ACUTE TOXICITY AND HYPOGLYCAEMIC ACTIVITY OF RHIZOME OF *COSTUS SPECIOSUS* (KOEN.) SM.

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Abstract

Costus speciosus (Koen.) Sm. is locally known as Phalan-taung-hmwe and belongs to the family Costaceae. The plant was collected from Hpa-an Township, Kayin State. In this study, the acute toxic study of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. was evaluated on albino mice by using method of OECD Guideline 423 (2001). There were no signs of toxicity and lethality of mice, even with the maximum dose of 5g/kg body weight of the extract during the observation period of 14 days. The hypoglycaemic activity of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. (1g/kg, 2g/kg and 4g/kg body weight) was also studied on adrenaline induced hyperglycaemic rats model by using the method of Gupta *et al.,* (1967). The results showed that the extract at dose of 4g/kg showed significant hypoglycaemic effect at 2 hr, 3hr and 4 hr ($p<0.001$) when compare with control. Therefore 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. had significant hypoglycaemic effect in rat model.

Key words: *Costus speciosus* (Koen.) Sm., Acute Toxicity and Hypoglycaemic Activity

Introduction

Costus speciosus (Koen.) Sm. belongs to the family Costaceae. It consists of 4 genera and about 150 species, pantropical in distribution, but best developed in the New World. The plants nearly always grow in wet, shady habitats (Cronquist, 1981).

The native of the plant is Indo-Malayan region, occurring from India to New Guinea (Rodriguez, 2005). *Costus speciosus* (Koen.) Sm. is locally known as Phalan-taung-hmwe in Myanmar and Indian spiral ginger in English (Hundley and Chit Ko Ko, 1987 and Kress *et al.*, 2003).

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Rhizomes are bitter, cooling and useful in aphrodisiac, anthelmintic, febrifuge, expectorant, tonic, constipation, pneumonia, rheumatism, asthma, urinary diseases, jaundice, mental disorders, skin disease and improves digestion (Malabadi *et al.*, 2016).

The most popular species in the genus is *Costus speciosus* (Koen.) Sm. which has emerged as an important antidiabetic plant (Rani *et al*., 2012). The fresh juice from the rhizomes of *Costus speciosus* (Koen.) Sm. are used by the local regions of easten Himalayan belt, Bangladesh in the treatment of diabetes (Rajesh, 2006).

Lijuan (2010) and Revathy *et al*., (2014) reported that ethanolic extract of rhizome of *Costus speciosus* (Koen.) Sm. exhibited hypoglycaemic activities on diabetic rats.

The Government of the Republic of the Union of Myanmar emphasizes on the treatment of six major diseases such as malaria, tuberculosis, diarrhoea, dysentery, hypertension and diabetes mellitus. Diabetes is one of the top priorities among these diseases. So, to fulfill one of the purposes, the present research has been carried out on this plant.

Costus speciosus (Koen.) Sm. is also claimed to possess hypoglycaemic activity. But there is no scientific research data of the acute toxicity and hypoglycaemic activity of this plant in Myanmar. Therefore, this study was aimed to evaluate the acute toxicity and to investigate hypoglycaemic activity from the rhizomes of *Costus speciosus* (Koen.) Sm.

Materials and Methods

Acute toxicity of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm.

The acute toxicity test on mice was carried out according to the method of OECD Guideline 423 (2001). Adult, 18 albino mice, Dutch Denken Yoken strain of female sex, weighting between 25 - 30g, were used for acute toxicity study. These animals were provided by the Laboratory Animal Services Division, Department of Medical Research,Yangon.

