Research Article Asian J Med Health Res. 2016;1(3) ISSN: 2455-8664

AJMHR

Asian Journal of Medical and Health Research

Journal home page: www.ajmhr.com

Studies on the Evaluation of Aqueous Extracts of the Selected Plant Parts and Polyherbal Mixture For Their Antihyperglycemic Activities

Vinay Kumar Theendra*[1], Shaik Abdul Rehaman [1]

1. Nirmala college of Pharmacy , Atmakuru Village, Mangalagiri MandalGuntur District, Pin Code -522 503 Andhra Pradesh, INDIA

ABSTRACT

In the present study the selected plant extracts and polyherbal mixture (PHM) were screened for their antihyperglycemic potential and thereby for their possible role in inhibition of the pathways leading to diabetic complications in STZ induced diabetic rats. Three herbs namely fruit pulp of *Citrullus colocynthis (CC),* leaves of *Stevia rebaudiana (SR),* and leaves of *Swertia Chirayata (SC).* Initially hypoglycemic studies were conducted without inducing of diabetes mellitus. Based on the results obtained PHM was prepared. The extracts which are showing % reduction of glucose between 30 -50% were selected in order to avoid hypoglycemic shock. All the selected plant extracts and PHM were found to decreased glucose levels at 3rd and 8th hrs and standard drug was found to decrease glucose levels at 1st & 6th hr significantly after every week (biphasic reduction). All the selected plant extracts and PHM were found to increase insulin levels at 3rd and 8th hrs and standard drug was found to increase levels at 1st & 6th hr significantly after every week (biphasic reduction).PHM was found to most potent.

Keywords: Diabetes mellitus, Diabetic complications, *Citrullus colocynthis, Stevia rebaudiana, Swertia Chirayata,* Polyherbal mixture (PHM)

*Corresponding Author Email: vinaykumartheendra@gmail.com

Received 13 April 2016, Accepted 27 April 2016

Please cite this article as: Theendra V., Studies on the Evaluation of Aqueous Extracts of the Selected Plant Parts and Polyherbal Mixture For Their Antihyperglycemic Activities. Asian Journal of Medical and Health Research 2016.

INTRODUCTION

Diabetes mellitus is group of syndrome characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins with an increased risk of complications such as retinopathy and nephropathy vascular disease etc., [1] it is of mainly two types

Type1Diabetes mellitus: It is also called as "Insulin Dependent Diabetes Mellitus"[IDDM] .or "Juvenile diabetes" This disease is characterised by the destruction of Beta cells. This leads to body fails to produce insulin. **Type2 Diabetes Mellitus**: It is said to be "Non – Insulin Diabetes Mellitus"[NIDDM] or "Adult Onset Diabetes". If the cells fail to utilize properly, this is said to be absolute insulin deficiency.

Prevalence of Diabetes:

Globally, as of 2010, an estimated 285 million people had diabetes, with type 2 making up about 90% of the cases.[2] In 2013, according to International Diabetes Federation, an estimated 381 million people had diabetes[3].Its prevalence is increasing rapidly, and by 2030, this number is estimated to almost double[4].

Role of herbal drugs in the treatment of DM:

Diabetes mellitus is a most common endocrine disorder, affecting more than 300million people worldwide. For this, therapies developed along the principles of western medicine (allopathic) are often limited in efficacy, carry the risk of adverse effects, and are often too costly, especially for the developing world. Therefore, treating diabetes mellitus with plant derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive. The field of pharmacology and therapeutics to develop evidence-based alternative medicine to cure different kinds of diabetes in man and animals. Isolation & identification of active constituents from these plants, preparation of standardized dose & dosage regimen can play a significant role in improving the hypoglycemic action[3].

Plant profiles:

Citrullus colocynthis:

Citrullus colocynthis belongs to the family Cucurbitaceae. The main chemical contain of fruit pulp colocynthin (the bitter principle upto 14 %), colocynthein (resin), colocynthetin, pectin gum. Seed contain a fixed oil (17 %) and albuminiods (6 %).[4]

Stevie Rheubaudiana:

Stevie Rheubaudiana belongs to the family Asteraceae. Stevie is well-known for its high content of sweet components. The dry extract of its leaves contains flavonoids, alkaloids, chlorophylls, xanthophylls, hydroxylcynnamic acids (caffeic, chlorogenic, etc), oligosaccharides, free sugars, amino acids, lipids and trace elements. [5, 6, 7]

Swertia Chirata:

Swertia Chirata belongs to the family Gentianaceae. The main chemical constituents are Swertinin, swertianin, swerchirin, decussating, isobellidifolin, friedelin and sitosterol isolated. Nine tetraoxy genated xanthones isolated from roots and aerial parts Gentianine, gentiocrucine and eniflavine isolated. Arghinine, leucine, methionine, threonine, tryptophan, aspartic acid and glutamic acid isolated [8, 9].

Antihyperglycemic studies:

The selected plant extracts have been evaluated for their Antihyperglycemic activity in the present study by estimation of blood glucose, serum insulin levels. A polyherbal mixture (PHM) was also prepared by mixing all the selected plant extracts in the ratio of 2:1:1 of CC, SR, and SC respectively based on their hypoglycemic activity. Streptozotocin (STZ; *N*-nitro derivative of glucosamine) is a cytotoxic chemical that is particularly toxic to the pancreatic, insulin producing beta cells in mammals[2] used for inducing diabetes. Normalization of blood glucose by intensive insulin therapy reduces the risk of development of diabetic complications. All the selected plant extracts, standard glibenclamide and PHM were administered once daily orally to STZ induced diabetic rats. The study period was maintained for 4weeks to get the induction of diabetic complications.[10] At the end of each week of 4 week study period change in body weight, food and water intake, serum glucose estimation and insulin levels were measured to evaluate Antihyperglycemic activity.

MATERIALS AND METHOD

Materials and Methods:

Induction of diabetes mellitus

The animals fasted overnight and diabetes was induced by a single intra peritoneal injection of freshly prepared STZ (60 mg/kg body weight of rats) in 0.1 M citrate buffer (pH 4.5). The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. The animal housing and handling were in accordance with CPSCEA guidelines. Our college was approved by CPSCEA for conducting animal experiments with the registration No. 516/01/A/CPCSEA. The prior permission for the study was obtained from our institutional Animal Ethical Committee (IAEC). Control rats were injected with citrate buffer alone. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dL on the third day after the STZ injection. The treatment started on the fourth day after the STZ injection and this day was considered the first day of treatment. The treatment was continued for 30 days by using above extracts through oral route. Since all the extracts are aqueous, water is used as vehicle for administration of extracts. After 30 days of

treatment, the polyherbal mixtures were formulated as fallows and they are considered as Diabetic treatment groups 22, 23 and 24. The same method was followed to induce diabetes in these groups. Human dose extrapolated to rats 10mg x 0.018 = 0.18mg / 200mg = 0.9 mg/kg.

Acute toxicity studies: Acute toxicity studies are performed.

Estimation of Glucose:

Estimation of glucose in blood was one of the first biochemical tests to be applied clinically and now it has become a routine in clinical biochemistry lab. In blood, quantitative estimation of glucose is done with either whole blood, plasma or serum and several methods have been in use. Whole blood values are 10-15% lower than plasma. Arterial blood values are higher than venous values. Glucose estimation was done in all the groups at the end of the day of each week of study i.e. 8^{th} ,15^{th} ,22^{nd} , and 29^{th} days at 0, 1,2,3,4,6,8,10 and 12 hour intervals. The term Blood Sugar is used synonymously with blood glucose but certain other substance like glutathione, glucuronic acid, threonine, uric acid, ascorbic acid, fructose etc. give erroneously high values (5-20%) when any reduction method is adopted.

Reagents

- Enzymatic (GOD / POD) Merck
- Distilled water
- Anhydrous glucose (500 gm) Merck
- Washing solution

Steps of measuring glucose oxidase method:

- Draw 2-3 ml of blood from retro orbital vein in a clean dry test tube.
- After 10 minutes centrifuge it at 3000 RPM for 3 minutes.
- Separate serum and within one hour perform glucose estimation.
- If it is not measured immediately after, collection preserves the serum into a clean, dry test at 4°C.

