

Original Research Article

Invivo 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. tinctorius* 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, Glibenclamide 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 kinobeaon 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 400 and 600 mg/kg, in alloxan induced diabetic rats.

Experimental Animals:

Healthy adult (8 weeks) male albino rats of Wistar strain (*Rattus norvegicus*) 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 chart 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. (chart 1)

Chart 1. BODY WEIGHTS OF THE RATS.

		20-02-	23-02-	24-02-	25-02-	26-02-	29-02-	01-03-	04-03-	11-03-	14-03-	21-03-
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	10A	248	192	190	186	188	194	202	209	209	218	221

600	10B	231	20 2	215	23 1	229	25 0	22 9	232	230	236	247
SSF-658 sAqueous 400	11A	241	24 5	278	27 5	279	28 5	28 7	295	238	245	242
	11B	253	22 0	218	21 3	206	21 3	21 9	226	230	233	240
SSF-658 Aqueous 600	12A	256	23 7	234	23 6	232	22 5	22 1	216	223	237	245
	12B	233	22 6	178	19 2	194	14 4	16 8	163	171	174	180
SSF-658 Methanol 400	13A	298	27 9	271	26 7	275	28 2	26 9	285	290	239	288
	13B	270	24 3	241	23 9	234	23 0	24 3	249	256	260	262
SSF-658 Methanol 600	14A	260	24 3	219	21 0	215	20 4	21 0	215	216	218	226
	14B	298	27 8	271	26 3	265	27 5	25 2	263	269	272	278
Co-A Aqueous 600	15A	243	23 1	246	24 4	247	23 7	23 6	216	220	227	232
	15B	234	21 2	220	22 0	219	22 3	23 2	235	227	230	221
Co-A Methanol 600	16A	232	22 7	230	22 1	220	20 6	19 4	197	195	182	169
	16B	300	29 3	293	27 9	294	27 7	27 4	265	264	276	265
Manjira Aqueous 600	17A	251	23 5	230	22 4	214	21 2	22 1	226	228	234	226
	17B	235	21 7	223	22 0	225	16 0	15 7	163	171	184	168
Manjira Methanol 600	18A	224	22 3	232	23 4	236	23 4	24 1	244	247	253	245
	18B	255	25 7	190	18 3	179	18 4	19 9	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 shown in below tables. (1a to 6b)

A significant reduction in glucose level was observed on 30th day in the experimental animals

treated with carthamin pbns-12((Table-2a) and ssf-658 extract(table-3a) as compared to control. The levels of blood glucose on 30th day were 109.2 ± 8.289 mg/dl IN Pbn-12 carthamin, 123.2 ± 8.843 IN SSF-658 carthamin((table-3a)) Extracts shown in animals treated with methanolic extract of safflower(carthamin) (400mg/kg and 600mg/kg) and aqueous extract of carthamin (400mg/kg and 600mg/kg), and commercial drug Glibenclamide respectively. The activity was better in aqueous extract of safflower (carthamin) 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.

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Table 1a. 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

Blood glucose level at hrs(mg/dl)

