



EVALUATION OF ANTI DIABETIC ACTIVITY OF *ICHNOCARPUS FRUTESCENS* L.

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ABSTRACT:

The plant *Ichnocarpus frutescens* L. is from the family Apocynaceae, which extensively cultivated in most regions of the world and common avenue tree, commonly called as black creeper in English. The literature survey reveals that *Ichnocarpus frutescens* L. roots has been used as tonic, diuretic, demulcent, diaphoretic and anti diabetic. Young stems and leaves are also used for diabetes. Young stems and leaves contain triterpenoids, α -amyrin, acetalupeol and its acetates, friedelin, epifriedelinol and β -sitosterol. Flowers and fruits contain flavonoids-Quercetin, Kaepferol-3-glucoside. The Ethanolic and Aqueous extracts of dried flowers of *Ichnocarpus frutescens* L. were screened for anti-diabetic activity in normal rats, STZ induced diabetic rats and on serum glucose levels in glucose over loaded rats. The effect on the insulin level with treatment by aqueous and ethanolic extract of *Ichnocarpus frutescens* L. flowers suggest that the mode of action is similar to that of glibenclamide. Oral administration of both the extracts for 21 days significantly reduced blood glucose level in STZ induced diabetic rats. Both the extracts exhibited antihyperglycemic effect in glucose loaded rats and STZ induced rats.

Key words: Diabetes mellitus, antidiabetic, *Ichnocarpus frutescens* L., OGTT, streptozotocin, hypoglycaemia

1. INTRODUCTION

Now days, herbal drugs are gaining popularity in the treatment of diabetes and its complications. Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used and have considerable importance in international trade⁽¹⁾. The major merits of herbal medicines seem to be their efficacy, low incidence of side effects, and low cost.

The plant *Ichnocarpus frutescens* L. belongs to the family Apocynaceae, is a large evergreen,

lactiferous, woody creeper with red appearance, found throughout India, up to an altitude of 4000feet.

Young stems and leaves contain triterpenoids, α -amyrin and its acetalupeol and its acetate, friedelin, epifriedelinol and β -sitosterol. Flowers and fruits contain flavonoids - quercetin, kaempferol-3-glucoside, sorbopyranoside. Roots contains flavonoids, sterols, terpenoids⁽²⁾. The root shows the presence of phenyl propanoids, phenolic acid, coumarins, pentacyclic terpenoids⁽³⁾. The plant contains wide range of irridoid glycosides⁽⁴⁾.

2. MATERIALS AND METHODS

Preparation of extract:

The flowers were dried in shade at room temperature. The dried flowers were coarsely powdered, stored in an airtight container until used.

Extraction⁽⁵⁾:

Extraction of *Ichnocarpus frutescens* L. flowers was carried out by using soxhlet apparatus by using solvents like petroleum ether, chloroform, ethanol and water.

Determination of acute toxicity⁽⁶⁾:

LD₅₀ value was determined in 15 days. Ethanolic and aqueous extracts were used to determine the LD₅₀ value. The doses were selected according to OECD guideline 420. The doses selected were 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg. Study was carried out for 15 days. For this purpose Swiss albino female mice were used

Experimental animals:

Albino Wistar rats weighing 150 – 250 gm and albino mice (20 – 25 g) were used throughout the experiment. Standard environmental conditions such as temperature (26 ± 2°C) relative humidity (45 – 55%) and 12 hrs dark / light cycle were maintained in the quarantine.

Ethical clearance for performing the experiments on animals was obtained from institutional animal ethics committee (IAEC). (Ref. No 346/CPCSEA)

Animal models in experimental diabetes mellitus⁽⁷⁾:

Anti diabetic activity on diabetic rats

The rats with body weight 150 – 250 gm were selected for the diabetogenic activity. The animals were deprived for food for 18 hours prior to administration of streptozotoin. Streptozotocin was freshly dissolved in freshly prepared 0.01 M citrate buffer (pH 4.5). Streptozotocin was given intra peritoneal (50 mg/kg body weight).