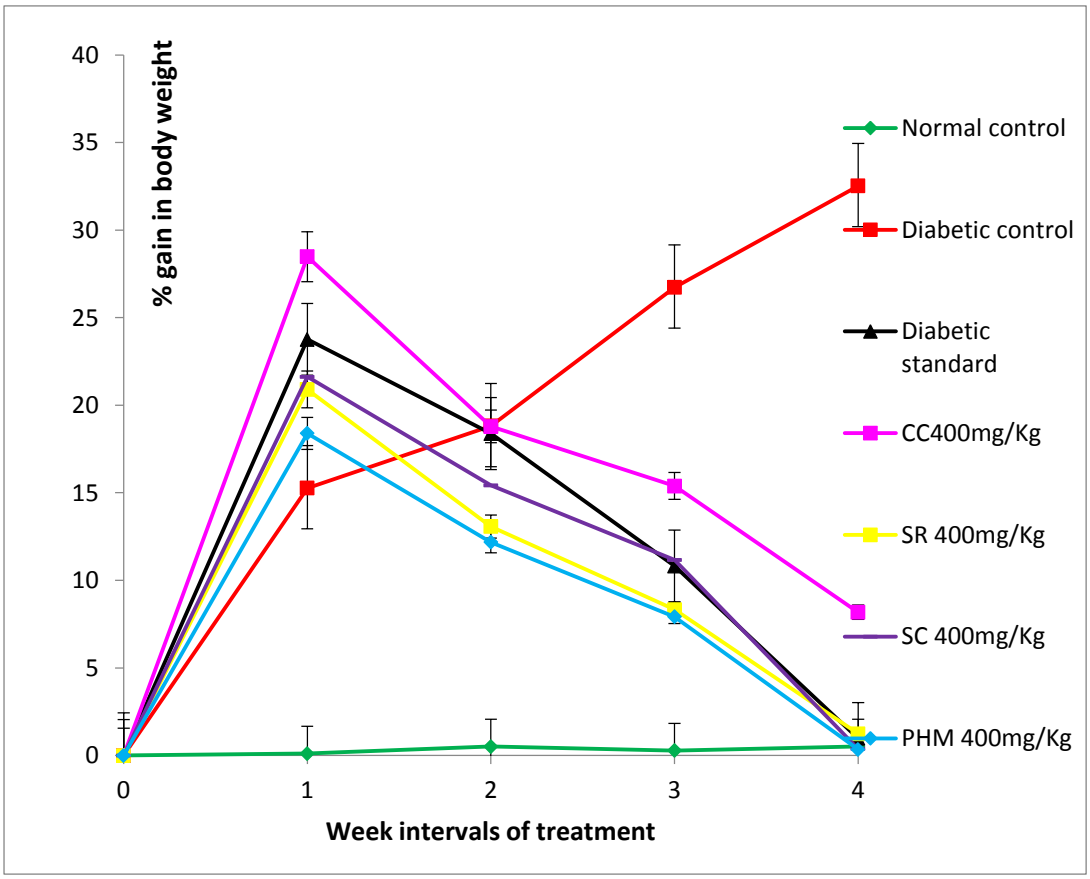
Estimation of serum Insulin:

Serum Insulin estimation was done at the time intervals where there is a peak % reduction in blood glucose levels and also at final hour. The estimations were carried out at the end of every week of treatment. For standard, control and disease control groups insulin was estimated at zero, 1^{st}, 6^{th}, and final hour intervals. All the treatment groups shown the peak % reduction in glucose levels at $3^{rd}$and 8^{th} levels. So, insulin levels were estimated at zero, 3^{rd}, 8th, and final hours. Insulin estimation was done using ELISA method by Mercoida rat insulin assay kit.

RESULTS AND DISCUSSION

Table: 1 Effect of selected plant extracts on percentage increase of body weight in rats

S.No	Group	1st week	2nd week	3rd week	4th week
1.	Normal control	0.11± 0.03	0.71± 0.94	0.28± 0.21	0.51± 0.08
2.	Diabetic control	**15.27±**	**18.81± 0.84$^{@}$**	**26.73± 1.25$^{@}$**	**32.52± 1.18$^{@}$**
3.	Diabetic standard	23.76± 0.14	**18.38± 0.93^{x}**	**10.82± 0.94^{y}**	**0.97± 0.08^{cz}**
4.	CC (100 mg/Kg)	27.99± 0.94	23.26± 0.94	19.47± 0.84	15.89± 1.15
5.	CC (200 mg/Kg)	28.51± 1.12	24.06± 1.23	19.03± 0.92	13.10± 1.13
6.	CC (400 mg/Kg)	28.48± 1.02	18.79± 1.04	15.39± 0.84	8.18± 0.84
7.	SR (100 mg/Kg)	21.06± 0.35	**16.66± 1.12^{x}**	**12.87± 0.94^{x}**	**9.29± 0.90^{a}**
8.	SR (200 mg/Kg)	20.93± 0.35	**16.77± 0.94^{x}**	**12.29± 1.14^{y}**	**2.94± 0.21^{by}**
9.	SR (400 mg/Kg)	20.90± 1.12	**13.07± 1.13^{y}**	**8.33± 0.94^{z}**	**1.24± 0.24^{cz}**
10.	SC (100 mg/Kg)	22.64± 0.92	**18.41± 1.19^{x}**	**14.46± 1.83^{x}**	**3.38± 0.87^{by}**
11.	SC (200 mg/Kg)	24.29± 1.13	**16.93± 1.84^{x}**	**12.63± 1.93^{x}**	**1.19± 0.10^{cz}**
12.	SC (400 mg/Kg)	21.62± 0.94	**15.42± 0.94^{x}**	**11.16± 1.14^{y}**	**0.37± 0.03^{cz}**
13.	PHM (100 mg/Kg)	21.14± 1.12	**15.18± 1.19^{x}**	**11.28± 1.0 ^{y}**	**2.74± 0.25^{by}**
14.	PHM (200 mg/Kg)	21.02± 0.94	**13.66± 0.94^{x}**	**9.36± 0.94^{z}**	**0.69± 0.08^{cz}**
15.	PHM (400 mg/Kg)	18.39± 1.23	**12.18±0.95^{y}**	**7.92± 0.87^{z}**	**0.34± 0.04^{cz}**

