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Hypoglycaemic and anti-hyperglycaemic effect of Jamboola, a polyherbal ayurvedic formulation in normal and alloxan induced diabetic rats

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ABSTRACT

The incidence of diabetes mellitus is on the rise and it is associated with disturbance of carbohydrate, fat and protein metabolism. Many herbal preparations and plant extracts are used with varying degree of success in the management of type 2 diabetes. Jamboola is one such polyherbal formulation composed of 5 medicinal plants which is used as an ayurvedic formulation in Andhra Pradesh, India to treat diabetes and claims no scientific evidence for its effectiveness as a polyherbal formulation. The herbal constituents of jamboola are known to possess anti-diabetic, antioxidant properties, and are used in indigenous system of medicine for the treatment of diabetes mellitus. According to the traditional system of Indian medicine, a combination of substances is used to enhance the desired activity and to eliminate unwanted side effects. In view of the above considerations the present study has been under taken to evaluate the hypoglycaemic, anti-hyperglycaemic activity of jamboola and to add a scientific proof to its efficacy. Glibenclamide at a dose of 0.45 mg/kg exhibited significant hypoglycaemic and anti-hyperglycaemic activity. Jamboola exhibited significant hypoglycaemic and anti-hyperglycaemic activity at 3 ml/kg than 1.5 ml/kg.

Key words: Jamboola, glucose tolerance test, alloxan monohydrate, hypoglycaemia, anti-hyperglycaemic.

INTRODUCTION

Diabetes mellitus is a known metabolic disorder of varied etiology, characterized by hyperglycaemia together with biochemical alterations of glucose and lipid metabolism [1-3]. It can also be defined as a disease which is characterized by altered insulin production or resistant to its action leading to micro and macro angiopathy [4]. This disorder is the single most important metabolic disorder that affects every organ system in the body and is therefore considered as a serious threat to human race. The number of patients with diabetes mellitus is

increasing worldwide with one third of the affected population living in India and China [5]. According to the census of WHO, around 171 million individuals were affected with diabetes mellitus in the year 2000 and it is expected that this number would reach 366 million by the year 2030.India is estimated to have 32 million affected individuals currently and is expected to reach 80 million by 2030 [6,7]. Oral hypoglycaemic treatment tends to fail over a period of 10-15 years from the time of diagnosis of the disease and the patient will have to rely on insulin along with other alternative medications [8]. Since oral hypoglycaemic agents are prone to side effects, there is a growing interest in herbal remedies. New compounds from plant sources provide a useful aid in the development of pharmaceutical entities or as a dietary adjuvant to existing therapies. In spite of the presence of known allopathic anti-diabetic medicines, remedies from medicinal plants are used with success due to their less toxicity and side effects [9].Poly herbal formulations are used most commonly rather than formulation containing single active ingredient. In this present investigation, jamboola was evaluated for its hypoglycaemic and anti-hyperglycaemic potential in normal and diabetic rats. Though enormous data is available on various other poly herbal formulations, no scientific study has been cited on this poly herbal formulation and hence this study is an authentic proof of its therapeutic potential.

MATERIALS AND METHODS

Animals

Adult female rats of wistar strain weighing 150-260 g, were obtained from the animal house of Bapatla College of Pharmacy (1032/ac/07/CPCSEA); Bapatla. The animals were maintained at a constant temperature of 26 ± 2^{o}C, humidity 30-40% and 12 h light/dark cycle, throughout the experiments. The animals were fed with commercial rat feed (Rayan's Biotechnologies Pvt. Ltd, Hyderabad, India) and sterile water was given *ad libitum*. The animals were housed in clean cages in an air conditioned animal house. The experimental protocol (IAEC/II/04/BCOP/2009) was approved by the institutional animal ethics committee (IAEC) of Bapatla College of Pharmacy; Bapatla and was in accordance with the guidelines of the committee for the purpose of control and supervision of experimentation on animals.

Drugs and Chemicals

Glibenclamide was procured as gratis sample from Zydus Cadila, Ahmedabad, India. Jamboola syrup, manufactured by Ratnakar Pharmaceuticals, Pathuru, A.P, India, was procured from the local ayurvedic drug store. Alloxan Monohydrate was procured from Sigma Aldrich, USA.

Experimental Design

Anti-hyperglycaemic activity of jamboola in normal rats

The animals were acclimatized to laboratory conditions prior to the study. The animals were divided in to four groups of six animals each. Group 1 was treated as glucose control and was administered with glucose at a dose of 3 mg/kg, p.o. Group 2 was treated with glibenclamide at a dose of 0.450 mg/kg, p.o. Group 3 and 4 was treated with 1.5 and 3 ml/kg, p.o of jamboola. The animals were fasted for a period of 12 hours before the experiment. Group 2-4 was treated with the herbal formulation and glibenclamide 30 minutes before the administration of glucose. The blood samples were collected from the tail vein of rats and were estimated for glucose levels at 30, 90, 150 minutes after administration of glucose with a glucometer (Roche Diagnostics, Germany) [10,11].