Materials

Test animals	-	18 female albino mice (ddy strain, body weight 25-30g)
Test agents	-	70% ethanolic extracts from rhizome of *Costus speciosus* (Koen.) Sm.
Apparatus	-	Mice cages, animal balance, "18" gauge intragastric needle, disposal syrings, rubber glove and mask.
Dose Schedule	-	2g/kg and 5g/kg (body weight) of 70% ethanolic extract of rhizome of *Costus speciosus* (Koen.) Sm.
Period of observation	-	Two weeks

Methods

Acute toxicity Test of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. was evaluated by the methods of OECD Guidelines 423 (2001). The animals were randomly selected and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. According to the test description, total number of 18 female albino mice (ddy strain), weighing between 25 - 30g were selected and divided into three groups. Each group contained six mice and kept in the each mouse cage. At first, the mice were individually marked with picric acid staining on the parts of the body and weighed. Required doses were calculated which based on the body weight of the mice. They were fasted for 18 hours before experiment but were allowed with free access to water. Group (I) mice served as a control group and they were administered 10ml/kg of distilled water orally. In this study, starting dose 2g/kg was chosen. Seventy percent ethanolic extract of *Costus speciosus* (Koen.) Sm. rhizome was dissolved in distilled water and the required doses were administered orally by using intragastric needle to every mouse. There were no lethality and toxic signs at the dose of 2g/kg body weight of the extract.

Thus, another 6 mice were administered 5g/kg body weight. The mice were observed for toxic sign by using the method described above. All the mice were observed to detect the delayed toxicity up to 14 days. The mortality and toxic signs during this period were recorded.

Figure 1. Weighing albino mice in

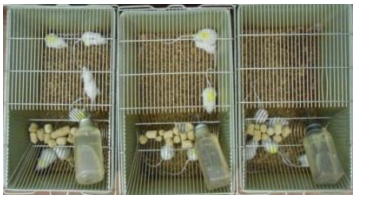

Figure 2. Groups of three mice cages

Figure 3. Administration of extract suspension

Hypoglycaemic activity of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm.

The hypoglycaemic activity of 70% ethanolic extract was also studied on adrenaline induced hyperglycaemic rats model by using the method of Gupta *et al.*, (1967) at Department of Medical Research (DMR), Yangon.

Materials

Test animals - 8 Wistar albino rats of both sexes (body weight 180 - 250g)

Test agent - Distilled water, 70% ethanolic extract, Glibenclamide tablets 5mg, India), Adrenaline injection (1mg/ml) (Myanmar Pharmaceutical Factory)

Apparatus - Aluminium cages, Animal balance, Spirit cotton wools, disposable syringes with needle (1 ml, 5 ml), Glucometer, Test strips, 18 gauge dosing needle, rubber glove and mask

Dose Schedule - 70% ethanolic extracts, 1g/kg, 2g/kg and 4g/kg body weight

Methods

Test animal profile

The study of hypoglycaemic effect of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. was performed by using the method of Gupta *et al*., (1967). Both sexes of eight adult healthy albino rats of Wistar strains weighing (180-250g) obtained from Department of Medical Research were used in this experiment. They were kept in clean and dry cages to allow for acclimatization to the laboratory conditions one week before starting the experiment. The rats were fasted overnight for 18 hours before the experiment but water was allowed freely. Firstly, they were served as control group and only distilled water was given orally to them during experiment. The study design used in this study was cross over study design in albino rats.

Preparation and administration of drug suspension

Before the experiment, individual rats were marked with picric acid, weighed and kept without food for 18 hours. The dosage was calculated according to the body weight of rat. Control animals were administered orally with 10ml/kg of distilled water. The drug suspension (i.e distilled water) was given orally to each rat by using an intragastric needle connected to a plastic syringe containing the calculated dosage. The syringe was put into the mouth about 5 cm until it reached the stomach. Then, the piston was pushed to deliver the test agents into the stomach. Immediate sneezing and coughing indicated injecting into the lungs and in such condition, the syringe was withdrawn.

Collection of blood sample and induction of hyperglycaemic of rats

Before the drug administration, the blood sample was collected by cutting about 1 mm at the tip of the tail as the base line blood sample (0hr). The glucometer test strip was inserted into the glucometer and then, one drop of the blood sample was dropped on this strip. Blood glucose concentration was measured by glucometer at 0 hour. The results were expressed in

(mg/dL). Then, these rats were orally given with distilled water (10 ml/kg) by using "18" gauge intragastric needle. Thirty minutes after administration of distilled water, these rats were subcutaneously injected with 0.4 ml/kg body weight of adrenaline to the back of the neck. Then, blood was taken from tail vein and blood glucose levels were determined hourly up to 4 hours with glucometer. After taking the blood sample, the tail of the rat was rubbed with cotton wool soaked in absolute alcohol to protect the puncture against infection.

