EFFECT OF AQUEOUS LEAF EXTRACT OF *B.TOMENTOSA* ON GTT OF NORMAL AND DIABETIC RATS

K.Devaki[1], U.Beulah[1], G.Akila[1], M.Sunitha[1], R.Narmadha[1] and V.K.Gopalakrishnan[1]*

[1]Department of Biochemistry, Karpagam University, Coimbatore-641 021, India.

*Corresponding author : Dr.V.K.Gopalakrishnan
Telephone number: 091-0422- 6453777
Fax number : 091-0422-2611043
E mail address : vkgopalakrishnan@gmail.com

Summary

Hypoglycemic effect of medicinal plant *Bauhinia tomentosa* was tested in Wistar albino rats. The oral glucose tolerance test in overnight fasted normal as well as in alloxan induced diabetic rats (150 mg/kg, of alloxan, i.p.) conducted with the administration of 100,200,300,400 mg/kg of b.wt of *B.tomentosa*. The blood glucose level at 0, 60, 120, 180 min indicated the hypoglycemic nature of the *B.tomentosa* leaf extract and it also identified the most effective dose as 300mg/kg b.wt.

Keywords: *B.tomentosa*, Hypoglycemia, Alloxan mono hydrate, GTT

Introduction

Diabetes is a pandemic in both developed and developing countries. The incidence of diabetes is considered to be high world wide [1]. According to the International Diabetes Federation, there are 246 million people with diabetes on the globe and this figure will rise to 380 million by the year 2025 [2]. In 2000, there were 175 million people with diabetes world wide and by 2030, the projected estimates of diabetes is 354 million. The greatest relative increase is predicted in the developing countries of Middle Eastern crescent, Sub-Saharan Africa and the Indian sub continent. By the year 2030, over 85% of the world's diabetics will be in developing countries [3].

GTT is the most commonly used test for the diagnosis of diabetes. According to American Diabetes Association, fasting glucose level between 110-125mg/dl are borderline (impaired Fasting glycemia) and fasting level at repeatedly, at or above 126mg/dl are diagnosis of diabetes. Glucose level above 200mg/dl at 2 hours confirms the diagnosis of diabetes [4].

Herbal remedies have greater advantages because of their effectiveness, minimal side effects in clinical experience and relatively low costs hence there is a growing interest in this field. Such a traditional medicinal plant is *Bauhinia tomentosa* L. well known for its folklore use. There are about 600 species of *Bauhinia* were found in the tropical regions. It is a fast-growing, medium to large shrub to a small tree, grows up to 4m in height. Leaves are divided into two lobes, light green in colour, with a leathery texture. Infusion of fresh flowers and barks were used for dysentry. Decoction of root

bark was used for liver problems and leaves were externally applied to the forehead for fevers. Pharmacogological and preliminary phytochemical studies of *Bauhinia tomentosa* (Linn) flower revealed the presence number of the biologically active photochemicals [5].

Large number of *Bauhinia* species were analysed for their antidiabetic effect but the antidiabetic effect of *B.tomentosa* is scarce. There was only one study reported the anti-hyperglycemic and anti-lipidemic activity of ethanolic extract of *Bauhinia tomentosa* (linn) flower in normal and streptozotocin-induced diabetic rats [6]. The availability of flower throughout the year is difficult, so this study aimed in finding the hypoglycemic potential of *B.tomentosa* leaf extract.

Materials and methods

Plant material preparation

B.tomentosa leaves were collected from a local farm and authenticated by Dr.G.V.S.Moorthy, Botanical Survey of India, Tamilnadu Agricultural University Campus, Coimbatore. The Voucher No is BSI/SRC/73/5/23/09-10/Tech.-723) and the specimen was deposited in the department herbarium for future reference. The leaves were washed; shade dried and coarsely powdered using a mixer. Then they were weighed and kept in an airtight container and stored in the refrigerator for future use.

Extraction and phytochemical Screening

The powdered plant material (50g) was taken in a conical flask and subjected to successive solvent extraction with solvents (250ml) of increasing order of polarity. The order of the solvent were petroleum ether, chloroform, ethyl acetate, ethanol and water for 16 hrs in a shaker. Each time before extracting with the next solvent the residue was dried thoroughly to remove the solvent used. After extraction the extracts were dried and the yield was calculated. Various extracts of *B. tomentosa* leaves were subjected to phytochemical screening using standard methods specified by [7].

Animals

The albino rats of both sexes, weighing about 150-180 g were procured from animal house of Karpagam University, Coimbatore and used for the study. Rats were housed at a constant temperature of 22+5°C with a 12-hour light, 12-hour dark cycle. The rats were fed on pellets with free access to tap water. All the experiments were carried out according to the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Induction of diabetes

Diabetes was induced by single intra peritoneal injection of alloxan monohydrate (150 mg/kg) in saline. Hyperglycemia, after 72 hrs was confirmed and the behavioural changes related to diabetes was also observed (Excess thirst and frequent urination).The rats with blood glucose level more than 250 mg/dl were used for the study. Urine sugar was checked and taken as a support for the selection of diabetic rats.