Table 1b. 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

Blood glucose level at hrs(mg/dl)
methanol extract

Table 2a. Genotype PbnS-12 carthamidin

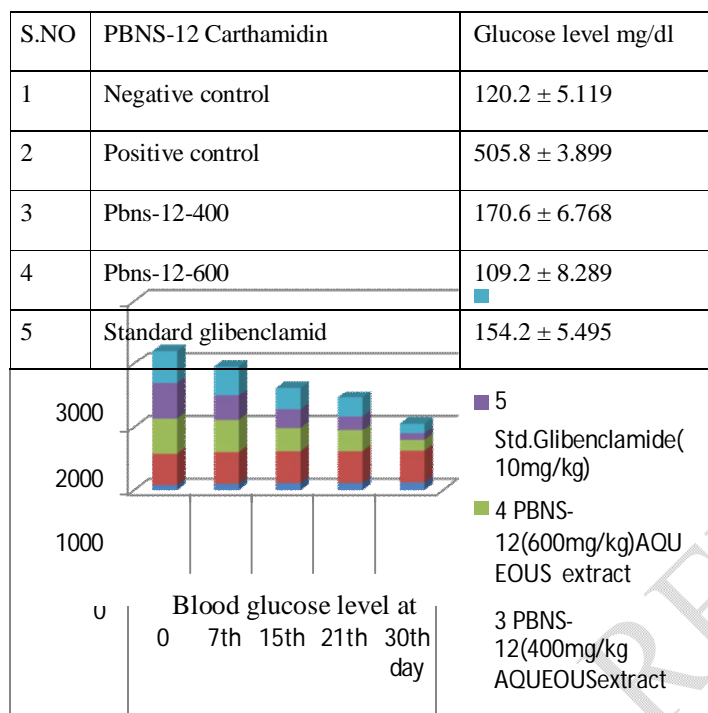


Table 2b. Genotype pbnS-12 carthamin

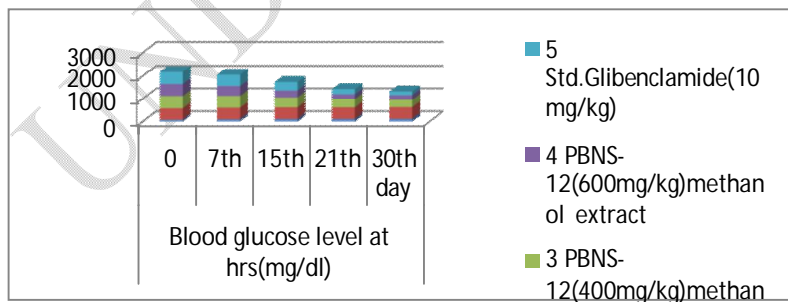
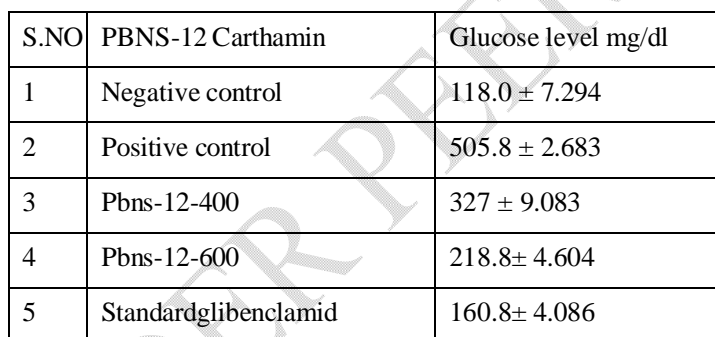