Determination of hypoglycaemic activity of 70% ethanolic extracts (1g/kg, 2g/kg and 4g/kg body weight) from the rhizomes of *Costus speciosus* (Koen.) Sm. on adrenaline induced Hyperglycaemic rats

After one week washout period, the same 8 rats were used again and these rats were kept without food for 18 hours before experiment. Only water was allowed orally to them. After that, these rats were orally given ethanolic extract 1g/ kg body weight by using "18" gauge intragastric needle. After 30 minutes, these rats were subcutaneously injected with 0.4ml/kg body weight of adrenaline. Fasting blood was taken from tail vein and blood glucose levels were determined at 0hr, 1hr, 2hr, 3hr and 4 hours with glucometer.

Then, all the animals were allowed to rest for one week of drug free period. After washout period for one week, the same 8 rats were also tested for determination of blood glucose level with 70% ethanolic extracts, 2g/kg body weight. Determinations of blood glucose levels were performed as above procedures. After washout period of one week, the same 8 rats were tested with 70% ethanolic extracts, 4g/kg body weight for determination of blood glucose level as above procedures.

Determination of hypoglycaemic activity of standard drug (glibenclamide) on adrenaline induced hyperglycaemic rats

After drug free interval of one week, the same 8 rats were used again and these rats were kept without food for 18 hours before experiment. Fasting blood glucose levels (0hr) were taken from venous blood obtained by cutting

about 1 mm at the tip of the tail and measured by glucometer. After that, these rats were orally given with standard drug glibenclamide, 4mg /kg body weight by using "18" guage intragastric needle. After 30 minutes, these rats were subcutaneously injected with 0.4ml/kg body weight of adrenaline. Then, blood was taken from tail vein hourly at 1hr, 2hr, 3hr and up to 4 hours and determination of blood glucose levels were done with glucometer.

Data management and analysis

Standard statistical methods were used in the calculation of arithmetic mean ($\overline{X}$) standard deviation (SD) and standard error (SE). Paired student "t" tests were used to analyze the significant differences between means of control and experimental groups (Gupta *et al*., 1967).

Determination of blood glucose concentration

Percent reduction was calculated by the following formula;

$$\text{Percent reduction} = \frac{\text{Difference between rises in blood glucose level of control and test}}{\text{Blood glucose level rise in control}} \times 100$$

$$= \frac{C - T}{C} \times 100$$

C = rise in blood glucose level of control, T = rise in blood glucose level of test

Figure 4. Albino rats in cages

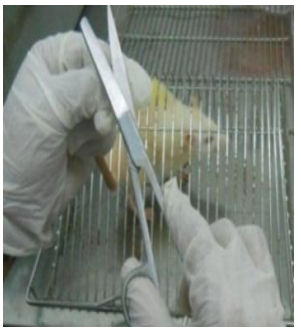

Figure 5. Cutting the tip of tail of the rat

Figure 6. Determining of blood glucose level by using glucometer

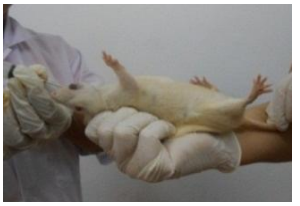

Figure 7. Administration of distilled water to rat

Figure 8. Administration of extracts suspension to rat

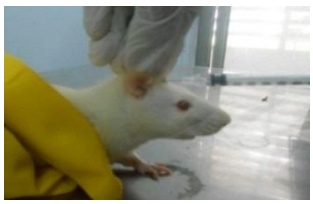

Figure 9. Adrenaline injection into nape of neck of albino rat

Results

Acute toxicity of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. on albino mice

In this study, the mice were administered with the dose of 2g/kg (body weight) and 5g/kg (body weight) of 70% ethanolic extract of rhizome of *Costus speciosus* (Koen.) Sm. Each group of mice was still alive and did not show any signs of toxicity. Even with the maximum dose of 5g/kg body weight of 70% ethanolic extracts, there was no lethality and toxic effect up to two weeks of observation period. Therefore, the extract was observed to be nontoxic. The results were shown in Table 1.