1. Acute study on normal rats:

For acute anti diabetic study, animals were divided into six groups of six rats in each group. The animals were deprived of food for 18 hours before the experiment, and water was allowed to them, but on the day of experiment water was withdrawn.

Group I – Received vehicle 5% aqueous gum acacia p.o. (control)

Group II – Received standard drug 10mg/kg of Glibenclamide p.o.

Group III – Received 250mg/kg of ethanolic extract p.o.

Group IV – Received 500mg/kg of ethanolic extract p.o.

Group-V – Received 250mg/kg of aqueous extract p.o.

Group-VI – Received 500mg/kg of aqueous extract p.o.

Blood glucose level was determined at 0 hour i.e. before drug administration, 0.5, 1, 2, 4, 8, 12, 16 and 24 hours after drug administration. Blood was collected from the tail vein by snipping tail with a sharp razor. The collected blood was centrifuged at 2000 rpm for 15 minute and serum glucose level

was determined by glucose oxidase peroxidase method in semi autoanalyzer.

The results are tabulated in **Table No. 1**

2. Oral glucose tolerance test⁽⁸⁾ on normal rats (OGTT):

The rats of all the groups were loaded with glucose (2 gm/kg p.o) 30 minutes after drug administration. Blood samples were collected from the tail just prior to drug administration and at 30, 90, 150 minute after glucose loading. Serum glucose levels were measured immediately. The blood is than centrifuged at 2000 rpm for 15 minutes. Glucose level was estimated by GOD-POD kit using semi autoanalyzer.

Six fasted animals were used in each group.

Rats were divided into following groups.

Group I - Received 5% aqueous gum acacia p.o. (control -ve)

Group II - Received 2 gm /kg glucose p.o. (control +ve)

Group III - Received standard drug glibenclamide (10 mg/kg), p.o.

Group IV - Received ethanolic extract of *Ichnocarpus frutescens* L. flowers, dose 250mg/kg, p.o.

Group V - Received ethanolic extract of *Ichnocarpus frutescens* L. flowers, dose 500mg/kg, p.o.

Group VI - Received Aqueous extract of *Ichnocarpus frutescens* L. flowers, dose 250mg/kg, p.o.

Group VII - Received Aqueous extract of *Ichnocarpus frutescens* L. flowers, dose 500mg/kg, p.o.

The results are tabulated in **Table No. 2**

3. Acute study on STZ induced diabetic rats:

Diabetogenic rats, having blood glucose level ≥ 250 mg/dl were selected for the study. After fasting overnight, the diabetic treated animals were divided into 6 groups, each group contained 6 rats (n=6). Blood samples were collected at 0, 0.5, 1, 2, 4, 8, 12, 16, and 24 hours after extracts/GLB administration (**Single dose one day study**) and blood sugar levels were measured by GOD/POD kit using a semi autoanalyser.

Following groups were made for the determination of acute study in streptozotocin induced diabetic rats.

Group I - Consisted of six streptozotocin induced diabetic rats which served as a control group and were given 5% aqueous gum acacia p.o.

Group II -Consisted of six streptozotocin induced diabetic rats which were treated orally with standard drug glibenclamide (10 mg/kg).

Group III - Consisted of six streptozotocin induced diabetic rats which were treated orally with ethanolic extract at a dose of 250 mg/kg.

Group IV - Consisted of six streptozotocin induced diabetic rats which were treated orally with ethanolic extract at a dose of 500 mg/kg.

Group V - Consisted of six streptozotocin induced diabetic rats and were treated orally with aqueous extract at a dose of 250 mg/kg.

Group VI - Consisted of six streptozotocin induced diabetic rats and were treated orally with aqueous extract at the dose of 500 mg/kg.

