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Research Article

ANTIHYPERGLYCAEMIC EFFECTS OF HERBAL EXTRACTS ON ALLOXAN INDUCED HYPERGLYCAEMIC MICE

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ABSTRACT

Diabetes is a group of metabolic disorders that result in hyperglycaemia due to decreased insulin production (Type-I) or insufficient insulin utilization (Type-II). Of these, type II (Non insulin dependent-*Diabetes mellitus*) is the most common and major problem of today. In the present study, leaf extracts of *Aegle marmelos* (Linn), *Annona squamosa* (Linn.), *Piper betle* (Linn.), *Syzygium cuminii* (Linn.) skeels and *Mangifera indica* (Linn.) were studied for their antihyperglycaemic potential. All the leaf extracts when administered to alloxan induced hyperglycaemic mice brought about a significant decline in the blood glucose levels. Rosiglitazone, a PPARγ agonist, was taken as a standard drug.

Keywords: Antihyperglycaemic , *Aegle marmelos* , *Annona squamosa* , *Piper betle* , *Syzygium cuminii* and *Mangifera Indica*

INTRODUCTION

Diabetes mellitus is a chronic endocrine disorder that causes impairment in body metabolic activities, characterised by elevation of fasting blood glucose level due to insulin insufficiency or complete cessation of insulin synthesis or insulin resistance[1]. In fact, at a recent conference of the International Diabetic Federation held in Paris, India has been declared as the Diabetic Capital of the world since one-fifth of the diabetics in the world are Indian[2]. Further, *Diabetes mellitus* ranked seventh among the leading causes of death and it is even considered third when its fatal complications are taken into account[3]. Plants have always been an excellent source of drugs but this fact has not gained enough momentum in the scientific community as these drugs have wide variations in their contents, quality and safety. Although, the history of Herbal medicine is as old as human civilization, of late there is a herbal renaissance occurring all over the globe. As early as in 1972, it was reported that there are many plants which are found to have antidiabetic potential[4]. Similar observations were also made by other authors [5]. The following plants reported in their exhaustive study were selected for the present investigation.

Syzygium cuminii (*Myrtaceae*) is a tall tree. Leaves are petiolate with a broadly elliptical leaf blade and are leathery with glands on both the surfaces. The bark of the tree has astringent, carminative, diuretic and digestive properties and is also used to cure sore throat, bronchitis, blood impurities and ulcers [6]. It was also reported that the leaves and bark have an anti-inflammatory activity [7, 8]. An ethanolic seed extract (100 mg/kg body weight) fed to a streptozotocin diabetic rats was found to improve glucose tolerance level [9]. Similarly, aqueous and ethanolic extracts of seeds showed an antihyperglycaemic effect in severely diabetic rabbits[10].

Piper betle (*Piperaceae*) is a perennial dioecious, semi woody climber. Leaves are simple alternate, and yellowish to bright green in colour[11, 12]. The juice from the leaves is given to the children for cough and also administered to the adults for night blindness[11]. Leaves are used against lung disorders in children and applied to purulent ulcers[13]. Several experiments have indicated that leaves of *P.betle* possess gastro protective, hepatoprotective [14] and antioxidant properties [15, 16].

Aegle marmelos (Rutaceae) is a popular medicinal plant and is used to treat a wide variety of ailments. The tree is slender, aromatic, perennial, 6.0-7.5 m tall and 90-120 cm in girth. Leaves, alternate, pale green, trifoliate; the terminal leaflet, 5.7 cm long, 2.8 cm broad, having a long petiole; the two lateral leaflets, almost sessile, ovate to lanceolate having reticulate pinnate venation; petiole, 3.2 cm long.

The roots are used for treating diarrhoea, dysentery and dyspepsia[17]. The leaf is used for opthalmia and asthmatic complaints[18]. The unripe fruit is useful for treating diarrhoea, dysentery and stomachalgia. As early as in 1954 it was reported that the aqueous extract is used to treat fever, jaundice and skin diseases such as ulcers, urticaria and eczema[19].The aqueous leaf extract and methanolic extract of root bark also showed preventive effect on myocardial disease[20]. The aqueous leaf extract significantly controlled blood glucose, urea, body weight, liver glycogen and serum cholesterol[21,22]. It showed histopathological alterations in the pancreatic, liver and the kidney tissues indicating the hypoglycaemic potential of the extract[23]. Earlier the methanolic leaf extract of *A.marmelos* was found to be effective in inducing hypoglycaemia in rabbits[24] and this was correlated with the antioxidant properties of the plant species [25].

