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Research Article

ANTIHYPERGLYCAEMIC AND ANTIDIABETIC EFFECT OF THE LEAF EXTRACTS OF *ALBIZZIA LEBBECK* LINN.(BENTH) AND *PSIDIUM GUAJAVA* LINN. ON ALLOXAN AND STREPTOZOTOCIN INDUCED DIABETIC MICE

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ABSTRACT

Diabetes mellitus is a metabolic disorder. The leaf extracts of *Albizzia lebbeck* (Benth), *Psidium guajava* (Linn), and *Trigonella foenum-graecum* (Linn), were tested for their antihyperglycaemic and antidiabetic potential on alloxan and streptozotocin induced diabetic models of mice. All leaf extracts tested showed a positive trend in regulating blood glucose levels in Swiss albino mice. Rosiglitazone was taken as a standard drug.

Keywords: Antihyperglycaemic, Antidiabetic, *Albizzia lebbeck*, *Trigonella foenum- graecum* and *Psidium guajava*.

INTRODUCTION

The progressive development of insulin resistance in the liver and peripheral tissues, accompanied by defective insulin secretion from pancreatic beta cells leading to overt hyperglycaemia (an abnormally high amount of glucose levels in blood)are some of the complexities involved in the pathogenesis of type 2 diabetes[1]. Despite genetic predisposition, the risk of developing type 2 diabetes in humans increases with age, obesity, cardiovascular disease (hypertension, dyslipidaemia) and a lack of physical activity[2].*Albizzia lebbeck (Mimosaceae)* is a medium to large size of tree distributed throughout India. Stem is large erect and branched. Leaves are bipinnate ,rachis 70-90 mm, rachillae 1-5 pairs, Leaflets 3-11 pairs, oblong to elliptical, asymmetrical, glabrous, entire, initially bright green and folding at night, maturing to a duller glaucous green and fixed rachis[3]. It was reported that the leaves are used in opthalmia[4].The plant possesses anxiolytic[5] and antidiarrhoeal activity[6]. Leaves have nootroopic [7] and anticonvulsant property[8]. Flavonoids like quercetin and Kaemferol have also been isolated and identified from the leaves[9].

*Trigonella foenum- graecum (Fabaceae)*is an annual herb,30-60cm in height, leaves are light green, pinnately trifoliate, leaflets toothed, flowers are white or yellowish white, papilionaceous and axillary[10]. An infusion of the seeds is a good cool drink for small pox patients. Powdered seeds find application in veterinary medicine. An aqueous extract of the seeds possesses antibacterial property[11, 12]. It contains several hypoglycemic and hypolipidemic constituents and has been the object of clinical trials confirming its beneficial action in diabetes[13].Treatment with a crude seed extract was shown to have an antioxidant effect in liver of diabetic rats[14]. The soluble dietary fibre from fenugreek seed produces a beneficial effect in dyslipidemia[15]. Seed powder was observed to repair the liver and kidney damage caused by alloxan[16]. Further, it was reported that the crude seed extract showed an antihyperglycaemic effect in diabetic rats[17].

Psidium guajava (Myrtaceae) small tree 3-10 m high with many branches and having generally a superficial but extensive root system, leaves opposite, simple, exstipulate with short petioles, oblong to elliptic, apex obtuse to bluntly acuminate and entire margin. Leaves are thick and leathery with prominent veins and dotted with glands. Flavonoids and saponins combined with oleanolic acid were isolated from the leaves[18].Guava has antioxidant properties attributed to polyphenols found in its leaves[19]. Besides, *Psidium guajava* is an excellent anti-LDL glycative agent whose potential therapeutic uses can be extended to the prevention of a variety of cardiovascular and neurodegenerative diseases associated with glycations [20].

In the present study the Swiss Albino Mice model was used and induction of hyperglycaemia /diabetes was effected through the use of alloxan or streptozotocin. Alloxan is an older model as compared to streptozotocin .However, the action of alloxan is of short duration and reversible whereas streptozotocin is used for a long term study Therefore, a comparative study was done using these chemicals to induce hyperglycaemia/diabetes and determine the possible mode of action of selected plant leaf extracts on these hyperglycaemic and diabetic models. The leaf extracts of *Albizzia lebbeck* and *Psidium guajava*,were tested for their antihyperglycaemic /antidiabetic potential. *Trigonella foenum-graecum* was used as a positive control while Rosiglitazone was used as the standard drug for comparison.

MATERIAL & METHODS

Plant Materials

The dried leaf powders of *A.lebbeck, P.guajava* and *T.foenum-graecum* (50 g each) were separately extracted in methanol using a soxhlet assembly. The filtrates were allowed to evaporate at 40°C to remove all traces of methanol. Appropriate quantity of the dried residues of each of the three plant species was suspended in 0.5 % CMC followed by sonication for one hour to obtain a uniform suspension with a concentration of 250 mg/kg b.wt. Dose for each group of 6 mice was calculated on the basis of their average body weight.

