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Research Article

EFFECT OF AQUEOUS LEAVES EXTRACT OF *CARISSA CARANDAS* LINN. ON BLOOD GLUCOSE LEVELS OFNORMOGLYCEMIC & ALLOXAN-INDUCED DIABETIC WISTER RATS

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ABSTRACT

The effect of the aqueous extract of alloxan induced and normoglycemic Wister rats has been investigated. Three doses of the extract (250 mg/kg; 500 mg/kg and 1000 mg/kg) were administered orally. The 250 mg/kg extract of Carissa carandas Linn. did not show any significant change in the blood glucose levels when compared to untreated control. The dose 500 & 1000 mg/kg of extract showed a significant ($p<0.5$) decrease in blood glucose levels after 4, 8 and 24 hours. in normoglycemic rats, the dose of 1000 mg/kg of the extract significantly ($p<0.05$) decrease the blood glucose levels at 8 and 24 hours. In conclusion, the doses of extract has shown both significant ($p<0.05$) hypoglycemic and antihyperglycemic effects in Wister rats.

Keywords: *Carissa carandas* Linn, Alloxan diabetes, Blood glucose.

INTRODUCTION

Diabetes mellitus (DM) is a major health problem all over the world. Globally, the number of people that has been diagnosed with diabetes has exploded in the past two decades. In 2000, 151 million people in the world were diabetic. With the current rate of increase (6% per annum), it has been projected that 221 million people will be diabetic in 2010 and 324 million by 2025[1]. Several approaches were made to reduce the hyperglycemia, the hallmark of diabetes mellitus, with treatments such as sulfonylureas, which stimulates pancreatic islet cells to secrete insulin; meteoric, which acts to reduce hepatic glucose production; Glucosidase inhibitors, which interfere with glucose adsorption and insulin itself, which suppresses glucose production and augments glucose utilization[2]. The growing public interest and awareness of natural medicines have led the pharmaceutical industry and academic researchers to pay more attention to medicinal plants[3]. The apparent reversal of trend from western to herbal medicine is partly due to the fact that synthetic drugs have always shown adverse reactions and other undesirable side effects. This has led to the belief that natural products are safer because they are more harmonious with biological systems. In addition, the cost of administering modern antidiabetic drugs is beyond the reach of people in the low income group and those living in the rural areas. In Nigeria, hundreds of plants are used traditionally for the management of diabetes mellitus. To date, however, only a few of these medicinal plants have received scientific scrutiny, despite the fact that the World Health Organization has recommended that medical and scientific examinations of such plants should be undertaken[4].

Table 1: **Epidemiological data for distribution of diabetes (in millions) and the percentage increase over last 10 years**.

	Year-2000	Year-2010	% Increase
World	15.1	22.1	46%
North America	14.2	17.5	23%
South America	15.6	22.5	44%
Africa	9.4	14.1	50%
Europe	26.5	32.9	24%
Asia	84.5	132.3	57%
Australia	1.0	1.3	33%

The global figure of people with diabetes is set to rise from current estimate of 150 millions to 220 millions in 2010, and 300 million in 2025. Most cases will be of type 2 diabetes, which is strongly associated with sedentary lifestyle and obesity.

Carissa carandas from apocynaceae is a tall, much branched thorny woody shrub distributed throughout drier part of India ascending to 900 m in hill, frequently cultivated. *Carissa carandas* Linn. contain various chemical constitutents i.e carissol, carissic acid, ascorbic acid, lupeol, β-sitosterol, glucose, galactose, serine, glutamine, alinine, valine, phenylalanine and glycine etc[5]. *Carissa carandas* Linn. demonstrated DPPH redical scavenging activity and inhibitory effects towards the in-vitro reaction of hypoxanthine and xanthin oxidase (XO) was also carried out in the presence of plant extract, aglycones quercetin, kaempferol and apigenin along with allopurinol. The C*arissa carandas* have the potential hypoglycemic and antihyperglycemic effects and also used to treat liver disease and jaundice.

Carissa carandas also show advance pharmacology activities i.e. antibacterial, scavengers of free radicals and inhibitors of xanthine oxidase, antioxidant, cardiotonic & blood pressure, anti convulsant activity, hepatoprotective, analgesic & anti-inflammatory activity and it also useful in the hypoglycemic conditions[6, 7].

MATERIALS AND METHODS

Plant collection and identification

Leaf part of *Carissa carandas* was collected from Jalandhar Cant., Jalandhar in the month January 2010. The plant material was identified by Dr. H. B. Singh of Raw Material Herbarium & Museum, NAISCARE, New Delhi where a voucher specimen (No.NISCAIR/RHMD/Consult/-2009-10/326/128) has been deposited at the herbarium unit.

