

**HYPOGLYCEMIC AND ANTIDIABETIC ACTIVITY OF TUBEROUS ROOTS OF
DECALEPIS HAMILTONII IN NORMAL AND STREPTOZOTOCIN INDUCED
DIABETIC RATS**Venkata Suresh J^{1*}, Ganapaty S¹ and Venkat Rao N²¹Department of Pharmacognosy and Phytochemistry, College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, AP, India²Department of Pharmacology, V. L. College of Pharmacy, Raichur, Karnataka, India***Corresponding author e-mail:** vsjilakara@yahoo.co.in**ABSTRACT**

Alcoholic and aqueous extract of tuberous roots of *Decalepis hamiltonii* (DH) were prepared and given individually orally at different doses to different groups of rats fasted for 18 h (both normal and streptozotocin (STZ) induced diabetic albino rats). The serum glucose levels were measured initially at 0 h (before treatment) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 h after the treatment. The alcoholic extract of tuberous roots of *D. hamiltonii* (AETRDH) at higher dose (200 mg/kg) produced maximal serum glucose lowering effect in both normal and STZ induced diabetic rats. The aqueous extract of tuberous roots of *D. hamiltonii* (AQETRDH) produced maximal percent reduction in serum glucose levels with higher dose (400 mg/kg). AETRDH and AQETRDH produced hypoglycemic and antidiabetic activities at 3 h in normal and STZ induced diabetic rats in a dose dependent manner. The effect produced by AETRDH was found better than that of standard gliclazide (2 mg/kg) an oral hypoglycemic agent. The AETRDH has exhibited higher and better hypoglycemic and antidiabetic activity for a prolonged period than that of the AQETRDH.

Keywords: *Decalepis hamiltonii*, tuberous roots, Hypoglycemic, Gliclazide and Streptozotocin; Antidiabetic**INTRODUCTION**

Diabetes mellitus (DM) is a complex metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion or action or both¹. Although currently available therapeutic modalities can control many aspects of diabetes, serious complications are still encountered in addition to the side effects of the commonly used antidiabetic drugs². Contraindication to this, many traditional treatments have been recommended in the alternative system of medicine for treatment of DM³⁻⁵. This leads to continuous search for alternative drugs; hence it is prudent to look for options in herbal medicines for DM as well. Traditional/folklore medicines are only alternatives in such conditions. The traditional practitioners, folklore herbalists and local tribes of Tirumala Hills region (which lie geographically in the South-Eastern Ghats of Andhra Pradesh state,

India) claims that tuberous herbs are of great use in controlling the DM. The available literature shows that there are about 248 species, listed in the Flowering Plants of Chittoor District for treating DM⁶. Though some of the plants are reputed in the indigenous systems of medicine for their activities, it requires scientific evaluation.

Decalepis hamiltonii belongs to the family Asclepiadaceae (Periplocaceae). Locally known as Madina kammulu, Mavillinga kammulu and Neemam teega^{7, 8}. It is distributed in Deccan Peninsula and common in the forest areas of Western Ghats. The plant DH is reported to possess antidiabetic activity⁹. The survey of literature revealed that, no systematic and scientific studies have been carried out on DH. Hence in the present study attempts are made to investigate the hypoglycemic and antidiabetic effects of different

doses of alcoholic and aqueous extracts of tuberous roots of *D. hamiltonii* (TRDH) in normal and STZ induced diabetic rats.

MATERIALS AND METHODS

Collection of plant material: Tuberous roots of DH collected from Tirumala hills and were identified by a botanist Prof. P. Jayaraman Ph.D, Director, Plant Anatomy Research Center (PARC) West Tambaram, Chennai-45, India. A voucher specimen was deposited in the museum of the PARC, Chennai (voucher number PARC/2008/182). The tuberous roots were washed thoroughly to remove adhering soil and earthy matter, later on sliced to thin chips and dried in shade at room temperature and ground to optimal coarse powder.