Table-3a. Genotype SSF-658 carthamidin

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

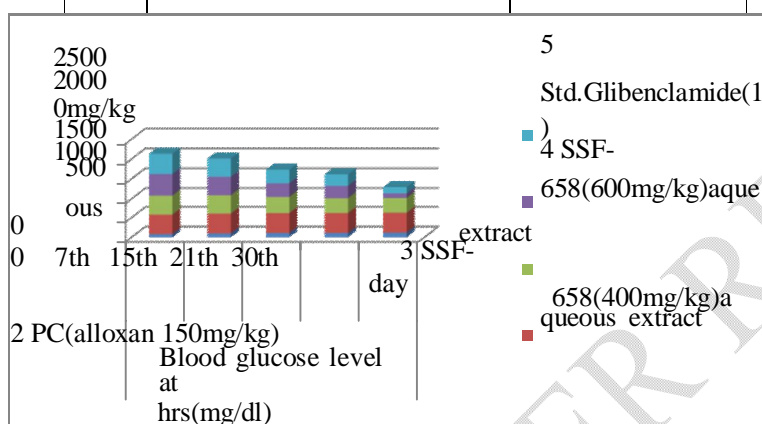


Table 3b. Genotype SSF-658 carthamin

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

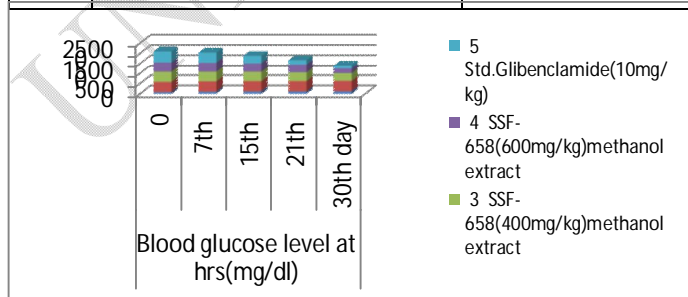


Table 4a. Genotype Nari-6 carthamidin

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

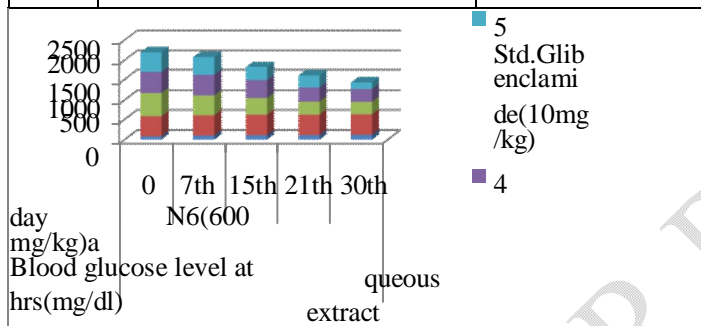
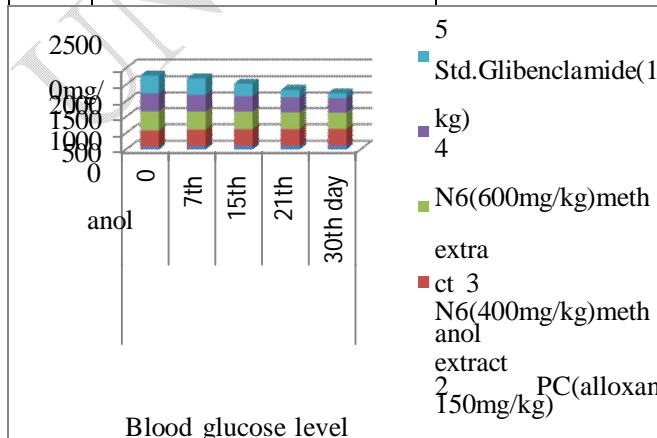


Table 4b. Genotype Nari-6 carthamin

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



at
hrs(mg/dl)

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Table 5a. Manjira carthamidin