Mangifera indica (Anacardiaceae) is a large evergreen tree that grows to a height of 10-45 m, with dense foliage, typically heavy branched with stout trunk. The stem bark extract also showed an antispasmodic and antipyretic in mice [26]. The aqueous extract of leaves possess hypoglycaemic properties [27]. It is also reported that the plant is used in the management and control of painful, arthritic and other inflammatory conditions[28] and immunomodulatory activity [29].The seeds of the plant also showed anti diarrhoeal activity[30].

Annona sqamosa (Annonaceae) is a medium size tree which is distributed throughout India. The plant is cultivated mainly for its edible fruit. As early as in 1972, it was reported that the plant possesses insecticidal properties[31].The leaves have a cardiotonic activity[32]. Earlier, in 1969, it was observed that the ethanolic extract of leaves and stem have anticancer activity[33].The aqueous extract of the plant has also been reported to ameliorate hyperthyroidism[34].

MATERIAL AND METHODS

Collection of Plant Material

Leaves of *P. betle, S. cuminii*, *A. marmelos*, *M . indica* and *A. squamosa,* were collected from the local area and were authenticated by a botanist. Voucher specimens of the plant species have been kept at the Research Centre.

Plant Material

The dried leaf powders of *P. betle, S. cuminii*, *A. marmelos*, *M. indica* and *A. squamosa*, (50 g each) were separately extracted in methanol using a soxhlet assembly. The extracts were cooled and filtered through two folds of muslin cloth. The filtrates were allowed to evaporate at 40^{o}C to remove all traces of methanol. The dried residues were used for further studies.

Experimental animals

Swiss Albino mice, procured from Haffkine Institute, Parel, Mumbai and weighing about 25-30 g were selected for study. The animals were housed in standard environmental conditions 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).The animals were placed in propylene cages with stainless grill top and bedding of clean paddy husk. 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. All the cages were marked and labelled appropriately.

Oral Glucose Tolerance Test (www. chmd.com [35])

Normal mice were taken for the glucose tolerance test. The animal had free access to water during the experimental period. A 75 mg/ml glucose stock solution was prepared by dissolving 0.75 g of anhydrous D-glucose in 10 ml sterile distilled water. The initial weight and baseline levels of glucose for these mice were recorded. To estimate blood glucose levels, caudal vein was used to draw the blood and a small drop of blood was placed on the glucometer strip. The blood glucose level was recorded using a (one touch ultra-2) glucometer. The experimental mice were then separately fed with leaf extracts of *P. betle,*

S. cuminii, A. marmelos , M. indica and *A. squamosa* at a dose of 250 mg/kg b.wt. After a gap of half an hour these mice were then challenged with 1.5 mg glucose/g body weight, glucose load. The glucose solution was injected intra-peritoneally by using 1 ml syringe and a 25 gauge needle. The time of injection was noted and two additional blood glucose readings were noted at 20 minutes and 40 minutes post challenge. The data obtained are recorded in Table 1.

Alloxan administration

Hyperglycaemia was induced through administration of alloxan (80 mg/kg b. wt.) intra peritoneally[36].

Antihyperglycaemic Study

The leaf extracts of *A. squamosa*, *A. marmelos*, *M. indica*, *P. betle* and *S. cuminii* at a dose of 250 mg/kg b. wt. were used for the antihyperglycaemic study. Dose for each group of six mice was calculated on the basis of the average body weight of the animals and was prepared by suspending the desired amount of each extract separately in 0.5% CMC followed by sonication so as to obtain a uniform suspension. Each group of mice was administered a single oral dose of the respective extract, by means of intragastric intubation, following which the antihyperglycaemic effect was monitored.

Experimental Design

The animals were randomly allocated into seven groups, each group comprising a mixed population of 6 mice.

Group I Alloxan induced hyperglycaemic mice + 0.5% CMC, orally.