Experimental animals

Swiss Albino mice, procured from Haffkine Institute, Parel, Mumbai and weighing about 25-30 g were housed under standard environmental conditions with the temperature ranging between 20-25°C,relative humidity was 60% and animals were exposed to natural day and night cycles. They were randomly allocated to different groups with six mice per group including both male and female (mixed group population). Mice were fed standard pelleted rodent feed (manufactured by Lipton Ltd.) and given filtered water (supplied by Brihan Mumbai Municipal Corporation) in glass bottles *ad libitum*. All the animals were taken care of and maintained as per guidelines of the CPCSEA with due approval from the Institutional Animal Ethical Committee.

Administration of Alloxan 21 /Streptozotocin[22]:

Animals were divided into non diabetic control, diabetic control and diabetic mice treated with respective leaf extracts and each group contained six mice. At zero hour, the animals were administered a single dose of alloxan (80 mg/kg body weight) intraperitoneally for inducing hyperglycaemia at the commencement of the experiment. Streptozotocin was administered in multiple low doses at an interval of 7 days for inducing diabetes in Swiss Albino mice as described in American Model of Diabetes Complications Consortium. This low dose administration of streptozotocin causes a partial damage to β cells.

Table 1: Blood glucose levels in alloxan induced hyperglycaemic mice administered a dose of 250 mg/kg b.wt. of leaf extracts of *P.guajava, A.lebbeck* and *T.foenum- graecum*.

Extracts	Normal mice	Diabetic Untreated	30 mins after treament	60 mins after treamen t	90 mins after treament	120 mins / 2 hrs after treament	240 mins/ 4 hrs after treament	360 mins /6 hrs after treament	1440 min/24 hrs after treament
P. guajava	67.66 ± 5.5	148.34± 2.52	158.33± 20.82	120.07± 18.87*	117.66± 9.07*	112.67± 9.29*	95.33± 9.6*	72±25.51*	123.67± 21.45
%change		100	6.73	-19.05	-20.68	-24.04	-35.73	-51.46	-16.63
A.lebbeck	65.67 ± 4.16	137.34± 5.13	155.34± 19.89	137.67± 25.42*	127±6.24*	115.34± 11.5*	90±22.92*	117.67± 22.48	126.67± 20.23
%change		100	13.10	-0.24	-7.52	-16.01	-34.46	- 14.32	-7.76
T. foenum graecum	59.34 ± 4.56	135.67± 8.5	155.67± 19.13	119.67± 11.84*	107.67± 13.57*	99.67± 17.38*	93± 6.55*	118.33± 5.68*	128± 14.93
%change		100	14.74	-11.79	-20.63	-26.53	-31.45	-12.78	-5.65
Rosiglita zone	62.47 ± 6.23	133.94± 6.69	143.54± 21.97	131.34± 16.1	126.34± 17.08	118± 11.78*	98.6± 13.72*	105.34± 20.83*	125.2± 18.72
%change		100	7.16	-1.94	-5.67	-11.90	-26.38	-21.35	-6.52
control	62.94 ± 7.12	143.2± 13.07	157.8± 21.16	148.27± 45.4	145.73± 20.19	159.4± 28.79	136.67± 25.3	140.06± 31.18	139.33± 34.78
% change		100	10.19	3.54	1.76	11.31	-4.56	-2.19	-2.70

* Significant in terms of decrease in blood glucose level ($p<0.05$).

Table 2: Blood glucose level in streptozotocin induced diabetic mice treated with leaf extracts (250 mg/kg b.wt.) of P.guajava, A.lebbeck & T. foenum-graecum.

Extracts	Normal mice	Diabetic Untreated	30 mins after treatment	60 mins after treatment	90 mins after treatment	120 mins / 2 hrs after treatment	240 mins/ 4 hrs after treatment	360 mins / 6 hrs after treatment	1440 min/24 hrs after treatment
P.guajava	61.67± 5.68	155.67± 8.62	183.67± 1.5	143± 20.95*	126.67± 3.05*	118.67± 11.01*	102.33± 15.82*	115± 31.22*	150± 29
%change		100	17.98	-8.13	-18.62	-23.76	-34.26	-26.12	-3.64
T.foenum-graecum	66 ± 14.73	176± 35.79	182.33±35.69	145.33± 17.38	124.67± 15.5*	112.33± 13.32*	90.65± 16.37*	107.15± 9.16*	161.67± 42.91
%change		100	3.88	-17.4	-29.16	-36.17	-48.49	-39.11	-8.14
A.lebbeck	65.34±7.76	165.67± 21	188± 34.59	137± 8.54*	121.33± 2.51*	115.33± 10.96*	101.33± 5.68*	123.33± 20.3*	190.67± 20.84
%change		100	13.47	-17.30	-26.76	-30.38	-38.83	-25.55	15.09
Rosiglitaz one	*65.56 ± 8.59*	*162.89± 27.3*	*177.56± 38.92*	*144.67±15.18**	*134.22±8.49**	*125.33± 9.69**	*108.78±10.14**	*121.56±12.57**	*156.67± 30.83*
% Change		100	9.00	-11.18	-17.60	-23.05	-33.2	-25.37	-3.8
Control	77.55± 13.92	168.55± 28.54	164.89±25.82	158± 28.27	148.78± 29.27	149.44± 20.24	136.11± 24.3	148.89± 18.85	175.89± 28.67
%change		100	-2.17	-6.25	-11.72	-11.33	-19.24	-11.66	4.35

* Significant in terms of decrease in blood glucose level ($p<0.05$).