Hypoglycaemic activity of jamboola in normal rats

The animals were acclimatized to laboratory conditions prior to the study. The animals were divided in to four groups of six animals each. The animals were fasted for a period of 12 hours

before the experiment. Group 1 was treated as normal control and was administered with water (0.2 ml). Group 2 was treated with the glibenclamide at a dose of 0.450 mg/kg, p.o. Group 3, 4 was treated with 1.5 and 3 ml/kg, p.o of jamboola. The blood samples were collected from the tail vein of rats before and after administration of drugs at time intervals of 0, 1, 2, 4, 6, 8 hours. The samples were estimated for glucose levels with a glucometer.

Anti-hyperglycaemic activity of jamboola in diabetic rats

Induction of experimental diabetes in rats

The animals were acclimatized to laboratory conditions one week before the induction of diabetes. The animals were fasted for a period of 12 hours before the induction. Alloxan monohydrate was administered through intra-peritoneal route at a dose of 150 mg/kg in ice cold normal saline. The animals were maintained with 10% glucose after one hour of alloxan treatment to avoid the mortality due to hypoglycaemic shock. Animals with blood glucose above 250 mg/dL were considered as diabetic and were selected for the study [12].

Anti-hyperglycaemic activity in diabetic rats:

The diabetic animals were divided in to four groups of six animals each. Group 1 was treated as diabetic control and was administered with water (0.2 ml). Group 2 was treated with glibenclamide at a dose of 0.450 mg/kg, p.o. Group 3, 4 was treated with 1.5 and 3 ml/kg, p.o of jamboola. The treatment was carried out for a period of 15 days. The blood glucose of the animals was recorded initially and on 5^{th}, 10^{th}, 15^{th} day of study. The glucose levels were measured after one hour of drug administration. The blood samples were collected from the tail vein of rats and were estimated for glucose with a glucometer [13].

Statistical significance

The values are represented as mean±S.E.M of six observations. The results obtained were statistically compared with control groups using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The value of $P<0.05$ was considered to be statistically significant.

RESULTS

Effect of jamboola on anti-hyperglycaemic activity in normal rats:

The glucose tolerance test was carried out in normal rats which were treated with 3 g/kg of glucose and the values are shown in Table 1. The peak glucose level after oral glucose load was observed at 30^{th} minute in the untreated group. Gradual decrease in the glucose level was observed in all the treated groups. High significant values were observed with group 2 and 4 ($P<0.001$) than group 3 ($P<0.05$, $P<0.01$) when compared to the untreated control group 1.

Effect of jamboola on the hypoglycaemic activity in normal rats:

The influence of jamboola on the blood glucose levels were studied for a period of 8 hours in normal rats and the values are depicted in Table 2. Group 2 animals treated with the glibenclamide, at a dose of 0.45 mg/kg exhibited significant ($P<0.001$) hypoglycaemic activity form the 1^{st} to 8^{th} hour of study. The hypoglycaemic activity of glibenclamide was observed at 2^{nd} hour. Jamboola at a dose 1.5 ml/kg (Group 3) exhibited insignificant decrease in blood glucose during the 1^{st}-4^{th} hour of study, whereas less significant ($P<0.01$) decrease was observed after 6 hours of drug treatment. Animals treated with jamboola at a dose of 3 ml kg (Group 4) showed significant decrease ($P<0.05$, $P<0.001$) in blood glucose after 4 hours of oral drug treatment.

Table 1: Anti-hyperglycaemic activity of jamboola in normal rats

Group	Treatment	Mean blood glucose levels (mg/dL)			
		0 min	30 min	90 min	150 min
1	Glucose 3 mg/kg	90.83±0.94	144.30±1.76	138.2±2.90	118.3±2.17
2	Glibenclamide 0.45 mg/kg	94.00±1.23	123.50±2.04a$^{\#}$	81.33±1.82 a$^{\#}$	61.50±2.17 a$^{\#}$
3	Jamboola 1.5 ml/kg	90.17±1.57	136.50±2.64a* b$^{\#}$	122.70±3.41 a** b$^{\#}$	103.3±4.24 a** b$^{\#}$
4	Jamboola 3 ml/kg	89.50±0.99	130.50±2.89 a$^{\#}$ b^{ns}	110.3±3.50 a$^{\#}$ b$^{\#}$	89.00±1.80 a$^{\#}$ b$^{\#}$

The values are Mean ± S.E.M of 6 observations. a- represents the probability of significance when compared to Group 1. b- represents the probability of significance when compared to Group 2.- p<0.05; ** - p<0.01; # - p<0.001; ns-non significant. (ANOVA followed by Dunnett's multiple comparison test)*

Effect of jamboola on the anti-hyperglycaemic activity in diabetic rats:

The anti-diabetic potential of jamboola was studied in alloxan induced diabetic rats for a period of 15 days and the data obtained are summarized in Table 3. The blood glucose levels of untreated diabetic rats were found to increase gradually till 15th day. Animals treated with glibenclamide and jamboola (3 ml/kg) exhibited high significant (P<0.001) decrease in blood glucose after 5 days of treatment whereas animals treated with jamboola (1.5 ml/kg) exhibited less significant decrease in blood glucose (P<0.01) when compared to untreated diabetic control.