Preparation of extracts: The above powdered material was subjected to a successive solvent extraction with petroleum ether (40-60^oc), chloroform, alcohol (95%) and water. Extraction was carried out for 16 h with each solvent by Soxhlet extractor. The yield of extracts was as follows, petroleum ether (2.5 %w/w), chloroform (1.33 %w/w), alcoholic (14.75 %w/w) and aqueous (12.36 %w/w). The alcohol and aqueous extracts were screened pharmacologically for hypoglycemic and antidiabetic activity.

Animals: Albino rats (Wistar strain) of either sex weighing between 150-200 g were procured from Sainath Agencies, Hyderabad-48. These animals were housed in standard environmental conditions in polyethylene cages (20x25x35cm) maintained at controlled room temperature (27±2^oc), relative humidity (45-55%) and light/dark cycle (12 : 12 h) and fed with standard commercial rat pellet diet (Amrut Laboratories, Pranav Agro industries Ltd. Sangli, India) and water *ad libitum*. The animals were acclimatized to the laboratory conditions. All the experimental procedures were performed according to the committee for the purpose of control and supervision of experiments on animals (Reg. number 557/02/c/CPCSEA) and approved by the Institutional Animal Ethical Committee (IAEC), of V.L. College of Pharmacy, Raichur, Karnataka, India.

Toxicity studies: The oral acute toxicity (LD₅₀) of the extracts was performed by using Albino mice of either sex weighing between 16 - 25 g as per the OECD guidelines No. 425 by fixed dose method. The LD₅₀ of AETRDH was found to be 1000 mg/kg. Therefore 1/20th (50 mg/kg), 1/10th (100 mg/kg) and 1/5th (200 mg/kg) were selected for the study. Whereas the LD₅₀ of AQETRDH was found to be

2000 mg/kg. Therefore 1/20th (100 mg/kg) 1/10th (200 mg/kg) and 1/5th (400 mg/kg) doses were selected for the present study.

Induction of diabetes: Streptozotocin (STZ) Biomol, International, U.S.A., was freshly prepared by dissolved in distilled water just before use. Diabetes was induced in 16 h fasted Albino rats (Wistar strain) of either sex (150-200 g) by intraperitoneal injection of STZ 60 mg/kg¹⁰⁻¹². The rats were then given 5 % w/v glucose solution in feeding bottles for the next 24 h to prevent hypoglycemia. After 72 h, rats with marked hyperglycemic fasting blood glucose > 250 mg/dl were selected and used for the study. All the animals were allowed free access to water and pellet diet and maintained at standard husbandry conditions.

Experimental design: Different groups of rats were used for studying the hypoglycemic and antidiabetic effects of AETRDH and AQETRDH. The rats were divided into 16 groups consisting of six rats each.

Group1: Normal rats treated with vehicle control (0.5 ml), p.o.
 Group2: Normal rats treated with gliclazide (2 mg/kg), p.o.
 Group3: Normal rats treated with 50 mg/kg of AETRDH, p.o.
 Group4: Normal rats treated with 100 mg/kg of AETRDH, p.o.
 Group5: Normal rats treated with 200 mg/kg of AETRDH, p.o.
 Group6: Normal rats treated with 100 mg/kg of AQETRDH, p.o.
 Group7: Normal rats treated with 200 mg/kg of AQETRDH, p.o.
 Group8: Normal rats treated with 400 mg/kg of AQETRDH, p.o.
 Group9: Diabetic rats treated with vehicle control (0.5 ml), p.o.
 Group10: Diabetic rats treated with gliclazide (2 mg/kg), p.o.
 Group11: Diabetic rats treated with 50 mg/kg of AETRDH, p.o.
 Group12: Diabetic rats treated with 100 mg/kg of AETRDH, p.o.
 Group13: Diabetic rats treated with 200 mg/kg of AETRDH, p.o.
 Group14: Diabetic rats treated with 100 mg/kg of AQETRDH, p.o.
 Group15: Diabetic rats treated with 200 mg/kg of AQETRDH, p.o.
 Group16: Diabetic rats treated with 400 mg/kg of AQETRDH, p.o.