The results are tabulated in **Table No. 3**

4. Sub acute study on STZ induced diabetic rats:

Sub acute study was carried out in streptozotocin induced diabetic rats. The study was carried out for 21 days. The animals were fasted for 18 hours prior to the experiment and blood glucose levels were checked. It was considered as a 0 day reading. The dose of *Ichnocarpus frutescens* L. flowers extracts were given orally daily to the animals for 21 days. The blood glucose levels were checked at 0, 7, 14 and 21 days intervals. The blood was collected by snipping the tail with a sharp razor in rats. The collected blood was centrifuged at 2000 rpm for 15 minutes and determination of blood glucose levels were carried out using GOD-POD method in semi-autoanalyzer.

The fasting blood glucose levels were determined before the experiment. The blood glucose levels were measured at day 0, 7, 14 and 21.

Group I - Normal control received 5% aqueous gum acacia p.o. (control -ve)

Group II - Diabetic control received 5% gum acacia p.o. (control +ve)

Group III - Received standard drug glibenclamide (10 mg/kg), p.o.

Group IV - Received ethanolic extract of *Ichnocarpus frutescens* L. flowers, dose 250 mg/kg, p.o.

Group V - Received ethanolic extract of *Ichnocarpus frutescens* L. flowers, dose 500 mg/kg, p.o.

Group VI - Received Aqueous extract of *Ichnocarpus frutescens* L. flowers, dose 250 mg/kg, p.o.

Group VII - Received Aqueous extract of *Ichnocarpus frutescens* L. flowers, dose 500 mg/kg, p.o.

The results are tabulated in **Table No. 4**

3. RESULTS

1. Acute study on normal rats:

The hypoglycemic effect of ethanolic and aqueous extracts of *Ichnocarpus frutescens* L. were investigated in normal rats and the results are expressed in **Table No. 1**

The ethanolic and aqueous extracts at the dose of 250 mg/kg and 500 mg/kg exhibited a significant reduction ($P < 0.01$ and $P < 0.001$ respectively) in SG level over a period of 12 hr. compared with the normal control group. 500 mg/kg of Aqueous showed maximum blood glucose level reduction at 12th hour ($38.46 \pm 0.49\%$, $P < 0.001$) compared to the normal control, While hours 1st, 2nd, 4th, 8th and 16th showed significant reduction in blood glucose level compared to the normal control. Further, treatment of 500 mg/kg with ethanolic and 250 mg/kg of ethanolic and aqueous extracts showed significant

($P < 0.01$ & $P < 0.001$ respectively) reduction compared to normal control group. Onset of action of aqueous extract started at 2nd hour after the treatment (21.22 ± 0.34 $P < 0.001$). Duration of action of aqueous extract (500 mg/kg) was for 16 hours.

Glibenclamide showed onset of action from 1st hour after the treatment. It reduced maximum blood glucose level at 12th hour (52.10 ± 0.09 , $P < 0.001$). Also glibenclamide significantly reduced the blood glucose level at 1st, 2nd, 4th, 8th and 16th hour, $P < 0.01$ as compared to the normal control group. The onset of action of glibenclamide starts from 1st hour after treatment (14.38 ± 0.15 , $P < 0.01$).

2. OGTT on normal rats:

The effect of the ethanolic and aqueous on glucose tolerance test in normal fasted rats are shown in **Table No. 2**

Most significant reduction was observed for aqueous extract (500 mg/kg) at 150 minutes (88.30 ± 0.10 mg/dl, $P < 0.001$) compared to the normal group. It also showed significant decrease in serum glucose level at 90 minutes compared to the control group (109.90 ± 0.19 , $P < 0.001$). Ethanolic extract (500 mg/kg) significantly decreased blood glucose level in glucose fed rats at 150 minutes (91.10 ± 0.71 , $P < 0.01$) compared with the normal group. It also decrease the elevated blood glucose at 90 min. (133.30 ± 0.10 , $P < 0.01$) after glucose administration. Ethanolic and aqueous extract at 250 mg/kg also showed marked decrease in glucose level (120.60 ± 0.40 , 110.80 ± 0.19 respectively).

