HYPOGLYCAEMIC EVALUATION OF ETHANOLIC EXTRACT OF RHIZOME OF *COSTUS SPECIOSUS* IN ALLOXAN INDUCED DIABETIC RATS

C.S. Deepak[1], P.T.A. Usha[2], Preethy John[3], S. Sujith[3] and V. Beena[4]
Department of Veterinary Pharmacology and Toxicology
College of Veterinary and Animal Sciences,
Mannuthy, Thrissur - 680 651.

Received : 01.07.2016
Accepted : 14.07.2016

Abstract

The present study was carried out to evaluate the hypoglycaemic effect of ethanolic extract of Costus speciosus. The extract was given at three different doses 250 mg/kg, 500 mg/kg and 750 mg/kg, and it was found that all the three doses significantly reduced (P<0.05) the blood glucose level. The reference drug (glibenclamide) and extract were administered orally in distilled water as vehicle for 45 days. All the treatment groups except the normal control was made diabetic by intraperitoneal injection of alloxan monohydrate at the dose rate of 130 mg/kg body weight for two consecutive days. The study confirmed that plant extract possessed the hypoglycaemic activity

Key words: *Costus speciosus, hypoglycemic and alloxan*

India is on the verge of becoming the diabetic capital of the world. Type 1 diabetes or insulin dependent diabetes is caused by the autoimmune destruction of the beta cells of the pancreas and represents approximately 10 per cent of all cases with diabetes and type 2 Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia, lipoprotein abnormalities, raised basal metabolic rate, defect in reactive oxygen species scavenging enzymes, high oxidative stress induced damage to pancreatic beta cells. For diabetes, pharmacological treatment is based on the two types of drugs e.g. insulin and oral hypoglycemic drugs such as Biguanide and sulfonylureas. Though these drugs are being used, their use is restricted due to their limited pharmacological action.

Now it has become important to look for economical as well as therapeutically effective treatments for diabetes mellitus especially for developing countries. Plants are well known in traditional herbal medicine for their hypoglycaemic activities and available literature indicate that there are more than 800 plant species showing hypoglycaemic activity. Literatures proved that rhizome of the *Costus speciosus* exert hypoglycaemic activity

1. MVSc Scholar
2. Professor and Head
3. Assistant Professor
4. Assistant Professor, Department of Veterinary Physiology

(Alamoudi *et al*. 2014). Hence, the current study was carried out the for the evaluation of hypoglycaemic activity of the rhizome of the *Costus speciosus* in diabetic rats

Materials and Methods

Collection, identification of plant materials and preparation of extracts

Plant materials were collected from the campus of the College of Veterinary and Animal Sciences, Mannuthy and identified by Dr. Anto (Taxonomist) from St. Thomas College. Thrissur (Kerala). Rhizomes of *Costus speciosus* were collected cleaned and shade dried. The material was pulverized to coarse powder using electrically operated plant sample grinder and kept in air tight container till used for extraction.The extracts was prepared using ethanol as solvent. A weighed quantity of the coarse powder was taken and extracted with ethanol in Soxhlet apparatus. The extract was concentrated in rotary vacuum evaporator and the concentrated extract was dried by keeping in the refrigerator. The yield of the extract was 3.33 per cent.

Drugs and Chemicals

Reference drug glibenclamide (Daonil) purchased from the Emacure Pharmaceuticals, Pune (Maharashtra) and alloxan monohydrate purchased from Sisco Research Laboratory (Maharashtra) were used for the study.

Animal used for study

The study was conducted in 36 female Sprague Dawely rats. The rats were procured from Small Animal Breeding Station, College of veterinary and animal sciences, Mannuthy. The animals were housed in appropriate cages in a well-ventilated room with temperature ranges from 21-24° C, relative humidity 65-68 per cent with 12 hours light and 12 hours dark cycle.

Collection of blood

Two milliliter of blood was collected from each rat under ether anaesthesia through retro orbital plexus. For serum separation blood was kept at refrigeration temperature for half an hour followed by room temperature for another half an hour. It was then centrifuged at 3200 rpm for 10 minutes and the clear serum obtained was pipetted out for the estimation blood glucose level. . Blood glucose levels were estimated using blood glucose estimation kit which was purchased from Agappe diagnostics Ltd, Ernakulam, Kerala.

