Evaluation of Anti-diabetic activity of Aqueous and Methanolic Extracts of C .tinctorius L. (Safflower Florets)

Abstract

Alloxan was one of the usual substances used for the induction of diabetes mellitus. It has a destructive effect on the β cells of the pancreas. Glibenclamide was an oral sulphonylurea antidiabetic preparation and widely used as standard drug in antidiabetic study. In the hypoglycaemic study of *C.tinctorius* L. petals extract in alloxan induced diabetic rats, significant increase in serum fasting blood glucose level with decrease in body weight were observed. On the other hand, petals extract treatment to animals produced a dose related hypoglycaemic effects. The increased blood glucose level was brought down and gain in body weight was seen.

Alloxan effectively induced diabetes in normal rats that are reflected by elevated levels of blood glucose, glycosylated Hb and reduced levels of body weight, liver, pancreas and kidney glycogen, insulin of the injected animals. Treatment with standard drug glibenclamide and methanolic and aqueous extracts of *C.tinct*orius L. petals reversed these conditions. While comparing plant extracts for antidiabetic study, aqueous extract of *C.tinctorius* L. petals showed better activity than the methanolic extract. Decrease in glycogen content of liver, pancreas and kidney were in diabetic control rats were due to the leakage of insulin in diabetic state. Prevention of glycogen depletion in the liver following administration of petals extract and standard drug could have been achieved by stimulation of insulin release. The highest improvement was recorded in 600 mg/kg b.w. dosage of *C. tinctorius L.* (petals).

Key words: Antidiabetic activity, Aqueous and Methanolic Extracts of C .tinctorius florets, cisplatin drug

Introduction

Nature made human and bestowed countless favours. Ironically, sickness, diseases, complications, inconsistencies and ailments grew slowly. The creator has not made any disease without any cure for it and graced the earth with numerous plants, especially for healing. It has been the necessity of man, which made him trace out the cure from the nature itself. Due to the safe status of herbal medicine, they are in great demand in the developing as well as developed

countries for primary and/or daily health care. Majority of Indian population have access to or practice various traditional medicines to maintain health or treat diseases. These include herbs that can be used either as mono-therapy or as add-on therapy. But the most important challenge faced by these formulations arises due to lack of standardization, identification and pharmacopoeia standards (Ali, M. 2009.).

Safflower, *Carthamus tinctorius* L. is a thistle herb belonging to the family Asteraceae. Safflower Plants are 30-150 cm tall with globular flower heads (capitula) and, commonly, brilliant yellow, orange or red flowers. It is one of humanity's oldest crops cultivated in India mainly for oil from the seeds and a dyes from the flowers. Though, safflower flowers have been used in preparations of Ayurvedic medicines in India and also merit mention in European and Japanese pharmacopoeia's, the interest in this crop has been rekindled in the last few years as the medicinal use of these flowers in china, has become more widely known. China has a significant area under safflower plantation, but is grown almost exclusively for its flowers, which are harvested for use in traditional medicines. Safflower flowers are used in china for the treatment of many illnesses as well as in the preparation of "tonic tea".

The dried flowers of *carthamus tinctorius* (safflower) have been used in traditional Asian medicine for thousands of years. The active compounds are red and yellow pigments, which have been experimentally shown to enrich blood, to decrease fatigue (Akihisa et al., 1994). Moreover, because of restrictions on using synthetic pigments for food colorants, there has been increasing interest in the use of natural pigments. Safflower pigments have been shown to be safe for use as natural pigments. Safflower pigments have been shown to be safe for use in processed foods and soft drinks. Kanehira et al. (2003) reported that kinobeon A, isolated from safflower, exhibited stronger effect on the oxidative stresses and could be a use fill cytoprotective reagent.

Material and methods

The selection of *C tinctorius* for this study was based on their traditional use for diabetes treatment. The plant part flowers were selected for the present studies albino rats, ACCU check glucometer, strips, alloxan monohydrate, glibenclamide, normal saline, distilled water.

