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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info/jasr

ISSN
0976-9595
Research Article

Evaluation of *Ageratum Houstonianum* Whole Plant For Its Anti Diabetic Activity

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ABSTRACT

In the present study the hydro alcoholic extract of whole plant of *Ageratum houstonianum* was investigated for its anti diabetic activity at two dose levels on both alloxan induced diabetic rats and normoglycaemic rats. The blood glucose levels were estimated in both alloxan induced hyperglycaemic and normoglycaemic rats. The acclimatized animals were kept fasting for 24 h with water *ad libitum* and injected a dose of 120 mg/kg of alloxan monohydrate in normal saline intraperitoneally. Test extract at 200 and 400 mg/kg and glibenclamide at 2.5 mg/kg was administered. Blood glucose level was estimated at 0 h, 1 h, 2 h, 4 h and 8 h respectively. Hydro alcoholic extract of *A. houstonianum* whole plant was found to produce significant ($p<0.01$) reduction in blood glucose concentration between 2-4 hours of administration in both alloxan induced hyper glycaemic and normoglycaemic rats at 400 mg/kg dose. When compared with the reference control glibenclamide, the extract caused noticeable reduction in the blood glucose level in both classes of animals which was demonstrated in above methods. Hence, from the above results it can be concluded that, *Ageratum houstonianum* whole plant extract showing good anti diabetic activity.

Keywords: *Ageratum houstonianum,* Asteraceae, alloxan, anti diabetic activity, glibenclamide

1. INTRODUCTION

Ageratum houstonianum (Mill.) a member of Asteraceae is an annual erect ornamental shrub of 30-80 cm height. It is commonly known as floss flower. It is native to southeastern mexico, central America and naturalized as a weed throughout India in planes and hills and in forests as under growth [1]. Traditionally, the plant is reported to be used as a poultice for tumors [2]. Several phytoconstituents have been reported earlier from *Ageratum houstonianum*. Flavones like agehoustin A, B, C, D, eupalestin, agecorynin C [3, 4] and Pyrrolizidine alkaloids like heliohoustine, lycopsamine, and retrohoustine [5] reported from the aerial parts of the plant. From the flowers flavones agehoustine E, F, G, lucidine dimethyl ether [6] reported. Triterpenes friedlin, friedelanol [7] and steroids sitosterol, Beta, stigmasterol [8] reported from the entire plant. Oxygen heterocycles ageratone, ageratum benzofuran [9-11] from the roots and Precocenes were reported from the leaf, stem, root, flowers and leaf essential oil of the plant [12]. The leaves and essential oil of the leaf reported for antifungal activity [13]. Since the plant contains many numbers of chemical constituents but it was not reported to any medicinal properties in a scientific way, the present study was under taken to evaluate anti diabetic activity of the whole plant of *Ageratum houstonianum*.

2. MATERIAL AND METHODS

2.1. Plant material

Fresh entire young matured plants of *Ageratum houstonianum* were collected from the rural belt of Pakala, Warangal district and authenticated by Prof V. S. Raju, Taxonomist, Kakatiya University, Warangal, Andhra Pradesh, India and a voucher specimen (Ucpsc/PCOG/KSR/05/2011) was deposited in the Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam for future reference. The collected plants were dried under shade and pulverized using a mechanical grinder.

2.2. Preparation of extract

The powdered plant material (500 g) was extracted with ethanol-water (1:1) for 8 h. The liquid extract was filtered and concentrated under vacuum to yield a dry extract (yield of extract was found to be 13.98 % w/w with respect to dry material). The extract designated as HAAH and was suspended

in 0.5% w/v sodium carboxy methyl cellulose in distilled water and used for the anti-diabetic screening.

2.3. Experimental animals

Adult Wistar albino rats weighing 180-200 g (for the hypoglycaemic and wound healing screening) and Swiss albino mice weighing 20-25 g (for the gross behavioural study) of either sex were used in the studies. The animals were kept in standard polypropylene cages at room temperature of 30 ± 2 ^{0}C and 60-65 % relative humidity. The study protocols were approved by Institutional Ethical Committee of Vaagdevi College of pharmacy, Hanamkonda, Andhra Pradesh, India vide approval number 1047/AC/09/CPCSEA.

