ANTIHYPERGLYCAEMIC ACTIVITY OF ETHANOL EXTRACT OF HELICTERES ISORA FRUITS IN ALLOXAN INDUCED DIABETIC MICE

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Summary

The antihyperglycaemic activity of the ethanol extract of Helicteres isora (EEHI) fruits was studied by administering three doses of EEHI (i. e.100, 200 and 400 mg/kg, p. o.) to alloxan (70 mg/kg, i. v.) induced diabetic mice. The serum glucose levels and body weights of mice were determined. The acute oral toxicity study showed no mortality up to 5000 mg/kg p. o. dose of EEHI. In acute study the maximum reduction in serum glucose level was observed at 2h (59.86 mg/dl), peak at 6 h (167.59 mg/dl) but antihyperglycaemic effect was vanished at 24 h (76.99 mg/dl). In glyburide treated mice the reduction in serum glucose level was observed at 2 h (94.04 mg/dl) and peak at 6 h (237.90 mg/dl) respectively. The subacute study was also carried out which showed maximum reduction in serum glucose level (221.42 mg/dl) at the dose of 400 mg/kg on 35th day. In glyburide treated mice maximum reduction in serum glucose level (312.28 mg/dl) was observed on 21st day. The oral glucose tolerance test (OGTT) was carried out after administration of EEHI in non-diabetic and diabetic mice previously loaded with (2.5 g/kg, p. o.) glucose. EEHI (400 mg/kg) showed increased glucose threshold in non-diabetic and diabetic mice. The EEHI(400 mg/kg) prevented the loss of body weight. These results indicated antihyperglycaemic activity of EEHI (400 mg/kg) in alloxan induced diabetic mice. The antihyperglycaemic activity of EEHI was comparable with glyburide.

Keywords: *Helicteres isora*, Alloxan, Antihyperglycaemic, Body weight, Oral glucose tolerance test.

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Introduction

Diabetes mellitus is a metabolic disorder characterized by a predisposition to developing significantly raised blood glucose. It is a world wide health problem affecting millions in both developed and developing countries. The incidence and prevalence of diabetes vary significantly with geographic location.Type-2 diabetes has high prevalence in Indians. About 31.7 millions cases of diabetes had been estimated in India in 2000.Many of the currently available treatments for type-2 diabetes aim to reduce hyperglycemia, but none have so far convincingly demonstrated that they can significantly alter the natural history of the progressive loss of pancreatic insulin secretion. Hence emphasis is on the development of drugs from plants for the treatment of diabetes mellitus (1).

Helicteres isora Linn. (sterculiaceae) is a shrub or small tree growing gregariously throughout India at forest edges.The fruits of this plant are commonly called as Mrigashringa in Sanskrit; Kewan or Muradsing in Marathi; Bhendu, Marorphali in Hindi; and East Indian Screw tree in English.The fruits are greenish brown beaked, cork screw like 5-follicles;seeds numerous, angular and testa wrinkled.

Muradshing is commonly used plant in ayurvedic system of medicine. It has a variety of uses such as, the root bark possess expectorant, demulcent, astringent to bowels, antidysenteric, antidiarrhoeal, antidiabetic and antihyperlipidemic actions.The dried fruits are used in intestinal disorders, colic pains, flatulence, diarrhea and worms. The seeds, stem and root bark are useful in the treatment of diabetes(2,3). Since the systematic study of anti diabetic activity of *H. issora* fruits has not been still reported; hence study was undertaken to evaluate antihyperglycaemic activity of EEHI in alloxan induced diabetic mice.

Materials and Methods

Collection and authentication of plant

The dried fruits of Helicteres isora Linn.were procured from the crude drug market of Pune in Maharastra state and were authenticated by Dr. A.M. Mujumdar, Department of Botany, at Agharkar Research Institute,Pune and voucher specimen was deposited at that Institute as Voucher No.AHMA L-02 on 29-07-2006.