Table 1. Acute toxicity test of 70% ethanolic extracts from the rhizome of *Costus speciosus* (Koen.) Sm. on albino mice

No. of Group	Type of drug administration	No. of mice tested	Dosage	Observed period	No. of death
I	Control (distilled water)	6	10ml/kg	Two weeks	0/6
II	70% ethanolic extracts	6	2g/kg	Two weeks	0/6
III	70% ethanolic extracts	6	5g/kg	Two weeks	0/6

Hypoglycaemic activity of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. on adrenaline induced hyperglycaemic rat model

The hypoglycaemic activity of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. was tested by using adrenaline induced hyperglycaemic albino rats. Eight adult healthy Wistar strain albino rats of both sexes, weighing between 180-250 g body weights were used for this study. The results of hypoglycaemic activity were shown in Tables (2 to 7) and Figure (10).

Effect of distilled water on blood glucose levels on adrenaline induced hyperglycaemic rats model (control group)

The mean blood glucose level of the 8 albino rats given orally with distilled water 10 ml/kg body weight at 0hr, 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline 0.4 ml/kg were 70.88 $\pm$ 2.68 mg/dl, 219.75 $\pm$ 9.58 mg/dl, 249.25 $\pm$ 9.77 mg/dl, 226.88 $\pm$ 8.52 mg/dl and 210.38 $\pm$ 7.4 mg/dl respectively. It was found that blood glucose level significantly increased at 1hr, 2hr, 3hr and 4hr after injection of adrenaline 0.4 ml/kg as shown in Tables (2 and 7) and Figure (10).

Efffect of different dose levels of 70% ethanolic extracts (1g/kg, 2g/kg and 4g/kg body weight) of *Costus speciosus* (Koen.) Sm. rhizome on blood glucose level on adrenaline induced hyperglycaemic rats model

The mean blood glucose level of the 8 albino rats treated with 70% ethanolic extracts of *Costus speciosus* (Koen.) Sm. rhizome 1g/kg body weight at 0hr, 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline 0.4 ml/kg were 69.75 $\pm$ 1.52 mg/dl, 220.13 $\pm$ 7.93 mg/dl, 247.63 $\pm$ 7.24 mg/dl, 224.25 $\pm$ 7.79 mg/dl and 176.13 $\pm$ 5.79 mg/dl respectively. It was observed that the oral administration of 70% ethanolic extracts of *Costus speciosus* (Koen.) Sm. rhizome (1g/kg body weight) produced a significant decrease in glucose level at 4 hr ($p<0.001$) when compared with that of control group as shown in Tables (3 and 7) and Figure (10).

The mean blood glucose level of the 8 albino rats treated with 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. (2g/kg body weight) at 0hr, 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline 0.4 ml/kg were 68.25 $\pm$ 1.69 mg/dl, 218.75 $\pm$ 7.75 mg/dl, 244.25 $\pm$ 8.29 mg/dl, 188.5 $\pm$ 6.69 mg/dl and 161.88 $\pm$ 6.25 mg/dl respectively. It was observed that the oral administration of 70% ethanolic extracts of *Costus speciosus* (Koen.) Sm. rhizome (2g/kg body weight) produced a significant decrease in glucose level at 3hr ($p < 0.001$) and 4 hr ($p < 0.001$) when compared with that of control group as shown in Tables (4 and 7) and Figure (10).

