoglycaemic activity in alloxan induced diabetes in experimental rats.

Keywords: Diabetes mellitus, alloxan, *Psidium guajava*, hypoglycaemic activity, metformin, blood glucose.

INTRODUCTION

Diabetes mellitus is the name given to a group of disorders characterized by chronic hyperglycemia, polyuria, polydipsia, polyphagia, emaciation and weakness due to disturbances in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion and/or insulin action (1). Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with aberration in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. It has been reported that the chronic hyperglycemia of diabetes is associated with complications like renal failure, coronary artery disorder, neurological complications, cerebro-vascular disease, blindness, limb amputation, long term dysfunctions and failure of various organs and eventually premature death (2).Diabetes results in abnormal levels of glucose in the blood stream. This can cause severe short term and long term consequences ranging from brain damage to amputations and heart diseases (3). Diabetes mellitus associated with long term complications such as retinopathy, neuropathy and angiopathy (4).It has been predicted by world health organization that Indian people are more genetically susceptible to diabetes accounting about 30 to 33 million and would go up to 40 million by the end of 2010 which further will reach to maximum of 74 million by 2025 (5). Multiple biochemical impairments associate with micro and macro vascular complications

which are a major cause of morbidity and death in diabetes mellitus (6,7) Diabetes becoming the third killer of mankind, after cancer and cardiovascular diseases, because of its high prevalence morbidity and mortality (8)Diabetic have significant accelerated levels of oxidative stress and this contributes massively to most neurological, cardiovascular, retinal, renal diabetic complications (9). Hence to overcome the adverse effects like hematological effects, coma, disturbances of liver and kidney etc (10). Many traditional plant medicines are used throughout the world to treat the diabetic diseases (11). When compared with synthetic drugs, the plant drugs have less toxic effects with fever side effects (12). Available literature indicates that there are more than 800 plants species showing hypoglycemic activity (13). Medicinal plants used in the treatment of DM are of considerable interest because of their perceived little or no side effect thus making them to be more preferred to the synthetic therapeutic agents known to be associated with many side effects (14).The present study was carried out in rats to test the efficacy of flowers of *psidium guajava* on serum insulin, hyperglycemia and enzymatic changes associated with diabetes.

Guava (*Psidium guajava* Linn.) is a large tropical evergreen shrub or small shade tree. The pharmacological actions and the medicinal uses of aqueous extracts of guava leaves in folk medicine include the treatment of various types of gastrointestinal disturbances such as vomiting, diarrhea, inhibition of the peristaltic reflex, gastroenteritis, spasmolytic activity, dysentery, abdominal distention, flatulence and gastric pain (15). Moreover, aqueous extracts of guava leaves were described to be effective against a number of microbial strains (16) and anti-rotavirus activity(17). It has also been used extensively as a hypo-glycaemic agent. Many pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, hepato-protective, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflammatory and antinociceptive activities, supporting its traditional uses (18).

MATERIALS AND METHODS

The fresh flowers of *Psidium guajava* were collected from Thiruvarur District, Tamilnadu, South India. During February-March-2012. The plant was identified by Dr.S. John Britto, the Director, the Rapinat Herbarium and the centre for Molecular Systematics, St.Joseph's College, Thiruchirappalli and a voucher specimen was deposited in the Rapinat Herbarium of St. Joseph's, College Thiruchirappalli (Voucher No.GP001/2012).The collected fresh flowers of *Psidium guajava* was shade, dried and powdered. The aqueous extract of this is flower was prepared and used for oral administration in experimental rats.Qualitative phytochemical examination were carried out for aqueous extracts as per the standard methods (19).