All the animals were subjected to fasting for 18 h prior to experimentation and during the course of time the animals had free access to water. Different doses of both the extracts were suspended in distilled water and administered orally by gastric intubation, using a force feeding needle to the above mentioned groups of rats respectively. Groups 1 and 9 were served as normal and diabetic controls and received 0.5 ml vehicle (distilled water with few drops of 0.1N NaOH). Groups 2 and 10 received gliclazide 2 mg/kg orally. Blood samples were withdrawn from the tail vein initially at 0 h (before the treatment) and once again at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 h time intervals after the treatment. The collected blood samples were centrifuged (3000 rpm for 20 min) to get clear serum. The separated serum was used for estimation of glucose levels by GOD/POD method¹³ using commercial glucose kit (Span Diagnostics Ltd.,

Surat, India) by semi-auto analyzer (ERBA Mannheim, CHEM-5 plus v₂ Germany).

Statistical analysis: Results were expressed as mean±SD (n=6) and data were analyzed by one way ANOVA followed by Dunnett's test. The level of significance was setup at $p < 0.05^*$, 0.01^{**} and 0.001^{***} respectively.

RESULTS

The AETRDH and AQETRDH at different dose levels produced maximum percentage reduction in serum glucose levels at 3 h in normal and STZ induced diabetic Albino rats. Whereas the standard drug gliclazide (2 mg/kg) produced a biphasic response at 2 h and 8 h. In normal albino rats, AETRDH with different dose levels i.e, (50, 100 and 200 mg/kg) has reduced the serum glucose levels by (32.88±0.88%), (38.45±1.85%) and (46.91±1.54%) at 3 h respectively. Similarly AQETRDH with 100, 200 and 400 mg/kg doses has reduced the serum glucose levels by (28.09±1.87%), (32.06±1.15%) and (38.25±1.76%) at 3 h respectively. Gliclazide (2 mg/kg) has produced percentage reduction in serum glucose levels (40.41 ± 0.30) at 2 h and (35.57 ± 0.23) at 8 h intervals in normal healthy Albino rats. The detailed results of the percentage reduction in serum glucose levels of AETRDH and AQETRDH in normal rats are compiled in table 1 and figures 1 and 2 respectively.

In STZ induced diabetic rats, the AETRDH with different dose levels i.e.; (50, 100 and 200 mg/kg) has reduced the serum glucose levels by (39.23±1.91%), (44.19±2.93%) and (49.09±2.73%) at 3 h respectively. Similarly AQETRDH with 100, 200 and 400 mg/kg doses has reduced the serum glucose levels by (31.26±1.07%), (35.01±2.46%) and (39.48±2.91%) at 3 h respectively. Gliclazide (2 mg/kg) has produced percentage reduction in serum glucose levels (45.34 ± 0.41) at 2 h and (38.91 ± 0.20) at 8 h intervals. The detailed results of the percentage reduction in serum glucose levels of AETRDH and AQETRDH in STZ induced diabetic rats are compiled in table 2 and figures 3 and 4 respectively.

The hypoglycemic and antidiabetic activity produced by AETRDH at 200 mg/kg was better than that of the standard drug gliclazide (2 mg/kg). Whereas the medium (100 mg/kg) and low (50 mg/kg) doses of AETRDH and different doses (100, 200 and 400 mg/kg) of AQETRDH has produced no significant effect in normal and STZ induced diabetic rats when compared with standard drug gliclazide (2 mg/kg).