Drugs and Chemicals

Glyburide (Ranbaxy Pharma. Ltd. India), alloxan monohydrate (Spectrochem,India), glucose estimation kit (Accurex Biomedical Pvt. Ltd., India) and d -glucose(S.D.FineChem. Ltd, India) and ethanol (Merck,Mumbai,India) were purchased from respective vendors.

Extraction and preparation of EEHI

The dried fruits of Helicteres isora were powered in grinder. The air dried powder was subjected to hot continuous extraction with ethanol in a soxhlet extractor and filtered. The filtrate was evaporated at room temperature and the extract concentrated . The % yield of ethanol extract was 2.24 % w/w. The EEHI was dissolved in distilled water to prepare the drug solution of concentration of 100 mg/ml and used for pharmacological studies.

Experimental animals
Swiss albino mice (25-30 g) were purchased from the National Toxicology Centre, Pune, India. Animals were housed under standard condition of temperature 25 ± 1ºC and relative humidity of 45% to 55% under 12-h light: 12-h dark cycle. The animals had free access to food pellets (Chakan Oil Mills, Pune, India), and water was given *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Poona College of Pharmacy, Pune, India.

Acute oral toxicity studies
Healthy adult Swiss albino mice of female sex weighing between 18 to 23 g were subjected to acute toxicity studies as per guidelines (AOT no. 425) suggested by the Organization for Economic Cooperation and Development (5). The mice were observed continuously for 2 h for behavioral, neurological and autonomic profiles for any lethality or death for the next 48 h.

Induction of experimental diabetes
Diabetes was induced in mice by a single intravenous injection of aqueous alloxan monohydrate (70 mg /kg i.v.). After 48 h, the animals showing serum glucose levels above 200 mg /dl (diabetic) were selected for the study (6). All the animals were allowed free access to water and pellet diet.

Collection of blood and determination of serum glucose
Blood samples were collected by retro-orbital puncture (ROP) technique. The collected blood samples were analyzed for glucose levels by the glucose oxidase peroxidase (GOD/POD) method (7) and serum glucose levels were expressed in mg/dl.

Effect of EEHI on serum glucose in alloxan-induced diabetic mice
The diabetic mice were divided into five groups (n =6), viz.: group I-vehicle (distilled water, 10 ml/kg); group II-glyburide (10 mg/kg); group III EEHI (100 mg/kg); group IV- EEHI (200 mg/kg); group V- EEHI (400mg/kg). All drugs were given orally. The acute study involved estimation of serum glucose at 0, 2, 4, 6 and 24 h after drug administration (8)**.** The subacute study involved repeated administration of drug for 28 days at prefixed times and serum glucose levels were estimated on the 7^{th}, 14^{th}, 21^{st} and 28^{th} day. At the end of 28 days the drug administration was stopped and a rest period of 7 days was given to the animals to study effect of drug treatment on blood glucose after 7 days i.e. on 35th day. The data were represented as mean serum glucose level and standard error of mean (SEM). The mice were weighed daily during the study period of 35 days and their body weights were noted and presented as mean change in body weights.

Effect of EEHI on oral glucose tolerance test (OGTT) in non-diabetic and diabetic mice
The animals were fasted overnight before commencing the experiment.Non diabetic and diabetic mice were divided into three groups (n=6),viz.groupI-d-glucose (2.5 g/kg); group-II glyburide (10mg/kg);group-III EEHI(400 mg/kg). d-glucose (2.5 g/kg) was administered in non-diabetic and diabetic mice at the 4th h of pretreatment with EEHI and glyburide. Serum glucose levels were estimated before and 2 h after glucose loading.

Statistical analysis

The results are expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by *post hoc* Tukey's test (9).

Results

In acute oral toxicity study, EEHI was safe upto a dose level of 5000 mg/kg of body weight. No lethality or any toxic reactions were found upto the end of the study period.

