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HYPOGLYCEMIC EFFECT AND GENOTOXIC EVALUATION OF LEAF EXTRACT OF CROTON SPARCIFLORUS IN ALLOXAN INDUCED DIABETIC MICE *MUS MUSCLES*

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Abstract

Oral administration of the methanolic (80%) leaf extract of *Croton sparciflorus* at the rate of 50 mg (T_1), 100mg (T_2) and 250mg (T_3) per Kg body weight per day for 42 days to alloxan-induced (150mg/kg bw) diabetic mice *Mus muscles* significantly (P<0.01) reduced the blood glucose level by 22.0, 33.4, 51.1% at T_1, 48.7%, 48.9%, 57.2% at T_2 and 49.4%, 54.3%, 57.7% at T_3) in the 1st, 3rd as 6th week respectively. Considerable fall in elevated blood glucose level was also noted with reference drug Glucobay (10mg/kgbw/day) during the same period of treatment. The genotoxic potential of the leaf extract was also evaluated in the mitotically dividing chromosomes of bone marrow cells which showed no genotoxicity of its own. These results demonstrated that *C. sparciflorus* is genotoxically safe hypoglycemic agent.

Key Words: Alloxan, Hypoglycemia, Glucobay, Genotoxicity

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Introduction

Diabetes is a chronic metabolic disorder of multiple etiologies characterized by hyperglycemia, resulting from defects in insulin secretion and/or insulin action (WHO, 1999). Death due to diabetes is among 11 top killers out of 250 causes worldwide leaving behind malaria and tuberculosis (TOI, 2012). The incidence of diabetes is on rise all over the world affecting at least 285 million people and this figure is likely to be higher than double by 2030 (Shetti *et al.,* 2013) India today leads the world with its largest number of diabetic patients (Gupta *et al.* 2005). Currently several synthetic oral drugs used for the treatment of diabetes are also associated with serious side effects and in addition they are not suitable in pregnancy, bowel, hepatic and renal disorder (Naggar *et al*., 2010). However, there has been a resurgence of interest in medicinal plants with hypoglycemic potential in recent years (Hag, 2004). Ethnopharmacological surveys indicate that more than 1200 plants are used in traditional medical systems for their alleged hypoglycemic activity (Murthi, 1995 Day *et al*., 2002; Grover *et al*., 2002). The use of oral hypoglycemic agents is of particular concern because they are consumed by diabetics throughout life. Management of diabetes without any side effect is still a challenge to medical community.

Incidentally a plant may contain secondary metabolites that can act as a mutagen or antimutagen (Nakamura and Yamamoto, 1982; Hartman and Shankel, 1990; Villasenor and Edu, 1993; Villaseno *et al*., 1993). Since the disease is related with the genetic impairments therefore the drug and disease chronicity particularly the hypoglycemic property of the plant and its derivates is to be evaluated genotoxically.

Croton sparciflorus (Linn) Commonly known as Ban mirch is a wild herb widely distributed in tropical and subtropical regions. The plant and its parts are traditionally used against viral and inflammatory fever, twig sap on budding boils. The present work was undertaken to determine the hypoglycemic as well as genotoxic potential of the ethanolic extract of *C. sparciflorus* in normal and alloxan induced diabetic mice, *Mus musculus.*

Materials and Methods

Collection and Identification of Plants

The experimental plant *C. sparciforus* was collected from Pakur agricultural field in Sept'11 and was identified by Botany Dept. K. K. M. College and deposited there Voucher specimen No.KKMCB/321 Fresh leaves were separated from the plants washed in running tap water and dried in incubator at 60^0c.

Preparation of the Extract

The areal part of the Plant was ground into a coarse power with the help of a suitable grinder. The dried Powder (250g) of *C. sparciflorus* was than soxhlated with 80% (v/v) ethanol for about 85 hrs at 60^0c. to get (08g) the dry residue (Hashemnia *et al*., 2012).

Chemicals

Alloxan monohydrate (Spectrochem, india) Glucobay (Bayer Pharona, Bombay) Gulmohar (Hindustan Lever Ltd. Banglore)

Experimental Protocol

A Colony of swiss albino mice, *Musmusculus*, (cdri-s, n=40)was maintained on Gulmohar diet (Hindustan Lever, Banglore) at a temperature (25 ± 2^0c), relative humidity (45-55%) under 12 h light dark cycle with drinking water made available *ad libitum*. The experimental protocol was approved by the Internal Institutional Animal Ethics Committee of the Institution.