In this study antidiabetic activity of methanolic and aqueous extracts of safflower florets at the dose level of 400and 600 mg/kg, in alloxan induced diabetic rats.

Experimental Animals:

Healthy adult (8 weeks) male albino rats of Wistar strain (Rattusnorvegicus) weighing between 150 to 275 g were selected for the study from institute animal house facility(NIN). The experiments were conducted in accordance to the ethical norms approved by Institutional Animal Ethics Committee Guidelines (IAEC No1164/ac/08CPCSEA).The animals were acclimatised to the laboratory conditions for ten days before the experimental work. The animals were housed in polypropylene cages, maintained under standard conditions (12-hours light/12-hours dark cycle; 25±3 °C; 35%-60% humidity), were fed standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and water ad libitum.

Experimental Induction of diabetes and grouping of animals:

Diabetes was induced in overnight fasted animals by a single intraperitoneal injection of 60 mg/kg alloxan monohydrate (Himedia chemicals). The alloxan was dissolved in distilled water. Animals were given 5% glucose solution in drinking water for two 8 days to prevent drug induced hypoglycaemia. Hyperglycaemia was confirmed by the elevated blood glucose levels of 125 mg/dL and above using ACCU CHECK Glucometer. Animals with fasting blood glucose level above 125 mg/dL were classified as diabetic and used for further study. The animals were divided into six groups with six rats in each group. The different groups were grouped as Normal/negative control, Diabetic control treated with alloxan, Diabetic treated with standard drug glibenclamide, Diabetic treated with aqueous extract, Diabetic treated with methanolic safflower floral extract. These groups were treated and observed for 30 days. After this they were sacrificed and the blood and tissues were collected for further studies.

Collection of tissue and blood samples:

The animals were dissected on the 30th day after treatment and dissected under ether anaesthesia. Pancreas, liver and kidney were collected immediately after dissection and stored at -20o C. Blood was collected by retro orbital puncture using glass capillary (20 mm) and collected into test tubes containing EDTA (2mg/mL). All the analysis was conducted within 24 hours of sample collections.

Antidiabetic Activity:

Estimation of Body Weight The body weight changes in control and experimental groups were illustrated in Table 1. The body weight of diabetic rats significantly decreased when compared with control group. Supplementation of methanolic (carthamin) and aqueous (carthamidin) extracts of *C.tinctorius* showed a significant improvement in the body weight of diabetic rats.

There were no significant changes observed between control treated group animals which indicates that both plants have potential to retain the bodyweight of animals. (Table-1)

Table 1. BODY WEIGHTS OF THE RATS

		20-02-2016	23-02-2016	24-02-2016	25-02-2016	26-02-2016	29-02-2016	01-03-2016	04-03-2016	11-03-2016	14-03-2016	21-03-2016
Positive Control	1A	255	233	218	218	225	230	227	221	227	219	212
	1B	262	257	254	248	241	220	218	211	209	204	201
Glibenclamide	2A	219	211	200	188	179	182	180	184	160	147	133
	2B	256	263	240	231	221	216	219	218	215	192	169
A1 aqueous 400	3A	240	218	210	205	211	208	208	209	209	206	208
	3B	234	193	192	192	184	182	186	183	182	180	180
A1 Aqueous 600	4A	224	214	265	254	272	231	246	223	220	243	233
	4B	247	220	225	225	219	177	180	176	173	172	170
A1 Methanol 400	5A	236	198	199	206	203	209	211	215	217	212	216
	5B	281	291	248	244	248	247	231	225	221	222	230
A1 Methanol 600	6A	263	254	251	243	244	239	236	231	233	230	229
	6B	257	269	204	203	208	219	211	213	211	210	205
PBNS-12 Aqueous 400	7A	241	232	225	214	211	193	191	203	202	203	187
	7B	235	220	212	210	212	210	206	201	199	200	201
PBNS-12 Aqueous 600	8A	262	217	240	235	241	245	241	255	262	264	264
	8B	278	270	248	246	252	242	250	245	284	284	284
PBNS-12 Methanol 400	9A	259	213	213	211	210	212	219	221	227	230	234
	9B	270	251	242	240	255	257	254	260	263	266	269
PBNS-12 Methanol 600	10A	248	192	190	186	188	194	202	209	209	218	221
	10B	231	202	215	231	229	250	229	232	230	236	247
SSF-658 sAqueous 400	11A	241	245	278	275	279	285	287	295	238	245	242
	11B	253	220	218	213	206	213	219	226	230	233	240
SSF-658 Aqueous 600	12A	256	237	234	236	232	225	221	216	223	237	245
	12B	233	226	178	192	194	144	168	163	171	174	180
SSF-658 Methanol 400	13A	298	279	271	267	275	282	269	285	290	239	288

