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Hypoglycaemic Effect of Vriddhadaru [*Argyreia nervosa* (Burm. f.) Boj.] in Alloxan Induced Diabetic Rabbits

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Abstract Diabetes Mellitus is the most important non communicable disease, playing a notorious role in the devastating phase of public health. Ayurveda, the life science provides astonishing results in such life style disorders. Vriddhadaru an excellent Ayurvedic drug and *Argyreia nervosa* has been traditionally used as Vriddhadaru, in diabetic management with promising results. But, the anti-diabetic effect of the drug has not been scientifically appreciated. This study ascertains the hypoglycaemic effect of the drug in a pre-clinical model. In this study, alloxan induced 18 diabetic albino rabbits were divided into 3 groups with 6 rabbits in each group. On the day of experiment, after assessing the fasting blood sugar levels in all 18 rabbits, root powder of the test drug as CMC suspension in the dose of 0.56 g/kg body weight and the standard drug, metformin; in the dose of 0.024 mg/kg body weight were administered to the first group and second group respectively. The third group was kept as control and administered with distilled water alone. Later, the blood glucose values were taken at 1st, 3rd and 5th hour and the obtained values were compared with in the group and between the groups. The blood glucose levels were compared using paired t test for within the group analysis and by student t test for between the group analysis. Within the group comparison of blood sugar level in group 1 showed significant decrease in the level at 1st hour and 5th hour from the corresponding fasting blood sugar levels. In group 2, significant decrease of blood sugar level was seen between fasting blood sugar level and 1st hour value and also in fasting blood sugar value and 5th hour value. Between group comparison of group 2 showed significant difference in the reducing the blood sugar level at different time intervals. Ayurvedic literature proposes Pramehaghna property to the drug Vriddhadaru which is substantiated through this pre-clinical trial. But the effect is comparatively less with that of the standard drug. This may be due to the absence of strong antioxidant and antidiabetic chemical constituents in this plant. From the above study, it is inferred that *Argyreia nervosa* (Burm. f.) Boj. is less effective in reducing blood sugar levels in Alloxan induced diabetic rabbits against the standard drug Metformin.

Keywords *Alloxan Monohydrate; Argyreia Nervosa*

1. Introduction

Life style disorders and non-communicable diseases causing life distress, melancholy and untimely death now a days. Among all non-communicable diseases, metabolic syndrome stands apart and is of quite a concern. This condition mainly includes dyslipidemia and diabetes mellitus type II. The latter is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. It occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces [1].

Diabetes is in the top 10, and perhaps the top 5, of the most significant diseases in the developed world, and is gaining significance elsewhere. In 2006, according to the WHO, at least 171 million people worldwide suffer from diabetes. Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will double [2]. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Even though a large number of drugs are available for this condition, none ensure complete cure, especially in preventing complications [3].

Many traditionally using Ayurvedic drugs were possessed pramehaghna property still many of them are having no scientifically validated data. Vriddhadaru is one if such drug mentioned firstly in Rasayana prakarana of Ashtanga Samgraha [4]. Even though many controversies are there about the identification of the genuine source plant, *Argyreia nervosa* (Burm. f.) Boj. of Convolvulaceae family is considered as the mostly used plant source as Vriddhadaru [5]. *Argyreia nervosa* is a climbing shrub with woody tomentose stem, commonly known as elephant creeper in English. It is widely distributed in tropical regions of the world. In India it is seen upto an altitude of 900 m [6]. It is generally found growing in slightly moist localities like river banks, edges of lakes etc. The roots are varying in size as well as in thickness [7].

As per Ayurvedic classical texts, this plant is useful in Prameha [8], a condition in which a drug Pramehaghna with Rasayana property is very much essential. This aspect of Vriddhadaru has not been established till date. Moreover, search for new effective drugs are very much essential now a days as genuine Ayurvedic drugs in this picture are facing radical scarcity.

There are many studies are there about the analgesic [9, 10], anti-inflammatory [11, 12], antipyretic [13], antiviral [14], anticonvulsant [15], nootropic [16, 17], anthelmintic [18], aphrodisiac [19, 20, 21], antidiarrheal [22], antiulcer [23] and antimicrobial action [24, 25, 26]. But only a few are there about the hypoglycaemic activity. Hence in this study, it was decided to take *Argyreia nervosa* as a source plant for Vriddhadaru and to document the hypoglycaemic activity in alloxan induces diabetic rabbits against the standard drug metformin.