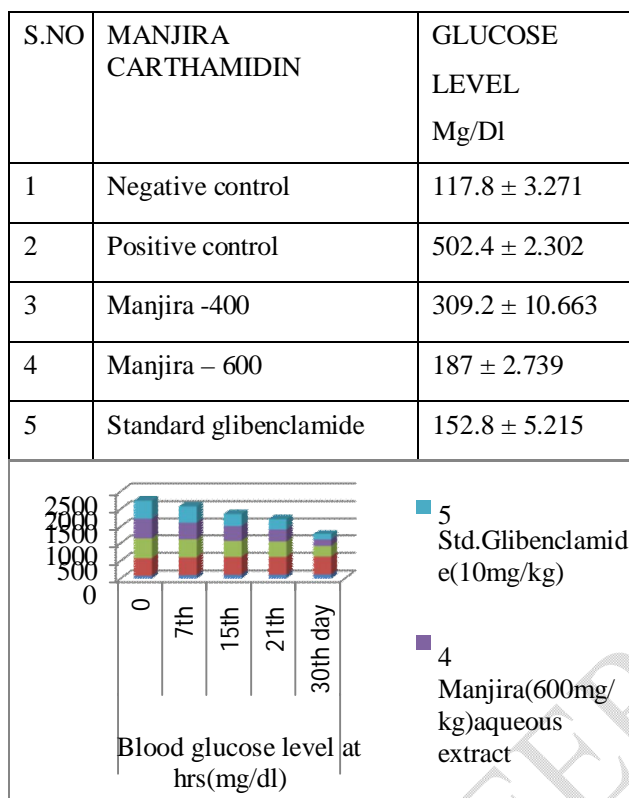


Table 5b. Manjira Carthamin

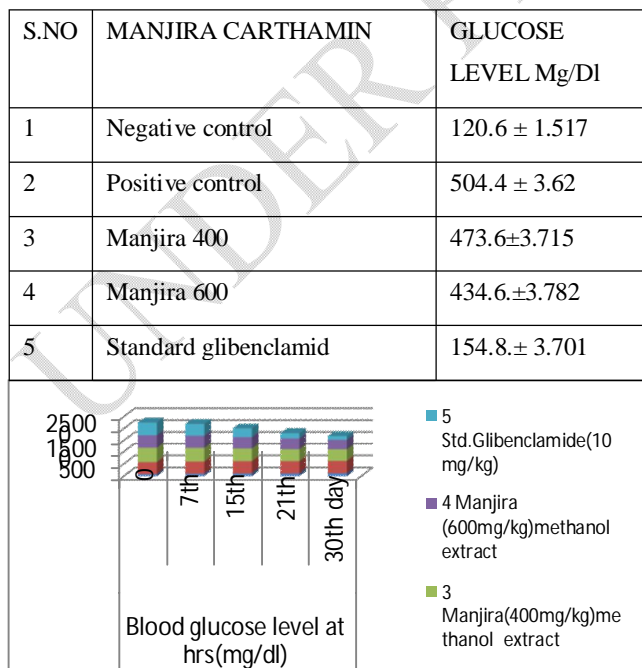


Table 6a. CO-1 carthamin

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

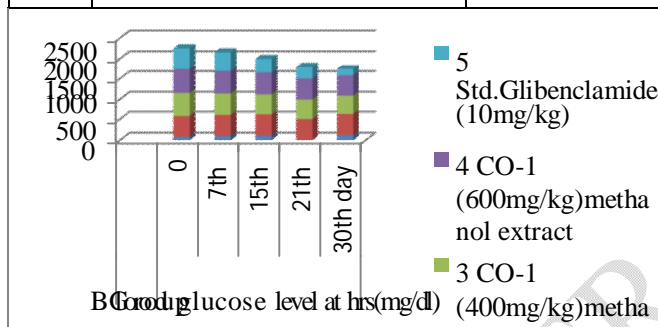
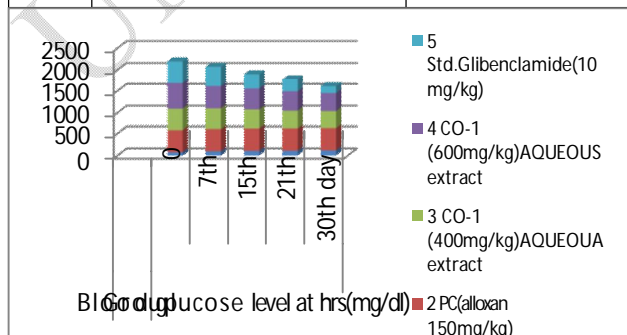


Table 6b. CO-1 Carthamidin

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 \pm 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

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.

Conclusion

The present review attempts to be useful to the scholars, scientists, and health professionals working in the field of pharmacology and therapeutics to develop antidiabetic drugs. In this work, we discussed traditional medicinal plants safflower florets for the treatment of DM. The antidiabetic effect of plants is attributed to the mixture of phytochemicals or single components of the plant extracts. The phytochemicals responsible for antidiabetic properties mainly are alkaloids, phenolic acids, flavonoids, glycosides, saponins, polysaccharides, stilbenes, and tannins. In the several animal studies

reported using different plants, there is a wide variety between the extraction methods, which is determinant in the phytochemical composition of the extracts. Moreover, phytochemical plant composition is highly dependent on several endogenous and exogenous factors, including genetic traits; plant organs used; and the growing, drying, and storing conditions. Stress factors, such as adverse climatology, and diseases affecting the plant also influence the phytochemicals obtained.

The antidiabetic activity of alloxan induced diabetic rats were shown 109.2 ± 8.289 PBNS-12, 123.2 ± 8.843 in SSF-658 activity in aqueous when the induction was given in aqueous and methanolic extracts of safflower petals when compared to standards Glibenclamide 152.4 ± 3.782 .

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