In acute study, EEHI (100,200 and 400 mg/kg) as well as glyburide (10 mg/kg) showed significant reduction of serum glucose levels at 2, 4, and 6 h. The onset of reduction of serum glucose of EEHI (100, 200 and 400 mg/kg) treated mice was observed at 2 h (54.43,55.13 and 59.86mg/dl respectively), peak effect at 6 h (96.70, 121.48 and 167.59mg/dl respectively) but effect was waned at 24 h. The onset of antihyperglycaemic effect of glyburide was at 2 h (94.04 mg/dl), the peak effect was at 6 h (237.90 mg/dl) (Table 1).

In the subacute study, repeated administration (once a day for 28 days) of the EEHI as well as glyburide caused significantly ($P < 0.001$) reduction in the serum glucose level as compared with vehicle treated group. Maximum reduction in serum glucose level was observed (221.42 mg/dl) on 35thday in the diabetic mice treated with EEHI (400 mg/kg). Maximum reduction in serum glucose level was observed (179.66 and 219.92 mg/dl respectively) on 35th day in the diabetic mice treated with EEHI(100 and 400 mg/kg respectively). Glyburide treated animals showed maximum reduction in serum glucose level (312.28 mg/dl) on 21stday (Table 2)

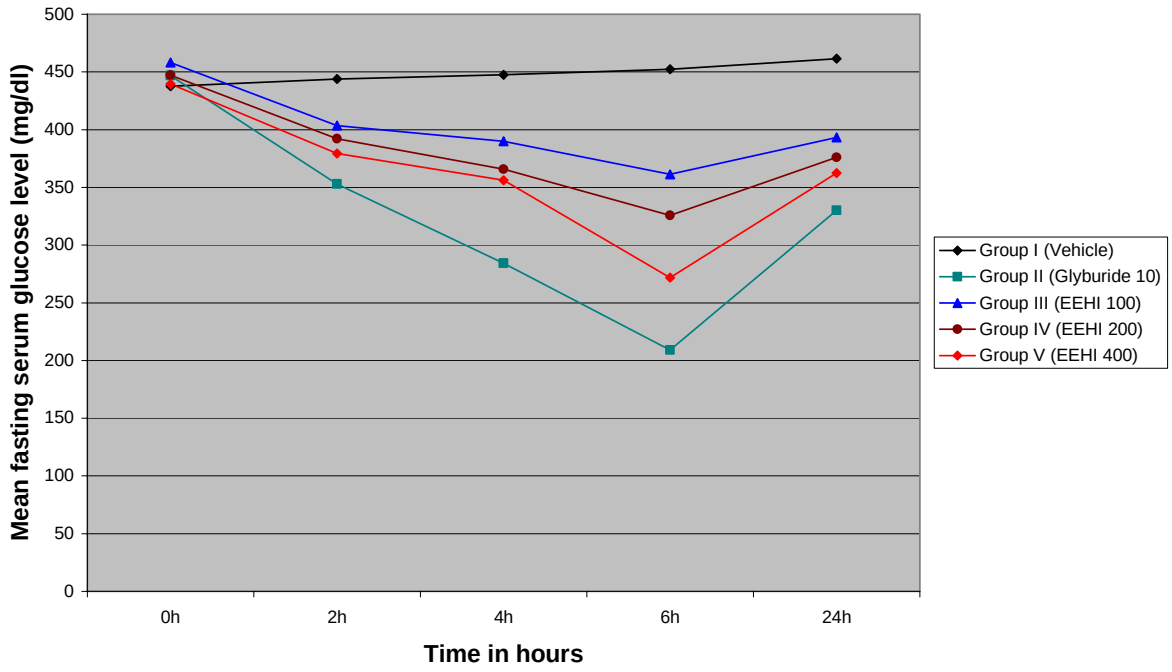
Body weight of vehicle and EEHI (100 and 200 mg/kg) treated diabetic mice decreased during study period. EEHI (400 mg/kg) and glyburide (10 mg/kg) prevented further loss of body weight in diabetic mice. On the other hand, mice gained body weight which indicated beneficial effect of EEHI (Table 3).