Healthy adult wistar strain of male albino rats two to three months old and weighing 150g-200g were obtained from Tamilnadu Veterinary and Animal Sciences University, Chennai and the animals were cared for in accordance with the principles and guidelines of Indian National Law on Animal care and use. The animals were allowed to acclimatize under laboratory conditions for a period of 5 days prior to the experiment. Animals were fed with standard rat chow pellet obtained from Sai Durga Foods and Feeds, Bangalore, India and water *ad libitum*. Diabetes mellitus was induced in a batch of normoglycemic albino rats, starved for 16 hours, 150mg/kg body weight of alloxan monohydrate was dissolved in physiological saline and injected intraperitoneally (20). This dose of alloxan produced persistent hyperglycemia after 4 days as revealed by determination of sugar levels. The diabetes induced rats were chosen and grouped for further studies.

EXPERIMENTAL DESIGN

Wistar strains of male albino rats weighing 150-200gm were used as the experimental models. The rats were divided into four groups comprising six rats each.

Group I : Normal control animals received normal diet and water adlibitum

Group II : Animals treated with alloxan in normal saline at a dosage of 150-mg/kg body weight IP.

Group III : Animals were treated as in Group II. After 4 days of alloxan induction, treated orally with flowers of *Psidium guajava* aqueous extract 100-mg/kg body weight.

Group IV : Animals were treated as in Group II. After 4 days of alloxan induction, treated with metformin 1-mg/kg body weight for 30 days orally.

After the experimental period, animals were sacrificed by cervical decapitation. Blood was collected; liver was dissected out and washed in ice-cold saline. Liver tissues were homogenized in 0.1 M Tris Hcl buffer, pH 7.4 and used for estimating enzyme parameters such as glucose-6-phosphatase, fructose1,6 bisphosphatase, glycogen synthase and hexokinase.

Statistical analysis

All the results were expressed as mean ± S.E. The data were statistically analyzed by one – way analysis of variance (ANOVA) and P values <0.05 were considered significant.

RESULTS

Table 1 represents phytochemical analysis of flower extract of *P.guajava*. *P.guajava* contains alkaloids, carbohydrate, glycosides, saponins, resins, phenols, tannins, flavonoids, proteins and amino acids, steroid and it was observed that fixed oils, fats, coumarins and chlorogenicacid were absent in *P.guajava*. As tested plants contains important secondary metabolites.The primary study support the further usage of *P.guajava* in experimental animal model received alloxan for evaluate antidiabetic efficiency.

Table 2 shows the effects of the aqueous extract of *P.guajava* on blood glucose, insulin, protein, AST, ALT, ALP, ACP, creatinine, urea catalase, SOD, LPO in alloxan induced diabetic rats.

Table 3 shows the effects of the aqueous extract of *P.guajava* on hexokinase, glucose6phosphatase, fructose1,6 bisphosphatase and glycogen synthase.

Table 1: Preliminary phytochemical constituents of flower extract of *Psidiumguajava* .

S.No.	**Phytochemical compound**	**Result of qualitative tests**
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Glycosides	+
4.	Saponins	+
5.	Fixed oils and fats	–
6.	Resins	+
7.	Phenols	+
8.	Tannins	+
9.	Flavonoids	+
10.	Proteins	+
11.	Aminoacids	+
12.	Steroids	+
13.	Coumarins	–
14.	Chlorogenicacid	–

(+) : presence (-) : Absence

Table 2: Effect of aqueous extract of *P.guajava* flowers on following biochemical parameters in alloxan induced experimental rats.