Treatment Protocol

All experimental animals were matched for their age and sex and 6-8 weeks old weaning male and female mice were divided in to 10 groups, comprising of six animals (3 male +3 female) per group. Solvent control (SC) received normal saline (@) 2 ml per kg body weight per day (2ml/kgbw/day). The other control groups were given alloxan monohydrate in similar amount of normal saline used for SC @ 150 mg/kg bw by single intraperitoneal injection (ip) and used as diabetic control (DC). The third group of control (GC) mice was fed glucobay only @ 50mg/kb bw/day dissolved in normal saline amount previously used. Three treatment groups (T_1, T_2 and T_3) were dosed with the plant expract @ 50, 100 and 250 mg/kg bw/day respectively. Four groups of diabetic mice (DC+T_1, DC+T_2, DC+T_3 and DC+G) were administered with the same amount of the plants extract for the Same priod barring the last one (DC+G) which was subjected to Glucobay treatment @ 50mg/kg bw/day, dissolved in the similar amount of normal saline used for all other experimental groups. The treatment lasted for 42 days.

Blood Glucose Monitoring

Weekly blood glucose levels were determined from each group from the blood samples taken from the tail end by puncturing, on one touch glucometer (Gluco check, Major Biosystem Corpn., Taiwan) strip (Aspen Diagnostic, Ltd. Delhi) at different time intervals viz; 1^{st}, 3^{rd} and 6^{th} week.

Chromosomal analysis

All the experimental mice were injected colchicines (4%) one and half hour prior to their sacrifice and the bone marrow was flushed out from both the femora. The slide preparation was done according to the method suggested by Preston *et al* 1982. Atleast 300 well spread metaphase plates @50 plates/animal were screened from the double coded slides. The abnormalition in mitotically dividing bone marrow cells if occurred were categorized into structural (chromatid break/gap, acentric fragment, centric fusion) and mitosis-disruptive(aneuploidy, polyploidy, c-mitosis, precocious separation, clumping, stickiness) types.

Statistical Analysis

Data obtained from blood glucose sampling were analyzed using t-test whereas data from chromosomal analysis were subjected to z-test at 5% level (Downie and Heath 1970).

Results and Discussion

Chromosome analysis

Total abnormality (Table 1 & 2) frequency showed significant (P< .05 to P < .001) increase in the alloxan-induced diabetic mice as compared to the solvent or glucobay control groups. The treatment with the plant extract of *C. sparciflorus* showed no genotoxicity of its own when compared with either of the controls. However, the same abnormality frequency in the extract fed to diabetic mice was found reduced insignificantly at T_1 and T_2 doses of the extract with respect to the diabetic control. Only T_3 dose of the extract reduced the incidence of abnormality frequency significantly to the level of control.

Table 1 : Abnormality frequency (% ± SE) in bone marrow cells in control and *C. Sparciflorus leaf* extract fed treated groups.

Experimental Groups	Days	Number of Met	Class No. % ± SE	Mit. Dis No. % ± SE	Total Abnormality No. % ± SE
DC + T1	1st	303	29 9.6 ±1.7	15 4.95 ±1.3	14 4.6 ±1.2
	8th	305	28 9.2 ±1.7	13 4.3 ±1.2	15 4.9 ±1.2[a]
	43rd	300	29 9.7 ±1.7	12 4.0 ±1.1	17 5.7 ±1.3
DC + T2	1st	308	27 8.8 ±1.6	12 3.9 ±1.1	15 4.9 ±1.2
	8th	312	25 8.0 ±1.5	10 3.2 ±0.99	15 4.8 ±1.2
	43rd	301	20 6.7 ±1.4	9 2.99 ±0.98	11 3.7 ±1.1
DC + T3	1st	315	18 5.7 ±1.3	8 2.5 ±0.9	10 3.2 ±0.99
	8th	309	18 5.8 ±1.3	8 2.6 ±0.9	10 3.2 ±1.0
	43rd	310	20 6.5 ±1.4	10 3.2 ±1.0	10 3.2 ±1.0
DC +G	1st	308	17 5.5 ±1.3	12 3.9 ±1.1	29 9.4 ±1.7
	8th	302	15 4.98 ±1.3	11 3.7 ±1.1	26 8.6 ±1.6
	43rd	305	16 5.3 ±1.2	9 2.96 ±0.97	25 8.2 ±1.6[ae]

Table 2: Abnormality frequency (% ± SE) in bone marrow cells in *control and C. sparciflorus leaf extract fed treated groups.*