Main items of observations

Mainitemsofobservationsincluded body weight at fortnightly intervals and blood glucose levels at days 0, 15, 30 and 45

Experimental Design

The rats were randomly divided into 6 groups comprising six animals in each group. All the animals were fasted overnight and their body weight and blood glucose were estimated on next day morning. Blood was taken before the experiment and on 5th day for estimation of blood glucose. Animal were considered diabetic, if the blood glucose values were approximately 300 mg/dl on the 5th day of alloxan administration.

The alloxan was accurately weighed and 10 per cent w/v solution in distilled water was prepared. All the treatment groups except the normal control group were made diabetic by intraperitoneal injection of alloxan monohydrate at the dose rate of 130 mg/kg body weight for consecutive two days (Balaji, S. 2005). Diabetic rats were administered with the extract and reference drug glibenclamide from 6th day onwards daily for 45 days. The drug and extract were administered orally with distilled water as vehicle. The experimental design was as follows:

Statistical Analysis

Body weight and blood glucose were statistically analysed using repeated ANOVA followed Duncan multiple comparison test usingSPSS version 20.1

T_1	Normal control with vehicle
T_2	Diabetic control
T_3	Diabetic rats administered with Glibenclamide at the dose rate of 0.25 mg/kg
T_4	Diabetic rats administered with ethanol extract of *Costus speciosus* 250 mg/kg
T_5	Diabetic rats administered with ethanol extract of *Costus speciosus* 500 mg /kg
T_6	Diabetic rats administered with ethanol extract of *Costus speciosus* 750 mg /kg

Results and Discussion

Body weight

The body weight of the rats was recorded on zero day and at fortnightly intervals. The value are presented in Table 1.The mean body weight on zero, 15, 30 and 45 days for group T_1 was 200.58 ± 0.13, 146.59 ± 2.92, 150.34 ± 4.16, 149.50 ± 4.58, 149.27 ± 3.15 and 149.33 ± 4.64 grams. The mean body weight on zero, 15, 30 and 45 days for group T_2 were 146.59 ± 2.92, 132.85 ± 1.87, 126.06 ± 4.06 and 112.80 ± 1.74 grams. The mean body weight on zero, 15, 30 and 45 days for group T_3 were 150.34 ± 4.16, 161.42 ± 3.51, 168.23 ± 4.52 and 188.46 ± 2.59 grams. The mean body weight on zero, 15, 30 and 45 days for group T_4 were 149.50 ± 4.58, 160.95 ± 3.87, 65.38 ± 4.63 and 174.56 ± 4.75 grams. The mean body weight on zero, 15, 30 and 45 days for group T_5 were 149.27 ± 3.15, 169.17 ± 1.86177.99 ± 2.63, 189.10 ± 2.33 grams. The mean body weight on zero, 15, 30 and 45 days for group T_6 were 149.33 ± 4.64, 170.54 ± 1.65, 182.53 ± 1.39 and 192.12 ± 1.32 grams.

The mean body weight of animals on zero day were 200.58 ± 0.13, 146.59 ± 2.92, 150.34 ± 4.16, 149.50 ± 4.58, 149.27 ± 3.15 and 149.33 ± 4.64 grams for groups T_1 - T_6 respectively. The mean body weight of animals on 15th day were 235.31 ± 5.86, 132.85 ± 1.87, 161.42 ± 3.51, 160.95 ± 3.87, 169.17 ± 1.86 and 170.54 ± 1.65 grams.The mean body weights of the animals on the 30th day were 254.30 ± 7.03, 126.06 ± 4.06, 168.23 ± 4.52, 165.38 ± 4.63, 177.99 ± 2.63 and 182.53 ± 1.39 grams for the groups T_1, T_2, T_3, T_4, T_5 and T_6 respectively. On 45 th day, the mean body weight were 315.49 ± 3.82, 112.80 ± 1.74, 188.46 ± 2.59, 174.56 ± 4.75, 189.10 ± 2.33 and 192.12 ± 1.32 grams for the groups T_1, T_2, T_3, T_4, T_5 and T_6 respectively.

All the treatment groups T_4, T_5 and T_6 showed gradual increase in mean body weight from day 15th to 45th day. Diabetic control group (T_2) showed reduction in mean body weight through-out the experimental period. Though the mean body weight of the groups T_4, T_5 and T_6 were progressively increased during the experiment, but they never attained the weight at the beginning of the experiment before inducing diabetes. This finding is similar to the findings of Xie *et al*. (2003) who described that body weight get reduced due to increased mobilization of the fatty acids from storage site to meet the energy demand.