Glibenclamide showed potent anti diabetic activity at 150 minutes (62.49 ± 0.51 , $P < 0.001$). Also the reduction in elevated blood glucose level at 30 and 90 minutes after the administration of glucose was significant compared to the normal control group.

This data suggested that treatment with ethanolic and aqueous extracts showed better tolerance to exogenously administered glucose.

3. Acute study on streptozotocin induced diabetic rats:

A single dose of ethanolic extracts (250 mg/kg, 500 mg/kg B.W) and aqueous extract (250 mg/kg, 500 mg/kg B.W) treatment exhibited reduction in serum glucose level at different time intervals compared to basal levels (0 Hr.) however aqueous and ethanolic extract (500 mg/kg B.W) treated animal showed significant percentage reduction ($P < 0.001$) in SG levels (51.39 ± 0.51 , 47.40 ± 0.390 respectively) over 12hr post treatment compared to their basal levels where as administration of GLB showed significant reduction ($P < 0.01$) in SG levels with maximum reduction (58.01 ± 0.15) at 12 hr compared to their basal level.

Furthermore single dose treatment of aqueous and ethanolic extract (250 mg/kg B.W) showed significant reduction ($P < 0.001$) in SG levels at different time intervals as compared to diabetic control group. This data suggested that the aqueous and ethanolic extracts (500 mg/kg B.W) exhibited greater hypoglycaemic activity than aqueous and ethanolic extract (250 mg/kg B.W) in streptozotocin induced diabetic rats.

The results are tabulated in **Table No. 3**

4. Sub acute study on STZ induced diabetic rats:

Repeated administration of aqueous and ethanolic extract (250 mg/kg B.W) for 21 days showed significant reduction in serum glucose levels (127.8 ± 0.1 , 125.8 ± 0.21 $P < 0.0001$ respectively) compared to basal values (0 day). However it was more marked in animals treated with a dose of 500 mg/kg of aqueous and ethanolic extracts (120.3 ± 0.28 , 115.0 ± 0.11 $P < 0.0001$ respectively). On 21st day higher doses of both the extract showed greater percentage reduction in glycemia compared to diabetic control and their potency is comparable to GLB treated diabetic rats (115.3 ± 0.41 $P < 0.000$)

Sub acute study of 21 days suggested that ethanolic and aqueous extract (500 mg/kg B.W) showed better antidiabetic activity.

The results are tabulated in **Table No. 4**

4. DISCUSSION

STZ is well known for its selective pancreatic islet β -cell cytotoxicity and has been extensively used to induce DM in animals. It interferes with cellular metabolic oxidative mechanisms. Higher doses of STZ (60 mg/kg, i.p.) effectively induced diabetes in normal rats as reflected by severe hyperglycaemia, glucosuria (>2%), polyphagia, polydipsia and body weight loss when compared with normal rat. Standardization of STZ dose experiment reveals that 40 mg/kg, i.p. did not produced significant hyperglycemia. Whereas, both 50 mg/kg and 60 mg/kg produced significant hyperglycemia and more

than 2% glucosuria, suggesting that these animals are under severe (type I) diabetic condition. We have selected 50 mg/kg of STZ for the screening of extracts because 60 mg/kg of STZ treated animals showed mortality and less response to GLB than STZ (50 mg/kg, i.p.).

The present study reveals the presence of flavonoids, tannins, saponins, carbohydrates, glycosides, alkaloids and steroids. Therefore it can be confirmed that the significant anti diabetic potential of *Ichnocarpus frutescens* L. flowers may be due to flavonoids, tannins, and saponin contents which were confirmed by preliminary phytochemical screening.

5. CONCLUSION

The present study was aimed to expose the antidiabetic property, of *Ichnocarpus frutescens* L. flowers in normal and in streptozotocin induced diabetic rats. The results demonstrate the strong hypoglycemic activity of *Ichnocarpus frutescens* L. flowers (aqueous and ethanolic extracts).