Table 2: Hypoglycaemic activity of jamboola in normal rats

Group	Treatment	Mean blood glucose levels (mg/dL)					
		0 h	1 h	2 h	4 h	6 h	8 h
1	Distilled water	93.33±1.02	94.33±1.11	95.50±1.17	93.67±1.05	92.17±0.94	93.17±1.07
2	Glibenclamide 0.45 mg/kg	91.67±0.88	74.00±3.51 a$^{\#}$	36.00±2.38 a$^{\#}$	60.50±3.09 a$^{\#}$	42.33±2.48 a$^{\#}$	37.00±2.73 a$^{\#}$
3	Jamboola 1.5 ml/kg	94.00±1.12	93.00±0.96 a^{ns} b$^{\#}$	94.00±0.68 a^{ns} b$^{\#}$	90.00±0.81 a^{ns} b$^{\#}$	81.67±2.64 a** b$^{\#}$	82.00±3.19 a** b$^{\#}$
4	Jamboola 3 ml/kg	94.33±1.28	92.00±1.57 a^{ns} b$^{\#}$	93.17±1.77 a^{ns} b$^{\#}$	87.33±1.66 a* b$^{\#}$	75.00±2.12 a$^{\#}$ b$^{\#}$	77.00±2.32 a$^{\#}$ b$^{\#}$

*The values are Mean ± S.E.M of 6 observations. a- represents the probability of significance when compared to Group 1. b- represents the probability of significance when compared to Group 2. *- p<0.05; ** - p<0.01; # - p<0.001; ns-non significant. (ANOVA followed by Dunnett's multiple comparison test)*

Table 3: Anti-hyperglycaemic activity of jamboola in diabetic rats

Group	Treatment	Mean blood glucose levels (mg/dL)				
		Initial	Day 0	Day 5	Day 10	Day 15
1	Distilled water	94.67±1.49	400.00±4.07	405.30±3.51	412.20±4.03	415.00±4.38
2	Glibenclamide 0.45 mg/kg	95.17±1.44	398.30±4.40	320.00±6.45 a$^{\#}$	295.00±7.07 a$^{\#}$	250.20±11.20 a$^{\#}$
3	Jamboola 1.5 ml/kg	93.50±1.43	405.50±5.96	375.00±6.83 a** b$^{\#}$	375.00±10.33 a** b$^{\#}$	358.30±9.18 a** b$^{\#}$
4	Jamboola 3 ml/kg	92.00±2.44	408.3±7.61	339.00±7.68 a$^{\#}$ b^{ns}	320.50±5.43 a$^{\#}$ b^{ns}	265.00±15.06 a$^{\#}$ b^{ns}

The values are Mean ± S.E.M of 6 observations. a- represents the probability of significance when compared to Group 1. b- represents the probability of significance when compared to Group 2.- p<0.05; ** - p<0.01; # - p<0.001; ns-non significant. (ANOVA followed by Dunnett's multiple comparison test)*

DISCUSSION

Earlier studies reveal that the knowledge on diabetes existed during the fourth centuries and specific treatments were adopted using plants and minerals as a source for the therapy. Based on the availability of enormous research data's, it is well understood that plants and plant based therapies have a vital role to play in the treatment regimens of diabetes mellitus. India is a potential source of various indigenous medicinal plants of high therapeutic value. The constituents of Jamboola include medicinal plants like *Eugenia jambolana, Cassia auriculata, Emblica officinalis, Momordica charantia and Tinospora cordifolia.* In the present study, the hypoglycaemic and anti-hyperglycaemic activity of Jamboola was studied in normal and alloxan induced diabetic rats. Alloxan induced diabetes mellitus is due to the damage caused to the pancreatic beta cells leading to poor utilization of glucose by the liver and other extra-hepatic tissues due to altered insulin secretion and action [14]. It is widely considered as a standard

model for the evaluation of anti-diabetic drug entities. The elevation of blood glucose levels in the alloxan treated animals is a significant outcome of the damage caused to the pancreatic islets. The results obtained from the anti-hyperglycaemic and hypoglycaemic studies of jamboola in normal and diabetic rats were found to be significant and was in accordance with the available literature on the individual constituents of jamboola [15-19]. The scientific data generated is an evidential proof of the efficacy of the herbal formulation. The detail study on the active constituents of these plants reveals the presence of glycosides, alkaloids, triterpenoids, flavonoids , steroids, peptides and these active principles share the anti-diabetic potential to a great extent and is widely reported [20,21]. The mechanism existing behind the reduction of blood glucose level by jamboola may due to its preventive effect against the oxidative stress caused by alloxan, influence on insulin sensitivity and reduction in insulin resistance or stimulation of insulin secretion.

CONCLUSION

The anti-diabetic effect of these herbs may be attributed due to the presence of potent phytochemicals. Further investigations are underway to estimate the influence of jamboola on the insulin levels and to evaluate the anti-hyperlipidemic potential of the poly herbal formulation.

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