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DETERMINATION OF ANTI HYPERGLYCEMIC EFFECT OF ANDROGRAPHIS PANICULATA LEAVES AND FLOWER EXTRACTS

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ABSTRACT

The aim of present study was to investigate the potential roles of Methanolic extracts of Andrographis paniculata leaves and flower on reducing the blood sugar level (BSL) in alloxan- induced rats. Oral administration of Methanolic (2 g/kg b. w.) extracts of Andrographis paniculata showed the antidiabetic properties and decreased the blood glucose level by 33.71% ($p<0.0001$) (leaves) and 39.69% ($p<0.0001$) flowers respectively in alloxan induced (40mg/kg b.w.) rats. The results were also compared with that of diabetic control rats.

KEYWORDS: Andrographis paniculata, alloxan-induced, antidiabetic rats, blood sugar level

INTRODUCTION

Andrographis paniculatais a member of the family Acanthaceae and has been used for centuries in Asia to treat not only one, but several types of illness. Extensive research has revealed that the plant has a wide range of pharmaceutical activities. Hepatoprotective, antiviral, antimalarial, antithrombotic, anti-clottingand cardiovascular activitiesof Andrographis paniculata have been identified. The plant also acts as anti-inflammatory and immune stimulant.

The plant is also safe and efficacious treatment for the relief of symptoms of uncomplicated upper respiratory tract infection. The gastroprotective effect was observed due to the presence of flavonoids in the hydroalcoholic extract of Andrographis paniculata. The presence of diterpenoids such as 14-deoxyandrographolide and 14-deoxy-11, 12-didehydroandrographolide is responsible for the cardioprotective activity of Andrographis paniculata.

The leaves contain the highest amount of andrographolide (2.39%), the most medicinally active phytochemical in the plant, while the seeds contain the lowest. The formation of free radicals such as superoxide, hydroxyl radicals, lipid peroxidation and nitric oxide in in-vivo system was inhibited by the plant. The antihyperglycemic property of Andrographis paniculata have been reported in streptozotocininduced hyperglycemic rats but enough evidence was not available to confirm the hypoglycemic activity of the various extracts on different hyperglycemic conditions. It is the aim of the present study to investigate the potential roles of Methanolic extract of Andrographispaniculata in lowering blood sugar level in alloxan-induced diabetic rats.

METHODOLOGY

SAMPLE COLLECTION

The plant samples were randomly collected from the nurseries. Representative sampling should be done of specific plant parts at the growth stage that is most closely associated with critical values as provided by research data. Sampling criteria and procedures for individual samples are similar to those of soil testing in that the sample should be representative of the field. A predetermined, representative number of plants from a homogenous sampling unit contribute to the composition of bulk sample.

EXTRACTION

1. 2 kg dried powder of AP leaves was extracted by percolation with ethanol and methanol.
2. The alcoholic filtrate was concentrated under reduced pressure to yield a 200 g gummy residue.

ANIMAL AND DIET

Adult male and female albino rats weighing 180 to 200g were used in the entire study. The animals were kept at standard laboratory conditions (temperature 24±1°C, relative humidity 55 ± 5%, and a 12 hour photoperiod) for one week before the commencement of the experiment. During the entire period of study, the rats were supplied with semi purified basal diet water ad libitum.

INDUCTION OF DIABETES IN RATS

In alloxan-induced experiment, Alloxan (40 mg/kg b.w.) was injected intraperitonially in order to induce diabetes in rats and after that, the rats were fasted for 18 hours.

ESTIMATION OF BLOOD SUGAR LEVEL (BSL)

The level of glucose in blood samples from each of the experimental and control rat was determined by using standard glucose kit essentially following the glucose oxidase-peroxidase (GOD-POD) method.The blood was centrifuged to get a clear supernatant Antidiabetic Activity of Andrographis paniculata. 2 µl of serum was taken in 2 ml test solution in a separate test tube. The intensity of the color of the solution was measured spectrophoto metrically at 546 nm for quantification of the glucose initially present in the blood specimen.

FOR GLUCOSE-LOADED EXPERIMENTS

28 rats were randomly divided in equal number into four groups (marked I, II, III, IV). One group (Gr-I, 7 rats) received only distilled water and termed as vehicle control group. The three experimental groups (Gr-II, III, IV) were orally administered with 1.5 g/kg b.w glucose solution. Gr-II rats were considered as diabetic control (only glucose), while Gr-III rats received 4 mg/kg b.w. Daonil [Glibenclamide BP tablet, 5mg, a standard market drug for non-insulin dependent diabetes mellitus (NIDDM, type-2) treatment] and served as the positive control (drug treated). Gr-IV was given with either the hot water or ethanol extract at different experimental regimen and was considered as sample treated.