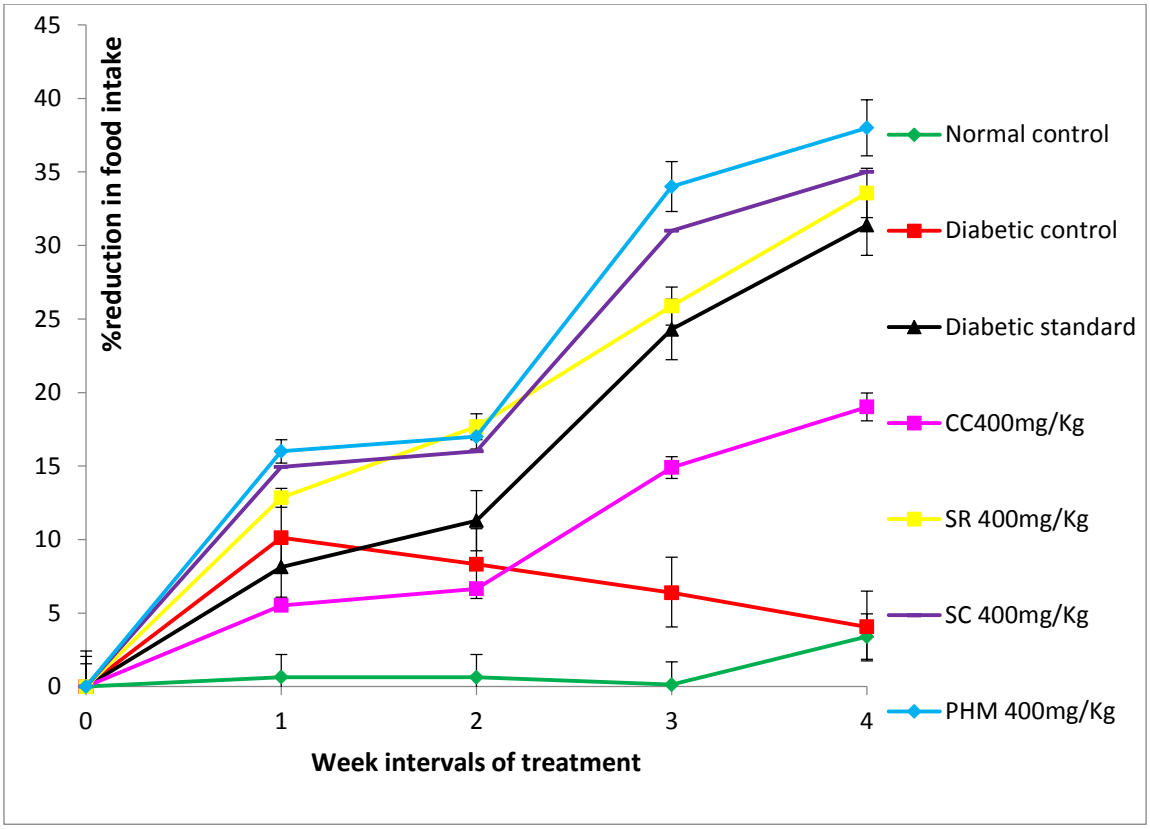


Graph: 1 Effect of selected plant extracts on percentage increase of body weight

Table: 2 Effect of extracts on percentage reduction in food intake

S.No	Group	0	1st week	2nd	3rd week	4th week
1.	Normal control	00.00	0.64	0.63	0.13	3.40
2.	Diabetic control	00.00	**10.12$^{@}$**	**8.32$^{@}$**	**6.38$^{@}$**	**4.06$^{@}$**

3.	Diabetic standard	00.00	8.13	**11.92^{x}**	**24.29^{y}**	**31.38^{cz}**
4.	CC (100 mg/Kg)	00.00	3.12	4.27	14.69	15.69
5.	CC (200 mg/Kg)	00.00	3.67	4.73	14.81	17.62
6.	CC (400 mg/Kg)	00.00	5.52	6.65	14.90	19.02
7.	SR (100 mg/Kg)	00.00	**7.95^{x}**	**13.63^{x}**	**21.80^{x}**	**29.42^{a}**
8.	SR (200 mg/Kg)	00.00	**11.83^{x}**	**16.25^{x}**	**24.46^{y}**	**31.78^{by}**
9.	SR (400 mg/Kg)	00.00	**12.85^{y}**	**17.68^{y}**	**25.88^{z}**	**33.57^{cz}**
10.	SC (100 mg/Kg)	00.00	**10.95^{x}**	**11.78^{x}**	**23.76^{x}**	**29.10^{by}**
11.	SC (200 mg/Kg)	00.00	**11.43^{x}**	**12.59^{x}**	**26.39^{x}**	**30.17^{cz}**
12.	SC (400 mg/Kg)	00.00	**14.94^{x}**	**16.14^{x}**	**30.68^{y}**	**34.46^{cz}**
13.	PHM (100 mg/Kg)	00.00	**7.45^{x}**	**9.09^{x}**	**27.87^{y}**	**31.69^{by}**
14.	PHM (200 mg/Kg)	00.00	**12.45^{x}**	**14.75^{x}**	**29.55^{z}**	**32.96^{cz}**
15.	PHM (400 mg/Kg)	00.00	**15.35^{y}**	**16.23^{y}**	**33.59^{z}**	**37.68^{cz}**

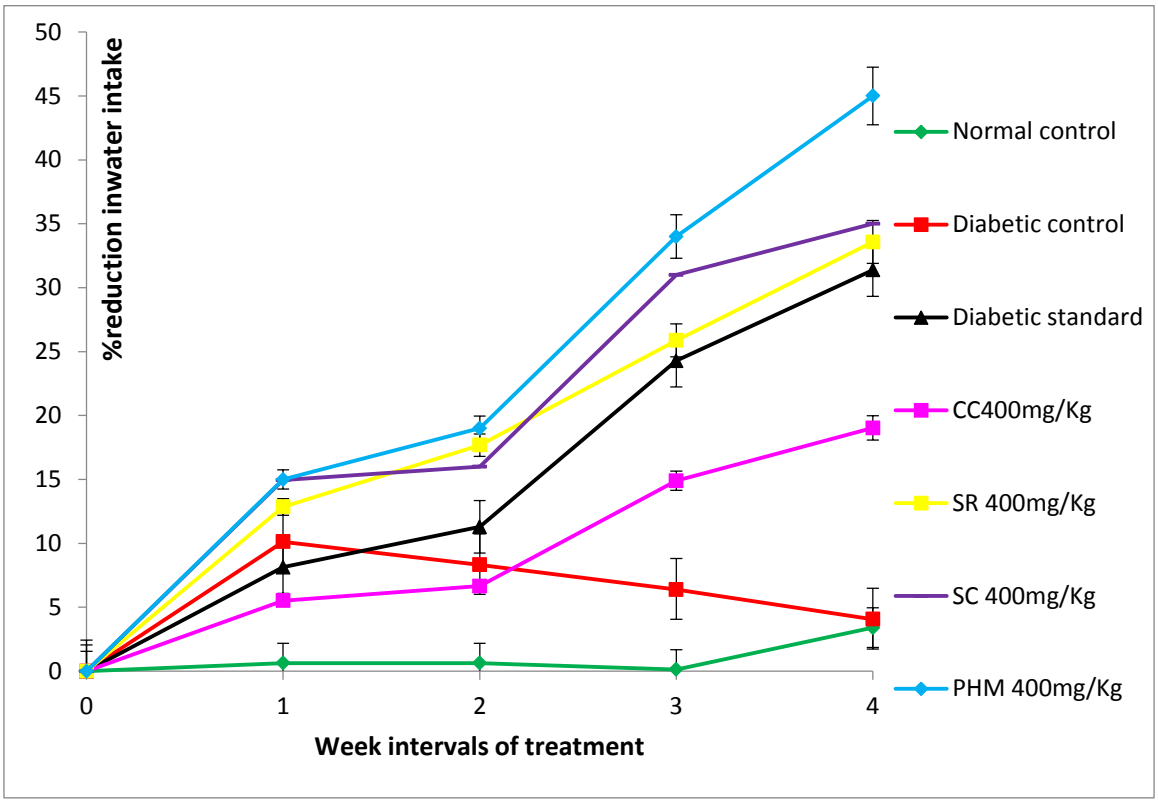


Graph: 2 Effect of selected plant extracts on percentage reduction of food intake

Table: 3 Effect of extracts on percentage reduction in water intake

S.No	Group	0 week	1st week	2nd week	3rd week	4th week
1.	Normal control	00.00	1.21	1.28	0.78	4.05
2.	Diabetic control	00.00	**8.04$^{@}$**	**7.97$^{@}$**	**6.03$^{@}$**	**5.71$^{@}$**
3.	Diabetic standard	00.00	**11.94^{x}**	**12.57^{x}**	**24.94^{y}**	**32.03^{cz}**
4.	CC (100 mg/Kg)	00.00	3.12	4.92	15.34	16.34
5.	CC (200 mg/Kg)	00.00	4.32	5.38	15.46	18.27
6.	CC (400 mg/Kg)	00.00	5.03	7.30	15.55	19.67
7.	SR (100 mg/Kg)	00.00	**11.29^{x}**	**14.28^{x}**	**22.45^{x}**	**30.07^{a}**
8.	SR (200 mg/Kg)	00.00	**13.29^{x}**	**16.90^{x}**	**25.11^{y}**	**32.43^{by}**
9.	SR (400 mg/Kg)	00.00	**16.94^{y}**	**18.33^{y}**	**26.53^{z}**	**34.22^{cz}**

10.	SC (100 mg/Kg)	00.00	**11.04^{x}**	**12.43^{x}**	**24.41^{x}**	**29.75^{by}**
11.	SC (200 mg/Kg)	00.00	**12.05^{x}**	**13.24^{x}**	**27.04^{x}**	**30.82^{cz}**
12.	SC (400 mg/Kg)	00.00	**14.56^{x}**	**16.79^{x}**	**31.33^{y}**	**35.11^{cz}**
13.	PHM (100 mg/Kg)	00.00	**8.43^{x}**	**9.74^{x}**	**28.52^{y}**	**32.34^{by}**
14.	PHM (200 mg/Kg)	00.00	**13.43^{x}**	**15.40^{x}**	**30.20^{z}**	**33.61^{cz}**
15.	PHM (400 mg/Kg)	00.00	**14.93^{y}**	**16.88^{y}**	**34.24^{z}**	**38.33^{cz}**