DISCUSSION

In the present study, the hypoglycemic and antidiabetic activity of vehicle, gliclazide (2 mg/kg), different doses of AETRDH (50, 100 and 200 mg/kg) and AQETRDH (100, 200, and 400 mg/kg) were evaluated in normal and STZ induced diabetic rats. STZ [2-deoxy-2-(3-methyl-3-nitrosourea) 1-D-glucopyranose], a broad spectrum antibiotic, produced from *Streptomyces achromogens*. The diabetogenic property of STZ was first described by Rakietyen., et al¹⁴ and is well known for its selective cytotoxicity of pancreatic islet of β -cells and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms. STZ has shows a triphasic response on serum glucose levels. Initially, serum glucose is increased after 3 h, reaching values of 150-200 mg%. 6-8 h after STZ induction, the serum insulin values are increased up to 4 times, resulting in a hypoglycemic phase which is followed by persistent hyperglycemia by 24-48 h after STZ administration^{15, 16, 10-12}.

In normal and STZ induced diabetic Albino rats, single dose treatment of gliclazide a sulphonylurea drug produced biphasic (maximum reduction in serum glucose levels at 2 different time intervals) response on serum glucose levels at 2 h and 8 h. The biphasic response of gliclazide in rat model may be due to its enterohepatic circulation in rats^{17, 18}. Gliclazide acts by both pancreatic (Insulin release by K^+ channel inhibition in the β -cells) and extra pancreatic (tissue uptake of glucose) mechanisms. The target for sulphonylurea activity is ATP sensitive K^+ channel (K^+ ATP channel). The sulphonylureas and related drugs used in type II diabetes stimulate insulin by closing K^+ ATP channels in pancreatic β -cells.

The sulphonylureas target the sulphonylurea receptor (SUR) subunit of K^+ ATP channels, which exists in several isoforms expressed in different tissues, SUR 1 in pancreatic β -cells, SUR 2A in cardiac muscle and SUR 2B in vascular smooth muscle¹⁹. The pancreatic β -cells ATP increase when plasma glucose level rises resulting in the closure of K^+ ATP channel in plasma membrane, allows the cells to depolarize, triggering Ca^{2+} entry and insulin release^{20, 21}. The AETRDH at higher dose (200 mg/kg) in normal and STZ induced diabetic Albino rats exhibited good monophasic hypoglycemic and antidiabetic activity at 3 h than that of gliclazide (2 mg/kg). Whereas the AETRDH at 50 and 100 mg/kg showed moderate hypoglycemic and antidiabetic activity at 3 h. The

AQETRDH at 200 and 400 mg/kg was exhibited moderate hypoglycemic and antidiabetic activity at 3 h in normal and STZ induced diabetic Albino rats. But these effects are found to be lower than that of gliclazide (2 mg/kg). Whereas the low dose (100 mg/kg) of AQETRDH produced marginal hypoglycemic activity at 3 h. It was observed that both the AETRDH and AQETRDH showed hypoglycemia in a dose dependent manner. The AETRDH has higher and better hypoglycemic activity for a prolonged period than that of AQETRDH in normal and STZ induced diabetic albino rats.

It is evident from the literature that voluminous amount of research has been done on various species and has established the high usefulness of it in controlling diabetes. *M.Charantia* aqueous extract (2.5 g/kg b.w.) produced 45% of hypoglycemic activity in normal rats after 4 h of treatment²². Higashino *et al*²³ showed 34% of hypoglycemic activity of water soluble fraction of *M.Charantia* after 3 h in streptozotocin induced diabetic rats. Welihinda *et al*²⁴ demonstrated that an aqueous extract from the *M.Charantia* was a potent stimulator of insulin release from β -cells rich pancreatic islets isolated from obese-hyperglycemic mice.

Some of the plants reported for their antidiabetic activity by increasing serum insulin levels significantly, those includes *Gymnema sylvestre* increases insulin secretion probably by regeneration of pancreatic β -cells²⁵, *Aegle marmelos*²⁶, *Prunella vulgaris* L²⁷, are reported to regenerate atrophied pancreatic islets, restore the secretion of insulin and thus corrected hyperglycemia. *M.Charantia* fruit is reported to have insulin secretagogue and Insulinomimetic activity²⁸. *Trigonella foenumgraecum* and *Allium sativum* L²⁹ are reported to act by stimulating insulin secretion. One of the main

constituent of *M.Charantia* is a steroidal saponins, charantin and is responsible for the antidiabetic effect of the fruit; It is also contains momordicine and insulin like steroidal saponin³⁰.