Experimental design

Effect of aqueous extract of *B.tomentosa* on GTT of normal rats

By Bonnerweir [8] method, the oral glucose tolerance test was performed in normal rats. After overnight fast, the animals were divided into five groups of five animals each. Group 1 was treated with normal saline. Group 2-5 receives 100,200,300,400 mg/kg of *B.tomentosa*. Before the treatment, fasting blood samples were collected and the respective doses of *B.tomentosa* were given to the treatment groups. After 30 min, blood was again drawn that gives the 0 h value. The animals were given a glucose solution (4 g/ kg b.wt) orally and blood samples were withdrawn from the retro orbital sinus by capillary puncture at 1, 2, 3 hrs after glucose administration which gives the 1, 2, 3 hr values. From the collected sample the glucose level was estimated using Barham and Trinder method and the percent decrease in glucose was calculated.

$$\% \text{ decrease in glucose level} = \frac{(\text{Before treatment} - \text{After treatment}) \times 100}{\text{Before treatment}}$$

Effect of aqueous extract *of B.tomentosa* on GTT of diabetic rats

GTT was also performed on diabetic rats with the aqueous extract of *B.tomentosa*. As mentioned above GTT was carried out in over night fasted diabetic rats. The animals were divided into seven groups with five animals each. Group 1 was treated with normal saline. Group 2 was diabetic control and group 3 was diabetic treated with glibenclamide (5mg/kg), group 4-7 receives 100,200,300,400 mg/kg of *B.tomentosa*. Before the treatment, the fasting blood samples were collected and the respective doses of *B.tomentosa* were given to the various groups. After 30 min, blood was again drawn that gives the 0 h value. The animals were given a glucose solution (4g/ kg b.wt) orally and blood samples were collected at 1, 2, 3 hrs after glucose administration which gives the 1, 2, 3 hr values.

Results and Discussion

Plants are considered as the repository of phytochemicals which are all responsible for large number of biological activities observed. Phytochemicals saponins, terpenoids, flavonoids, tannins. Steroids and alkaloids have anti-inflammatory effects [9] Glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities [10] Rupasinghe *et al.* [11] reported that saponins possess hypocholesterolemic and antidiabetic properties.

Table1 indicated the presence of tannin, phenols, flavonoids, steroids and cardiac glycosides in the aqueous leaf extract of *B.tomentosa* and the yield 3.11% obtained in the aqueous extract was found to be maximum (Table 2).

Table: 1- Phytochemical screening of *Bauhinia tomentosa* L.

Extracts	AL	SA	TP	FL	ST	CG	OF	TN	AP	CH
Petroleum Ether	-	-	-	-	+	-	-	-	-	-
Chloroform	-	-	-	+	+	-	-	-	-	-
Ethyl acetate	+	-	-	+	-	+	-	-	-	+
Ethanol	+	-	+	+	-	-	-	-	-	+
Water	-	-	+	+	+	+	-	-	-	+

AL	Alkaloids	CG	Cardioglycosides
SA	Saponin	OF	Oils & Fats
TP	Tannin & Phenolic compounds	TN	Terpenoids
FL	Flavonoids	AP	Aminoacids & Proteins
ST	Steroids	CH	Carbohydrates

'+' Present '_' Absent

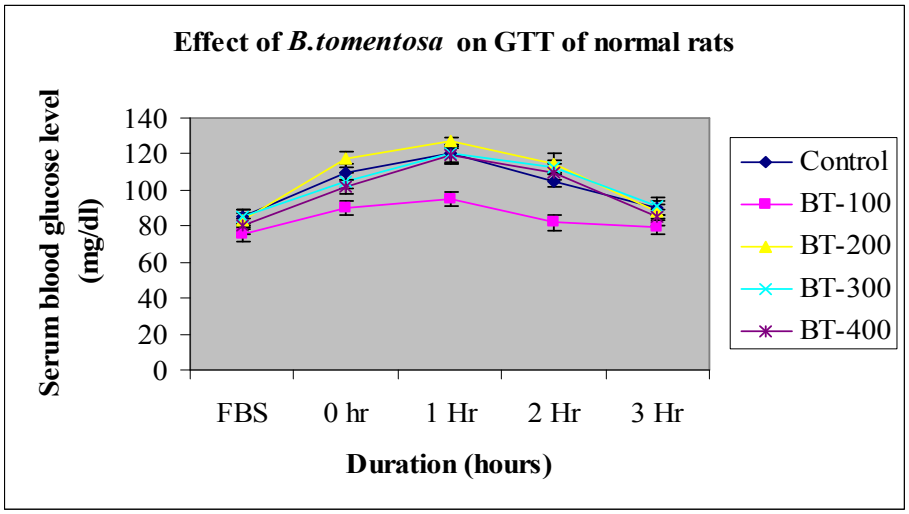
Table:2- Percentage Yield for various extract of *B.tomentosa* L.