Experimental Groups	Days	Number of Met	Class No. % ±SE	Mit. Dis No. % ±SE	Total Abnormality No. % ±SE
Solvent Control (SC)	1st	305	7 2.3 ±0.9	7 2.3 ±0.9	14 4.6 ±1.2
	8th	335	8 2.4 ±0.8	6 1.8 ±0.7	14 4.2 ±1.1
	43rd	321	6 1.9 ±0.8	6 1.9 ± 0.8	12 3.7 ±1.1
Diabetic Control (DC)	1st	310	13 4.2 ±1.1	12 3.9 ±1.1	25 8.1 ±1.6
	8th	301	19 6.3 ±1.4	15 4.98 ± 1.3	34 11.3±1.8
	43rd	296	21 7.1 ±1.5	11 3.7 ±1.1	32 10.8 ±1.8
Glucobay (GC)	1st	309	6 1.9 ±0.8	6 1.9 ±0.8	12 3.9 ±1.1
	8th	301	7 2.3 ±0.9	6 1.99 ±0.8	13 4.3 ±1.2
	43rd	304	6 1.97 ±0.8	10 3.3 ±1.0	16 5.3 ±1.3
C. sperciflorus ¼ mtd (T1)	1st	307	13 14.2 ±1.2	5 1.6 ±0.7	8 2.6 ±0.9
	8th	311	15 4.8 ±1.2	6 1.9 ±0.8	9 2.9 ±0.95
	43rd	303	14 4.6 ±1.2	5 1.7 ±0.7	9 2.97 ±0.98
C. sperciflorus ½ mtd (T2)	1st	301	16 5.3 ±1.3	7 2.3 ±0.9	9 2.99 ±0.98
	8th	305	14 4.6 ±1.2	6 1.97 ±0.8	8 2.6 ±0.9
	43rd	309	13 4.2 ±1.1	6 1.9 ±0.8	7 2.3 ±0.9
C. sperciflorus Full mtd (T3)	1st	300	12 4.0 ±1.1	5 1.7 ±0.8	7 2.3 ±0.9
	8th	306	14 4.6 ±1.2	6 1.96 ±0.8	8 2.6 ±0.9
	43rd	308	16 5.2 ±1.3	5 1.6 ±0.7	11 3.6 ±1.1

Symbols Used : a, b and c and d, e and f indicate significant values at 0.05, 0.01 and 0.001 levels respectively in comparison to SC (or GC) and DC.

Table 3 : Weekly blood sugar level (mg/dl) in control and diabetic mice blood samples when fed with *C. sperciflorus* alcohlic leaf extract.

Variant		Time		WEEK					
		0.0 hrs	72.0 hrs	1st	2nd	3rd	4th	5th	6th
Control (C)	Solvent (SC)	104.2 ±15.75	102.83 ±4.86	92.5 ±3.12		97.83 ±5.29			100.0 ±4.71
	Glucobay (GC)	96.0 ±4.17	93.83 ±5.199	84.17 ±16.099		96.67 ±6.28			98.83 ±3.41
	Diabetic (DC)	192.0 ±12.80	216.33 ±14.98	262.67 ±19.42		240.0 ±23.58			237.3 ±21.86
C Sperciflorus Treated (T)	T1	102.67 ±4.28	98.5 ±4.09	107.5 ±4.19		103.67 ±4.04			98.83 ±3.97
	T2	101.67 ±4.25	105.67 ±4.53	99.67 ±3.22		103.5 ±3.95			97.5 ±4.10
	T3	108.33 ±4.38	105.83 ±4.38	104.0 ±3.69		95.83 ±3.79			93.33 ±3.63
*Sperciflorus*Treated (T)	DC + T1	204.83 ±4.88	174.83 ±4.007	138.5 ±3.66		110.83 ±6.68			94.67 ±4.13
	DC + T2	144.67 ± 4.48	134.33 ±4.91	112.5 ±3.68		99.6 ±6.48			99.0 ±4.597
	DC + T3	132.8 ±3.89	120.0 ±4.03	111.17 ±2.54		104.83 ±4.98			95.0 ±2.45
	DC + G	181.17 ±12.44	137.17 ±47.89	127.5 ±12.44		100.83 ±5.15			102.17 ±3.995

Hypoglycemic Investigation

Blood Glucose level was measured in all the normal and treated groups and the result indicated that the administration of plant extract to diabetic mice significantly ($P < .01$) reduced the level by 22.0%, 33.4%, 51.1% at T_1 48.7%, 48.9%, 57.2% at T_2 and 49.4%, 54.3%, and 57.7% at T_3 levels in the 1st, 3rd and 6th week respectively (Table 3). Thus the extract showed dose and time dependent hypoglycemic activity. The reference medicine glucobay was also able to reduce the blood sugar level significantly by ($P < .001$) in the corresponding weeks which showed higher efficacy of the drug in comparison to the extract.

Glucobay is a potent, second generation, oral hypoglycemic agent which is known as insulin secretogauge since it stimulates pancreatic islet's P - cells. The hypoglycemic action of *C. sparciflorus* could possibly be due to the similar increase in the insulin secretion. Further it has also been reported that plant extract exhibit their hypoglycemic action by inhibiting glucose uptake by intestine, inhibiting hepatic glucose production and/or enhancing glucose utilization by peripheral muscle and adipose tissues (*Eddouks et al*., 2003 and 2004)

Compound such as flavonoids and alkaloids present in the plant have been reported to be antihepatotoxic (Gadgoli and Mishra, 1999) and show insulin like effect (Males and Fransworth, 1995). These Compounds are also known as scavenger of excess free radicals (Pratt 1992). The decrease in total abnormality frequency of chromosomes in extract fed diabetic mice might be due to these substances. Thus on the basis of these results it can be postulated that *C. sparciflorus* is a

genotoxically safe hypoglycemic agent nonetheless works with isolated compounds of *C. sparciflorus* is to be needed, in future to elucidate the actual action mechanism.

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