	13B	270	243	241	239	234	230	243	249	256	260	262
SSF-658 Methanol 600	14A	260	243	219	210	215	204	210	215	216	218	226
	14B	298	278	271	263	265	275	252	263	269	272	278
Co-A Aqueous 600	15A	243	231	246	244	247	237	236	216	220	227	232
	15B	234	212	220	220	219	223	232	235	227	230	221
Co-A Methanol 600	16A	232	227	230	221	220	206	194	197	195	182	169
	16B	300	293	293	279	294	277	274	265	264	276	265
Manjira Aqueous 600	17A	251	235	230	224	214	212	221	226	228	234	226
	17B	235	217	223	220	225	160	157	163	171	184	168
Manjira Methanol 600	18A	224	223	232	234	236	234	241	244	247	253	245
	18B	255	257	190	183	179	184	199	210	212	221	216

Estimation of Blood glucose

Anti-diabetic activity was studied in alloxan induced diabetic rats .The blood glucose levels of normal and experimental rats shown in a significant increased level of blood glucose was observed in diabetes animals compared to the corresponding control group. Treatment with methanolic floral extracts of safflower increased the levels of blood glucose of diabetic group of rats and the effect was more pronounced in the group of rats treated with experimental plant.

A significant reduction in glucose level was observed on 30th day in the experimental animals treated with carthamidin pbns-12 and ssf-658 extract as compared to control(Table - 2a to 2b)). The levels of blood glucose on 30th day were 109.2+_8.289mg/dl IN Pbns-12 carthamidin **(Table-3a)** .,123.2+_8.843,in SSF-658 carthamidin **(Table-3b)** Extracts shown in animals treated with methanolic extract of safflower(carthamin*)* (400mg/kgand 600mg/kg) and aqueous extract of carthmidin (400mg/kg and 600mg/kg), and commercial drug Glibenclamide respectively. The activity was better in aqueous extract of safflower (carthamidin) than methanolic extract .

However, the group that received aqueous extract of *C.tinctorius* flowers showed maximum reduction in blood glucose level among the experimental groups.

Table 2a : Genotype A1 (Carthamidin)

S.NO	A1 CARTHAMIDIN	GLUCOSE LEVEL Mg/Dl
1	Negative control	124 ± 5.830
2	positive control	51.26 ± 11.392
3	A1 -400	233.6 ± 11.081
4	A1-600	216.2 ± 8.438
5	Standard glibenclamid	157.4 ± 0894

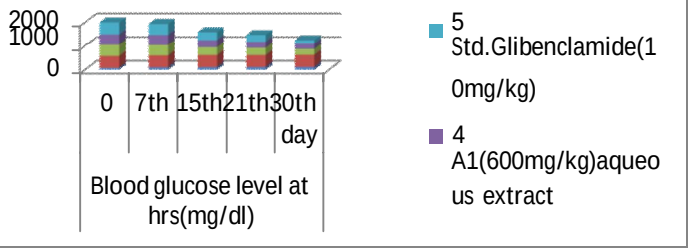


Table 2b : Genotype A1 Carthamin

S.NO	A1 Carthamin	Glucose level mg/dl
1	Negative control	121.2±5.932
2	Positive control	566±5.242
3	A1(400)	348±18.124
4	A1(600)	338±9.460
5	Standard glibenclamid	152.8±8.585