Extract preparation

The leaf were dried under the shade and ground into powder. The powder (500 g) was macerated in 205L of distilled water at room temperature for 24 h. it was then filtered using a filter paper (Whatmann size no. 1) and the filtrate evaporated to dryness in the water bath at 60^0C. A brownish residue weighing 20.6 g (yield of 4.12 % w/w) was obtained. This was kept in air tight bottles in a refrigerator until used.

Chemicals used

All chemicals and drugs were obtained commercially and were of analytical grade**.**

Acute toxicity study

The method of lorke[8] was adopted and a total of 24 rats weighing 140-155 g each were used for this study. The animals were fasted for 12 h before the study, but were allowed water *ad libitum*. In the initial phase, four groups (n= 3) were given normal saline as control group and 100, 1000 and 10,000 mg/kg of the extract orally for the remaining three groups respectively. They were then observed for 24 h for signs of toxicity or deaths. In the final phase, another four groups(n= 3) were given normal saline, 2000, 4000 and 8000 mg/kg of extract orally for the remaining groups respectively and were observed for 24 h for signs of toxicity or deaths. The median lethal dose (LD_{50}) was calculated from the final phase.

Animals and induction of diabetes mellitus

Fifty Wister rats of both sexes weighing 145-180 g were used for the study of the effects of aqueous extract of *Carissa Carandas* on the blood glucose levels of the animals. They were kept in standard cages at 25^0C and 12 h light/dark condition in the animal room of the Department of Pharmacology, CTIPS, Jalandhar. They were fed on commercial rats' feeds and were given water *ad libitum*. The animals were fasted from feeds for 12 h before the commencement of each experiment, but were allowed water *ad libitum*. The rats assigned to the diabetic groups were injected with a freshly prepared alloxan monohydrate dissolved in the sterile normal saline at a dose of 150 mg/kg body weight interaperitoneally. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic release of insulin, the rats were treated with 20% glucose solution interaperitoneally after 6 h. The rats were kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia. After a period of two weeks the rats with a blood glucose levels greater than 150 mg/dl were considered diabetic and used for this research work[9].

Experimental design

The alloxan-induced diabetic Wister rats were randomly assigned into five groups (1-5) of five rats (n= 5) each as follows, namely: Group 1- Received 250 mg/kg body weight of aqueous extract oral; Group 2- Received 500 mg/kg body weight of aqueous extract oral; Group 3- Received 1000 mg/kg body weight of aqueous extract oral; Group 4-1- Received metformin (300 mg/kg) orally; Group 5- Received with normal saline orally. The normoglycemic Wister rats were also randomly grouped into five (6-10) with five rats (n= 5) in the each groups as follows, namely: Group 6-Received normal saline oral; Group 7-Received 250 mg/kg body weight of aqueous extract oral; Group 8-Received 500 mg/kg body weight of aqueous extract oral; Group 9-Received 1000 mg/kg body weight of aqueous extract oral; Group 10-Recevied metformin (300 mg/kg) orally

Determination of blood glucose levels

All blood samples were collected by cutting the tail-tip of the rats. Blood samples for blood glucose determination were collected from the tail at intervals of 0, 2, 4, 8 and 24 h. determination of the blood glucose level was done by using glucometer (Accu chek) and results reported as mg/dl.

Statistical analysis

Blood glucose levels were expressed in mg/dl as mean±SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group by Dunnett's method. Values of $p<0.05$ or less were taken as significant. The alloxan-induced diabetic groups and normoglycemic groups were analyzed separately for statistical significance.

RESULTS

Acute toxicity study

Signs of toxicity were first noticed after 3-4 h of aqueous extract administration. There were decreased locomotors activity and sensitivity to touch and pain. Also there was decreased feed intake, tachynoea and prostration after 6-8 h of aqueous extract administration. In the final phase, the mortality rates for 2000, 4000 and 8000 mg/kg of the extract 0, 67 and 100% respectively. The LD_{50} was calculated as 3309 mg/kg by log-probit method.

Table 2: Effects of aqueous extract of *Carissa carandas* on blood glucose levels of alloxan-induced diabetic Wister rats.