TIME SCHEDULE FOR GLUCOSE-LOADED EXPERIMENT

All the animals were primarily fasted for 18 hours (given only distilled water) and then glucose solution was given through feeding needle. After 2 hours, distilled water, drug solution and A. paniculata extracts, prepared with water were given orally according to rats of respective group. Two hours later, all the animals were anesthetized with diethyl ether and blood sample were collected from cardiac vessel by syringe for every observation in each study.

FOR ALLOXAN INDUCED EXPERIMENTS

Rats were grouped in an identical manner to glucose-loaded classification. Gr-I rats received only distilled water. Rats of groups-II, III and IV were intraperitonially injected alloxan tetrahydrate (40 mg/kg b.w.). Gr-II rats were considered as diabetic control (only alloxan), Gr-III rats also received 4 mg/kg b.w. Diactin (Glipizide BP tablet 5 mg, a standard drug indicated as an adjunct to diet the control of hypoglycemia in NIDDM) and termed as positive control. Gr-IV rats were treated with hot water or ethanol extract at different experimental observation and were designated as the sample treated group.

TIME SCHEDULE FOR ALLOXAN INDUCED EXPERIMENT

All the animals were injected with alloxan and were fasted for 18 hours. Then standard drug and sample extract were given orally to the rats group wise in every experiment. Two hours later of treatment, blood samples were collected as described before.

ESTIMATION OF SUGAR BY DNS METHOD

PROCEDURE

1. Weigh 100mg of the sample and extract the sugars with the hot 80% ethanol twice (5mL each time)
2. Collect the supernatant and evaporate it by keeping it on a water bath at 80°C.
3. Add 10mL water and dissolve the sugars.
4. Pipette out 0.5 to 3mL of the extract in test tubes and equalize the volume to 3mL with water in all the tubes.
5. Add 3mL of DNS reagent.
6. Heat the contents in a boiling water bath for 5min.
7. When the contents of the tubes are still warm, add 1mL of 40% Rochelle salt solution.
8. Cool and read the intensity of dark red color at 510nm.
9. Run a series of standards using glucose (0 to 500mg) and plot a graph.

Graph 1: standard DNS graph

Concentration(mg/ml)	OD Values at 510nm
0.5	0.05
1	0.1
1.5	0.15
2	0.2
2.5	0.22

Table 1: standard DNS graph values

IN VIVO ANTI DIABETIC SCREENING OF CRUDE EXTRACTS

1. Diabetes was induced by a single intraperitonial injection of alloxan monohydrate 200mg/kg dissolved in distilled water and given to overnight fasted mice.

2. After 72 hours of alloxan injection those mice that had blood glucose level ≥250mg/dl were divided into 4 groups of 2 mice each.

3. Group 1 was administered oral lying feeding tube and syringe with aqueous extract andrographis paniculata

4. Group 2 administered alcoholic extract of AP at a dose of (250, 500, 1000mg/kg), respectively.

5. Group 3 received distilled water serving as negative control.

6. All groups were fasted for 16 hours before the experiment.

7. The glucose levels were estimated by using the blood from the tested groups.

RESULTS AND DISCUSSION

In this study, the hypoglycemic and anti hyperglycemic activity of aqueous and hydro alcoholic crude extracts of Andrographis paniculata were evaluated in normal and diabetic mice.

ANTI HYPERGLYCEMIC ACTIVITY OF ANDROGRAPHIS PANICULATA BY INVITRO METHOD

Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus in animals. It induces diabetes by dose dependent destruction of β -cells of islets of langrhans. Many natural active compounds have been isolated from plants of different species.

These active principles are complex carbohydrates, alkaloids, flavonoids, saponins, amino acids, steroids, peptides, terpinoids and others. These compounds were shown to produce potent hypoglycemic, anti-hyperglycemic and glucose suppressive activities. These effects might be achieved by facilitating insulin release from pancreatic ß-

cells, inhibiting glucose absorption in gut, stimulating glycogenesis in liver and/ or increasing glucose utilization by the body.