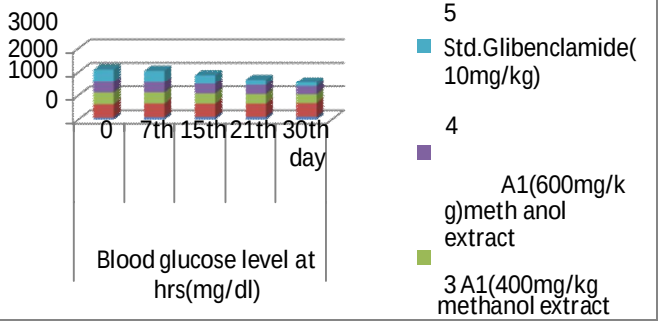


Table 3a : Genotype Pbns-12 carthamidin

S.NO	PBNS-12 Carthamidin	Glucose level mg/dl
1	Negative control	120.2 ± 5.119
2	Positive control	505.8 ± 3.899
3	Pbns-12-400	170.6 ± 6.768
4	Pbns-12-600	109.2 ± 8.289
5	Standard glibenclamid	154.2 ± 5.495

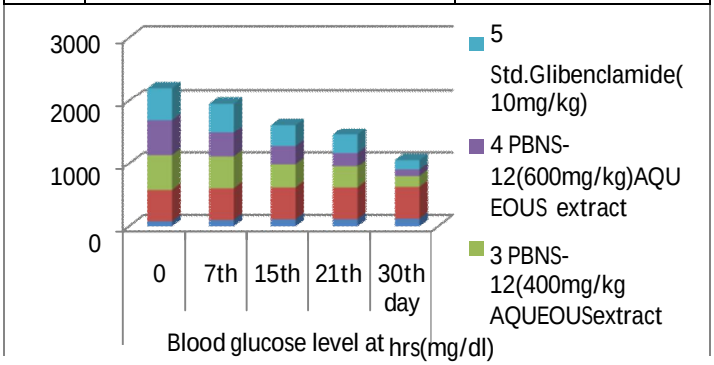


Table 3b : Genotype pbns-12 carthamin

S.NO	PBNS-12 Carthamin	Glucose level mg/dl
1	Negative control	118.0 ± 7.294
2	Positive control	505.8 ± 2.683
3	Pbns-12-400	327 ± 9.083
4	Pbns-12-600	218.8± 4.604
5	Standardglibenclamid	160.8± 4.086

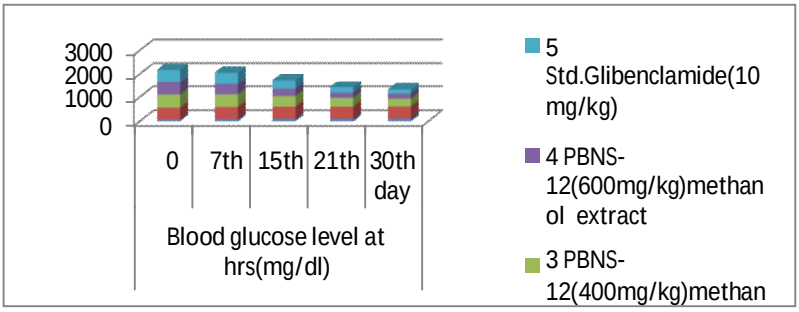


Table 4a : Genotype SSF-658 carthamidin

S.NO	SSF-658 Carthamidin	Glucose level mg/dl
1	Negative control	123 ± 6.403
2	Positive control	515.2 ± 9.121
3	SSF658-400	393.6 ± 10.968
4	SSF 658-600	123.2 ± 8.843
5	Standard glibenclamid	157.8 ± 3.564