6. REFERENCE

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TableNo. 1: Effect of *Ichnocarpus frutescens* flower extracts on blood glucose level in normal rats

Groups	Treatment	0 Hour Basal reading mg/dL	Percentage decrease in blood glucose level							
			0.5	1	2	4	8	12	16	24
Control	5% Aqueous gum acacia	89.97±0.71	1.73±0.01	3.63±0.05	4.61 ±0.15	3.500 ± 0.15	1.64± 0.10	2.38± 0.10	3.60± 0.14	5.36± 0.16
Standard	Glibenclamide 10 mg/kg	94.02±1.27	8.38±0.01	14.38*± 0.15	21.99***± 0.13	34.98***± 0.15	41.10***± 0.11	52.10***± 0.01	25.02***± 0.15	7.48±0.55
Ethanolic	250 mg/kg	92.11±1.89	3.39±0.43	7.56±0.46	13.97*±0.80	20.49***± 0.30	24.37***± 0.66	29.46*** ± 0.46	18.22**± 0.77	7.27±0.47
Ethanolic	500 mg/kg	97.13±0.60	3.15±0.42	9.09±0.48	14.43* ±0.43	19.40**± 0.16	24.89*** ± 0.32	34.49***± 0.17	19.49** ± 0.80	4.27±0.18
Aqueous	250 mg/kg	93.90±1.48	5.180±0.64	11.460*± 0.63	17.120**± 0.43	20.090***± 0.19	26.090*** ± 0.33	30.880*** ± 0.92	13.790*± 0.64	4.77± 0.14
Aqueous	500 mg/kg	94.82±1.32	6.37±0.70	8.27±0.21	21.22***± 0.34	29.45***± 0.64	33.55***± 0.57	38.46***± 0.49	19.70**± 0.20	6.73±0.23

Values are mean ± SEM; n=6 *P<0.05 **P<0.01 ***P<0.001 compared with normal control

Table No. 2: Effect of *Ichnocarpus frutescens* L. flower extracts on oral glucose tolerance test in normal rats

Groups	Blood glucose level mg/dl			
	0 min.	30 min.	90 min.	150 min.
Control -ve	86.0±0.73	87.28±0.34	88.63±0.35	89.53±0.05
Control +ve Glucose 2 gm/kg	93.7±0.97	169.0±3.09	198.0±0.55	224.6±0.81
Standard Glibenclamide 10 mg/kg	89.6±0.02	136.0±0.71***	84.91±0.32***	62.49±0.51***
Ethanolic 250 mg/kg	98.30±0.67	154.30±0.82	129.70±0.63***	120.60±0.40***
Ethanolic 500 mg/kg	100.70±0.21	143.90±0.13	120.90±0.10***	91.10±0.71***
Aqueous 250 mg/kg	99.20±0.29	180.30±0.31	126.70 ±0.31***	110.80±0.19***
Aqueous 500 mg/kg	87.70±0.42	165.50±0.93	109.90±0.19***	88.30±0.10***

Values are mean ± SEM; n=6 ***P<0.001 compared with the normal control.

Table No. 3: Effect of *Ichnocarpus frutescens* flowers extracts on blood glucose level in STZ induced diabetic rats (acute study)