Graph: 3 Effect of selected plant extracts on percentage reduction of water intake

Table: 4[a] Effect of selected plant extracts on percentage reduction values of glucose after 1st week

Treatment Group	Time intervals						
	1st Hr	2nd Hr	3rd Hr	4th Hr	6th Hr	8th Hr	10th Hr
Normal Control	-1.89± 0.46	8.17±0.47	6.13± 0.64	9.22± 0.59	15.76± 0.43	3.63± 0.46	16.93± 0.49
Diabetic control	1.11±0.20	2.12±0.52	3.50±0.53	5.03±0.65	**9.85±0.88[@]**	**10.72±1.01[@]**	**11.32±0.98[@]**
Diabetic standard	31.65 ±0.47	19.78 ± 1.26	12.13± 1.84	6.66± 1.19	30.08± 0.47	16.13± 0.95	7.41± 0.97
CC (100 mg/Kg)	6.01± 0.47	9.95± 0.82	18.54± 0.51	10.98± 1.27	5.25± 0.53	17.99± 0.71	6.64± 0.91
CC (200 mg/Kg)	6.01± 0.86	11.10± 0.70	25.53± 0.59	12.00± 1.04	6.08± 1.10	24.61± 0.96	10.13± 0.40
CC (400 mg/Kg)	6.43± 0.63	13.20± 1.08	30.05± 0.45	16.04± 1.67	8.85± 0.56	29.46± 0.79	12.24± 1.21
SR (100 mg/Kg)	7.47± 1.19	11.54± 1.58	20.89± 0.80	13.08± 1.30	7.14± 1.04	19.91± 0.77	8.58± 1.08
SR (200 mg/Kg)	8.85± 0.89	14.08± 0.72	29.20± 0.23	15.01± 1.07	9.83± 1.00	28.62± 0.37	13.09± 0.40
SR (400 mg/Kg)	9.85± 0.62	16.55± 1.04	34.49± 0.34	19.49± 1.72	12.06± 0.62	33.35± 0.76	15.57± 1.28
SC (100 mg/Kg)	8.98± 0.50	13.12± 0.88	21.79± 0.51	14.20± 1.32	7.74± 0.74	21.79± 0.50	9.63± 0.95
SC (200 mg/Kg)	9.09± 0.92	14.47± 0.74	30.83± 0.26	15.42± 1.09	9.17± 1.15	29.73± 0.38	13.45± 0.41
SC (400 mg/Kg)	9.85± 0.62	16.55± 1.04	34.49± 0.34	19.49± 1.72	12.06± 0.62	33.35± 0.76	15.57± 1.28
PHM (100 mg/Kg)	10.17± 0.64	17.38± 1.12	**40.18± 0.52[d]**	20.42± 1.84	12.75± 0.54	**41.04± 0.88[d]**	16.37± 1.42
PHM (200 mg/Kg)	10.36± 0.68	18.46± 1.92	**42.24± 0.52 [d]**	22.42± 1.94	17.48± 0.46	**43.04± 0.88[d]**	24.04± 1.93
PHM (400 mg/Kg)	10.17± 0.64	17.38± 1.12	**46.45± 0.52[b]**	20.42± 1.84	12.75± 0.54	**48.34± 0.31[b]**	16.37± 1.42

Table: 4[b] Effect of selected plant extracts on percentage reduction values of glucose after 2nd week

Treatment Group	Time intervals						
	1st Hr	2nd Hr	3rd Hr	4th Hr	6th Hr	8th Hr	10th Hr
Normal Control	3.76± 2.09	4.26± 0.73	4.03± 1.63	5.30± 1.79	6.13± 2.68	7.29± 2.61	3.34± 4.48
Diabetic control	1.02±0.20	2.04±0.49	2.89±0.44	3.69±0.51	7.23±0.80	**7.95±0.71[@]**	8.91±0.61
Diabetic standard	32.81± 0.66	21.26± 1.53	14.98± 1.56	10.14± 1.52	32.06± 1.64	16.78± 1.11	10.49± 1.60
CC (100 mg/Kg)	6.82± 0.36	10.21± 0.60	18.62± 0.25	10.25± 0.78	5.52± 0.99	18.39± 0.36	8.71± 0.89
CC (200 mg/Kg)	7.91± 1.12	15.01± 1.07	26.60± 0.25	7.92± 1.02	5.27± 0.67	25.56± 0.36	9.46± 0.30
CC (400 mg/Kg)	6.46± 0.48	19.07± 0.60	30.57±0.46	25.38± 0.69	16.01± 0.79	31.65± 0.44	19.10± 0.87
SR (100 mg/Kg)	9.92± 0.34	13.41± 0.61	**21.85± 0.25[a]**	13.45± 0.79	8.58± 1.02	**21.32± 0.42[a]**	11.86± 0.95

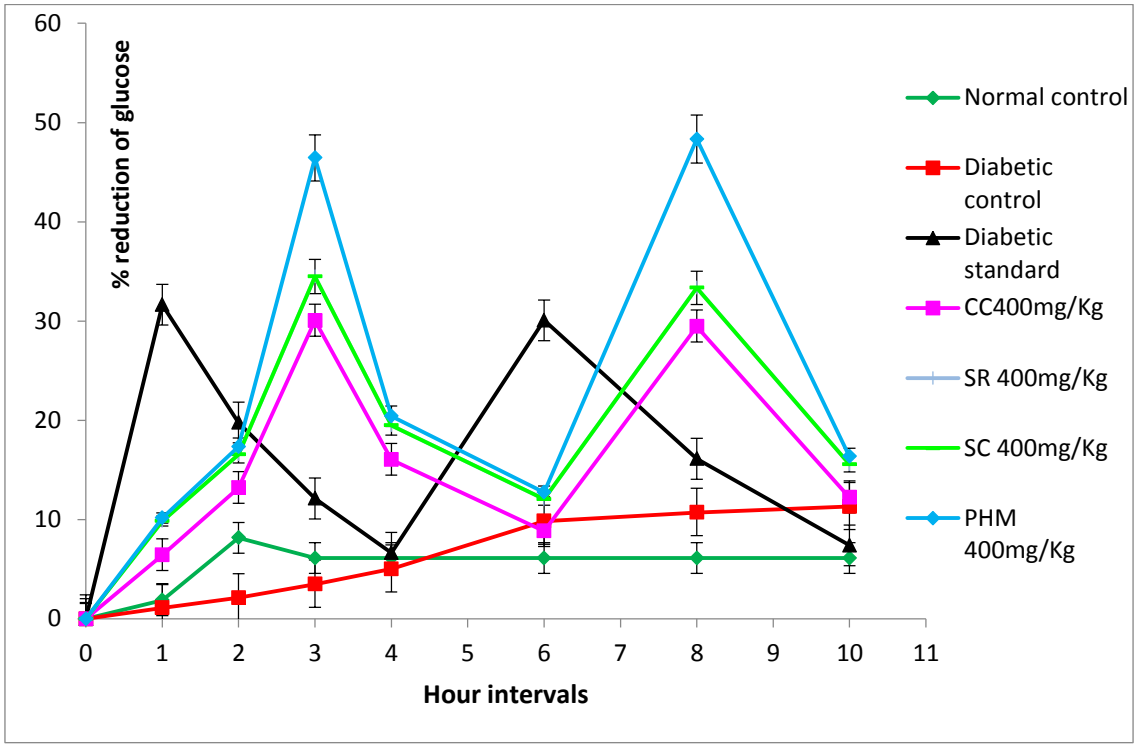
SR (200 mg/Kg)	10.75± 0.88	18.07± 0.76	**30.00± 0.40** [b]	9.30± 1.56	5.99± 1.47	**28.93± 0.56**[b]	10.92± 1.29
SR (400 mg/Kg)	10.17± 0.52	23.23± 0.56	**37.49± 0.38** [b]	29.75± 0.69	20.05± 0.86	**37.37± 0.12**[b]	23.26± 0.95
SC (100 mg/Kg)	10.22± 0.35	13.30± 0.49	**22.32± 0.38**[a]	13.85± 0.82	8.83± 1.05	**22.47± 0.40**[a]	11.71± 0.93
SC (200 mg/Kg)	13.00± 0.41	20.39± 0.65	**31.39± 0.59**[b]	11.56± 1.07	8.75± 0.71	**31.04± 0.63**[b]	13.21± 0.31
SC (400 mg/Kg)	10.17± 0.52	23.23± 0.56	**37.49± 0.38**[b]	29.75± 0.69	20.05± 0.86	**37.37± 0.12**[b]	23.26± 0.95
PHM (100 mg/Kg)	9.95± 0.94	24.06± 0.57	**46.04± 0.40**[d]	30.83± 0.71	20.78± 0.89	**45.34± 0.13**[d]	24.10± 0.99
PHM (200 mg/Kg)	11.85± 0.94	26.87± 0.57	**48.04± 0.40**[d]	33.65± 0.71	22.65± 0.89	**48.34± 0.13**[d]	27.09± 0.99
PHM (400 mg/Kg)	12.95± 0.94	27.31± 0.57	**52.05± 0.40**[d]	34.92± 0.71	23.94± 0.89	**53.34± 0.13**[d]	29.56± 0.99