Groups	Glucose (mg/dl)	Insulin (IU/ml)	Protein (g/dl)	AST (U/L)	ALT (U/L)	ALP (KA Unit)	ACP (KA Unit)	Creatinine(mg/dl)	Urea (mg/dl)	CAT (units/ mgprotein)	SOD (units/ mgprotein)	LPO (mol MDA/g wet tissue weight)
Group I	89.3± 0.3[a]	19.5± 0.95[a]	7.3± 0.49[a]	73.23 ± 0.85[a]	67.96 ± 0.55[a]	12.73 ± 0.2[a]	3.15± 0.61[a]	0.52± 0.10[a]	17.2± 0.32[a]	78.04 ± 2.28[a]	5.2± 0.36[a]	0.45± 0.05[a]
GroupII	243.1 ± 0.65[b]	9.3± 1.0[b]	5.13± 0.30[b]	194.53± 4.68[b]	248.9 ± 0.30[b]	21.1± 1.5[b]	7.6± 0.52[b]	1.77± 0.80[b]	36.3± 1.00[b]	55.3± 0.94[b]	1.9± 0.1[b]	2.23± 2.3[b]
GroupIII	110.9 ± 1.04[a]	15.4± 0.85[a]	6.93± 0.15[a]	70.23 ± 0.15[a]	85.66 ± 0.83[a]	17.1± 0.49[a]	6.9± 0.36[a]	1.06± 0.37[a]	15.2± 0.85[a]	64.6± 0.77[a]	4.6± 0.35[a]	0.66± 0.20[a]
GroupIV	84.8± 0.29[a]	19.3± 0.55[a]	7.4± 0.26[a]	64.26 ± 5.4[a]	83.3± 3.32[a]	12.9± 0.37[a]	5.46± 0.25[a]	0.89± 0.05[a]	16.2± 0.58[a]	72± 3.13[a]	4.5± 0.26[a]	0.61± 0.08[a]

Results were expressed as mean ± S.E. The data were statistically analyzed by one way analysis of variance (ANOVA) and p values <0.05 were considered significant.

Table 3: Effect of aqueous extract of *P.guajava* flowers on enzymes of carbohydrate metabolism in alloxan induced experimental rats.

Groups	Hexokinase (n moles of glucose 6phosphate formed/h/mg protein)	Glucose-6 phosphatase(moles of pi liberated/min/ mg protein)	Fructose 1,6bisphosphatase (moles of pi liberated/min/ mg)	Glycogen synthase (unit/mg of protein/min)
Group I	293± 7[a]	0.170± 0.002[a]	0.264± 0.013[a]	3.6± 0.2[a]

Group II	84.66± 15.97[b]	0.242± 0.008[b]	0.424± 0.004[b]	1.53± 0.47[b]
Group III	162.66± 14.18[a]	0.191± 0.002[a]	0.268± 0.013[a]	1.9± 0.1[a]
Group IV	191± 3.60[a]	0.168± 0.003[a]	0.288± 0.029[a]	2.0± 0.78[a]

Results were expressed as mean ± S.E. The data were statistically analyzed by one way analysis of variance (ANOVA) and p values <0.05 were considered significant.

DISCUSSION

In Ayurvedic and indigenous folk medicine system, the hypoglycemic plants have been used mostly in their natural forms, consisting of both inorganic and organic constituents of the concerned herbs. It is important to note that the inorganic part of medicinal plants containing mainly mineral, plays a contributory role in enhancing hypoglycemic activity (21,22) and their indirect role in diabetes management is increasingly recognize (23)Wide application of medicinal plant in the management of DM has been reported (24, 25, 26)

The flower extract of *psidium guajava* Linn.contains a range of active pharmacological agents such as alkaloids, carbohydrate, glycosides, saponins, resins, phenols, tannins, flavonoids, proteins and amino acids, steroids. Literature showed that saponins and flavonoids are good anti diabetic metabolites(27). Alkaloids, glycosides, carbohydrates, and saponins have similarly been implicated in the antidiabetic activities of plants(28,29).

Alloxan a beta cytotoxin, is widely used in animal models to induce chemical diabetes by damaging the pancreatic beta cells (30, 31). Consequently, there is reduced secretion of insulin leading to clinical conditions such as hyperglycemia, polyphagia, polydypsia, polyuria and weight loss (32). Alloxan has been observed to cause a massive reduction in the number of the β-cells of the islets of langerhans and induce hyperglycemia (33). The diabetogenic action of alloxan is mediated by reactive oxygen species, with simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of pancreatic β-cells which reduces the synthesis and the release of insulin (34, 35).

There is an increasing evidence that alloxan caused diabetes by rapid depletion of cells, by DNA alkylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus. Its leads to a reduction in insulin release there by a drastic reduction in plasma insulinconcentration leading to stable hyperglycemic states (36).