Solvents	*Bauhinia tomentosa* *(g %)*
Petroleum Ether	0.89
Chloroform	2.15
Ethyl acetate	1.18
Ethanol	2.50
Water	3.11

Biologically active phytochemicals in the petroleum ether, chloroform, acetone, ethanol and aqueous extract of *Bauhinia tomentosa* (Linn.) flowers were reported [5]. Presence of tannin, phenolic compounds and oils were also reported in various species of bauhinia. Pettit et al [12] have isolated new cancer cell growth inhibitor *Bauhinia statin* I and III from various parts of *B.purpurea* and reported the presence of phenol, flavonoid, saponins, glycosides and tannins in the same species. Presence of terpenoids, steroids and flavonoids in *B. forficata* [13] and high amount of phenols in the leaves of *B.racemosa* were also recorded [14].

The hypoglycemic potential of single oral administration of various doses of aqueous extract of *B.tomentosa* on GTT of normal rats is presented in Figure 1.

Figure1: Effect of aqueous extract of *B. tomentosa* on GTT of normal rats



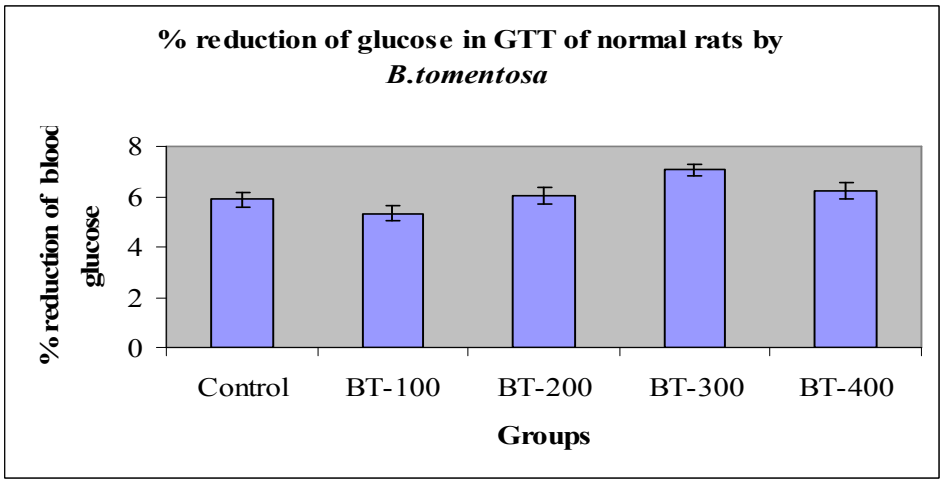
Values are expressed as Mean ± S.D of five individual experiments

PE-100-P.edulis 100mg/kg b.wt; PE-200-P.edulis 200mg/kg b.wt

PE-300-P.edulis 300mg/kg b.wt; PE-400-P.edulis 400mg/kg b.wt

The % reduction of glucose was found to be 5.33, 6.02, 7.05 and 6.25 with the doses of 100,200,300 and 400mg/kg body weight respectively (Figure 2).

Figure2: % Reduction of blood glucose by various doses of *B.tomentosa* in normal rats



Values are expressed as Mean ± S.D of five individual experiments

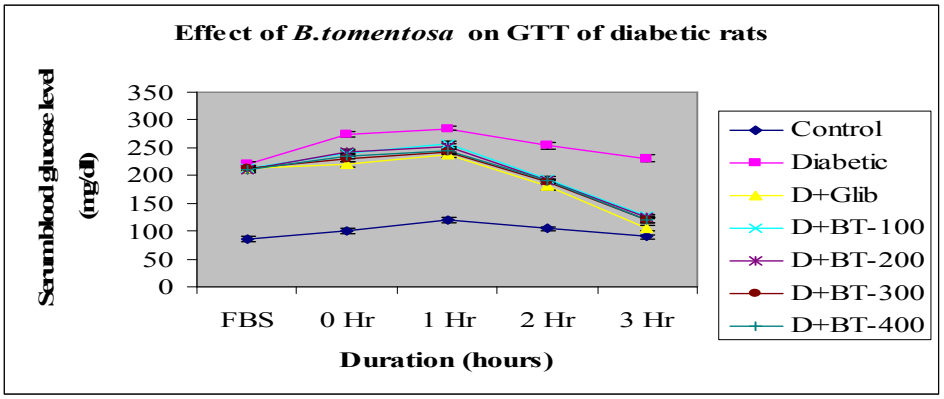
PE-100-P.edulis 100mg/kg b.wt; PE-200-P.edulis 200mg/kg b.wt

PE-300-P.edulis 300mg/kg b.wt; PE-400-P.edulis 400mg/kg b.wt

This indicates that the maximum fall in blood glucose was observed in the dose level of 300mg/kg. So this dose levels was identified as the most effective dose.