Table: 4[c] Effect of selected plant extracts on percentage reduction values of glucose after 3rd week

Treatment Group	**Time intervals**						
	1st Hr	**2nd Hr**	**3rd Hr**	**4th Hr**	**6th Hr**	**8th Hr**	**10th Hr**
Normal Control	1.16± 3.09	1.79± 2.19	-3.02± 2.03	2.45± 3.25	-5.27±3.23	2.94± 2.14	-0.40± 1.65
Diabetic control	0.98± 0.17	1.93± 0.18	2.51± 0.24	2.96±0.28	4.33± 0.28	**5.19±0.28**[@]	6.61±0.48
Diabetic standard	**44.94± 0.15**[d]	21.43± 0.92	12.83± 1.27	6.53± 1.03	**45.34± 0.20** [d]	20.71± 0.86	11.43± 0.49
CC (100 mg/Kg)	6.65± 0.67	11.68± 0.66	18.25± 0.40	12.68± 0.63	7.60± 0.64[a]	18.79± 0.28	8.45± 0.73
CC (200 mg/Kg)	9.27± 0.38	16.22± 0.64	26.58± 0.27	15.11± 0.84	9.52± 0.87	**26.24± 0.60** [d]	14.44± 0.42
CC (400 mg/Kg)	10.40± 0.52	22.18± 1.21	32.86± 0.36	14.37± 0.64	8.98± 1.22	**32.74± 0.12** [d]	17.52± 1.91
SR (100 mg/Kg)	10.38± 0.72	14.41± 1.17	**22.38± 0.38**[a]	16.62± 0.62	11.36± 0.62	**22.95± 0.34** [a]	12.24± 0.77
SR (200 mg/Kg)	17.38± 1.00	23.36± 0.78	**30.21± 0.58**[c]	19.40± 0.87	13.62± 0.90	**32.27± 0.52**[c]	18.72± 0.43
SR (400 mg/Kg)	15.06± 0.46	27.34± 1.14	**44.93± 0.34**[d]	19.21± 0.71	13.58± 1.18	**44.51± 0.33**[d]	22.49± 2.00
SC (100 mg/Kg)	10.18± 1.00	15.58± 0.82	**22.62± 0.67**[b]	16.08± 0.99	11.20± 0.54	**23.78± 0.37**[b]	12.11± 1.13
SC (200 mg/Kg)	18.05± 1.40	24.27± 0.81	**31.62± 0.59**[c]	20.16± 0.90	14.15± 0.98	**31.40± 1.00**[c]	19.44± 0.44
SC (400 mg/Kg)	15.06± 0.46	27.34± 1.14	**44.93± 0.34**[d]	19.21± 0.71	13.58± 1.18	**44.51± 0.33**[d]	22.49± 2.00
PHM (100 mg/Kg)	15.73± 0.47	28.55± 1.17	**52.94± 0.31**[d]	20.06± 0.76	22.04±1.15	**51.94± 0.30**[d]	23.48± 2.08
PHM (200 mg/Kg)	17.49± 0.47	32.45± 1.17	**55.94± 0.31**[d]	24.40± 0.76	24.05±1.03	**56.94± 0.30**[d]	25.83± 2.08
PHM (400 mg/Kg)	18.06± 0.47	34.94± 1.17	**61.94± 0.31**[d]	27.12± 0.76	25.09±1.10	**60.94± 0.30**[d]	27.12± 2.08

Table: 4[d] Effect of selected plant extracts on percentage reduction values of glucose after 4[th] week

Treatment Group	Time intervals						
	1[st] Hr	**2[nd] Hr**	**3[rd] Hr**	**4[th] Hr**	**6[th] Hr**	**8[th] Hr**	**10[th] Hr**
Normal Control	-1.80± 2.55	-0.51±2.82	-3.41±3.36	-2.26± 3.43	-2.09± 2.63	-9.17± 3.20	-5.35± 2.37
Diabetic control	0.79 ± 0.16	1.59± 0.22	2.35± 0.21	2.72± 0.23[b]	3.52±0.26[d]	4.36± 0.32[d]	5.01±0.35
Diabetic standard	**50.96± 0.98 [d#]**	23.19± 0.55	15.51± 0.86	7.39± 1.10	**49.29± 0.84 [d#]**	23.99± 0.89	12.44± 1.70
CC (100 mg/Kg)	5.92± 1.02	10.77± 0.96	19.21± 0.34	11.26± 0.77	5.30± 1.12	18.12± 0.37	7.45± 0.67
CC (200 mg/Kg)	13.15± 0.99	18.92± 0.78	**26.78± 0.57[b]**	18.17± 1.26	9.97± 1.27	**26.49± 0.39[b]**	14.41± 1.36
CC (400 mg/Kg)	10.55± 0.58	20.65±1.21	**36.49± 0.45 [d#]**	26.63± 0.64	16.91± 1.22	**37.25± 0.44[d#]**	15.75± 1.91
SR (100 mg/Kg)	10.43± 1.00	15.49± 0.95	**23.51± 0.38[b]**	15.26± 0.74	9.80± 1.17	**23.16± 0.42[b]**	12.04± 0.66
SR (200 mg/Kg)	13.56± 1.15	21.02± 1.05	**33.21± 0.28[d]**	24.35± 1.28	15.73± 1.29	**33.11± 0.37[d]**	20.40± 1.36
SR (400 mg/Kg)	16.31±0.85	26.01± 1.43	**46.96± 0.60[d#]**	33.23± 0.75	23.00± 1.21	**46.28± 0.44[d#]**	21.79± 2.29
SC (100 mg/Kg)	10.89± 1.04	16.18± 0.98	**24.57± 0.41[b]**	16.73± 0.80	10.23± 1.16	**24.20± 0.45[b]**	12.57± 0.69
SC (200 mg/Kg)	14.31± 1.21	22.18± 1.10	**35.05± 0.30[d]**	25.70± 1.34	16.59± 1.36	**34.00± 1.10[d]**	21.53± 1.43
SC (400 mg/Kg)	16.31±0.85	26.01± 1.43	**46.96± 0.60[d#]**	33.23± 0.75	23.00± 1.21	**46.28± 0.44[d#]**	21.79± 2.29
PHM (100 mg/Kg)	17.20± 0.91	28.41± 0.96	**57.73± 1.31[d]**	35.06± 0.80	24.26± 1.28	**54.94± 1.03[d]**	22.99± 2.43
PHM (200 mg/Kg)	19.34± 0.91	31.03± 0.96	**61.73± 1.31[d]**	36.94± 0.80	26.52± 1.28	**60.94± 1.03[d]**	23.45± 2.54
PHM (400 mg/Kg)	23.43± 0.91	33.46± 0.96	**63.73± 1.31[d#]**	38.73± 0.80	27.93± 1.28	**65.94± 1.03[d#]**	24.03± 2.63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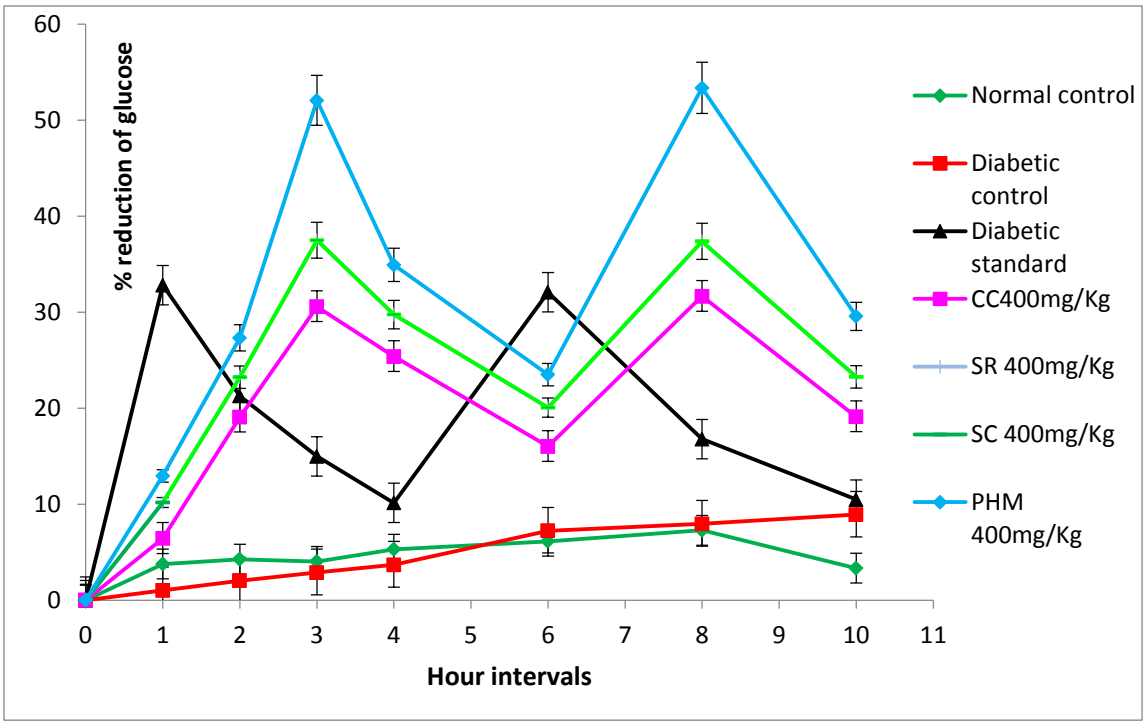
Hour intervals
Normal control
Diabetic control
Diabetic standard
CC400mg/Kg
SR 400mg/Kg
SC 400mg/Kg
PHM 400mg/Kg


