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Pharmacological Screening on Leaves of the Plant of *Hemionitis Arifolia* (Burm).T.Moore

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ABSTRACT

Hemionitis arifolia (Burm. f.) T. Moore, a folklore anti-diabetes fern, was evaluated for its anti-diabetic properties using rats. Glucose lowering effect and anti-diabetes activity were studied in normal rats and streptozotocin (60mg/kg) induced diabetic rats, respectively. 48 hours after STZ induction, diabetic rats received ethanolic and aqueous extracts of *Hemionitis arifolia* (Burm. f.) T. Moore orally at 250 mg/kg and 500mg/kg body weight daily for 15 days. Glibenclamide (5 mg/kg p. o) was used as reference drug. Blood glucose levels were measured on 0th, 2nd, 5th, 10th and 15th days of the study. Ethanolic and aqueous extracts were found to be to lower the levels of blood glucose in glucose fed rats. All the extracts were evaluated for preliminary phytochemical screening. When the ethanolic and aqueous extracts showed the presence of flavonoids, carbohydrates, phenolic compounds and sterols were the major phyto constituents present in the above fractions. This fraction containing flavonoids are believed to showed the anti-diabetic activity in streptozotocin induced diabetic rats as judged from blood glucose levels, body weight, biochemical parameters and histopathological studies.

Keywords: *Hemionitis arifolia* , diabetic, glibenclamide, STZ

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INTRODUCTION

Diabetes mellitus (DM) is an important medical problem and it is on the raise [1]. The available oral hypoglycaemic agents are insufficient and there is a need for discovering more effective and safe oral hypoglycaemic agents [2]. DM is known from ancient time onwards and numerous medicinal plants are used to control diabetes in traditional medicine. In south India, many medicinal plants are traditionally used to treat DM. Some of them were scientifically verified [3]. Previous works in this laboratory revealed the anti-diabetes properties of Artemisia pallens, Geophila reniformis and Cassia klenii [4-9]. Many anti-diabetes plants used in folk and tribal medicine in remote villages in Kerala state, India are not known to the main stream population.

*Hemionitis arifolia (*Burm. f.) T. Moore is found in the tropics. Within India, it is found commonly in the plain sand mountains of South India, up to an altitude of 900 m. and in Bengal, Bihar and Orissa. *Hemionitis arifolia (*Burm.) Moore (family: Hemionitidaceae) .The present study was undertaken to scientifically verify the traditional claim using experimental (normal and diabetic) rats.

MATERIAL AND METHODS

Collection of plant material

The fresh leaves of *Hemionitis arifolia* (Burm. f.) T. Moore was collected in the month of September 2009 from the foot hills of sheveroys, Salem district, Tamilnadu, India. The plant was identified and authenticated by the botanist Dr. A Balasubramanian (consultant central research) Executive Director ABS botanical garden, Salem, Tamilnadu.

Chemical and reagents

All chemicals and reagents used were analytical grade and purchased from Merck India Ltd., Mumbai and SRL, India. Streptozotocin (LOBA Chemie, Mumbai, India) was purchased, preserved at 25ºC and used for this study. Glibenclamide manufactured by Aventis Pharma Ltd. Goa, India, was collected from market and preserved at room temperature.

Preparation of *Hemionitis arifolia* extracts

The authenticated fresh leaves were dried under shade for two weeks. The plant leaves were coarsely powdered in a domestic grinder and passed through sieve no.40 and stored in air tight container in refrigerator for further use. Known weight of (500gm) coarsely powdered leaves of *Hemionitis arifolia* was extracted in a soxlet apparatus using petroleum ether, chloroform, acetone, Ethanol (hot percolation process) and aqueous extracts (cold maceration process) subsequently. The last trace of solvent was removed under reduced pressure then distillated and the crude extract was dried in a vacuum desiccators and the final residue was collected and stored in a refrigerator at 4^0c for further use.

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Preliminary photochemical screening

All the extracts of *Hemionitis arifolia* were subjected to qualitative test for the identification of various phytoconstituents. The major constituents are phenolic compounds, flavonoids, carbohydrates and sterols. The major constituents were found in aqueous and ethanolic fractions. Hence these fractions were selected for further studies.

Acute toxicity studies (OECD Guideline 423)

The acute toxicity of the extracts was determined according to the OECD guideline No. 423. Swiss Albino mice (female 20-25 gm) were used for this study. Ethanolic and aqueous extract of *Hemionitis arifolia* was given to four groups (n =3) of animals at 5, 50, 300 and 2000 mg/kg b.w. p. o. The treated animals were under observation for 15 days, for mortality and general behaviour. No death was observed till the end of the study. The test samples were found to be safe up to the dose of 2000 mg/kg b.w. The experimental protocols were approved by Institutional Animal Ethical committee (IAEC): ph.chem/24/2010/IAEC/VMCP after scrutinisation

Glucose tolerance test:

Rats were divided into indicated number of groups. Control group received the vehicle (2% gum acacia or 5% Tween 80; 1 ml, p.o.). The experimental groups received the herbal drug (water suspension or extracts or fractions) at indicated doses in an identical manner. In the screening study, a relatively high dose [500 mg (dry weight)/kg] of the water suspension of the plant powder was taken to detect activity, if any. The rats of all the groups were loaded with glucose (2 g/kg, p.o.) 30 min after herbal drug administration. Blood samples were collected by retro-orbital puncture, just 1 min prior to drug administration, and at 30, 90 and 120 min after glucose loading under mild ether anesthesia. Serum glucose levels were measured immediately. Six overnight fasted animals were used in each group.