The mean blood glucose level of the 8 albino rats treated with 70% ethanolic extracts of *Costus speciosus* (Koen.) Sm. rhizome (4g/kg body weight) at 0hr, 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline 0.4 ml/kg were 69.63 $\pm$ 2.15 mg/dl, 215.75 $\pm$ 10.66 mg/dl, 224.25 $\pm$ 10.79 mg/dl, 180.38 $\pm$ 6.53 mg/dl and 141.13 $\pm$ 4.04 mg/dl respectively. It was observed that the oral administration at 70% ethanolic extracts of *Costus speciosus* (Koen.) Sm. rhizome (4g/kg body weight) produced a significant decrease in glucose level at 2hr ($p < 0.001$), 3hr ($p < 0.001$) and 4 hr ($p < 0.001$) when compared with that of control group as shown in Tables (5 and 7) and Figure (10).

Efffect of standard drug, glibenclamide on blood glucose level in adrenaline induced hyperglycaemic rats model

The results of mean blood glucose level of the 8 albino rats treated with standard drug, glibenclamide (4mg/kg body weight) at 0hr, 1hr, 2hr, 3hr and 4hr after subcutaneous injection of adrenaline 0.4 ml/kg were 70.75 $\pm$ 2.8 mg/dl, 188.75 $\pm$ 5.25 mg/dl, 173.63 $\pm$ 4.56 mg/dl, 148.63 $\pm$ 5.28 mg/dl and 114.38 $\pm$ 4.24 mg/dl respectively. The results of the oral administration of standard drug, glibenclamide showed that the blood glucose level of adrenaline induced rats were significant decreased at 1hr ($p < 0.005$), 2hr ($p < 0.001$), 3hr ($p < 0.001$) and ($p < 0.001$) when compared with that of control group are shown in Tables (6 and 7) and Figure (10).

Comparison of percent reductions of blood glucose level with different dose of 70% ethanolic extracts from rhizome of *Costus speciosus* (Koen.) Sm. and standard drug, glibenclamide

The comparision of mean percent reductions of blood glucose levels with 70% ethanolic extracts from the rhizome of *Costus speciosus* (Koen.) Sm. and standard drug, glibenclamide are shown in Table (8) and Figure (11).

The mean percent reduction of blood glucose level with 70% ethanolic extracts (1g/kg body weight) were -1.44 ± 3.7%, -0.07 ± 1.61%, 0.91 ± 0.9% and 23.54 ± 2.42% at 1hr, 2hr, 3hr and 4hr respectively. The mean percent reduction of blood glucose level with 70% ethanolic extracts (2g/kg body weight) were -1.74 ± 3.05%, 1.11 ± 1.58%, 23.05 ± 1.06% and 33.1 ± 2.33% at 1hr, 2hr, 3hr and 4hr respectively. The mean percent reduction of 70% ethanolic extracts (4g/kg body weight) were 2.07 ± 1.75%, 13.37 ± 2.7%, 28.88 ± 2.0% and 47.98 ± 3.24% at 1hr, 2hr, 3hr and 4hr respectively. The mean percent reduction of blood glucose level with glibenclamide (4mg/kg body weight) were 19.62 ± 3.26%, 41.55 ± 2.5%, 49.67 ± 1.74% and 68.5 ± 1.65% at 1hr, 2hr, 3hr and 4hr respectively.

Table 2. Effect of distilled water on blood glucose concentration of adrenaline induced hyperglycaemic rats (Control group)

Rat Code No.	Blood glucose concentration (mg/dl)				
	0 HR	**1HR**	**2HR**	**3HR**	**4HR**
1	65	251	273	234	212
2	60	185	229	218	195
3	71	230	283	272	255
4	67	185	205	192	183
5	69	205	231	214	209
6	72	211	236	210	203
7	80	249	272	239	216

Rat Code No.	***Blood glucose concentration (mg/dl)***				
	0 HR	***1HR***	***2HR***	***3HR***	***4HR***
8	*83*	*242*	*265*	*236*	*210*
SUM	*567*	*1758*	*1994*	*1815*	*1683*
MEAN	*70.88*	*219.75*	*249.25*	*226.88*	*210.38*
SD	*7.59*	*27.1*	*27.63*	*24.09*	*20.93*
SE	*2.68*	*9.58*	*9.77*	*8.52*	*7.4*

n=8

Table 3. Effect of 70% ethanolic extract of *Costus speciosus* (Koen.) Sm. 1g/kg on blood glucose concentration of adrenaline induced hyperglycaemic rats