Group II Alloxan induced hyperglycaemic mice + 250 mg/kg b.wt, leaf extract of *A. squamosa*

Group III Alloxan induced hyperglycaemic mice + 250 mg/kg b.wt, leaf extract of *A. marmelos*

Group IV Alloxan induced hyperglycaemic mice + 250 mg/kg b.wt, leaf extract of *M. indica*

Group V Alloxan induced hyperglycaemic mice + 250 mg/kg b.wt, leaf extract of *P. betel*

Group VI Alloxan induced hyperglycaemic mice + 250 mg/kg b.wt, leaf extract of *S. cuminii*

Group VII Alloxan induced hyperglycaemic mice + 4 mg/kg b.wt. standard drug (Rosiglitazone)

Statistical Analysis

All values were expressed as mean ±SE. The results were analysed for statistical significance by one way ANOVA.

RESULT AND DISCUSSIONS

According to literature six biomolecules, namely, alkaloids, flavonoids, cardiac glycosides, tannins, saponins and carotenoids, were found to be responsible for antidiabetic activity [37] and antioxidant properties were attributed to them[38]. The Thin Liquid Chromatography screening of the extracts of *A. squamosa*, *S. cuminii*, *A. marmelos*, *P. betle* and *M. indica* as per methods described by Wagner *et al* [39] showed the presence of these six biomolecules, namely alkaloids, flavonoids, cardiac glycosides, tannins, saponins and carotenoids.

A spike in the blood glucose levels after oral administration of leaf extracts of *A. squamosa*, *S. cuminii*, *A. marmelos*, *P. betle* and *M . indica* was recorded 20 minutes post treatment in the glucose loaded mice (Table1). The blood glucose level decreased significantly as compared to that of the control in response to the treatment with the leaf extracts of *A. marmelos* (139 mg/dl), and *P. betle* (142 mg/dl). The latter showed the least reduction as compared with other leaf extracts.

Table 1: Blood Glucose Level In Normal Mice treated with Leaf Extracts of *A. Squamosa, S. Cuminii, A. Marmelos, P. Betle, M. Indica* and Subjected to a Glucose Tolerance Test

Extracts & Control	Untreated	20 mins after challenge	40 mins after challenge
Control	144±9.16	469±18.52	244±10.53
A .squamosa Linn.	130±6.72	156±7.16	140±5.64
S. cuminii Linn.	123±2.64	140±6.24	141±9.64
A .marmelos Linn.	141±5.52	175±9.53	139±9.49
P. betle Linn.	157±8.54	169±2.64	142±4.0
M. indica Linn	130±7.54	173±10.44	135±9.84

It was further reported that the aqueous suspension of *Picrorrhiza kurroa* (10%) extract, (75 and 150 mg/kg b.wt.) when administered to rats followed by glucose loading (p.o) reduces the glucose level in rats[40]. It was also observed that during the glucose tolerance test, the aqueous extract of the fruit pulp of *Eugenia jambolana* at a dose of 25 mg/kg b. wt brought about a decline in the blood glucose level in fasting rabbits.The blood glucose declined by 21% after 1 hour of administration of extract as compared to the control (1.3%) [41].

Table 2 depicts the blood glucose level in Swiss Albino mice monitored over a period of 24 hours following treatment with leaf extracts of *A. squamosa*, *A. marmelos*, *M. indica*, *P. betle* and *S. cuminii* at a concentration of 250 mg/kg b.wt. It was seen that the blood glucose level of euglycaemic mice increased to a statistically significant level, after administration of alloxan intraperitoneally, thereby confirming the hyperglycaemic state of mice. Administration of the leaf extract of *A. marmelos* showed a significant decrease in the blood glucose level ; similarly, treatment with the other leaf extracts also resulted in a decrease in blood glucose levels. This decline was in the decreasing order *M. indica*, *A. squamosa, P. betle* and *S. cuminii*. However at the end of 6 hours the blood glucose levels were more or less similar to the baseline values in all the treatment. It is pertinent to note that the antihyperglycaemic effect of all the leaf extracts used in the present study was similar to that of the standard drug, Rosiglitazone.