% reduction of glucose
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Diabetic control
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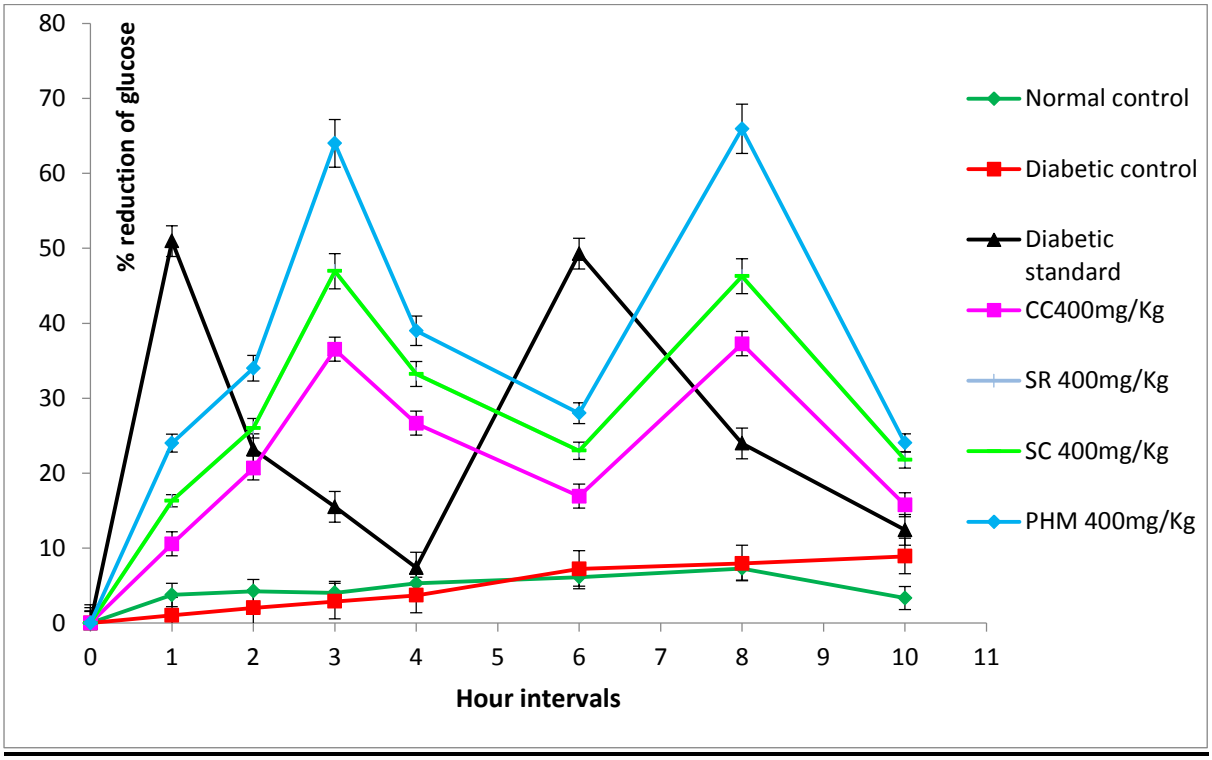
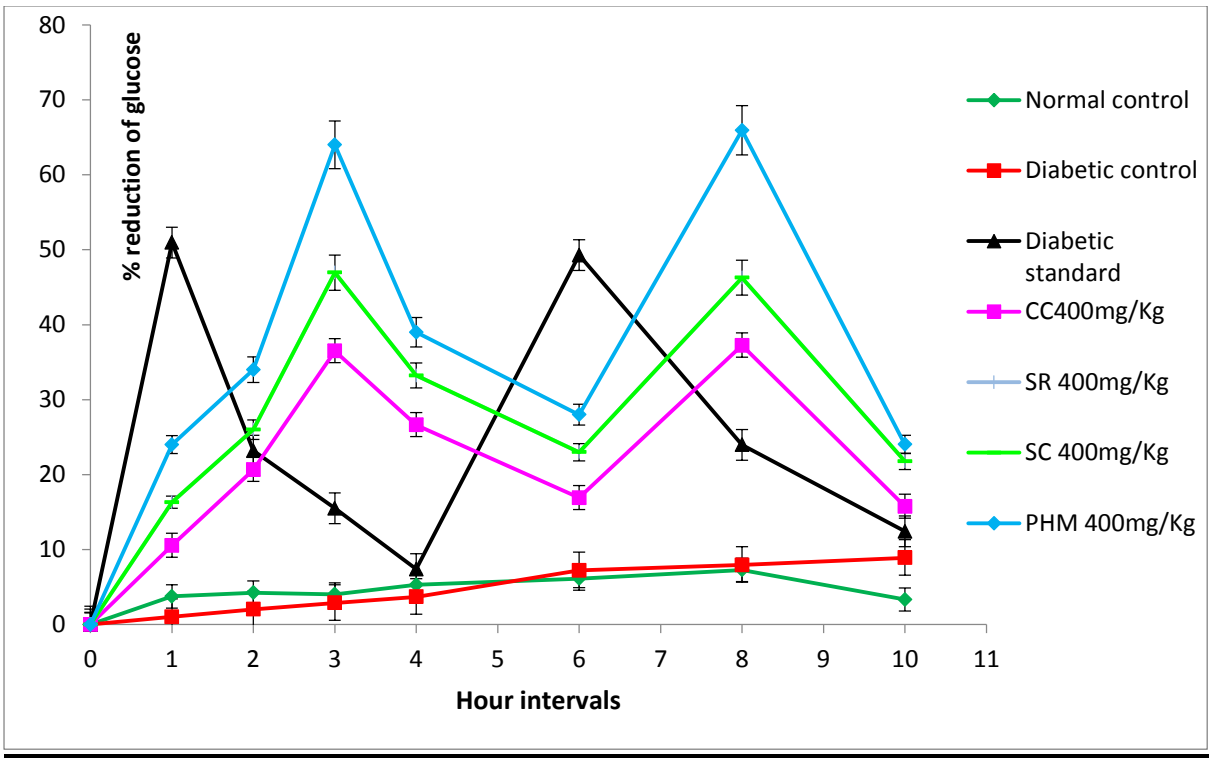