Rat Code No.	**Blood glucose concentration (mg/dl)**				
	0 HR	**1HR**	**2HR**	**3HR**	**4HR**
1	67	253	260	235	170
2	62	215	239	214	165
3	72	217	275	265	204
4	69	178	209	189	150
5	71	208	241	215	177
6	77	222	237	216	175
7	71	227	261	235	192
8	69	241	259	225	176
SUM	558	1761	1981	1794	1409
MEAN	69.75	220.13	247.63	224.25	176.13
SD	4.3	22.44	20.47	22.02	16.37
SE	1.52	7.93	7.24	7.79	5.79
P value	0.647	0.948	0.631	0.201	0.000
P value	NS	NS	NS	NS	< 0.001***

n=8, Statistical comparison was made between the test group and control group

Table 4. Effect of 70% ethanolic extract of *Costus speciosus* (Koen.) Sm. 2g/kg on blood glucose concentration of adrenaline induced hyperglycaemic rats

Rat Code No.	**Blood glucose concentration (mg/dl)**				
	0 HR	**1 HR**	**2 HR**	**3 HR**	**4 HR**
1	70	243	274	205	155
2	63	210	229	180	154
3	61	206	264	217	199

Rat Code No.	Blood glucose concentration (mg/dl)				
	0 HR	**1 HR**	**2 HR**	**3 HR**	**4 HR**
4	64	189	213	160	142
5	72	201	224	185	156
6	71	213	228	170	152
7	73	248	269	202	175
8	72	240	253	189	162
SUM	546	1750	1954	1508	1295
MEAN	68.25	218.75	244.25	188.5	161.88
SD	4.77	21.93	23.44	18.92	17.68
SE	1.69	7.75	8.29	6.69	6.25
P value	0.269	0.841	0.135	0.000	0.000
P value	NS	NS	NS	< 0.001***	< 0.001***

n=8, Statistical comparison was made between the test group and control group

Table 5. Effect of 70% ethanolic extract of *Costus speciosus* (Koen.) Sm. 4g/kg on blood glucose concentration of adrenaline induced hyperglycaemic rats

Rat Code No.	Blood glucose concentration (mg/dl)				
	0 HR	**1HR**	**2HR**	**3HR**	**4HR**
1	73	252	261	197	135
2	56	180	188	173	132
3	74	228	235	204	148
4	67	178	185	159	130
5	69	190	201	161	129
6	71	212	220	165	143
7	72	247	259	201	160
8	75	239	245	183	152
SUM	557	1726	1794	1443	1129

Rat Code No.	Blood glucose concentration (mg/dl)				
	0 HR	**1HR**	**2HR**	**3HR**	**4HR**
MEAN	69.63	215.75	224.25	180.38	141.13
SD	6.09	30.15	30.51	18.48	11.44
SE	2.15	10.66	10.79	6.53	4.04
P value	0.535	0.066	0.001	0.000	0.000
P value	NS	NS	< 0.001	< 0.001***	< 0.001***

n=8, Statistical comparison was made between the test group and control group

Table 6. Effect of standard drug, glibenclamide 4mg/kg on blood glucose concentration of adrenaline induced hyperglycaemic rats

Rat Code No.	Blood glucose concentration (mg/dl)				
	0 HR	**1HR**	**2HR**	**3HR**	**4HR**
1	72	190	178	155	121
2	75	185	173	158	120
3	83	205	190	175	137
4	57	162	153	127	101
5	66	174	162	134	105
6	65	192	170	145	109
7	73	198	172	143	104
8	75	204	191	152	118
SUM	566	1510	1389	1189	915
MEAN	70.75	188.75	173.63	148.63	114.38
SD	7.91	14.84	12.91	14.94	11.98
SE	2.8	5.25	4.56	5.28	4.24
P value	0.973	0.003	0.000	0.000	0.000
P value	NS	< 0.005**	< 0.001***	< 0.001***	< 0.001***

n=8, Statistical comparison was made between the test group and control group

Table 7. Mean blood glucose concentration (Mean ± SE) of 70% ethanolic extract of *Costus speciosus* (Koen.) Sm. (1g/kg, 2g/kg, 4g/kg) and glibenclamide 4mg/kg on adrenaline induced hyperglycaemic rats model