In oral glucose tolerance test, EEHI (400 mg/kg) produced nonsignificantly ($P<0.001$) increase in glucose threshold, 4th h post glucose loading in non-diabetic (Table 4) as well as diabetic (Table 5) mice respectively. These results suggest that EEHI possessed antihyperglycaemic activity in alloxan induced diabetic mice.

Table 1: Effect of EEHI on serum glucose level in alloxan-induced diabetic mice (acute study).

Groups (Treatment mg/kg p.o.)	Mean Fasting Serum Glucose Level (mg/dl) ± SEM				
	0h	2h	4h	6h	24h
Group I (Vehicle)	437.50 ± 9.66	443.74 ± 12.58	447.60 ± 13.26	452.40 ± 16.58	461.55 ± 12.67
Group II (Glyburide 10)	446.83 ± 12.63	352.79 ± 11.89***	284.24 ± 11.43***	208.93 ± 18.90***	330.09 ± 20.45***
Group III (EEHI 100)	458.00 ± 11.46	403.57 ± 11.70	389.80 ± 11.71	361.30 ± 13.33**	393.25 ± 13.15
Group IV (EEHI 200)	447.33 ± 14.03	392.20 ± 15.47	365.61 ± 15.50**	325.85 ± 17.38***	376.10 ± 16.98*
Group V (EEHI 400)	439.33 ± 13.24	379.47 ± 14.28*	356.38 ± 14.32***	271.74 ± 16.93***	362.34 ± 16.09**

CHART-1 EFFECT OF EEHI ON SERUM GLUCOSE LEVEL IN ALLOXAN INDUCED DIABETIC MICE (ACUTE STUDY)

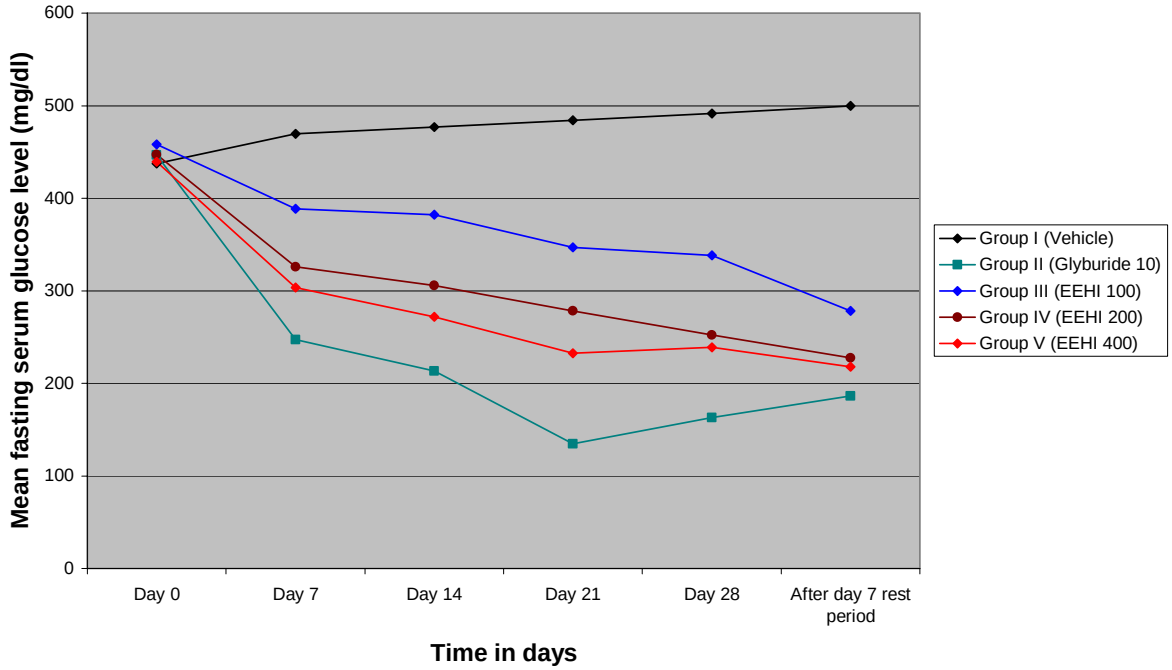


Values are mean ± SEM,n=6 in each group, data were analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software, *P<0.05, **P<0.01,***P<0.001 as compared with vehicle-treated group (distilled water, 10 ml/kg).