2. Materials and Methods

2.1. Selection of Animals

25 healthy albino rabbits of either sexes weighing 1500-2000gm were collected from the animal house of Agada tantra department, Government Ayurveda College, Thiruvananthapuram.

2.2. Preparation of Alloxan Solution

Alloxan monohydrate was obtained from Laboratory supplies Thiruvananthapuram, batch no: 43256. Using normal saline 0.5%, the solution was prepared.

2.3. Standard Drug - Metformin

A stock solution was prepared with 0.5 mg of metformin in 50 ml distilled water so that 1 ml contained 0.02 ml of metformin [27].

2.4. Preparation of Test Drugs

Form of preparation	-	Powder
Route of administration	-	Oral
Drug	-	Root of *Argyreia nervosa* (Burm. f.) Boj.

A Carboxy-methyl-cellulose (CMC) stock solution of strength 0.01g% of the test drug was prepared in distilled water.

2.5. Dose

The dose of the test drug was calculated from Sarngdhara samhitha; the dose being the common dose for choorna [28]. Effective dose for rabbit was calculated by using the table constructed by Paget G.E. and Barnes T.M. in the evaluation of drug activities. Based on this; corresponding doses were calculated.

2.6. Setting

Animal house working under the Drug Standardization Unit, Govt. Ayurveda College, Trivandrum, Kerala

2.7. Procedure [29]

25 albino rabbits of either sex (weighing 1500-2000gm) were used in the present study. The animals were kept under observation for one week. Normal fasting blood sugar values were noted before administration of alloxan. All rabbits were fasted for 18 hours and the samples were collected from marginal ear vein. Blood sugar was calculated by glucose peroxidase (GOD-POD) method. After that, normal rabbit feed was given. Alloxan monohydrate (150 mg/kg body weight) dissolved in normal saline was injected via intra peritoneal route in 18 h previously fasted animal to induce diabetes. After one hour of alloxan administration, the animals were fed with standard pellets and water at libitum. After 72 hours, the blood glucose levels were estimated, applying the glucose oxidase method and rabbits having blood glucose level more than double were selected for the study [30].

18 healthy diabetic rabbits were taken for the study. They were grouped into three, with each group containing 6 animals which were caged separately. Animals of each group were marked for individual identification. First group (G1) was treated with test drug i.e. 0.56 g/kg body weight of root powder of *Argyreia nervosa* (Burm. f.) Boj., second (G2) group was administered with standard drug metformin at the dose of 0.024 mg/kg and the third group (G3) was kept as control and treated with distilled water alone.

On the day of the experimental study, fasting blood samples were taken from all the rabbits and then; the drugs were administered orally using feeding cannula. After 1 hour blood samples were collected and all the animals were fed. Blood samples for glucose assessment were again collected from all animals at 3rd and 5th hours after the drug administration. The values were subjected to statistical analysis.

3. Observations and Results

The blood glucose levels were compared using paired t test for within the group analysis and by student t test for between the group analysis using the programme WinPepi version 11. Table number 1 shows the blood sugar values in G1 at different time interval.

Table 1: *Blood sugar values of G1 at different time interval*

	Mean	SD	Mean diff	p - Value
FBS and	232.0	29.4	36.2	< 0.01
1st hour	195.7	12.7		
FBS and	232.0	29.4	14.0	> 0.05
3rd hour	217.9	16.6		
FBS and	232.0	29.4	45.2	< 0.01
5th hour	186.7	19.1		
1st hour and	195.7	12.7	22.2	< 0.05
3rd hour	217.9	16.6		
1st hour and	195.7	12.7	9.0	> 0.05
5th hour	186.7	19.1		
3rd hour and	217.9	16.6	31.2	< 0.01
5th hour	186.7	19.1		

Table number 2 shows the comparison of the blood sugar level at different time of G2

Table 2: *Blood sugar values of G2 at different time interval*

	Mean	SD	Mean diff	p - Value
FBS and	246.6	34.1	85.2	< 0.01
1st hour	161.4	37.9		
FBS and	246.6	34.1	34.1	> 0.05
3rd hour	212.4	31.0		
FBS and	246.6	34.1	96.4	< 0.01
5th hour	150.2	34.3		
1st hour and	161.4	37.9	51.0	< 0.01
3rd hour	212.4	31.0		
1st hour and	161.4	37.9	11.2	> 0.05
5th hour	150.2	34.3		
3rd hour and	212.4	31.0	62.3	< 0.01
5th hour	150.2	34.3		

The comparison of the blood sugar level at different time of G3 is given in the table number 3 using paired t test.