Liver is the candidate organ invoved in glucose homeostasis. It is the main site for glycolysis, a process where glucose is degraded and gluconeogenesis, where glucose is synthesized from lactate, aminoacids and glycerol. These are the two important complementary events that balance the glucose load in our body (37).

The results of the present study indicated that the flower extract of *P. guajava* is capable of reducing blood glucose level associated with diabetes. Administration of alloxan increased plasma glucose when compared to normal animals and also induced persistent diabetes mellitus in rats. Diabetic rats treated with flower extract showed reduction in blood glucose in comparison to untreated diabetic rats and the results indicates the efficacy of aqueous extracts of *P.guajava* decrease the blood glucose level in alloxan induced diabetic rats.

Insulin influences the intracellular utilization of glucose in a number of ways. Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes

including glucokinase and phosphofructokinase. Alloxan (beta toxin) induced diabetes in a wide variety of animals, by damaging the insulin secreting beta cell resulting in a decrease in endogenous insulin release, which decreased the utilization of glucose by the tissues.The significant antidiabetic activity of flower extract of *Psidium guajava* may be due to the inhibition of subsequent tissue damage induced by alloxan or potentiation of serum insulin effect. Metformin does not stimulate insulin secretion and acts by reducing hepatic glucose production through inhibition of gluconeogenesis and to a lesser extent.

Renal disease is one of the most common and severe complications of diabetes. Insulin is a physiolgical factor, which plays an important role in the maintanence of protein synthesis, but also inhibits protein degradation (38). The increase in total protein may be due to changes in circulating amino acid levels, hepatic amino acids uptake and muscle output of amino acid concentrations (39). In the present study, the elevated level of serum proteins may related with increased level of plasma insulin in diabetic rats treated with the flower extracts of *Psidium guajava* and metformin.

The enzyme directly associated with the conversion of amino acids to keto acids are aspartate transaminase and alanine transaminase. These enzyme activities were increased in diabetic conditions (40) have reported the increase of AST and ALT in the liver of diabetic animals. Treatment with flower extracts of *P.guajava* normalized these enzyme activities. Increased levels of AST and ALT in diabetic liver of rats were reported by 41. The increased protein catabolism accompanying gluconeogenesis and urea formation that are seen in diabetic state might be due to result of elevation of tissue transaminases. The rise in ALT activity is almost always due to hepatocellular damage and is usually accompanied by rise in AST.

In addition to the assessment of AST and ALT levels during diabetes, the measurement of enzymatic activities of phosphatases such as acid phosphatase (ACP) and alkaline phosphatase (ALP) is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants (42) In the present study, serum ACP and ALP increased considerably in alloxan induced diabetic rats. Elevated level of these enzymes in diabetes may be due to extensive damage to liver in the experimental animals by alloxan. Treatment with aqueous extract of flower of *P.guajava* in alloxan-induced diabetic rats produces a more significant decline in these levels. From the present observation, it was evident that aqueous flower extract protects the adverse effects of lipid peroxide mediated tissue damage in alloxan induced diabetic rats.

There is significant increase in the level of creatinine which is the marker of renal dysfunction (43) in the diabetic groups compared to control level. After treatment of aqueous flower extract of *P.guajava* increased the total protein and lowered the serum creatinine level by enhancing the renal function that is generally impaired in diabetic rats.

For the detoxification of ammonia in mammals the urea cycle is the main biochemical pathway. Increased activity of the urea cycle enzymes in diabetic condition may be due to increased protein catabolism, which results in the increased elimination of urea nitrogen. In diabetic condition, urea synthesis was normalized by extract treatment. Administration of flower extract to diabetic rats reduced the elevated blood urea levels to normal levels.

Free radicals exert diverse biochemical effects mainly derived from univalent reduction of oxygen and giving rise to numerous by products through reactions with almost all the unsaturated double bonds found in natural living cells (44).The levels of potentially toxic superoxide radicals and hydrogen peroxide have been controlled by superoxide dismutase and catalase. The level of catalase was significantly reduced in alloxan induced rats. These adverse changes was reversed to near normal values in aqueous extract of *P.guajava* flowers treated rats. It is well known that catalase play an important role as protective enzymes against free radical formation in tissues (45)

Activities of superoxide dismutase and catalase are generally high in liver of normal rats (46). Like catalase, the activity of superoxide dismutase was decreased in liver of diabetic rats. And this decreased values was normalized by the aqueous flower extract of *P.guajava*.