Group of rats	Blood glucose concentration (mg/dl)				
	0 HR	1HR	2HR	3HR	4HR
Control	70.88 ± 2.68	219.75 ± 9.58	249.25 ± 9.77	226.88 ± 8.52	210.38 ± 7.4
Ethanolic extract 1g/kg	69.75 ± 1.52	220.13 ± 7.93	247.63 ± 7.24	224.25 ± 7.79	176.13 ± 5.79***
Ethanolic extract 2 g/kg	68.25 ± 1.69	218.75 ± 7.75	244.25 ± 8.29	188.5 ± 6.69***	161.88 ± 6.25***
Ethanolic extract 4 g/kg	69.63 ± 2.15	215.75 ± 10.66	224.25 ± 10.79***	180.38 ± 6.53***	141.13 ± 4.04***
Glibenclamide 4 mg/kg	70.75 ± 2.8	188.75 ± 5.25**	173.63 ± 4.56***	148.63 ± 5.28***	114.38 ± 4.24***

*P < 0.05, **P < 0.01, ***P < 0.001

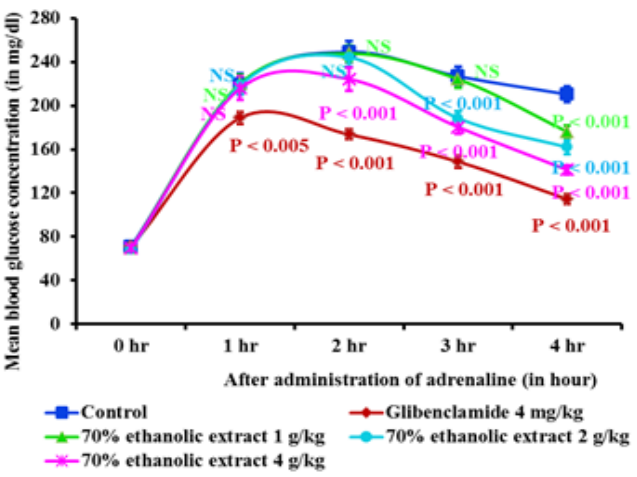

Figure10. Time course of the effect of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. (1 g/kg, 2 g/kg and 4 g/kg) and glibenclamide, 4 mg/kg on adrenaline induced hyperglycaemic rats

Table 8. Percent reduction (Mean ± SE) of hyperglycaemic with 70% extracts of rhizome of *Costus speciosus* (Koen.) Sm. and glibenclamide, 4 mg/kg on adrenaline induced hyperglycaemic rats model

Group of rats	**Percent reduction of hyperglycaemic**			
	1HR	**2HR**	**3HR**	**4HR**
Glibenclamide 4 mg/kg	19.62 ± 3.26	41.55 ± 2.5	49.67 ± 1.74	68.5 ± 1.65
Ethanolic extract 1 g/kg	-1.44 ± 3.7	-0.07 ± 1.61	0.91 ± 0.9	23.54 ± 2.42
Ethanolic extract 2 g/kg	-1.74 ± 3.05	1.11 ± 1.58	23.05 ± 1.06	33.1 ± 2.33
Ethanolic extract 4 g/kg	2.07 ± 1.75	13.37 ± 2.7	28.88 ± 2.0	47.98 ± 3.24