Koneri Raju and Balaraman, R¹⁶ studied the antidiabetic mechanisms of saponins of *M. cymbalaria* in STZ induced diabetic rats. In view of the above, the hypoglycemic and antidiabetic activity of tuberous roots of *D. hamiltonii* may be due to its stimulating effect on the remnant β -cells or improvement in insulin action at cellular level or it could also be due to the insulin like effect of the active principle(s) present in the extract. Steroidal saponins are reported for the antidiabetic activity and the preliminary phytochemical investigation of the above two extracts also revealed the presence of steroidal saponins, hence these can be accounted for hypoglycemic and antidiabetic activities.

CONCLUSIONS

From this study it can be concluded that AETRDH possess beneficial effects on serum glucose levels in normal and STZ induced diabetic Albino rats. Further pharmacological and biochemical investigations are under progress to elucidate the mechanism of the hypoglycemic and antidiabetic effect of extract.

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Table 1: Hypoglycemic activity of AETRDH and AQETRDH in normal albino rats

% Reduction in serum glucose levels at different time intervals								
Groups →	Control (0.1N NaOH)	Gliclazide	Alcoholic extract of tuberous roots of <i>D. hamiltonii</i>			Aqueous extract of tuberous roots of <i>D. hamiltonii</i>		
Dose (mg/kg) →	0.5 (ml)	2	50	100	200	100	200	400
Time (h)↓	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
0	-	-	-	-	-	-	-	-
0.5	0.79 ± 0.30	11.02± 0.15**	1.73 ± 1.67	3.98 ± 1.82*	6.71 ± 1.75**	0.79 ± 2.92	1.96 ± 1.67	4.11 ± 1.83*
1	0.30 ± 0.66	23.24± 0.19**	11.17 ± 3.01**	15.60 ± 2.68**	20.11 ± 0.62**	7.11 ± 2.09**	10.27 ± 2.61**	16.70 ± 1.07**
2	0.01 ± 0.19	40.41± 0.30**	20.45 ± 1.61**	27.25 ± 1.54**	34.61 ± 1.06**	16.97 ± 1.69**	21.72 ± 1.71**	25.13 ± 1.60**
3	1.11 ± 0.87	26.04± 0.27**	32.88 ± 0.88**	38.45 ± 1.85**	46.91 ± 1.54**	28.09 ± 1.87**	32.06 ± 1.15**	38.25 ± 1.76**
4	-0.07 ± 0.89	7.52 ± 0.16**	29.77 ± 2.73**	36.13 ± 2.71**	44.33 ± 1.94**	20.14 ± 2.87**	25.65 ± 2.74**	31.48 ± 2.90**
6	0.06 ± 0.46	19.86± 0.31**	26.46 ± 2.87**	35.70 ± 1.60**	41.92 ± 2.99**	14.90 ± 2.78**	21.40 ± 2.46**	26.37 ± 2.04**
8	1.55 ± 0.87	35.57± 0.23**	20.93 ± 1.86**	33.58 ± 2.15**	39.16 ± 2.25**	9.07 ± 1.59**	16.70 ± 1.70**	20.39 ± 2.78**
12	0.18 ± 0.96	19.86± 0.39**	16.19 ± 0.04**	28.70 ± 1.72**	36.57 ± 2.76**	4.98 ± 2.72**	8.98 ± 2.70**	11.48 ± 3.39**
16	-0.07 ± 0.48	10.45± 0.28**	10.58 ± 1.52**	21.37 ± 1.63**	25.75 ± 2.13**	2.13 ± 2.71	4.01 ± 1.87**	6.98 ± 2.67**
20	2.37 ± 0.28	4.39 ± 0.25	8.14 ± 1.15**	11.14 ± 2.00**	17.09 ± 1.81**	0.98 ± 1.94	1.28 ± 1.74	2.52 ± 2.25
24	1.03 ± 0.08	1.90 ± 0.19**	5.09 ± 1.06**	7.01 ± 1.65**	10.98 ± 1.48**	0.11 ± 2.83	0.91 ± 2.76	1.89 ± 1.58

n=6 significant at p<0.05*, 0.01** and 0.001***.