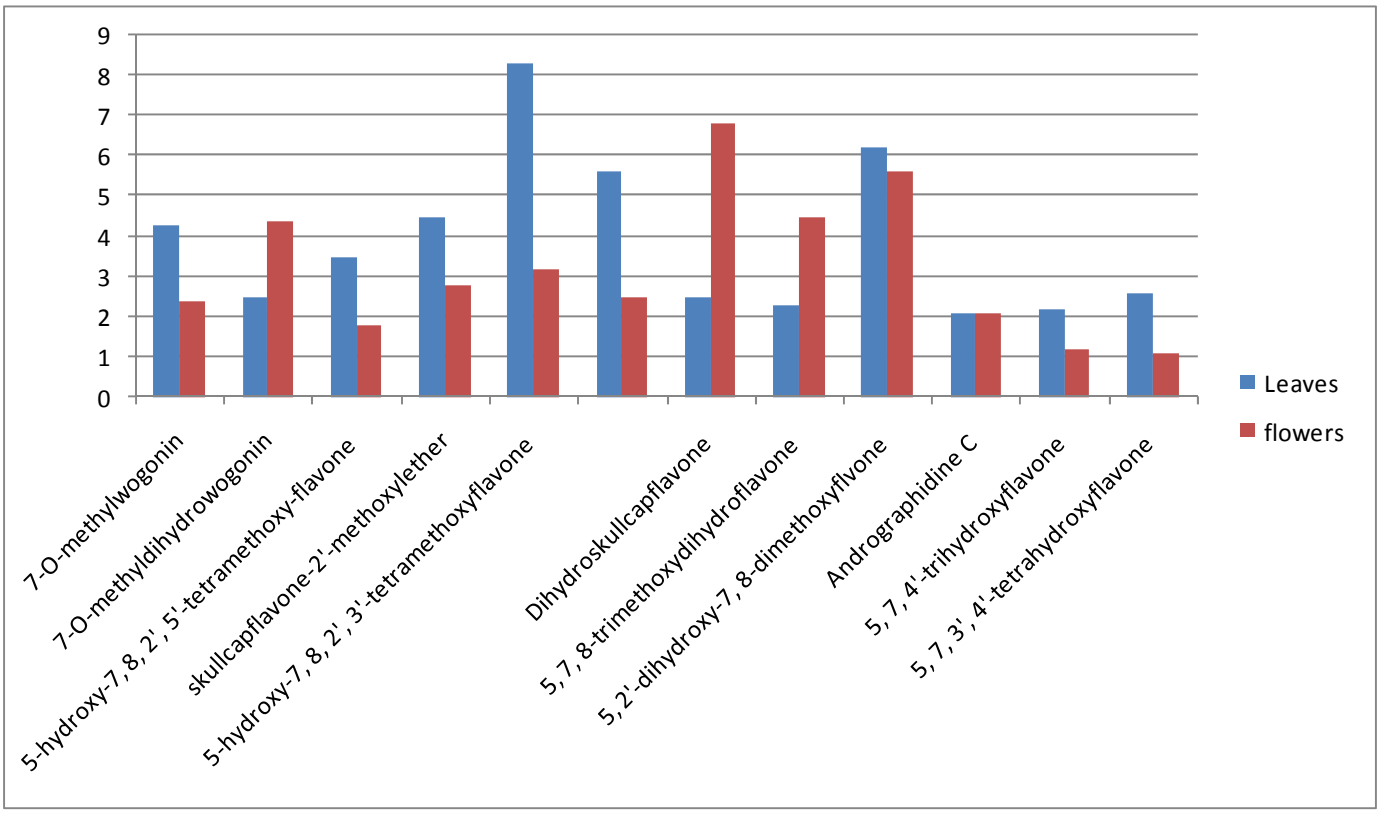
These compounds also exhibited antioxidant, hypolipidemic and anticataract activities, and restored enzymatic functions, repair and regeneration of pancreatic islets and the alleviation of liver and renal damage. Some active constituents have been obtained from plants having insulin like activity and could be provide alternative for insulin therapy. But hypoglycemic constituents may present with hyperglycemic constituents in the same species

Hydro alcoholic crude extract of Andrographis paniculata at a dose of 250mg and 500mg/kg have no any significant effect on normal glycemic mice. But it significantly reduced the blood glucose level of diabetic mice at one and two hours after treatment. But after three hours the blood glucose level started to rise. This shows that the extract may have short acting compounds and/or there could be antagonistic compounds in the extract that have longer duration of action and cause increase in blood sugar level. If the compound found in andrographis paniculatais short acting antihyperglycemic, it would be a good candidate to study to get a drug for treatment of postprandial hyperglycemia.