Table 2: Blood Glucose Level in Alloxan Induced Hyperglycaemic Mice Treated with Leaf Extracts (250 mg/kg b.wt.) of *A. marmelos, A. squamosa, P. betle, S. cuminii* and *M. indica.*

Extracts	Normal mice	Diabetic pre treatment	30 mins post treatment	60 mins post treatment	90 mins post treatment	120mins post treatment	240mins /4hrs post treatment	360 mins /6hrs post treatment	1440 min/ 24 hrs post treatment
A.squamosa	62.66 ± 5.3	149.34± 3.52	160.31± 17.83	123.07± 16.88*	116.67± 10.07*	113.67± 9.29*	90.33±9.72*	104±23.47 *	129.67± 19.45
%change		-	7.38	-17.44	-21.8	-23.88	-39.51	-30.36	-13.17
A.marmelos	65.61 ± 4.13	135.23± 4.13	153.34± 14.89	136.67± 25.40*	120±6.22*	115.31± 9.45*	87±21.86*	110.57± 20.45	121.62± 20.19
%change		-	13.39	-1.06	-11.26	- 11.32	-35.65	- 18.23	-10.06
S.cuminii	59.34 ± 4.56	139.67± 8.5	155.67± 19.13	129.67± 11.84*	117.67± 13.57*	114.67± 17.38*	101±6.55*	118.33±5.68*	128±14.93
%change		-	11.45	-7.15	-15.75	-17.89	-27.68	-15.27	-8.35
P.betle	61.56 ±5.54	138.43±7.68	157.64±14.28	128.27±18.63	110±17.14*	105.62±16.15*	87.31±25.64*	108.35±21.34*	132.53±19.53
%change		-	13.87	-7.33	-20.53	-23.70	-36.92	-21.72	-4.26
M.indica	60.32 ±7.76	132.67±65	158.54+4.97	132.45±9.18	127.35±6.45*	117.65±10.98*	82.34±28.68*	106.43±13.45*	129.38±17.77
%change		-	19.49	-0.16	-4.00	-11.32	-37.93	-19.77	-24.79
Rosiglitazone	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		-	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

It was earlier noted that the administration of a seed extract of *P. kurroa* reduces the blood glucose level after 2 hours in alloxan induced hyperglycaemic rats[42]. The aqueous extracts of leaves and shoots of *Chrysanthemum coronarium* and *Morus alba* were found to effectively reduce the blood glucose level in alloxan induced diabetic rats when compared to the effect of glibenclamide [43]. The lipophilised powder of *Clitoria ternatea* and *Tinospora cordifolia* was shown to potentiate insulin release from the pancreatic β cells in a diabetic rat model[44]. It was further observed that the antihyperglycaemic effect of an extract of a bark of *A. lebbeck* proved to be effective in a dose and time-dependent manner in alloxan induced diabetic mice[45]. The intraperitoneal administration of the extract (250 mg/kg body weight) , one and a half hours prior to oral glucose load to overnight fasted alloxan induced diabetic mice, showed a maximum reduction of glucose level by 76% over that of control at 480 minutes (8 hours). In two recent articles the antidiabetic activity of the seed extract of *Strychnos nuxvomica* [46], and the fruit extract of *Abelmoschus esculentus*[47] has been linked to the hypoglycaemic and free radical scavenging activity, while a third article has established the antidiabetic potential of extracts of the rhizome of *Smilax chinensis*[48].

The statistically significant decline in the blood glucose level observed at 4 hours suggests that extracts either take time to reach the target tissues in the body or they gets metabolized and the metabolites are active.

In the present study , among the five leaf extracts *A. marmelos* was found to be most effective in reducing the blood glucose level in the hyperglycaemic mice model; similarly, treatment with other leaf extracts also has the same effect but in the order, *M. indica* > *A. squamosa* > *P. betle* > *S. cuminii*. The biomolecules present in all these leaf extracts possibly contribute to the reduction in the blood glucose level. Further, at the concentration of alloxan used in the present set of experiments, it is known to cause only partial and short term damage to the β cells in the pancreas. As proposed by Lenzen[49] the residual β cells therefore result in a partial stimulation of insulin release , which could account for lowering of blood glucose level following treatment with the leaf extracts as observed during the present study.

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