Table 2: Effect of EEHI on serum glucose level in alloxan-induced diabetic mice (subacute study).

Groups (Treatment.mg/kg p.o.)	Mean Fasting Serum Glucose Level (mg/dl) ± SEM					
	Day 0	Day 7	Day 14	Day 21	Day 28	After day 7 rest period
Group I (Vehicle)	437.50 ± 9.66	469.48 ± 14.67	476.93 ± 14.81	484.01 ± 15.59	491.67 ± 15.91	499.67 ± 15.72
Group II (Glyburide 10)	446.83 ± 12.63	247.23 ± 17.56***	213.42 ± 16.70***	134.55 ± 20.02***	163.11 ± 19.59***	186.05 ± 19.21***
Group III (EEHI 100)	458.00 ± 11.46	388.33 ± 13.32*	381.97 ± 13.56**	346.77 ± 14.23***	338.37 ± 13.64***	278.34 ± 14.16***
Group IV (EEHI 200)	447.33 ± 14.03	325.88 ± 17.73***	305.75 ± 17.70***	278.37 ± 17.79***	252.13 ± 17.24***	227.41 ± 17.54***
Group V (EEHI 400)	439.33 ± 13.24	303.37 ± 17.00***	271.78 ± 17.14***	232.28 ± 17.07***	239.13 ± 17.07***	217.91 ± 16.95***

CHART-2 EFFECT OF EEHI ON SERUM GLUCOSE LEVEL IN ALLOXAN INDUCED DIABETIC MICE (SUBACUTE STUDY)