Table 1. Effects of ethanolic extracts of *Costus speciosus* on body weight (g.)

Groups	Days			
	0	15	30	45
T_1	200.58 ± 0.13^{Aa}	235.31 ± 5.86 ^{Ba}	254.30 ± 7.03^{Ca}	315.49 ± 3.82 ^{Da}
T_2	146.59 ± 2.92^{Ab}	132.85 ± 1.87 ^{Bb}	126.06 ± 4.06^{Cb}	112.80 ± 1.74^{Ab}
T_3	150.34 ± 4.16^{Ab}	161.42 ± 3.51^{Bc}	168.23 ± 4.52^{Cc}	188.46 ± 2.59^{Dd}
T_4	149.50 ± 4.58^{Ab}	160.95 ± 3.87 ^{Bc}	165.38 ± 4.63 ^{Bc}	174.56 ± 4.75^{Cd}
T_5	149.27 ± 3.15^{Ab}	169.17 ± 1.86^{Bc}	177.99 ± 2.63^{Ccd}	189.10 ± 2.33 ^{Dc}
T_6	149.33 ± 4.64 ^{Ab}	170.54 ± 1.65^{Bc}	182.53 ± 1.39^{Cd}	192.12 ± 1.32^{Dd}

(Mean ± SE, n= 6) with common superscript (A –D within row, a-d within column) does not differ significantly at 5 % level

On 45th day the highest increase in body weight was observed in T_6 (1.92 ± 1.32 g) compared to diabetic control (T_2). The results of the present study indicate that rhizome extract of *Costus speciosus* showed dose dependent gain in body weight. Similar increase in body weight was also observed by Alarcon – Aguilar *et al*. (2005) by the administration of dichloromethane extract of *Ibervilleasonorae* for 41 days at the dose rate of 300 mg /kg body in alloxan induced diabetic rats.

Blood Glucose

The mean blood glucose levels are presented in Table 2. The blood glucose levels on zero, 5, 15, 30 and 45 for group T_1 were 84.96± 1.99, 84.03 ± 2.22, 84.58 ± 2.28, 83.53 ± 2.19 and 84.44 ± 2.16 mg/dl. The blood glucose levels on zero, 5, 15, 30 and 45 for group T_2 were 84.88 ± 5.19, 305.67 ± 2.71, 307.15 ± 4.91 322.11 ± 6.64 and 323.21 ± 4.86 mg/dl. The blood glucose levels on zero, 5, 15, 30 and 45 for group T_3 were 84.88 ± 5.19, 300.14± 3.04, 229.23 ± 7.85, 161.96 ± 2.05 and 111.43 ± 3.34 mg/dl. The blood glucose levels on zero, 5, 15, 30 and 45 for group T_4 were 87.56 ± 1.23, 310.39± 4.02, 270.86 ± 2.88, 167.67 ± 4.68 and 118.64 ± 5.88 mg/dl. The blood glucose levels on zero, 5, 15, 30 and 45 for group T_5 were 87.80 ± 1.94, 307.67± 2.47, 258. 83± 3.13, 182.96 ± 2.46 and 122.01 ± 1.29 mg/dl. The blood glucose levels on zero, 5, 15, 30 and 45 for group T_6 were 91.62 ± 2.87, 307.20 ± 3.29, 274.96 ± 5.13, 139.92 ± 24.99 and 95.51 ± 3.06 mg/dl.

The blood glucose levels before administration of the alloxan were 84.96 ± 1.99, 84.88 ± 5.19, 84.88 ± 5.19, 87.56 ± 1.23, 87.80 ± 1.94 and 91.62 ± 2.87 mg/dl for the groups T_1, T_2, T_3, T_4, T_5 and T_6 respectively. The groups T_3, T_4, T_5 and T_6 showed blood glucose levels of 229.23 ± 7.85, 270.86 ± 2.88, 258. 83 ± 3.13 and 274.96 ± 5.13 mg/dl respectively on 15th day. On 30th day the levels of blood glucose were 161.96 ± 2.05, 167.67 ± 4.68, 182.96 ± 2.46 and 139.92 ± 24.99 mg/dl for the groups T_3, T_4, T_5 and T_6 respectively. The levels of blood glucose on 45th day were 111.43 ± 3.34, 118.64 ± 5.88, 122.01 ± 1.29 and 95.51 ± 3.06 mg/dl for the groups T_3, T_4, T_5 and T_6 respectively.