Groups	Treatment	0 Hour Basal reading mg/dL	Percentage decrease in blood glucose level							
			0.5	1	2	4	8	12	16	24
Control	5% aqueous gum acacia	257.6 ± 0.33	0.51 ± 0.04	0.97 ± 0.02	1.14 ± 0.04	1.48 ± 0.16	2.66 ± 0.13	2.44 ± 0.14	1.67 ± 0.11	0.81 ± 0.60
Standard	Glibenclamide 10mg/kg	266.1 ± 0.26	23.22** ± 0.01	34.13*** ± 0.12	38.93*** ± 0.1	49.03*** ± 0.12	50.44*** ± 0.1	58.01*** ± 0.15	35.67*** ± 0.11	9.46 ± 0.13
Ethanollic	250 mg/kg	292.2 ± 0.25	2.310 ± 0.06	9.98 ± 0.71	17.190** ± 0.810	23.56*** ± 0.140	31.780*** ± 0.100	44.70*** ± 0.390	15.600* ± 0.310	4.680 ± 0.540
Ethanollic	500 mg/kg	312.2 ± 0.21	3.97 ± 0.11	11.38* ± 0.30	19.20** ± 0.68	29.78*** ± 0.13	38.38*** ± 0.69	47.40*** ± 0.03	39.60*** ± 0.37	9.37 ± 0.53
Aqueous	250 mg/kg	307.7 ± 0.32	1.180 ± 0.47	13.290* ± 0.86	18.360** ± 0.79	26.780*** ± 0.33	33.710*** ± 0.25	41.500*** ± 0.06	17.860*** ± 0.73	2.800 ± 0.10
Aqueous	500 mg/kg	279.6 ± 0.466	8.64 ± 0.40	17.34** ± 0.49	23.59*** ± 0.14	32.18*** ± 0.96	43.30*** ± 0.14	51.39*** ± 0.51	41.93*** ± 0.26	17.30** ± 0.68

Values are mean ± SEM; n=6 *P<0.01 **P<0.001 ***P<0.0001 compared with the diabetic control.

Table No. 4: Effect of *Ichnocarpus frutescens* L. flower extracts on blood glucose level in STZ induced diabetic rats (sub acute study)

Groups	Blood glucose level mg/dL			
	0 day	7 th day	14 th day	21 st day
Control -ve	89.97±0.71	90.25±0.62	91.99±0.38	93.06±0.28
Control +ve	257.6±0.33	265.2±0.25	265.7±0.79	272.3±0.62
Std. Glibenclamide (10 mg/kg)	266.1±0.26	212.4±0.32***	150.7±0.50***	115.3±0.41***
Ethanollic extract (250 mg/kg)	268.4±0.25	201.3±0.12	164.0±0.01***	125.8±0.21***
Ethanollic extract (500 mg/kg)	268.6±0.21	210.0±0.11***	160.0±0.32***	115.0±0.11***
Aqueous extract (250 mg/kg)	256.9±0.32	219.6±0.20	178.9±0.60***	127.8±0.1***
Aqueous extract (500 mg/kg)	260.9±0.46	202.3±0.35	169.3±0.24***	120.3±0.28***

Values are mean ± SEM; n=6 *P<0.01 **P<0.001***P<0.0001 compared with the diabetic control.