Hypoglycemic study in normal fasted rats

To investigate hypoglycaemic effect, if any, of the ethanol extract, the overnight fasted rats were divided into two groups of six each. Control group received 1 ml of 5% Tween 80 and the experimental group received 250 and500 mg/kg ethanolic extract. Blood samples were collected at 0, 120 and 180 min after the extract administration and glucose levels were measured as described above.

Induction of experimental diabetes

Diabetes is to be induced in overnight fasted adult Wister albino rats weighing 150-200 gm by single i.p. injection of 60 mg/kg streptozotocin. Streptozotocin was dissolved in cold citrate buffer (p^H 4.5). Animals were fed with 5 % glucose solution in order to prevent hypoglycemic shock for 18 hrs. Hyperglycemia is to be confirmed by elevated blood glucose

levels in plasma, determined at 72 hrs and then on day 0 after injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as >200mg/dL. Only rats found with permanent diabetes were used for the ant diabetic study.

Statistical treatment

Statistical comparison was done using one-way ANOVA followed by Dunnets' post hoc comparison when more than two groups were involved. P values <0.05 were considered significant. When the number of groups was 2, Student's t-test was used for comparison.

RESULTS AND DISCUSSION

As shown in Table 1, in glucose tolerance test, the ethanolic and aqueous extracts of *Hemionitis arifolia* (250&500 mg/kg), when administered 30 min before glucose loading(2.5gm/kg), significantly reduced the rise in blood glucose levels at 90 and 120 min after glucose administration and % reduction was more at 120 min compared to that at 90 min. When different extracts of the plant was tested for their glucose lowering effects, using glucose tolerance test, the ethanolic and aqueous extract (500 mg/kg) showed significant activity at 90 and 120 min after glucose loading. Whereas the ethanolic & aqueous extract (250mg/kg) showed a moderate activity.

Table no 1: Effect of extracts of *Hemionitis arifolia* on Glucose tolerance test

Treatment	Blood glucose level(mg/dl)			
	0min	30 mins	90 mins	120 mins
Normal (control)	78.16±0.60	154.92±0.64	142.67±1.52	110.83±0.75
Glibenclamide 5mg/kg	76.33±0.88	105.22±0.34***	90.83±0.60***	72.75±0.66***
EEHA 250mg/kg	78.16±0.70	149.5±0.72*	138.5±0.43**	105.16±0.31**
EEHA 500mg/kg	76.83±0.87	146.33±0.33***	135.33±0.33**	98±0.364***
AEHA 250mg/kg	78.83±0.3	152.5±0.43ns	141.5±0.43ns	103.25±0.31**
AEHA 500mg/kg	81.83±1.09	148.08±0.33*	136±0.52**	101.75±0.25**

Values are given as mean ± SEM from six rats in each group.
*** Represents statistical significance Vs control (P <0.001)
** Represents statistical significance Vs control (P < 0.01)
* Represents statistical significance Vs control (P < 0.05)
ns represents non significance

As shown in Table 2, in antihyperglycemic effect of H.arifolia in STZ induced rat models showed the significant reduction of blood glucose level. The ethanolic extract of H. arifolia showed a significant activity at a dose level 250&500mg/kg where as aqueous extract showed an optimum activity at a dose level 250&500mg/kg in the 15th day of experiment.

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Table no.2: Effect of anti hyperglycemic activity of *Hemionitis arifolia* on STZ induced rat models

Treatment	blood glucose level(mg/dl)				
	0days	2days	5days	10days	15days
normalcontrol	81.16±0.602	82.16±1.196	82.33±0.56	83.5±0.429	82.16±0.705
diabetic control	82.5±1.54	293.67±1.09	321.4±0.38	375±0.82	391.33±1.23
Glibenclamide 5mg/kg	81.16±0.602	255.67±0.99**	209.83±1.4***	157±0.61***	118.33±0.56***
EEHA 250mg/kg	79.16±0.48	289.41±0.56*	275.58±0.42**	227.25±0.75**	165.5±0.43**
EEHA 500mg/kg	81.5±0.621	278.55±0.26*	247.08±0.42**	212±0.52**	148.08±0.38**
AEHA 250mg/kg	81.66±0.49	291.5±0.43^{ns}	285.16±0.54	221.83±0.4**	180.83±0.60**
AEHA 500mg/kg	79±0.732	287.75±0.63*	268±0.73	210.5±0.43**	177±0.58**

Group II is compared with group I control (P <0.001)
Group III,IV,V,VI and VIII compared with group II control
Values are given as mean ± S.E.M from six rats in each group.
*** Represents statistical significance Vs control (P <0.001)
** Represents statistical significance Vs control (P < 0.01)
* Represents statistical significance Vs control (P < 0.05)
ns represents non significance

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