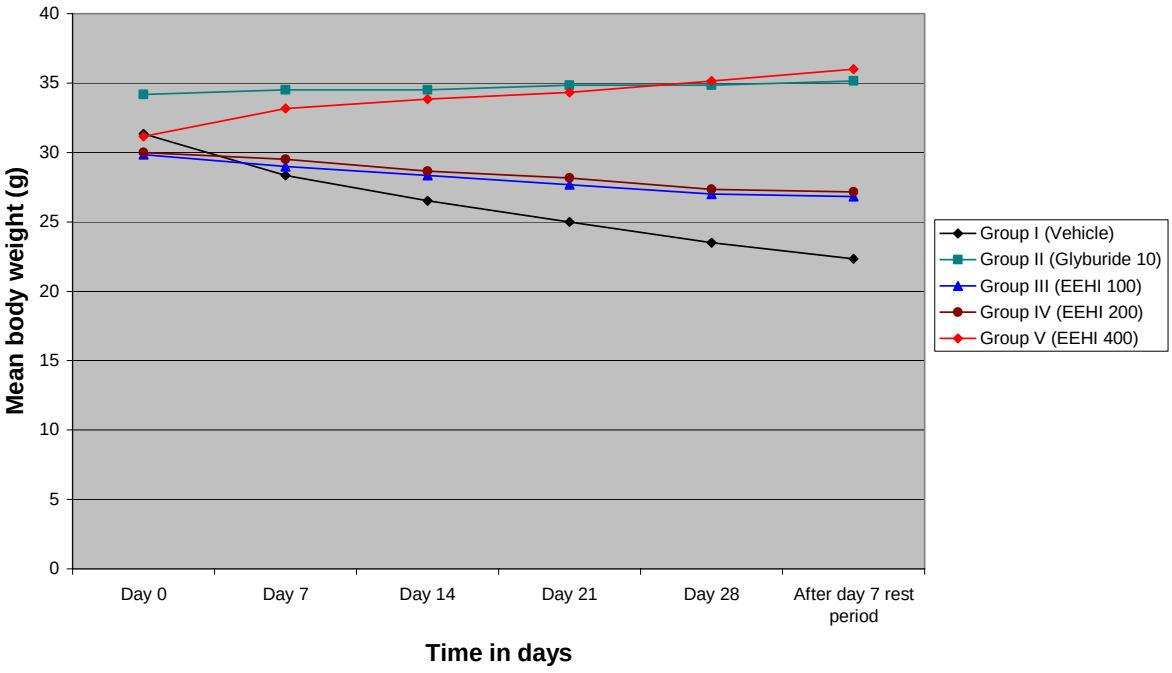


Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one way ANOVA followed by Tukey's test using Graphpad Instat software, ns- not significant, **P<0.01.All other values are significant (P<0.001) as compared with vehicle-treated group (distilled water, 10 ml/kg).

Table 3: Effect of EEHI on body weight in alloxan-induced diabetic mice.

Groups (Treatmentmg/kg p.o.)	Mean Body Weight (g) ± SEM					
	Day 0	Day 7	Day 14	Day 21	Day 28	After day 7 rest period
Group I (Vehicle)	31.33 ± 1.14	28.33 ± 1.05	26.50 ± 0.99	25.00 ± 1.06	23.50 ± 1.11	22.33 ± 1.14
Group II (Glyburide 10)	34.16 ± 0.94	34.50 ± 0.61**	34.50 ± 1.20***	34.83 ± 0.90***	34.83 ± 1.13***	35.16 ± 1.19***
Group III (EEHI 100)	29.83 ± 0.65	29.00 ± 0.57	28.33 ± 0.88	27.66 ± 0.95	27.00 ± 0.85	26.83 ± 0.79
Group IV (EEHI 200)	30.00 ± 0.81	29.50 ± 0.84	28.66 ± 0.88	28.16 ± 1.10	27.33 ± 0.84	27.16 ± 0.83
Group V (EEHI 400)	31.16 ± 1.47	33.16 ± 0.87*	33.83 ± 0.79***	34.33 ± 0.71***	35.16 ± 0.70***	36.00 ± 0.63***