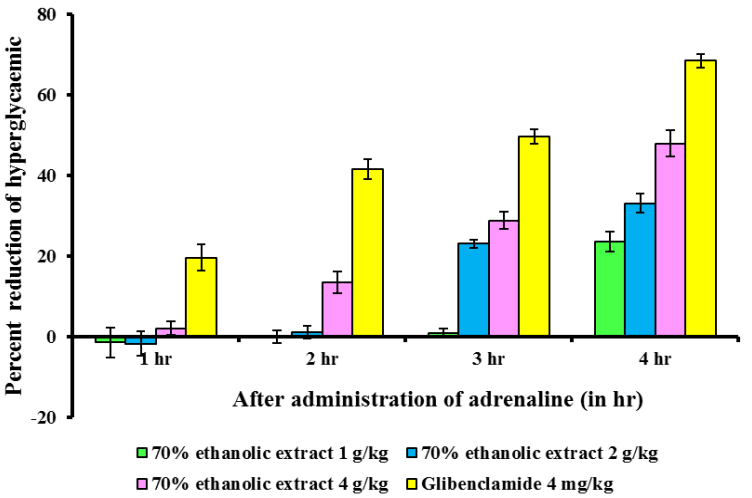

Figure11. Percent reduction of hyperglycaemic with ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. (1g/kg, 2g/kg and 4g/kg) and glibenclamide 4mg/kg on adrenaline induced hyperglycaemic rats model. n=8, Each point represents the mean of observations and the vertical bars indicate standard errors of the means

Discussion and Conclusion

In this research, acute toxicity of 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. was evaluated by the methods of OECD

Guidelines 423 (2001). In this study, the mice were administered with the dose of 2g/kg (body weight) and 5g/kg (body weight) of 70% ethanolic extract of rhizome of *Costus speciosus* (Koen.) Sm. All the animals did not show any signs of toxicity and lethality during observation period of 14 days. Therefore, 70 % ethanolic extract of rhizome of *Costus speciosus* (Koen.) Sm. had no acute toxic effect on mice.

In this study, the hypoglycaemic effect of 70% ethanolic extracts from rhizomes of *Costus speciosus* (Koen.) Sm. (1g/kg, 2g/kg and 4g/kg) were also investigated on adrenaline induced hyperglycaemic rats model by using the method of Gupta *et al*., (1967). 70% ethanolic extracts 1g/kg significantly decreased the blood glucose concentration of the rats at 4 hours ($p<0.001$), 2g/kg at 3 hours and 4 hours ($p<0.001$) and 4g/kg at 2 hours up to 4 hours ($p<0.001$) after subcutaneously injection of adrenaline. These results demonstrated that 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm. was able to reduce blood glucose levels in adrenaline induced hyperglycaemic rats. The hypoglycaemic effect of standard drug, glibenclamide at the dose level of 4mg/kg showed a significant reduction in blood glucose level at 1hour ($p<0.005$) and at 2 hours, 3 hours and 4 hours ($p<0.001$) after the administration of drugs on adrenaline induced hyperglycaemic rats. Hypoglycaemic effect of glibenclamide 4mg/kg was more than 70% ethanolic extracts of rhizome of *Costus speciosus* (Koen.) Sm.

The results showed that percent reductions of hyperglycaemic with 70% ethanolic extract of *Costus speciosus* (Koen.) Sm. rhizomes were 23.54% at the doses of 1g/kg, 23.05- 33.1% at 2g/kg and 13.37- 47.98% at 4g/kg. The percent reductions of hyperglycaemic with standard drug glibenclamide 4 mg/kg was 41.55-68.5%. Therefore, hypoglycaemic effect of 70% ethanolic extracts from rhizomes of *Costus speciosus* (Koen.) Sm. 4g/kg had the most effective hypoglycaemic activity among 3 doses of the extracts.

Therefore, 70% ethanolic extract of rhizome of *Costus speciosus* (Koen.) Sm. possessed significant hypoglycaemic activity. These results are agreed with those mentioned by Lijuan (2010) and Revathy *et al*., (2014).

In conclusion, 70% ethanolic extract of rhizome of *Costus speciosus* (Koen.) Sm. possessed significant hypoglycaemic activity although hypoglycaemic effect of the extract is less effective than glibenclamide. Therefore, *Costus speciosus* (Koen.) Sm. rhizome could be beneficial for the treatment of diabetes mellitus.

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