Graphs: 4[a] – 4[d] Effect of selected plant extracts on percentage reduction of glucose levels after 1[st] week to 4[th] week

Table: 5[a] Percentage increase values of insulin levels after 1[st] week

S.No.	Treatment groups	Time intervals		
		First peak	Second peak	Final hour
1.	Normal Control	0.15± 0.11	0± 0.00	0.60± 0.11
2.	Diabetic control	7.28± 0.04	**10.53± 0.09[@]**	2.19± 0.15
3.	Diabetic standard	37.14± 0.54	57.63± 0.77	1.70± 0.14

4.	CC (100 mg/Kg)	22.93± 0.05	28.04± 0.43	2.62± 0.07
5.	CC (200 mg/Kg)	25.84± 0.13	32.07± 0.21	1.84± 0.09
6.	CC (400 mg/Kg)	31.63± 0.06	42.94± 0.11	2.31± 0.11
7.	SR (100 mg/Kg)	32.84± 0.32	**45.03± 0.27^{x}**	10.24± 0.27
8.	SR (200 mg/Kg)	38.03± 0.31	**49.14± 0.31^{x}**	12.45± 0.47
9.	SR (400 mg/Kg)	42.94± 0.26	**57.04± 0.21^{x}**	14.94± 0.04
10.	SC (100 mg/Kg)	34.65± 0.14	**46.94± 0.16^{x}**	11.32± 0.14
11.	SC (200 mg/Kg)	40.64± 0.34	**54.42± 0.15^{x}**	13.42± 0.03
12.	SC (400 mg/Kg)	44.84± 0.35	**61.73± 0.18^{x}**	16.83± 0.13
13.	PHM (100 mg/Kg)	41.95± 0.53	**60.74± 0.21^{x}**	15.43± 0.14
14.	PHM (200 mg/Kg)	44.41± 0.35	**63.93± 0.24^{x}**	18.95± 0.23
15.	PHM (400 mg/Kg)	48.83± 0.37	**66.02± 0.73^{y}**	20.83± 0.32

Table: 5^{b} Percentage increase values of insulin levels after 2nd week

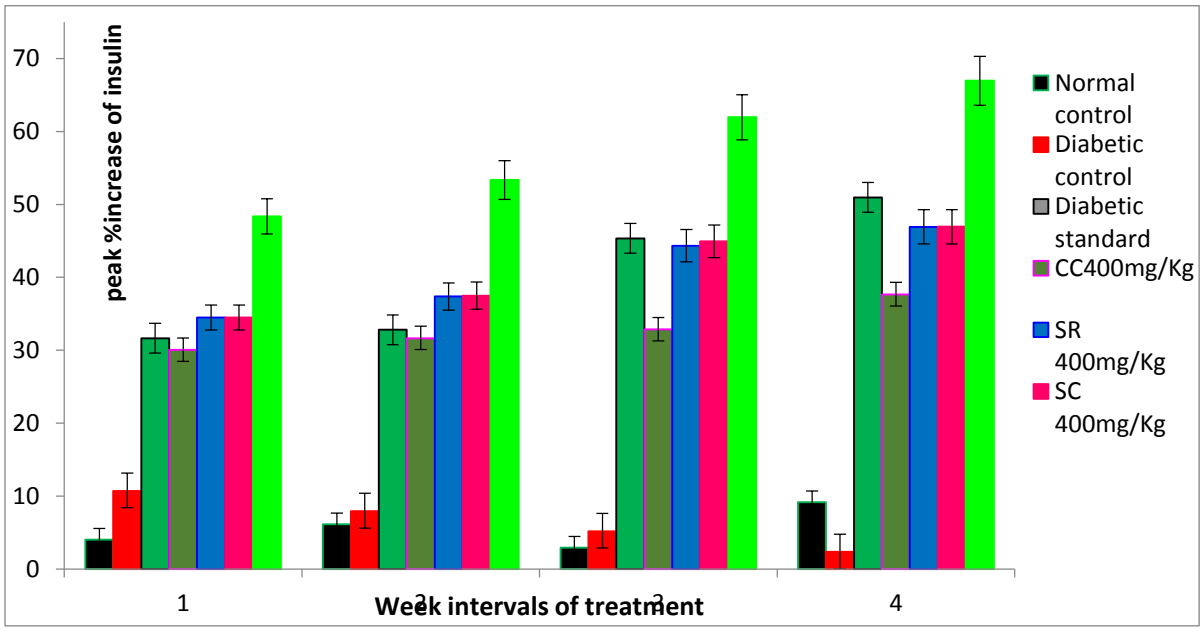
S.No.	Treatment groups	Time intervals		
		First peak	Second peak	Final hour
1.	Normal Control	0.13± 0.02	0.24± 0.03	0.51± 0.04
2.	Diabetic control	**5.21± 0.11$^{@}$**	4.32± 0.10	2.54± 0.17
3.	Diabetic standard	41.84± 0.06	**61.42± 0.31^{a}**	2.84± 0.12
4.	CC (100 mg/Kg)	25.09± 0.15	31.45± 0.41	3.21± 0.21
5.	CC (200 mg/Kg)	29.42± 0.35	36.43± 0.09	2.36± 0.42
6.	CC (400 mg/Kg)	34.61± 0.18	46.31± 0.28	3.16± 0.39
7.	SR (100 mg/Kg)	36.41± 0.09	**48.04± 0.32^{a}**	11.50± 0.32
8.	SR (200 mg/Kg)	43.29± 0.38	**54.32± 0.24^{ax}**	13.51± 0.45
9.	SR (400 mg/Kg)	46.25± 0.26	**61.04± 0.49^{ax}**	16.36± 0.83
10.	SC (100 mg/Kg)	38.36± 0.31	**50.95± 0.13^{ax}**	13.52± 0.21
11.	SC (200 mg/Kg)	44.85± 0.25	**58.03± 0.84^{ax}**	14.93± 0.39
12.	SC (400 mg/Kg)	48.30± 0.14	**65.85± 0.07^{ay}**	17.37± 0.82
13.	PHM (100 mg/Kg)	45.26± 0.41	**66.24± 0.32^{ay}**	17.92± 0.78
14.	PHM (200 mg/Kg)	48.84± 0.09	**67.46± 0.31^{ay}**	19.54± 0.54
15.	PHM (400 mg/Kg)	51.52± 0.18	**71.42± 0.21^{by}**	22.40± 1.03

Table: 5^{c} Percentage increase values of insulin levels after 3rd week

S.No.	Treatment groups	Time intervals		
		First peak	Second peak	Final hour
1.	Normal Control	0.34± 0.2	0.45± 0.94	0.60± 0.30
2.	Diabetic control	5.02± 0.05	**7.53± 0.04$^{@}$**	1.19± 0.43
3.	Diabetic standard	41.84± 0.06	61.42± 0.31	3.57± 0.10
4.	CC (100 mg/Kg)	30.19± 0.34	35.61± 0.10	3.94± 0.25
5.	CC (200 mg/Kg)	34.52± 0.93	40.59± 0.95	3.09± 0.84
6.	CC (400 mg/Kg)	39.71± 0.83	50.47± 0.93	3.89± 0.94
7.	SR (100 mg/Kg)	41.51± 0.84	**52.20± 0.81^{ax}**	12.23± 0.72
8.	SR (200 mg/Kg)	48.39± 0.41	**58.48± 0.14^{bx}**	14.23± 0.95
9.	SR (400 mg/Kg)	51.35± 0.54	**65.20± 0.26^{bx}**	17.03± 0.29
10.	SC (100 mg/Kg)	43.46± 0.92	**55.11± 0.54^{ax}**	14.23± 0.94
11.	SC (200 mg/Kg)	49.95± 0.64	**70.01± 0.21^{ax}**	15.63± 0.09
12.	SC (400 mg/Kg)	53.40± 0.92	**72.19± 0.64^{by}**	18.03± 0.93
13.	PHM (100 mg/Kg)	50.36± 0.14	**70.40± 0.58^{by}**	18.63± 0.74
14.	PHM (200 mg/Kg)	53.94± 0.84	**71.62± 0.48^{by}**	20.23± 0.72
15.	PHM (400 mg/Kg)	56.62± 0.93	**75.58± 0.14^{cz}**	23.13± 0.72