Treatment	Blood glucose levels (mg/dl)				
	0 hour	2 hour	4 hour	8 hour	24 hour
Control(N/Saline)	268.2±16.98	245.7±25.11	310.1±22.89	331.5±26.25	324.3±21.88
Metformin	270.4±18.10	210.1±24.89^{ns}	180.9±15.9^{ns}	158.7±21.86^{ns}	152.7±3.09^{ns}
250 mg/kg	277.2±15.01	323.8±20.17^{ns}	356.6±18.6^{ns}	382.1±14.55^{ns}	398.5±10.70^{ns}
500 mg/kg	238.0±22.90	200.4±26.23^{ns}	145.1±10.70^{a}	92.7±25.45^{a}	126.4±15.05^{a}
1000 mg/kg	267.9±17.63	203.0±19.52^{ns}	157.2±16.89^{a}	94.5±19.56^{a}	117.6±17.83^{a}

^{a}=P <0.05= Significant, ^{ns}=not significant n= 5

Blood glucose levels of alloxan-induced diabetic Wister rats

Table 2 showed the results of the effects of three doses (250, 500 and 1000 mg/kg) of the aqueous extract of *Carissa carandas*, metformin and control groups in alloxan diabetic Wister rats. The doses of metformin and 250 mg/kg of the extract did not show any significant change in the blood glucose levels when compared to untreated control. However, the doses of 500 and 1000 mg/kg of extract showed a significant ($p<0.5$) decrease in the blood glucose levels after 4, 8 and 24 h.

Table 3: Effects of aqueous extract of *Carissa carandas* on blood glucose levels of Normoglycemic Wistar rats

Treatment	Blood glucose levels (mg/dl)				
	0 hour	2 hour	4 hour	8 hour	24 hour
Control(N/Saline)	68.3±2.44	76.8±1.23	82.8±3.89	90.1±2.45	79.5±5.70
Metformin	70.8±5.20	46.4±1.44^{a}	52.9±6.23^{ns}	61.7±5.00^{a}	82.6±1.20^{ns}
250 mg/kg	67.6±6.50	74.2±6.52^{ns}	70.5±4.63^{ns}	68.3±3.63^{ns}	77.8±4.30^{ns}
500 mg/kg	65.2±1.99	92.8±3.95^{ns}	51.6±1.28^{ns}	46.9±2.45^{a}	74.5±6.50^{ns}
1000 mg/kg	65.0±5.56	98.5±4.22^{ns}	49.8±6.60^{ns}	39.7±3.11^{a}	47.3±1.60^{a}

^{a}=P <0.05= Significant, ^{ns}=not significant n= 5

Blood glucose levels of normoglycemic Wister rats

Table 3 showed the results of the effects of three doses (250, 500 and 1000 mg/kg) of the aqueous extract of *Carissa carandas*, metformin and control groups in normal Wister rats. The dose of metformin showed a significant ($p<0.05$) decrease of blood glucose levels at 2 and 8 h. the extract did not significantly alter the blood glucose levels at a dose of 250 mg/kg body weight. The dose of 500 mg/kg did significantly ($p<0.05$) decrease the blood glucose levels after 8 h of treatment only. However, the dose of 1000 mg/kg did significantly ($p<0.05$) decrease the blood glucose levels after 8 and 24 h of treatment.

DISCUSSION AND CONCLUSION

Alloxan monohydrate is one of the chemical used to induce diabetes mellitus. It induces diabetes by partial destruction of the β-cell of islets of Langerhan's[10]. This results in decreased insulin levels and hyperglycemia leading to diabetes mellitus. However, animal models of diabetes differ significantly from each other and can be taken, without reservation, to reproduce the essentials of human diabetes[11].

Carissa carandas Linn have been shown to have hypoglycemic potentials in normal and glucose loaded mice and rats. In another study, reported the antihyperglycemic effects of *Carissa carandas* Linn in the glucose induced hyperglycemia[12].

Thus, the results of this study of the aqueous crude extract of *Carissa carandas* Linn. on the blood glucose levels of normal and alloxan induced diabetic Wister rats were in consonant with the findings of earlier researchers. The dose of 250 mg/kg of the extract did not significantly alter the blood glucose levels of both normal and alloxan induced diabetic Wistar rats. However, the doses of 500 and 1000 mg/kg of the extract did significantly ($p<0.05$) decrease the blood glucose levels of alloxan diabetic Wistar rats at 4, 8 and 24 h. in normal rats, the dose of 1000 mg/kg of extract significantly ($p<0.05$) decrease the blood glucose levels at 8 and 24 h only. In conclusion, the dose 1000 mg/kg of the extract has shown both significant ($p<0.05$) hypoglycemic and anti-hyperglycemic effects in Wistar rats.

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