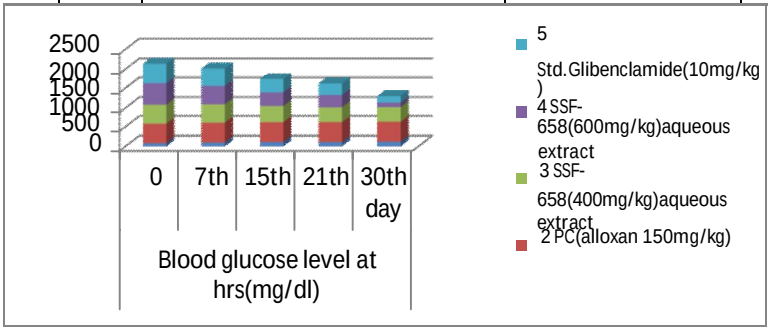


Table 4b : Genotype SSF-658 carthamin

S.NO	Ssf-658 Carthamin	Glucose level mg/dl
1	Negative control	123±6.403
2	Positive control	513.6±10.807
3	SSF-658(400)	382.6±12.3
4	SSF-658 (600)	232.8±8.585
5	Standard glibenclamide	157.8±3.585

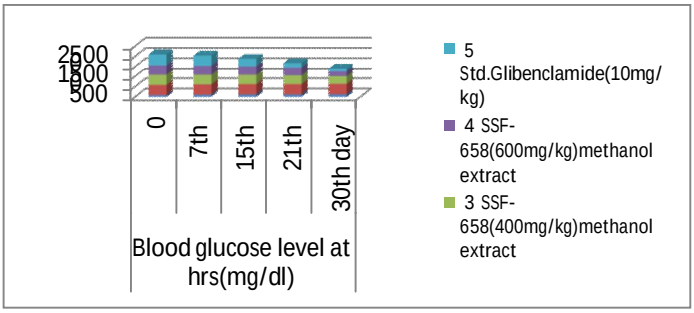


Table 5a : Genotype Nari-6 carthamidin

S.NO	Nari-6 carthamidin	Glucose level mg/dl
1	Negative control	120.6 ± 3.975
2	positive control	506 ± 3.937
3	Nari-6 -400	327.8 ± 17.065
4	Nari-6 – 600	313.6 ± 4.775
5	Standard glibenclamide	156.4 ± 3.647

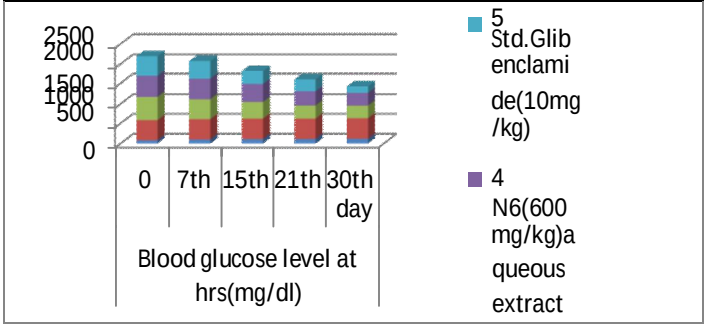


Table 5b : Genotype Nari-6 carthamin

S.NO	NARI-6 CARTHAMIN	GLUCOSE LEVEL Mg/Dl
1	Negative control	120.6 ± 1.517
2	Positive control	506.2 ± 2.864
3	Nari-6-400	495.2 ±4.147
4	Nari- 6-600	462.8±3.114
5	standard glibenclamid	152.4± 3.782

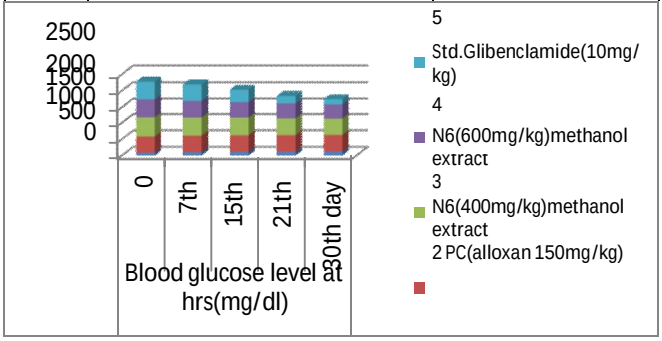


Table 6a : Manjira carthamidin

S.NO	MANJIRA CARTHAMIDIN	GLUCOSE LEVEL Mg/Dl
1	Negative control	117.8 ± 3.271
2	Positive control	502.4 ± 2.302
3	Manjira -400	309.2 ± 10.663
4	Manjira – 600	187 ± 2.739
5	Standard glibenclamide	152.8 ± 5.215