Table 2: Antidiabetic activity of AETRDH and AQETRDH in streptozotocin induced diabetic albino rats

% Reduction in serum glucose levels at different time intervals								
Groups →	Control (0.1N NaOH)	Gliclazide	Alcoholic extract of tuberous roots of <i>D. hamiltonii</i>			Aqueous extract of tuberous roots of <i>D. hamiltonii</i>		
Dose (mg/kg) →	0.5 (ml)	2	50	100	200	100	200	400
Time (h)↓	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
0	-	-	-	-	-	-	-	-
0.5	0.64 ± 0.15	15.81± 0.15**	3.54 ± 1.7	6.81 ± 2.78**	9.13 ± 2.83**	2.72 ± 2.05	4.28 ± 2.78*	7.18 ± 2.73**
1	0.13 ± 0.39	30.25± 0.91**	15.25 ± 2.61**	20.64 ± 2.94**	24.61 ± 2.63**	10.98 ± 2.61**	15.12 ± 1.13**	19.97 ± 2.25**
2	0.29 ± 0.40	45.34± 0.41**	26.95 ± 1.62**	32.88 ± 2.64**	35.09 ± 2.50**	22.13 ± 2.95**	28.96 ± 2.71**	31.25 ± 1.72**
3	0.62 ± 0.54	32.34± 0.53**	39.23 ± 1.91**	44.19 ± 2.93**	49.09 ± 2.73**	31.26 ± 1.07**	35.01 ± 2.46**	39.48 ± 2.91**
4	0.18 ± 0.51	16.08± 0.50**	36.92 ± 0.04**	39.54 ± 2.71**	46.73 ± 1.75**	26.02 ± 1.60**	30.63 ± 1.69**	35.62 ± 1.87**
6	0.40 ± 0.03	22.85± 0.03**	34.63 ± 1.87**	37.82 ± 2.67**	44.35 ± 0.02**	21.84 ± 1.76**	26.39 ± 2.82**	29.10 ± 2.66**
8	-0.14 ± 0.96	38.91± 0.20**	32.20 ± 2.90**	34.78 ± 0.07**	42.07 ± 2.76**	16.73 ± 1.74**	20.84 ± 1.82**	25.79 ± 1.60**
12	0.04 ± 0.74	23.82± 0.34**	29.99 ± 1.25**	32.01 ± 1.70**	39.86 ± 0.04**	10.16 ± 2.06**	14.33 ± 2.61**	18.01 ± 2.71**
16	0.41 ± 0.60	16.78± 0.49**	19.57 ± 1.18**	26.72 ± 2.86**	30.42 ± 2.02**	6.27 ± 1.96**	8.99 ± 2.78**	12.54 ± 2.49**
20	0.07 ± 1.01	7.30 ± 0.12**	13.18 ± 2.67**	19.98 ± 1.87**	22.60 ± 2.88**	3.83 ± 2.73	5.03 ± 1.87	7.02 ± 1.71**
24	0.28 ± 0.28	0.96 ± 0.06**	9.81 ± 1.83	15.44 ± 1.74	19.30 ± 1.73	1.10 ± 2.92	2.10 ± 1.67	2.91 ± 2.87

n=6 significant at p<0.05*, 0.01** and 0.001***.