2.4. Gross behavioural and toxicity studies

The gross behavioural and toxicity study was screened as per the method of Seth *et al*[14]. Male Wistar Albino mice, weighing between 20-25 g were divided into nine groups of six animals each. The control group received 2 ml /kg distilled water orally. The other groups received the HAAH, at dose levels of 100, 200, 400, 800, 1000, 2000, 3000 and 4000 mg/kg in distilled water through oral route. After administration of the dose the animals were observed continuously for first four hours for behavioural changes and for mortality if any at the end of 72 h[14]. However, there was no mortality was observed in any of the test doses. Hence, HAAH was selected for anti diabetic evaluation at dose level of 200 and 400 mg/kg body weight.

2.5. Anti diabetic evaluation

The anti-diabetic screening of the hydro alcoholic extract of *A. houstonianum* entire plant was studied on both alloxan induced diabetic rats and normoglycaemic rats.

2.5.1. Study in hyperglycaemic rats

The acclimatized animals were kept fasting for 24 h with water *ad libitum* and injected a dose of 120 mg/kg of alloxan monohydrate in normal saline intraperitoneally. After one hour, the animals were provided feed *ad libitum*. The blood glucose level was checked before alloxanisation and 24 h after alloxanisation by withdrawing blood from the tip of the tail of each rat under mild ether anesthesia. The blood glucose level was measured with haemoglucostrips supplied by M/s Pulsatum Health Care Pvt. Ltd., Bangalore, India with the help of a Pulsatum blood glucose monitor.

Animals were considered diabetic when the blood glucose level was raised beyond twice the value of normal. This condition was observed at the end of 48 h after alloxanisation. The animals were randomly segregated into four groups of six rats in each. Group-I served as control and received vehicle (2 ml/kg) through oral route. Group-II received glibenclamide (2.5 mg/kg). Group-III and IV received the HAAH at doses of 200 and 400 mg/kg in a similar manner. Blood samples were collected from each rat by cutting the tip of the tail under mild ether anaesthesia. Blood glucose level was estimated at 0 h, 1 h, 2 h, 4 h and 8 h respectively [14, 15]. The results were shown in table 1.

2.5.2. Study in normoglycaemic rats

The animals were fasted for 18 h, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn from the tip of the tail of each rat under mild ether anaesthesia and the blood glucose was estimated as above. The normal rats were then divided into three groups of six animals each. Group-I served as control and received vehicle (2 ml/kg) through oral route. Group-II received glibenclamide (2.5 mg/kg). Group-III and IV received the extract at doses of 200 and 400 mg/kg in a similar manner. Blood glucose levels were measured after 1, 2, 4 and 8 h of administration of single dose of test samples [15, 16]. The results were presented in Table-2.

2.6. Statistical Analysis

The results are expressed as mean ± S.E.M. Significance of difference between control and treated groups was determined using Student's *t*-test and the statistical significance was set accordingly.

3. RESULTS

3.1. Gross behavioural and toxicity studies

The gross behavioural and toxicity study of the extract was studied at eight dose levels using Male Wistar Albino Mice. This study revealed no mortality with the tested doses at the end of 72 h. The animals were then kept under supervision upto 14 days after drug administration and no mortality was observed.

3.2. Anti diabetic Activity

Anti diabetic activity of the hydro alcoholic extract of the whole plant of *Ageratum houstonianum* was performed at two dose levels that is 200 and 400 mg/kg in alloxan induced diabetic model and normal rats in a scientific way. The present studies revealed that the hydro alcoholic extract of the whole plant of caused significant reduction in the blood glucose levels in the rats.

In alloxan induced diabetic rats model, standard i.e. glibenclamide group, the blood glucose level was 86.3 mg/dl at 2nd hour where as at 4th hr it was reduced to a value of 63.4 mg/dl and the percentage reduction was found to be 48.35 and 62.03 respectively and in test group at 200 and 400 mg/kg the blood glucose levels were found to be 146.5, 128.9 mg/dl and 138.2, 101.8 mg/pl at 2nd and 4th hour respectively. The percentage reductions were 20.14, 31.65 and 24.15, 45.17 at 2nd and 4th hour respectively. The results were depicted in table no. 1.

In normal rats model, standard i.e. glibenclamide group, the blood glucose level was 48.17 mg/dl at 2nd hour where as at 4th hr it was reduced to a value of 44.33 mg/dl and the percentage reduction was found to be 26.26 and 32.14 respectively and in test group at 200 and 400 mg/kg the blood glucose levels were found to be 65.0, 61.35 mg/dl and 63.21, 58.33 mg/pl at 2nd and 4th hour respectively. The percentage reductions were 4.41, 7.5 and 7.04, 12.06 at 2nd and 4th hour respectively. The results were depicted in table no. 2.