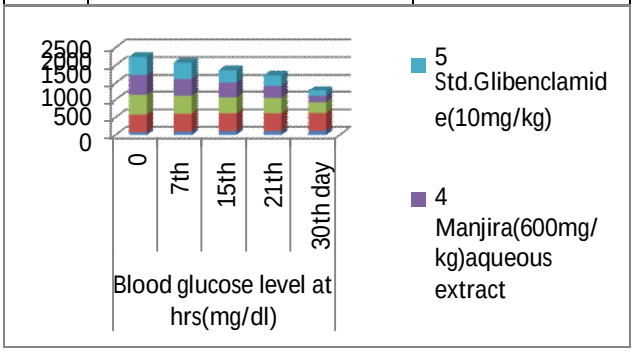


Table 6b : Manjira Carthamin

S.NO	MANJIRA CARTHAMIN	GLUCOSE LEVEL Mg/Dl
1	Negative control	120.6 ± 1.517
2	Positive control	504.4 ± 3.62
3	Manjira 400	473.6±3.715
4	Manjira 600	434.6.±3.782
5	Standard glibenclamid	154.8.± 3.701

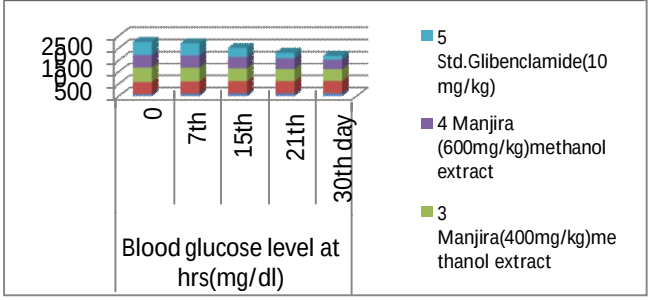


Table 7a : CO-1 carthamin

S.NO	CO-1 CARTHAMIN	GLUCOSE LEVEL Mg/Dl
1	Negative control	118.2 ± 3.194
2	Positive control	504.8 ± 3.701
3	CO-1 400	467.6.±5. 639
4	CO-1 600	498.6.±5.177
5	Standard glibenclamid	156.± 2.345

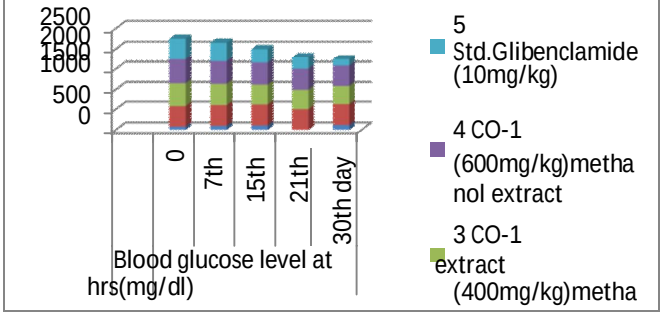
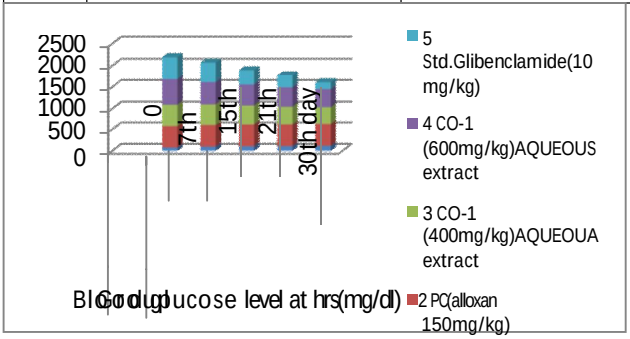