At the highest dose tested there was no significant reduction in blood glucose level. The reduced hypoglycemic activity could be explained in terms of the presence of non hypoglycemic substances that may obscure the hypoglycemic effect of the components present at higher doses or the presence of other antagonistic components in the extract. Practical feasibility of the effect of A. paniculata hydroalcoholic extract is questionable. Because it reduced the blood sugar level very significantly but it did not reduce to the normal blood sugar level of the mice Even though glibenclamide had antihyperglycemic effect and there are remnant β –cells in the pancreas of diabetic mice, the effect of A.paniculata may not be due to stimulation of insulin secretion because it did not reduce the blood glucose level of normal glycemic mice.

Secondary metabolite	Leaves	flowers
7-O-methylwogonin	4.3	2.4
7-O-methyldihydrowogonin	2.5	4.4
5-hydroxy-7, 8, 2', 5'-tetramethoxy-flavone	3.5	1.8
skullcapflavone-2'-methoxylether	4.5	2.8
5-hydroxy-7, 8, 2', 3'-tetramethoxyflavone	8.3	3.2
5, 4'-dihydroxy-7, 8, 2', 3'-tetramethoxyflavone	5.6	2.5
Dihydroskullcapflavone	2.5	6.8
5, 7, 8-trimethoxydihydroflavone	2.3	4.5
5, 2'-dihydroxy-7, 8-dimethoxyflvone	6.2	5.6
Andrographidine C	2.1	2.1
5, 7, 4'-trihydroxyflavone	2.2	1.2
5, 7, 3', 4'-tetrahydroxyflavone	2.6	1.1

Table 2: Antidiabetic activity of secondary metabolites from Androgrphis paniculata values



Graph 2: Antidiabetic activity of secondary metabolites from Andrograrphis paniculata

CONCLUSIONS AND RECOMMENDATIONS

Green plants synthesise and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. However, a sustained supply of the source materialoften becomes difficult due to the factors like environmental changes, cultural practices, diverse geographical distribution, labour cost and selection of the superior plant stock and over exploitation by pharmaceutical industry.

The alcoholic extract of Andrographis paniculata showed significant antidiabetic activity with minimal toxicity. Out of the fractions of alcoholic extract, has potent anti diabetic effect. Therefore the crude extract or fractionated components could be new sources of development of new plant based therapy for management of diabetes .It also supports the traditional use of the plant for diabetes mellitus.

Based on the present study the following studies are recommended to be undertaken prior to subject it to clinical trial.

• Multiple dose tests for consecutive days or chronic antidiabetic effect.

• Oral glucose tolerance test.

• Isolation and characterization of the active principles responsible for antidiabetic action.

• Sub acute and chronic toxicity test.

• Mechanism of antidiabetic action.

On the other hand, traditional medicinal plants with various active principles and properties as discussed in this article have been used since ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes, coronary heart disease and cancer.