Hypoglycemic activity of AETRDH and AQETRDH in normal Albino rats

Fig. 1: % Reduction in serum glucose levels of AETRDH at different time intervals

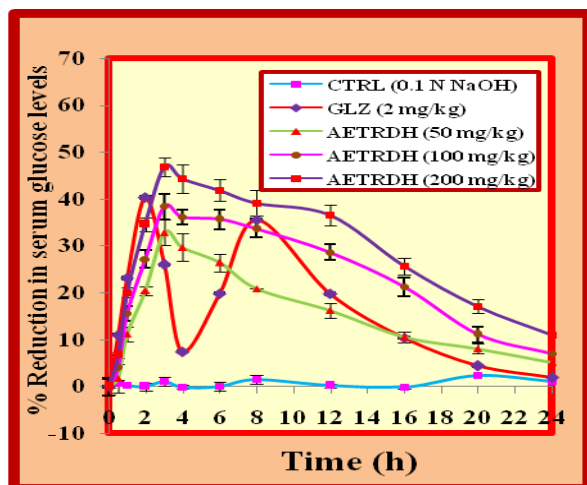
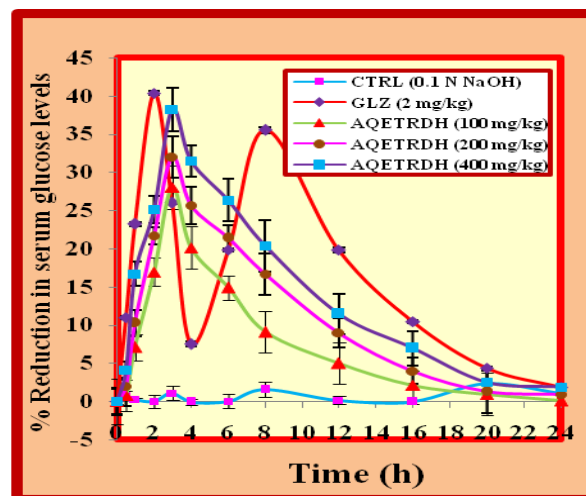


Fig. 2: % Reduction in serum glucose levels of AQETRDH at different time intervals



Antidiabetic activity of AETRDH and AQETRDH in STZ induced diabetic Albino rats

Fig. 3: % Reduction in serum glucose levels of AETRDH at different time intervals

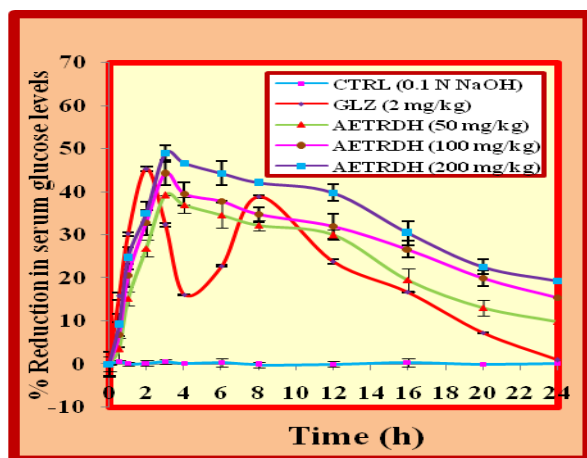
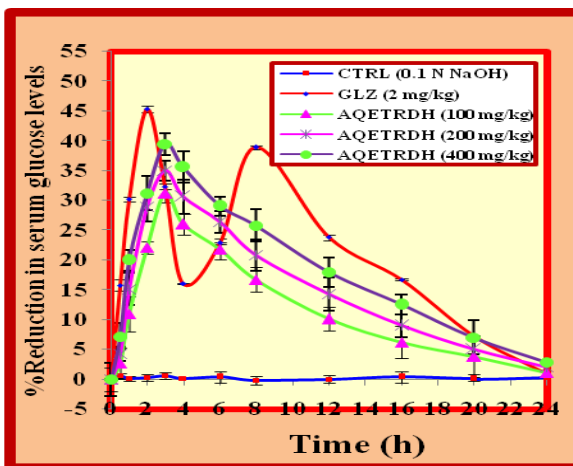


Fig. 4: % Reduction in serum glucose levels of AQETRDH at different time intervals



CTRL- Control, GLZ- Gliclazide, AETRDH- Alcoholic extract tuberous roots of *D. hamiltonii*, AQETRDH- Aqueous extracts tuberous roots of *D. hamiltonii*.

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