Production of lipid peroxides by free radicals such as superoxide hydroxyl radical and hydrogen peroxide causing cellular injury (47). Increased lipid peroxidation was observed in liver of diabetic rats. The increased levels were reverted to near normal after flower extract treatment of *Psidium guajava*. Alloxan induced lipid peroxidation in rat erythrocytes through the generation of hydrogen peroxide was observed by48. These increased lipid peroxidation in tissues suggested the presence of oxidative cellular injury (49).

Hexokinase is the prime enzyme which catalyses the phosphorylation of glucose by adenosine triphosphate in the utilization of glucose by glycolysis, and it plays an important role the entry of glucose in to cells and channeling it in to various metabolic routes (50)Liver which is known to be dependent on insulin for glucose metabolism has been found to have a significantly lower hexokinase activity in diabetes. The decreased activity of glucokinase was correlated with the decreased level of glucokinase. Administration of *Psidium guajava* flower extract treatment to diabetic rats produced an increase in the level of glucokinase and thereby enhancing the activity of the hexokinase enzyme.

The activity of gluconeogenic enzyme, glucose-6-phosphatase in liver was found significantly elevated in diabetic rats when compared with control rats. Oral administration of flower extracts of *Psidium guajava* for 30 days brought back the activity of the glucose-6-phosphatase to the near normal level.

The enzyme fructose-1,6-bisphosphatase which catalyses the hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate is an essential step in the gluconeogenic pathway (51). The activity of the enzyme fructose-1,6-bisphosphatase was found to have increased in diabetic rat liver (52,53) Administration of flower extract of *Psidium guajava* to diabetic rats normalized the increased liver fructose-1,6-bisphosphatase activity.

Glycogen synthase, a highly phosphorylated protein is the rate limiting enzyme of glycogen synthesis. Decreased activity of glycogen synthase in liver of diabetic mice has been reported (54). The activity of glycogen synthase was induced by *Psidium guajava* flower extract and reverted the enzyme values to normal in rat hepatocytes. The abnormal activity of glycogen synthase in the liver of diabetic rats returned to normal by flower extract of *P.guajava*.

The studies on hypoglycaemic activity in alloxanised rats, significant reduction of blood glucose was observed from the 2nd day of the study. The comparable effect of the extract with metformin may suggest similar mode of action since alloxan permanently destroys the pancreatic B cells and the extract lowered blood sugar level in alloxanised rats, indicating that the extent possesses extra pancreatic effect. From the phytochemical analysis it was found that the major chemical constituents of the extract and some of this active principle including flavonoids are known to be used for the treatments of diabetes (55, 56) on the basis of the above evidences it is possible that the presence of flavonoids and tannins are responsible for the observed Antidiabetic activity (57).

CONCLUSION

Although numerous oral hypoglycemic drugs exist alongside insulin, still there is no promising therapy to cure diabetes. India has a rich emporium of various potent herbs and herbal components for treating various diseases including diabetes. In recent years, numerous traditional medicinal plants were tested for their antidiabetic potential in the experimental animals. Oral administration of *Psidium guajava* flower extract reversed the above biochemical parameters and improved towards normalcy. From this study it was concluded that the aqueous extract of *Psidium guajava* flower extract possesses good glycaemic control properties in alloxan induced experimental rats. This study justifies the traditional use of *Psidium guajava* flower in diabetes management.

REFERENCES

[1] Deb L, and Dutta A, Diabetes mellitus its possible pharmacological evaluation techniques and naturopathy,*Int J Green Pharmacy.,* (2006) V.1(7): P.28

[2] Lyra R M, Oliveria D, Lins N, Cavalcanti N, Arquivos Brasileiros de *Endocrinol Metabol*., (2006) V.50: P 239-249.