Table 3: *Blood sugar values of G3 at different time interval*

	Mean	SD	Mean diff	p - Value
FBS and 1st hour	252.6 233.1	26.0 21.7	19.5	< 0.05
FBS and 3rd hour	252.6 291.2	26.0 21.0	38.6	< 0.05
FBS and 5th hour	252.6 273.4	26.0 18.5	20.8	> 0.05
1st hour and 3rd hour	233.1 291.2	21.7 21.0	58.1	< 0.01
1st hour and 5th hour	233.1 273.4	21.7 18.5	40.3	< 0.05
3rd hour and 5th hour	291.2 273.4	21.0 18.5	17.8	< 0.01

Comparison of average changes in blood sugar level between group 1 and group 2 was done using unpaired t test and the results were given in the table number 4.

Table 4: *Comparison of changes in the blood sugar in different groups*

Duration	Group 1		Group 2		p value
	Mean	SD	Mean	SD	
FBS & 1st hour	14.91	7.42	34.45	12.83	< 0.01
FBS & 3rd hour	5.13	10.23	13.07	13.54	> 0.05
FBS & 5th hour	19.01	7.12	38.38	14.98	< 0.05
1st hour & 3rd hour	11.52	7.57	33.86	12.84	< 0.01
1st hour & 5th hour	4.51	8.30	5.92	16.09	> 0.05
3rd hour &5th hour	14.39	4.67	29.45	11.98	< 0.05

Within the group comparison of blood sugar level in group 1 showed significant decrease in the level at 1st hour ($p<0.01$) and 5th hour ($p<0.01$) from the corresponding fasting blood sugar levels. No significant difference was there between fasting blood sugar level and 3rd hour value ($p > 0.05$). Significant increase in the blood sugar level was noted from 1st hour to 3rd hour ($p < 0.05$) then gradually decreased, but not statistically significantly ($p>0.05$) while comparing it with 1st hour value. A significant decrease at $p <0.01$ was observed between 3rd hour and 5th hour blood glucose level. Hence, initially there was a significant decrease in the blood sugar at 1st hour. Then in 3rd hour there was a significant increase of value than that of 1st hour followed by a gradual decrease in 5th hour.

In group 2, significant decrease of blood sugar level was seen between fasting blood sugar level & 1st hour value ($p<0.01$) and fasting blood sugar value & 5th hour value ($p<0.01$). No significant change was observed between fasting blood sugar level and 3rd hour value ($P>0.05$). Significant increase in the blood sugar level was noted between 1st and 3rd hour value ($p<0.01$) but no difference was observed between 1st and 5th hour value ($p > 0.05$). But 3rd and 5th values are different significantly ($p < 0.01$).

In group 3, the blood sugar levels increased significantly ($p < 0.05, 0.01$) in most of the cases and in few cases there was no statistical difference ($p > 0.05$)

Between group comparison of group 1 and group 2 showed significant difference in the change of the blood sugar level at different time intervals. Mean values showed that standard drug is more effective than the test drug.

4. Discussion

Statistical evaluation shows that the blood glucose level in group 1, treated with *Argyreia nervosa* shows a gradual decrease but less effective than that of standard drug metformin.

Previous studies show that *Argyreia nervosa* contains 1-tricontanol, epifriedelinol acetate, epifriedelinol and β-sitosterol [25] and the hexane extract of the root yielded tetradecanyl palminate, 5, 8-oxidotetracosan-10-one26 and two novel aryl esters characterized as stigmasteryl phydroxycinnamate and hexadecanyl p-hydroxycinnmate along with scopoletin [26]. Even though some of these were told to be having antioxidant, a prominent hypoglycaemic activity has not proven for any of these constituents. This may be the reason for the weak hypoglycaemic activity than the standard drug.

5. Conclusion

From the above study, it is inferred that *Argyreia nervosa* (Burm. f) Boj. is effective in reducing the blood glucose level in reducing blood sugar levels in Alloxan induced diabetic rabbits but less effective while comparing with that of the standard drug Metformin. Effectiveness of the plant and advantages if any over the existing therapy can only be assessed by a long term study in more than one type of rodents. Clinical efficacy in human subjects should also need to be proved for establishing it as an effective single drug therapy in Diabetes mellitus.

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