BIBLIOGRAPHY

1. Adachi, Y., Yoshida, J., Kodera, Y., Katoh, A., Takada, J. and Sakurai, H. (2006). Bis(allixinato)oxovanadium(IV) complex is a potent anti-diabetic agent: studies on structure-activity relationship for a series of hydroxypyrone-vanadium complexes. J. Med. Chem., 49: 3251-3256.
2. Ahmad, F., Khan, M.M., Rastogi, A.K., Chaubey, M. and Kidwai, J.R. (1991). Effect of (-)-epicatechin on cAMP content, insulin release and conversion of proinsulin to insulin in immature and mature rat islets in vitro. Ind. J. Exp. Biol., 29: 516-520.
3. Ahmad, M.S. and Ahmad N. (2006). Antiglycation properties of aged garlic extract: possible role in prevention of diabetic complications. J. Nutr., 136: 796-799.
4. Ahmed, S. I. 1988. Potential of using the neem tree (Azadirachta indica) for pest control and ruraldevelopment. Neem Newsl., 5:49-55.
5. Aiyer, K. N. and Kolammal , M., 1963. Pharmacognosy of Ayurvedic Drugs, Dept of Pharcognosy, Uty. OfKerala, Trivandrum.
6. Aiyer, K. N. and Kolammal, M. 1960-1966. Pharmacgnosy of Auyrvedic drugs, Trivandrum Nos.4-9
7. Baser, K. H. C. and Bisset, N. G. 1982. Alkaloids of Sri Lankan Strychnos nux-vomica. Phytochemistry ,21 (6):1423-1429.
8. Baser, K. H. C., Bisset, N. G. and Hylands, P. J. 1979. Protostrychnine, a new alkaloid from Strychnos nuxvomica.Phytochemistry, 18 (3):512-514.
9. Chakroborty, B.K., Gupta, S., Gambhir, S.S. and Gode, K.D. (1980). Pancreatic ß-cells regeneration- a novel anti-diabetic mechanism of Pterocarpus marsupium Roxb. Ind. J. Pharmacol., 12: 123-127.
10. Chandola, H.M., Tripathi, S.N. and Udupa, K.N. (1980). Hypoglycemic response of Cinnamonum tamala in patients of maturity onset (NIDDM) diabetes. J. Res. Ayurv. Sidha, 1: 275-290.
11. Chandrasekar, B., Bajpai, M.B. and Mukherjee, S.K. (1990). Hypoglycemic activity of Swertia chirayita (Roxb. ex Flem.) Karst. Ind. J. Exp. Biol., 28: 616-618.
12. Chandrasekar, B., Mukherjee, B. and Mukherjee, S.K. (1989). Blood sugar lowering potentiality of selected Cucurbitaceae plants of Indian origin. Ind. J. Med. Res., 90: 300-305.
13. Gibson, M. R. 1978. Glycyrrhiza in old and new perspective. Lloydia, 41:348-354.
14. Gopimony, R. 1991. Sasyasabdhavali (Malayalm). Directorate of Extension, KAU, Thrissur, India. p.99.
15. Govindachari, T. R., Pal, B. R. Srinivasa, M. and Kalyanaram, P. S. 1969. Chemical examination ofAndrographis paniculata. Indian J. chem., 7:306: Chem. Abstr., 1969,70.
16. Graf, E. and Wittlinger, C. 1985. Assay of a alkaloids in vegetable drugs and galenicals of Strychnos nuxvomicaand Strychnos ignatii. Dtsch. Apoth. Ztg., 125 (46):2417-22.
17. Graves, G. 1996. Medicinal Plants-An illustrated guide to more than 180 herbal plants. Bracken Books,London. p.91.
18. Grieve, M. and Leyel, C. F. 1992. A Modern Herbal. Tiger Books International, London. pp.169-172.
19. Mathews, H. W. D., Luu, B. and Ourisson, G. 1980. Chemistry and biochemistry of Chinese drugs. Part. VI.Cytotoxic components of Zingiber zerubet, Curcuma zedoaria and C. domestica. Phytochemistry.,19:2643.

20. Matthew, K. M. 1993. The Flora of the Tamil Nadu Carnatic. Vol.3 part II. The Rapinat Herbarium,Thiruchirappalli, Tamil Nadu. p.1471.
21. Matthew, K. M. 1995. An Excursion Flora of Central Tamil Nadu, India. Oxford and IBH Publishing Co.Pvt. Ltd. New Delhi. p.273.
22. Mayer, W., Gorner, A. and Andral, K. Punicalagin and punicalin, two tannins from pomegranate peels. JutusLeibig Ann. chem., 1977, 1976.
23. Mehta, B. K., Bokadia , M. M and Mehta , S. C.,1980. Study of root oil: compound fatty acids of Curculigoorchioides roots. Indian Drugs, 18 (3) :109-110.
24. Mehta, B. K., Gawarikar, R. 1991. Characterization of a novel triterpenoid from Curcligo orchioidesGaertn. Indian J. Chemistry V. 30B (10) : 986 –988.
25. Nigam, K. B. and Kandalkar, V. S. 1995. Ashwagandha. In Chadha, K. L. and Gupta, R. (Eds.) 1995.NRF (Nagarjuna Research Foundation). Chengazhuneer Kizhangu. Express week dt. 2/5/98.NRF (Nagarjuna Research Foundation). Neermaruthu. Express week dt. 6/5/98.
26. Ogura M., Kolke, K., Cordell, G. I. and Farnsworth, N. R. 1978. Potential anticancer agents. VIII.Constituents of Baliospermum montanum (Euphorbiaceae). Planta med., 33:128.
27. Porwal, M. and Mehta, B. K., 1985. Curculigo orchioides a medicinally important plant. Nagarjun 29(3):12-13.
28. Porwal, M., Batra, A. and Mehta, B. K. 1988. Some new compounds from the rhizome of Curculigoorchioides Gaertn. Indian Journal of Chemistry. 27B :856-857.
29. Prakash, K. S. 1997. Indian Ginseng. Science Express dt. 17 June 1997. p.8.
30. Raghunathan, S. and Mitra, R. (Eds), 1982. Pharmacognosy of indigenous drugs. Vol. I and II, New Delhi.