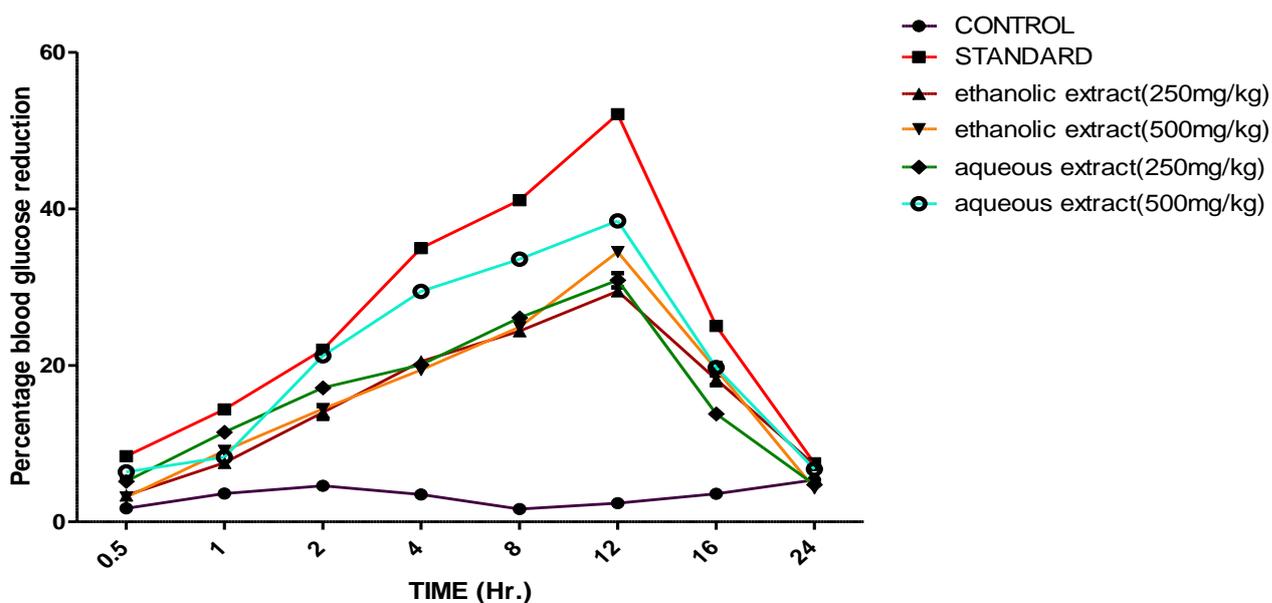


Fig. No. 1: Effect of *Ichnocarpus frutescens* flowers extracts on blood glucose level in normal rats

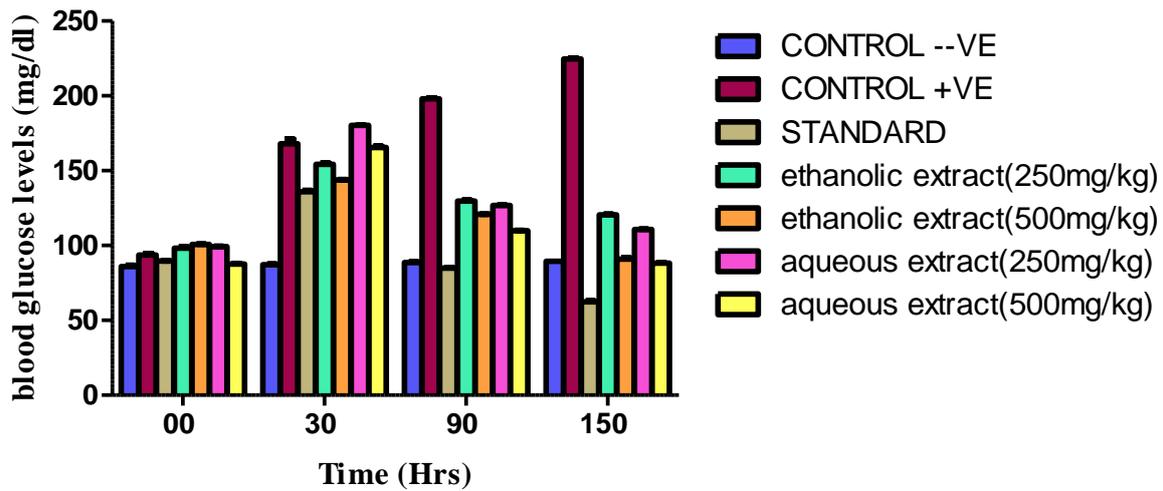


Fig. No. 2: Effect of *Ichnocarpus frutescens* L. flower extracts on oral glucose tolerance test in normal rats