CHART-3 EFFECT OF EEHI ON BODY WEIGHT IN ALLOXAN INDUCED DIABETIC MICE

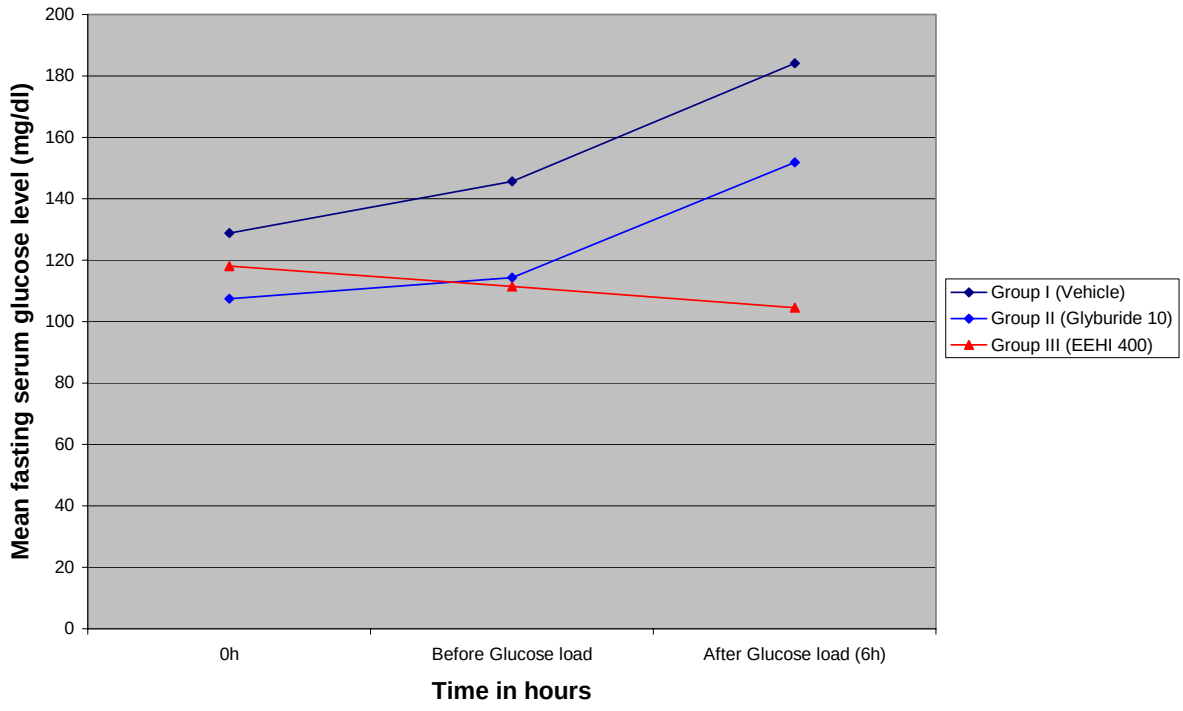


Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software, *P<0.05, **P<0.01, ***P<0.001.All other values are not significant as compared with vehicle-treated group (distilled water, 10 ml/kg).

Table 4: Effect of EEHI on oral glucose tolerance test (OGTT) in non-diabetic mice.

Groups (Treatment.mg/kg p.o.)	Mean Fasting Serum Glucose Level (mg/dl) ± SEM		
	0h	Before Glucose load	After Glucose load (6h)
Group I (Vehicle)	128.84 ± 5.03	145.67 ± 8.28	184.19 ± 11.44
Group II (Glyburide 10)	107.50 ± 5.37	114.39 ± 4.82***	151.85 ± 6.77
Group III (EEHI 400)	118.07 ± 14.10	111.46 ± 16.73	104.51 ± 14.91