[3] American Diabetes Association, Diagonosis and classification of diabetes, *Diabetes care 30.,* (2007) suppl. l: S 42-7. [Medline].

[4] Kristova V, Liskoya S, Sotnikova S, Vojtko R, Kurtansky A, Sulodexide improve endothelial dysfunction in streptozotocin induced diabetes in rats. *Physiol. Res.,* (2008) P.491-494

[5] Porter J R, Barrett T G, *J Med Genetics.,* (2005) V.42: P.893-902.

[6] Xie W, Zhao Y, Gu D, Du L, Cai G, Zhang Y, Scorpion in combination with gypsum: novel antidiabetic activities in streptozotocin induced diabetic mice by up regulating pancreatic PPARg and PDX-1 expressions, *Evidence based complementary and alternative medicine.,* (2011) P.9.

[7] Berger G., Stenstr" OM., Sundkvist G., (1999) Incidence, prevalence and mortality of diabetes in a large population: A report from the skaraborg diabetes registry. *Diabetes Care.,* V.22(5): P.773-777.

[8] Li W L, Zheng H C, Bukuru J, De kimpe N, Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus, J. *Ethnopharmacol.,* (2004) V.92: P.1-21

[9] Mohanty P, Hamouda W, Garg R, Aljada A, Ghanim H, Dandona P, Glucose challenge stimulates reactive oxygen species (ROS) generation by leucocytes.

Journal of clinical endocrinology and metabolism.. (2000) V.85: P.2970- 297]

[10] Larmer J, Insulin and oral hypoglycemic drugs, Glucagon In: Gilman A G, Goodman L S, *The pharmacological basis of therapeutics*.7th edition, NewYork: Macmillan publishing.,(1985) P.1490.

[11] Syed Mansoor Ahmed, Vrushabendraswamy B M, Gopkumar, Dhnapal R, Chandrashekar V M, Iranian J. *Pharmacol Therapeautics.,* (2005) V.4(1): P.36-39.

[12] Moming A, Role of indigenous medicine in primary health care. Proceeding of first international seminar on Unani medicine., Newdelhi, (1987) P. 54.

[13] Rajagopal K, Sasikala K, *Singapore Med J.,* (2008) V.49: P.137-141.

[14] Venkatesh S, Reddy G D, Reddy B M, Antihyperglycemic activity of *Helictres isora* roots in alloxan induced diabetic rats, *Pharmacuetical Biology.,* (2003) V.41(5): P.347-350

15] Lutterodt G D, Innhibition of Microlax – induced experimentel diarrhea with narcotic-like extracts of *Psidium guajava* leaf in rats, *Journal of Ethno Pharmacology*., (1992) V.37(2): P. 151-157.

[16] Chulasiri M, Suthienkul O, Pavaro C, Wongkrajang Y, Herbal extracts for diarrheal treatment: Antibacterial activity in vitro, *Journal of public health.,* (1986) V.16: P.21-35.

[17] Goncalves J L S, Lopes R C, Oliveira D B, Costa S S, Miranda M M F S, Romano M T V *et al*., In vitro-rotavirus activity of some medicinal plants used in Brazil against diarrhea, *Journal of Ethnopharmacology.,* (2005) V.99(3): P.403-407.

[18] Gutierrez R M, Mitchell S, Solis R V, *Psidium guajava* : A review of its traditional uses, phytochemistry and pharmacology, *J.Ethnopharmacol.,* (2008) V.117(1): P. 1-27.

[19] Kokate C K, Purohit A P, and Gokhale S B, *Pharmacognosy,* 3rd edition, Niraliprakashan, Pune. (1995) P.214.

[20] Jadhav J K, Masirkar V J, and Deshmuk h V N, Antihyperglycemic effect of *Diospyros melanoxylon*. Roxb bark against alloxan induced diabetic rats, *International Journal of Pharm Tech Research.,* (2009) V. 1(2): P.196-200.