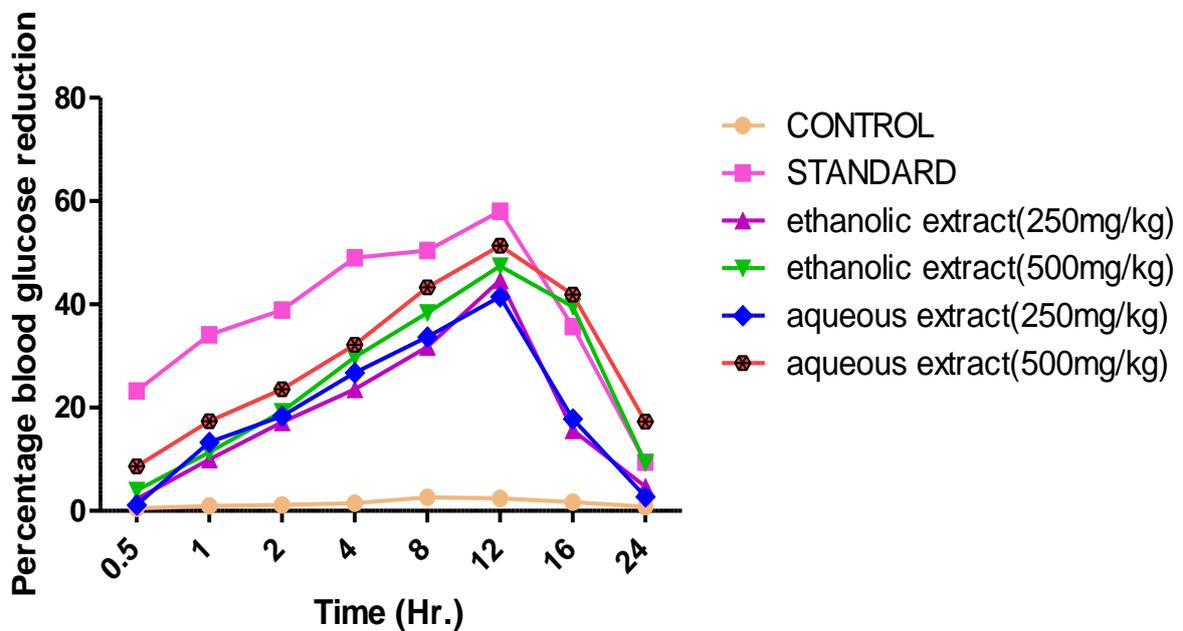


Fig. No. 3: Effect of *Ichnocarpus frutescens*. L flower extracts on blood glucose level in STZ induced diabetic rats (acute study)

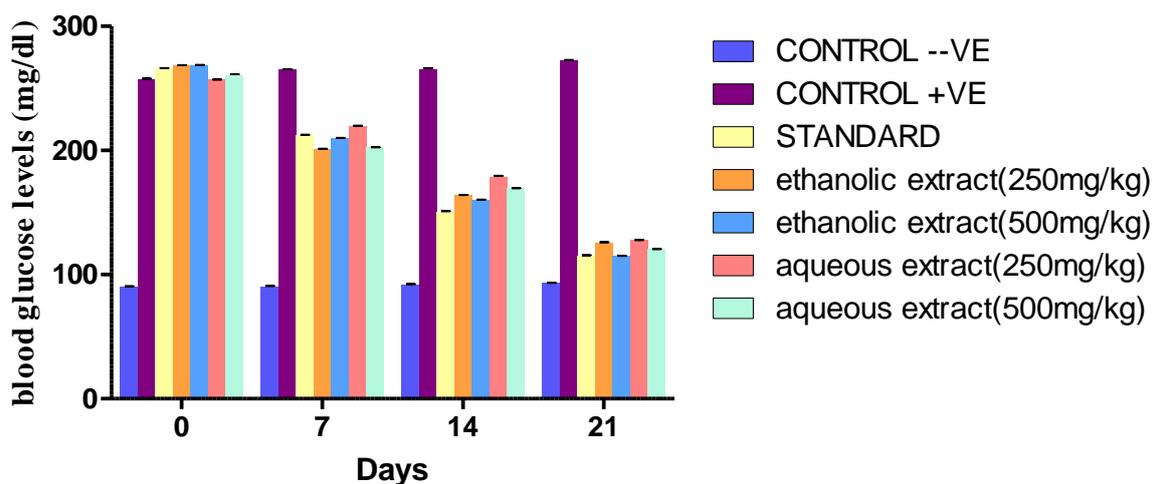


Fig. No. 4: Effect of *Ichnocarpus frutescens* L. flower extracts on blood glucose level in STZ induced diabetic rats (sub acute study)

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