Experimental Design

(For Antihyperglycaemic/ Antidiabetic study)

Animals were divided into 11 groups, each containing a mixed population of 6 mice. Group I: Normal non diabetic mice, without any treatment. Group II: mice treated with alloxan (80 mg/kg b.wt). Group III to V: alloxan treated hyperglycaemic mice + 250 mg/kg b.wt. leaf extract of *P.guajava, A. lebbeck* and *T.foenum-graecum* respectively. Group VI: alloxan treated hyperglycaemic mice +4 mg/kg b.wt. standard drug (Rosiglitazone). Group VII: Streptozotocin induced diabetic mice + 0.5% Carboxy Methyl Cellulose(CMC) , oral dose. Group VIII to X: Streptozotocin induced diabetic mice + 250 mg/kg b.wt. leaf extract of *P.guajava, A.lebbeck* . Group XI: Streptozotocin induced diabetic mice + 4 mg/kg b.wt. standard drug (Rosiglitazone) respectively.

RESULT AND DISCUSSIONS

After administration of leaf extracts of *P.guajava, A.lebbeck* and *T.foenum-graecum* (250 mg/kg b.wt) to alloxan induced hyperglycaemic mice there was a sudden surge in the levels of blood glucose in all the cases but after a lapse of 60 minutes the blood glucose level continued to decline upto 240 minutes (Table 1).While in mice treated with a leaf extract of *P.guajava*, where the blood glucose level continued to decline upto a 6 hours period, where as in all other cases the blood glucose levels showed an increase. This suggests that the effect of the leaf extract of *P.guajava* lasts over a longer period as compared to that of the other two plants. Similarly, at the end of 24 hours , the leaf extract of *P.guajava* continued to exert its effect in reducing the blood glucose level but the effect was less pronounced as compared to the 6 hours stage. In all other cases, the blood glucose levels, although slightly higher as compared to the respective values at the end of 6 hours, they were more or less similar to the baseline values. It was reported that the aqueous extract of *E.jambolana* administered at a concentration of 200 mg/kg body weight brought about a hypoglycaemic effect at 30 minutes in alloxan induced diabetic rabbits[23].Table 1, shows the percentage change in the blood glucose level at 60 minutes after administration of leaf extracts of *P.guajava, A.lebbeck,, T.foenum-graecum* respectively in hyperglycaemic model of mice. A statistically significant decrease in blood glucose level was observed at 240 minutes and 360 minutes after treatment. This suggests that the extracts either take time to reach the target tissues in the body or are metabolized and its the metabolites which exhibit activity.

After administration of streptozotocin, the blood glucose level showed a more than two fold increase (Table 2). After a 30 minutes period the blood glucose levels reached their peak values but began to decline thereafter. Overall, the blood glucose levels declined to their lowest levels after an interval of 4 hours. From the table it is

apparent that the effect of *P.guajava* in reducing the blood glucose level was more or less similar to that of the standard drug Rosiglitazone. While the extract of *A.lebbeck* seemed to have a somewhat better effect than the standard drug, the effect of the extract of *T.foenum- graecum* was more pronounced than that of rosiglitazone. From the table it is also clear that leaf extracts of all the three plants ceased to have an antidiabetic effect after a 4 hour period as is evidenced by the rise in the blood glucose level over a 6 hour period. By the end of 24 hours, the glucose levels were restored and their values were somewhat similar to those obtained at the commencement of the experiment. Statistical analysis of the data indicated that the changes in the blood glucose level were not significant at $P<0.05$.

Administration of extracts of *P.guajava , T.foenum- graecum* and *A. lebbeck* at 250 mg/kg b.wt to streptozotocin induced diabetic mice and alloxan induced hyperglycaemic mice showed that percentage reduction in the blood glucose level was greater in the former as compared to the latter (Table 1 and 2). In the present study two different models were used i.e alloxan and streptozotocin. There were significant differences in the blood glucose levels in the alloxan treated hyperglycaemic mice when administered with natural leaf extracts and the standard drug as that of streptozotocin induced diabetic model of mice treated with leaf extracts and standard drug. (Table 1). However, overall result showed a significant decrease in the blood glucose level in the streptozotocin induced diabetic mice as compared to alloxan treated hyperglycaemic mice. The study reveals, streptozotocin is a better model than alloxan as a wider range of studies can be done due to its prolonged effect and also because it is known to cause partial damage to the β cells leading to the induction of diabetes when a low dose protocol is adopted.

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