CHART-4 EFFECT OF EEHI ON OGTT IN NONDIABETIC MICE

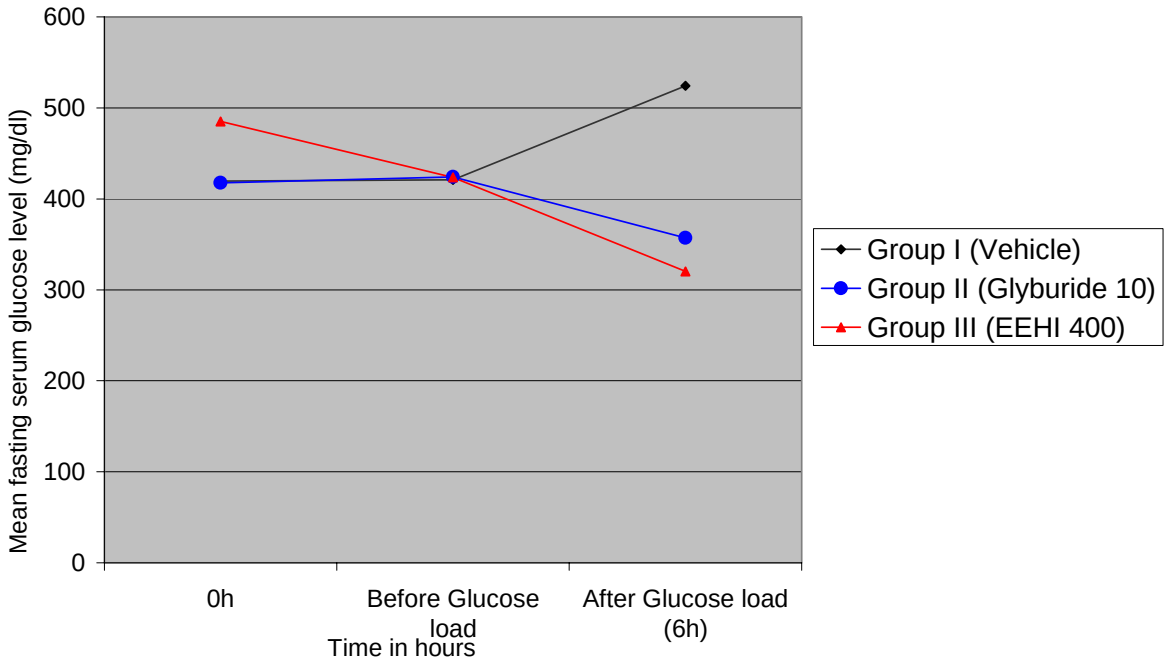


d-glucose (2.5 g/kg) was administered in non-diabetic mice at the 4th h of pretreatment with EEHI and glyburide. Serum glucose levels were estimated before and 2 h after glucose loading. Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software, ***P<0.001 as compared with vehicle-treated group (distilled water, 10 ml/kg).

Table 5: Effect of EEHI on oral glucose tolerance test (OGTT) in diabetic mice.

Groups (Treatment.mg/kg p.o.)	Mean Fasting Serum Glucose Level (mg/dl) ± SEM		
	0h	Before Glucose load	After Glucose load (6h)
Group I (Vehicle)	419.52 ±4.99	420.86 ± 5.37	523.93 ± 5.84
Group II (Glyburide 10)	417.44 ± 9.45	424.22 ± 6.18	357.19 ± 9.41***
Group III (EEHI 400)	484.90 ± 10.36***	423.55 ± 8.70	320.32 ± 16.87***

CHART-5 EFFECT OF EEHI ON OGTT IN DIABETIC MICE



d-glucose (2.5 g/kg) was administered in diabetic mice at the 4th h of pretreatment with EEHI and glyburide. Serum glucose levels were estimated before and 2 h after glucose loading. Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one- way ANOVA followed by Tukey's test using Graphpad Instat software, *P<0.05, ***P<0.001 as compared with vehicle-treated group (distilled water, 10 ml/kg).

Discussion

Various chemical constituents like alkaloids, flavonoids, tannins, glycosides, reducing sugars,terpenes, anthraquinones are reported (4).

EEHI (100, 200 and 400 mg/kg) showed significant ($P<0.001$) decrease in serum glucose level at 2, 4 and 6 h. Continuous treatment with EEHI (100, 200 and 400 mg/kg) for a period of 35 days showed a significant ($P<0.001$) decrease in the serum glucose level in diabetic mice. Maximum reduction of serum glucose level in acute and subacute study occurred at the dose of 400 mg/kg. p. o. The EEHI showed short onset and short duration of antihyperglycaemic action

Subacute treatment for 35 days with the EEHI in the treated doses brought about improvement in body weights indicating its beneficial effect in preventing loss of body weight in diabetic mice.The ability of EEHI to prevent body weight loss seems to be due to its ability to reduce hyperglycaemia.

OGTT study indicated that EEHI enhanced glucose utilization in non- diabetic & diabetic mice. Administration of EEHI effectively prevented the increase in serum glucose level without causing a hypoglycaemic state. The effect may be due to restoration of the delayed insulin response. In this context, other medicinal plants, such as *Ficus racemosa* (10), *Ficus religiosa* (11) and Psidium guajava (12) have been reported to possess similar effect.

Kameswararao et al reported that flavonoids ,alkaloids, tannins and phenolics as bioactive antidiabetic principles.Helicteres isora have been reported to contain chloroplast pigments, phytosterol, hydroxycarboxylic acid, saponins,sugars, phlobatannins and lignin (4). The fruits contain α and β-amyrins,friedelin,lupeol and taraxerone.Seeds contain diosgenin and roots contain cucurbitacinB,isocucubritacin B,malatyamine and sitosterol (13)

Preliminary phytochemical analysis indicated that the fruit extract of Helicteres isora contain alkaloids, flavonoids ,tannins, glycosides,reducing sugars,anthraquinones and terpenes

In glucose loded animals, the drug has reduced the serum glucose levels. It is possible that the drug may be acting through potentiating the pancreatic secretion or increasing glucose uptake. Thus it is apparent that EEHI possesses antihyperglycaemic activity.The further study of isolation of active constituent by TLC and flash chromatography and antihyperglycaemic activity of isolatd constituent is ongoing

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