Table: 5[d] Percentage increase values of insulin levels after 4[th] week

S.No.	Treatment groups	Time intervals		
		First peak	Second peak	Final hour
1.	Normal Control	0.14± 0.04	0.64± 0.08	0.81± 0.15
2.	Diabetic control	**4.24± 0.14[@]**	2.35± 0.14	1.53± 0.23
3.	Diabetic standard	50.99± 0.73	**68.70± 0.81[a]**	3.86± 0.03
4.	CC (100 mg/Kg)	34.24± 0.94	53.59± 0.03	4.23± 0.07
5.	CC (200 mg/Kg)	38.57± 0.14	53.73± 0.92	3.38± 0.13
6.	CC (400 mg/Kg)	43.76± 0.93	58.59± 0.03	4.18± 0.09
7.	SR (100 mg/Kg)	45.56± 0.73	**55.32± 0.02[ax]**	12.52± 0.16
8.	SR (200 mg/Kg)	52.44± 0.93	**61.60± 0.84[bx]**	14.52± 0.17
9.	SR (400 mg/Kg)	55.40± 0.40	**68.32± 0.26[by]**	17.32± 0.07
10.	SC (100 mg/Kg)	47.51± 0.94	**58.23± 0.05[ax]**	14.52± 0.11
11.	SC (200 mg/Kg)	54.00± 0.83	**73.13± 0.94[bz]**	15.92± 0.19
12.	SC (400 mg/Kg)	57.45± 0.72	**75.31± 0.83[bz]**	18.32± 0.28
13.	PHM (100 mg/Kg)	54.41± 0.94	**73.52± 0.94[bz]**	18.92± 0.06
14.	PHM (200 mg/Kg)	57.99± 0.85	**74.74± 0.92[bz]**	20.52± 0.14
15.	PHM (400 mg/Kg)	60.67± 0.79	**78.70± 0.93[cz]**	23.42± 0.18



Graph: 5 Effect of selected plant extracts on peak percentage increase of insulin levels

Data were expressed as Mean± SEM (gm) and analyzed by performing two ways ANOVA with significant value at P > 0.05. 1[st] week values are compared with 2[nd], 3[rd] and 4[th] weeks in every group. For each week, % of change in parameters of every group is compared with control group, and a non-parametric test (Dunntte's multiple comparison test) was applied for multiple comparisons.

- @ = Normal control group is compared with disease control
- a = *, b = ** and c = ***, within each group changes in parameters after 2[nd], 3[rd]and

4th weeks were compared.

- x = #, y = ## and z = ###, whereas changes in parameters of all groups were compared with control group after every week of treatment.

The % reduction in body weight of normal rats was found to be 0.11± 0.03 and 0.51± 0.08 after 1st and 4th weeks respectively. In STZ induced diabetic rats % reduction in body weight was found to be significantly ($p< 0.05$) increased from 15.27± 0.73 to 32.52± 1.18 when compared with normal rats while, in rats treated with Glibenclamide % reduction in body weight was decreased significantly ($p< 0.05$) from 23.76± 0.14 to 0.97± 0.08 after 4th week of treatment. In SR, SC & PHM treated diabetic rats % reduction of body weight was found to be significantly ($p< 0.05$) decreased from 1st to 4th week of treatment.

The % of reduction food and water intake in STZ induced diabetic rats was found to be decreased significantly ($p< 0.05$) from 10.12 to 4.06 while, in rats treated with Glibenclamide it was increased significantly ($p< 0.05$) from 8.13 to 31.38 after 4th week of treatment. In CC, SR, SC & PHM treated diabetic rats % reduction of food and water intake was found to be increased significantly ($p< 0.05$).

The percentage of glucose reduction was found to be peak at 1stand 6th hours in Glibenclamide treated diabetic rats and at 3rd&8th hours in diabetic rats treated with extracts and PHM. There is no % glucose reduction in STZ induced diabetic rats while diabetic rats treated with Glibenclamide, CC, SR, SC & PHM shown to significantly ($p< 0.05$) decrease the % of glucose levels from 1st week to 4th week of treatment.

The percentage of insulin increase was found to be peak at 1st and 6th hours in Glibenclamide treated diabetic rats and at 3rd& 8th hours in extracts and PHM treated diabetic rats. There is no % of insulin increase in STZ induced diabetic rats while diabetic rats treated with Glibenclamide, CC, SR, SC & PHM shown to significantly ($p< 0.05$) increase % of insulin levels from 1st week to 4th week of treatment.

DISCUSSION

In STZ induced diabetic rats body weight was found to be decreased and food and water intake were found to be increased. Treatment with all the selected plant extracts and PHM significantly increased the body weight and decreased food and water intake in a dose dependent manner. The PHM, the aqueous extracts of SC and SR were shown more activity than standard glibenclamide. Among the selected plant extracts SC was found have better activity in increasing body weight and decreasing food and water intake than all other extracts. The order of potency of extracts was PHM>SC>SR>Glibenclamide>CC. The increase in food and water intake in diabetic rats might be due to excessive thirst and hunger (Polyphagia and Polydypsia). Excessive food and water intake in diabetes might be due to

glucose sensors that are present on hypothalamus are directly linked to the nerves which regulates energy equilibrium. The glucose sensors on hypothalamus are connected to nerves of the portal vein and carotid body, which can increase eating and drinking habits in diabetes (Levin B *et al*., 1999).Weight loss in diabetic rats might be due to increase in muscle wasting as described by Chakravarti B.K *et al.,* 1981, catabolism of fats and proteins, due to insulin deficiency and decreased protein content in muscular tissue by proteolysis (Swanston Flat, S.K. *et al.,* 1990).

The decrease in food intake, water intake and increase in body weight with the treatment of selected plant extracts, PHM and standard glibenclamide might be due to decreased muscle wasting, decreased catabolism of fats and proteins, and due to increased protein content in muscular tissues.

CONCLUSION

In this study the aqueous extracts of three herbs namely fruit pulp of *Citrullus colocynthis (CC),* leaves of *Stevia rebaudiana (SR),* and leaves of *Swertia Chirayata (SC)* were evaluated for their antihyperglycemic activity and further for their role in the inhibition of development of diabetic complications. The literature review revealed that the active constituents (polyphenols) were more in their respective selected parts. Hence in the present study, respective parts of selected plants were evaluated for their beneficial role in diabetes and diabetes induced complications.

In the present study the selected plant extracts and polyherbal mixture (PHM) were screened for their antihyperglycemic potential and thereby for their possible role in inhibition of the pathways leading to diabetic complications in STZ induced diabetic rats. The doses of the selected plant extracts were fixed by performing acute toxicity studies according to OECD 425 guidelines and same doses were fixed as low dose (100mg/Kg), mid dose (200mg/Kg) and high dose (300mg/Kg) for all selected plant extracts. Polyherbal mixture (PHM) was prepared by selecting the doses of the selected plant extracts which were shown optimal percentage of blood glucose reduction (30–40%) in albino Wister rats. The selected plant extracts were mixed to prepare PHM in the ratio of 2:1:1 of CC, SR and SC respectively. Three doses of PHM low dose (100mg/Kg), mid dose (200mg/Kg) and high dose (300mg/Kg) were also evaluated for their antihyperglycemic activities and for their role in preventing diabetic complications in STZ induced diabetic rats.

Initially hypoglycemic studies were conducted without inducing of diabetes mellitus. Based on the results obtained PHM was prepared. The extracts which are showing % reduction of glucose between 30 -50% were selected in order to avoid hypoglycemic shock.

All the selected plant extracts and PHM found to be inhibited increase in body weight and among them PHM was found to be most effective than standard and remaining extract. All the selected plant extracts and PHM found to be reduction in water intake and among them PHM was found to be most effective than standard and remaining extract. All the selected plant extracts and PHM were found to decreased glucose levels at 3rd and 8th hrs and standard drug was found to decrease glucose levels at 1st & 6th hr significantly after every week (biphasic reduction). All the selected plant extracts and PHM were found to increase insulin levels at 3rd and 8th hrs and standard drug was found to increase levels at 1st & 6th hr significantly after every week (biphasic reduction).PHM was found to most potent.

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