Table 7b : CO-1 Carthamidin

S.NO	CO-1 CARTHAMIDIN	GLUCOSE LEVEL Mg/Dl
1	Negative control	121.2±5.932
2	Positive control	566±5.242
3	A1(400)	348±18.124
4	A1(600)	338±9.460
5	Standard glibenclamide	152.8±8.585



Statistical analysis:

Results obtained are expressed as means ± standard error. To determine differences among the treated groups, data were statistically analysed using a computerized one-way ANOVA (social science statistics) followed a by t-test. Differences between means were considered significant with a value of $P \leq 0.05$.

Plate 1. Study protocol

Discussion and conclusion

The results of the present study indicated that safflower petal extracts reduced the glucose level in alloxan diabetic rats. Alloxan is known to induce free radical production and cause tissue injury, and the pancreas is especially susceptible to the action of alloxan induced free radical damage. The aqueous and methanolic extract exhibited significant ($p < 0.05$) antihyperglycemic activity in alloxan – induced hyperglycaemias without causing hypoglycaemia. It has been suggested that regeneration of islet beta cell following destruction by alloxan may be the primary mechanism of the recovery of alloxan-injected rats following drug administration. Therefore, safflower florets could be inducing pancreatic cell regeneration. Similar effects in alloxan treated diabetic animals were reported for "Pancreas Tonic". The phytochemical result showed that ME, AQE were very rich in terpenoids, fatty acids, flavonoids, .Terpenoids and flavonoids, fatty acids are good antidiabetic metabolites. The non- significant ($p > 0.05$) effect on normoglycemic rats suggest that unlike insulin and other common hypoglycaemic agent overdose of the extract may not result in hypoglycaemia... The result of this present study therefore justifies the local use of petals of safflower florets in the treatment of diabetes mellitus.

Acknowledgment

The authors are grateful to Professor S.Y Anwar for botanic identification, Dr Uttam and Professor Roja rani, Osmania university Hyderabad for providing all facilities to carry out the experiment genetics and biotechnology department Hyderabad. For providing the microorganisms and testing antimicrobial activity.

Ethical Approval:

Animal Ethic committee approval has been collected and preserved by the author(s)

References

1. Grover et al., 2002.Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. J Ethanopharmacol 2002; 81:81-100.
2. Vohraand Parasar, 1970;Vohra SB, Parasar GC. Antidiabtic studies on Ficus bengalensis Linn. Ind J Pharm 1970; 32: 68-69
3. Shukla et al., 1994; Prabhu KM, Murthy PS. Hypoglycaemic effect of the water extract of ficus bengalensin in alloxan recovered, mildly diabetic and severely diabetic rabbits. Intl Diabetes Dev Count 1994; 14: 78-81.
4. Grover et al., 2002, Grover et al., 2002, Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. J Ethanopharmacol 2002; 81:81-100.
5. Saxena and Vikram, 2004. 111. Saxena A, Vikram NK. Role of selected Indian plants in management of type 2 diabetes: a review. J Alt Comple Med. 2004; 10:369–378. [PubMed
6. Mukherjee et al., 2006.Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ. Leads from Indian medicinal plants with hypoglycemic potentials. J Ethnopharmacology. 2006; 106:1–28. (PubMed)
7. Alaa- Eldin Salah-Eldin, Marwan A. Ibrahim, Mohamed M.Soliman 2015., Alaa-Eldin Salah-Eldin, Marwan A. Ibrahim, Mohamed M. Soliman. RT-PCR analysis of genes expression to evaluate the biomedical importance of medical-herbal extracts in diabetes treatment, European Scientific Journal, 2015
8. Mohamed Mohamed Soliman, 2, 4 Mohamed Mohamed Ahmed and 2, 5 Samir Ahmed El-Shazy 2013. Cinnamon extract regulates gene expression of lipid and carbohydrate metabolism in streptozotocin induced diabetic wistar rats. American Journal of Biochemistry and Biotechnology 2013; 9 (2): 172-182.