GTT of diabetic rats (Figure 3) demonstrated the antidiabetic effect of single administration of various doses of aqueous extract of *B.tomentosa*.

Figure: 3 Effect of aqueous extract of *B. tomentosa* on GTT of diabetic rats



Values are expressed as Mean ± S.D of five individual experiments

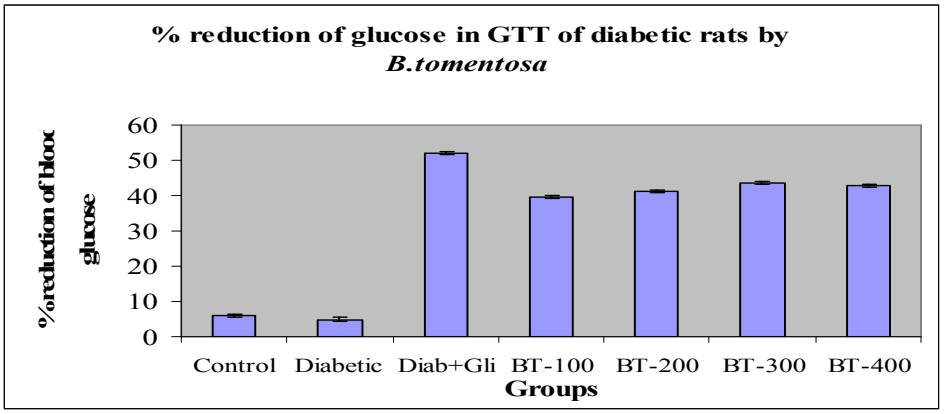
Diab+gliben -Diabetic ats treated with glibenclamide

PE-100 -P.edulis 100mg/kg b.wt; PE -200-P.edulis 200mg/kg b.wt

PE -300-P.edulis 300mg/kg b.wt; PE -400-P.edulis 400mg/kg b.wt

Here also the dose level of 300mg/kg of *B.tomentosa* produced the maximum fall of 43.66 % in blood glucose after 3 hours of glucose administration. The fall of blood glucose in non treated diabetic was very minimum (5%), but the antidiabetic drug glibenclamide treated rats were maximum (52%). A fall of 39.62, 41.23 and 42.80 % was observed with the administration of 100,200 and 400 mg/kg of *B.tomentosa* (Figure 4).

Figure: 4 % Reduction of blood glucose by different doses of *B.tomentosa* in diabetic rats.



Values are expressed as Mean ± S.D of five individual experiments

Diab+gliben -Diabetic rats treated with glibenclamide (5mg/kg b.wt)

PE-100 -P.edulis 100mg/kg b.wt; PE -200-P.edulis 200mg/kg b.wt

PE -300-P.edulis 300mg/kg b.wt; PE -400-P.edulis 400mg/kg b.wt

This study again confirms that 300mg of *B.tomentosa* / kg b.wt was the most effective dose.

Antioxidant enzymes such as SOD, CAT, GPx and GST too showed enhanced activities on administration of ethanolic extract of the *Bauhinia tomentosa* (Linn.) flower. [15]. Excess glucose in diabetic patients induces the production of free radicals, this leads to increased oxidative stress in diabetes. Oxidative stress is responsible for micro and macro vascular damage that occur in diabetes. Phenolic compounds are the major group of compounds that acts as primary antioxidant because of it reaction with oxygen free radicals such as hydroxyl, superoxide anion radicals and lipid peroxyl radicals [16]. There is high correlation between antioxidant activity and phenolic compounds[17]. Phenols and flavonoids inhibit amylase, sucrase and also Glucose Transporter-1(S-GLUT-1) of intestinal brush border cells that reduce the absorption of glucose which ultimately reduce the hyperglycemia [18].

Conclusion

This study support that the leaves of *Bauhinia tomentosa* exhibit hypoglycemic activity and this due to the presence of active compounds like phenols and flavonoids and other phytochemicals. Significant blood glucose lowering effect of *Bauhinia tomentosa leaf extract* in alloxan diabetic rats will be of more important in maintaining the blood sugar level in diabetic patients and validate its traditional use in the treatment of diabetes**.**

Acknowledgement

The authors express their sincere gratitude to the Chairman and Chancellor, Advisor, Vice Chancellor and Registrar of Karpagam University, Coimbatore, India for providing fund and lab facilities to carry out this study.

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