[21] Kar A, Choudhary B K, Important mineral content of a few ayurvedic herbs with a discussion on medicinal aspects, *Indian Drugs.,* (1994) V. 31(3):P.127-30.

[22] Kar A, Choudhary B K, Bandyopadhyay N G, Preliminary studies on the inorganic constituents of some indigenous hypoglycemic herbs on oral glucose tolerance test, *J.Ethnopharmacol.,* (1999) V. 64(2): P.179-84.

[23] Gurson C T, Saner G, Effect of chromium on glucose utilization in marasmic protein calorie mal nutrition, *Am J Clin Nutr.,* (1971) V.24: P.1313-19.

[24] Latha M, Pari L Effect of an aqueous extract of *Scoparia dulcis* on blood glucose, plasma insulin and some poly pathway enzymes in experimental rat diabetes, *Braz. J. Med. Biol. Res.*(2004)V.37(4):P.577- 586.

[25] Pareek H, Sharma S, Khaija B S, Jain K, Jain G C, Evaluvation of hypoglycemic and antihyperglycemic potential of *Tridax Procumbens* BMC component, *Altern.Med.,* (2009) P. 9:48.

[26] Bera T K, De D, Chatterjee K, Ali K M, Ghosh D, Effect of diashis, a poly herbal formulation , in streptozotocin induced diabetic male albino rats, *Int . J.Aurveda Res.,* (2010)V.1(1): P.18-24

[27]Sherma R D, Sarkhar D K, and Hazra M B, Toxicological evaluation of fenugreek seeds: a long term feeding experiment in diabetic patients, *Phytother. Res.,* (2010) V.10: P.519-520.

[28] Reher G, Slijepcevic M, and Krans L, Hypoglycaemic activity of triterpenes and tannins from *Sarcopterium spinosum* and two sanguisorba species, *Planta Med.* (1991)V.(57):P.57-58.

[29] Sikarwar M S, and Patil M B, Antidiabetic activity of *Cratevanurvala* stem bark extracts in alloxan-induced diabetic rats, *J. Pharm.Bioallied. Sci.,* (2010) V.2:P.18-21.

[30] Ho E, Chen G, Bray TM, Supplementation of N-acetylcysteine inhibits NFkB activation and protects against alloxan-induceddiabetes in CD-1 mice, *FASEB Journals.,* (1999)V.13**(**13): P.1845-1854

[31]Saravanan R, Pari L , Antihyperlipidemic and antiperoxidative effect of Diasulin, a polyherbal formulation in alloxan induced hyperglycaemic rats, BMC Complement *Altern. Med*., (2005) V.5(10): P.1186/1472-6882-5-14.

[32] Braganca L A R, *Plantas Medicinais Antidiabeticas.,* UFF: Niteroi. (1996).

[33] Goldner M, Gomori G, Alloxan induced diabetes, *Endocrinology,* (1943)V.33, P.297-299.

[34] Szkudelski T, The mechanism of Alloxan and strepotozotocin action in -cells of rat pancreas, *Physiol Re.s,* (2001) V.50 P. 537-546.

[35] Sakurai K, Katoh M, Someno K, Fujimoto Y, Apoptosis and mitochondrial damage in INS-1 cells treated with alloxan, *Biol. Pharm. Bull,* (2001) V.24, P.876-882.

[36] Yasodha Krishna janapati, Jayaveera KN, Ravindra Reddy K, Rupesh K,Raghavendra D, Siddaiah M, *J of PharmacyandChemistry.,*(2008) P.156-160.

[37] Prakasam S, Sethupathy S, Pugalendi K V, *Pharmazie.,* (2002) V.57:P.11.

[38] Pathak A, Dhawan D Effects of lithium on the levels of blood urea and creatinine in diabetic rats, *Med. Sci. Res.,(*1988) V. 26: P.855-859.

[39] Felig P, Wahren J, Sherwin R, Palaiologos G ,Amino acid and protein metabolism in diabetes mellitus, *Arch. Int. Med.,* (1977) V.137: P.507-513.