Table 1 Effect of hydro alcoholic extract of the A. houstonianum whole plant on blood glucose concentration in alloxan induced hyperglycaemic rats

Group	Treatment	Dose	Blood glucose conc. (mg/dl.)				
			0h	1h	2h	4h	8h
I	0.5% w/v Sodium CMC (Vehicle)	2 ml/kg	172.4±10.8	179.4± 9.8	182.2±11.26	186.5±11.3	190.5±8.5
II	Glibenclamide	2.5 mg/kg	167.2±9.7	121.38±11.2* (27.42%)	86.3±10.4** (48.35%)	63.4±9.7** (62.03%)	58.2±6.2** (65.19%)
III	HAAH	200 mg/kg	183.4±14.2	174.4±12.3 (4.94%)	146.5±11.6 (20.14%)	138.2±10.3 (24.65%)	128.4±8.4* (30.01%)
IV	HAAH	400 mg/kg	187.6±16.6	166.8±15.2 (11.08%)	128.2±9.64 (31.65%)	101.8±11.7* (45.71%)	98.36±7.3** (47.57%)

*Results expressed as Mean ± SEM from six observations. Figures in parentheses represent percentage reduction in blood glucose concentration, HAAH= Hydro alcoholic extract of A. houstonianum, *p < 0.01, ** p < 0.001.*

Table 2 Effect of hydro alcoholic extract of A. houstonianum whole plant on blood glucose concentration in normoglycaemic rats

Group	Treatment	Dose	Blood glucose conc. (mg/dl.)				
			0h	1h	2h	4h	8h
I	0.5% w/v Sodium CMC (Vehicle)	2 ml/kg	69.8±1.9	68.17±2.68	70±2.24	68.6±2.1	70.17± 2.02
II	Glibenclamide	2.5 mg/kg	65.3±2.0	52.83±2.5* (19.13%)	48.1±1.8** (26.26%)	44.3±1.41** (32.14%)	42.83±1.9** (34.44%)
III	HAAH	200 mg/kg	68±1.4	67.12±1.7 (1.29%)	65±1.9 (4.41%)	63.2±2.2 (7.04%)	60.5±2.3 (11.02%)
IV	HAAH	400 mg/kg	66.3±1.7	63.1±2.1 (4.77%)	61.3±1.9 (7.5%)	58.3±2.69* (12.06%)	54±3.1* (18.58%)

*Results expressed as Mean ± SEM from six observations. HAAH= Hydro alcoholic extract of A. houstonianum and Figures in parentheses represent percentage reduction in blood glucose concentration. * p < 0.01, **p< 0.001.*

4. DISCUSSION

Management of diabetes with the agents devoid of any side effects is still a challenge in the medicinal system. This concern has led to an increased demand for natural products as anti diabetic agents. Alloxan induces diabetes with a single dose of administration by selective necrotic action on the beta cells of pancrease leading to the insulin deficiency [16]. Hydro alcoholic extract of *A. houstonianum* whole plant was found to produce significant ($p<0.01$) reduction in blood glucose concentration between 2-4 hours of administration in both alloxan induced hyper glycaemic and normoglycaemic rats at 400 mg/kg dose. When compared with the reference control glibenclamide, the extract caused noticeable reduction in the blood glucose level in both classes of animals which was demonstrated in above methods.

5. CONCLUSION

Our *in vivo* study showed administration Hydro alcoholic extract of *A. houstonianum* whole plant produced a significant reduction in blood glucose level. However the higher dose 400 mg/kg was found to be more effective than the 200 mg/kg. The results of the study strongly suggest that Hydro alcoholic extract of *A. houstonianum* whole plant is useful in the treatment of Diabetes mellitus. Further studies are needed to isolate and characterize the phytoconstituent responsible for the anti diabetic activity.

6. ACKNOWLEDGEMENT

The authors express their gratitude to the principal and the management of Vaagdevi College of Pharmacy, Hanamkonda, Andhra Pradesh, India for the facilities and encouragement. The authors are thankful to Prof. V. S. Raju, Department of Botany, Kakatiya University, Warangal, Andhra Pradesh, India for authentication of the plant.

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