Mean blood glucose levels before alloxan administration were similar between the groups. After 5 days of alloxan administration rat with the blood glucose levels 300 mg/dl selected for study. The administration of alloxan led to about three fold elevation of blood glucose level. The glucose levels of diabetic control rats were maintained at a higher level through-out the study. The mean blood glucose value increased from 84.88 ± 5.19 mg/dl to 323.21 ± 4.86 mg/dl for T_2 after 45 days which is accordance with the findings of Babu *et al.* (2002).

The highest reduction in blood glucose level was observed in T_6 group than diabetic control group. Similar dose dependent decrease in mean blood glucose levels was reported by Krishna. (2007) who found that the decrease in mean blood glucose level was obtained with

Table 2. Effects of ethanolic extracts of *Costus speciosus* on blood glucose level (mg/dl)

Groups	Days				
	0	5	15	30	45
T_1	84.96 ± 1.99	84.03 ± 2.22	84.58 ± 2.28	83.53 ± 2.19	84.44 ± 2.16
T_2	84.88 ± 5.19^{A}	305.67 ± 2.71^{Bb}	307.15 ± 4.91$_{Cb}$	322.11 ± 6.64$_{Dbc}$	323.21 ± 4.86 ^{Eb}
T_3	84.88 ± 5.19^{A}	300.14± 3.04$_{Bbc}$	229.23 ± 7.85^{Cb}	161.96 ± 2.05$_{Db}$	111.43 ± 3.34^{Eb}
T_4	87.56 ± 1.23^{A}	310.39± 4.02^{Bc}	270.86 ± 2.88^{Cc}	167.67 ± 4.68^{Dc}	118.64 ± 5.88 ^{Ac}
T_5	87.80 ± 1.94^{A}	307.67± 2.47^{Bc}	258. 83± 3.13^{Cd}	182.96 ± 2.46$_{Dbc}$	122.01 ± 1.29 ^{Eb}
T_6	91.62 ± 2.87^{A}	307.20 ± 3.29^{Bc}	274.96 ± 5.13^{Be}	139.92 ± 24.99^{Bd}	95.51 ± 3.06^{Cd}

(Mean ± SE, n= 6) values with common superscript (A –E within row, a-d within column) does not differ significantly at 5 % level.

Pleuritico stereatus at doses of 250, 500 and 100 mg/kg body weight in diabetic induced rats at the end of the study.

The study concluded that the ethanolic extract of *Costus speciosus* possessed hypoglycaemic activity in alloxan induced rats

References

Alamoudi, E. F., Khalil, W. K. B., Ghaly, I. S., Hassan, N. H. A and Ahmed, E. S 2014. Nanoparticles from *Costus Speciosus* extract improves the antidiabetic and antilipidemic effects against STZ-induced diabetes mellitus in albino rats. *Int. J. Pharmcol. Sci. Rev. Res.* **29**: 279-288.

Alarcon – Aguilar *et al*. 2005. Alarcon – Anguilara, F. J. Roman Ramos, R., Perez-Guiteterez, S., Anguilar –Conterras, A., Conterras- Weber, C. C and Florez- Sanez, J. L. 1998. Study of the hypyperglcaemic effect of plant used as antidiabetic. *J. Ethanopharmacol*. **61**: 101-110.

Babu, V., Gangadevi, T and Subramonium, A. 2002. Antihyperglycaemic activity of *Cassia kleina*in glucose fed normal rats and alloxan induced diabetic rats. *Indian J. Physiol. Pharmacol*. **34**: 409-415.

Balaji, S. 2005. Hypoglyceamic effect of *Scopariadulcis* and *costus* species in albino rats. *M.V.Sc.Thesis,* Kerala Veterinary and Animal Sciences University, Thrissur, 103p.

Krishna, S. 2007. Hypoglycaemic effect of *Pleuritico stereatus* in Sprague Dawley rats. *M.V.Sc. Thesis,* Kerala Veterinary and Animal Sciences University, Thrissur, 66p. ■