[40] Begum N, and Shanmugasundaram K R, Transaminase in experimental Diabetes, *Arogya.,* (1978) V.4: P.116-122.

[41] Jorda A, Cabo J, and Grisolia S, Changes in the levels of urea cycle enzymes and in metabolites there of in diabetes, *Enzyme.,* (1981) V.26: P.240-244.

[42] Som Nath Singh, Praveen Vats, Shoba Suri, Radhey Shyam M M L., Kumria, S.Ranganathan, K. Sridharan, Effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats, Journal of Ethnopharmacology, Issue 3, August (2001)V.76: Pages
269-277.

[43] Alarcon A F J, Calzada B F, Hernandez G E, Ruiz A C and Roman R R, Acute and chronic hypoglycaemic effect of Ibervillea sonorae root extracts-II, *J Ethnopharmacol.,* (2005) V.97: P.447

[44] Remacle J, Lambert D, Raes M, Pigeolet E, Michiels C, and Toussiant O, Importance of various antioxidant enzymes for cell stability, Confrontation between theoretical and experimental data, *Biochem. J.,* (1992) V.286: P.41-46. Roglic G, Wild S, Green A, Sicree R, King H, Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030, *Diabetes Care.,* (2004) V.27(5): P. 1047-1053.

[45]Oberly W R, and Buettner R G, Role of superoxide dismutase in cancer, *Cancer Research.,* (1974) V.35: P.1141-1149.

[46] Wohaieb S A, and Godin D V, starvation related alterations in free radical tissue defense mechanisms in rats, *Diabetes.,* (1987) V.36: P.169-173.

[47] Ashwood- smith, M J, current concepts concerning radioprotective and cyto protective properties of dimethyl sulphoxide in cellular system, *Ann. NY. Acad . sci.,* (1975) V.243: P.246-256.

[48] Yadav P, Sarkar S, and Bhatnagar D, Protective effect of glutathione and selenium against alloxan induced lipid peroxidation and loss of antioxidant enzymes in erythrocytes, *J. Biosci.,* (1994) V.10: P.19-25.

[49]Asayama K, Hajashibe H, Dobashi K, Niitsu T, Miyao A, Katok, Antioxidant enzyme status and lipid peroxidation in various tissue of diabetic and starved rats, *Diabetes Res.,* (1989) V.12: P.85-91.

[50] Gonzalez A M, Sochor M, Hother sall, J S, Mc lean P, Effect of aldose reductase inhibitor (sorbinil) on integration of polyol pathway, Pentose phosphate pathway and glycolytic route in diabetic rat lens, *Diabetes.,* (1986) V.35: P.1200-1205.

[51] Horecker, B L, Melloni, E, and Pontremoli S, Fructose-1,6-bisphosphatase:properties of the neutral enzymes and its modification by proteolytic enzymes, *Adv. Enzymol.,* (1975)V.42:P.193-226.

[52] Weber G, Singhal R L, and Srivastava S K Insulin suppressor of biosynthesis of hepatic gluconeogenic enzymes, *Proc.Natl.Acad.Sci.* USA, (1965) V.53: P.96-104.

[53] Mazzotta M Y, and Veneziale C M, Concentration of liver and kidney fructose-1,6-diphosphatase determined by specific radio immunoassay,*Biochem, Biophys, Acta*., (1980) V.611: P.156-167.

[54] Roesler W J and Khandelwal R L, Quantitation of glycogen synthase and phosphorylase protein and enzyme activity, *Arch. Biocem, Biophys.,* (1986) V.244: P.397-407

[55] T, The mechanism of Alloxan and strepotozotocin action in -cells of rat pancreas, *Physiol Re.s,* (2001) V.50 P. 537-546.

[56] Meiselman H L, Halparrn B P, Dateo G P, *Physiology and Bhahaviour*., (1976) V.17: P.313-317

[57] Suba V, Murugesan J, Arunnchalam G., Mandal SC., Sahu B P., Antidiabetic potential of *Borleria Lupilina* extract in rats, *Phytomed.,* (2004) V.11: P.2027.