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Antidiabetic, Antioxidant and Antibacterial Activities of Leaf extracts of *Adhatoda zeylanica*. Medic (Acanthaceae)

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Abstract

The present work is aimed at exploring the antidiabetic, antioxidant and antimicrobial activities of leaf extracts of *Adhatoda zeylanica*, Medic (Acanthaceae). The hexane (HE), chloroform (CE) and methanolic (ME) extracts were studied for their antioxidant and antidiabetic activities at two different doses (100mg and 200mg/kg body weight). The study of antidiabetic activity involves induction of diabetes to rats of all the groups using alloxan (100mg/kg i.p) followed by subsequent treatment with HE, CE and ME at two different doses each. The blood glucose level was then monitored by means of GOD/POD method and results were compared with standard drug glibenclamide (10mg/kg per day for one week). The antioxidant activity was evaluated using ferrozin reagent and compared with standard drug α- tocopherol. The influence of extract on cholesterol level was also evaluated using ferric chloride sulphuric acid reagents and cholesterol as standard.The antimicrobial activity was studied using various organisms by means of agar diffusion method. The extracts were found to exhibit 24% (HE), 36.2 % (CE) and 67.85 % (ME) of antioxidant activity. The two extracts were also found to decrease the blood glucose level and blood cholesterol levels significantly at same doses indicating the antidiabetic activity and the hexane extract was found to have an antibacterial activity. The result obtained confirms the antidiabetic, antioxidant and antimicrobial activities of the extracts thus making the extract more valuable in treating not only diabetes but also the associated secondary disorders.

Key words: Diabetes, antihyperlipidemic, antioxidant, alloxan, *Adhatoda zeylanica*

INTRODUCTION

Diabetes is one of the most prevalent metabolic disorders characterized with increased blood sugar level and improper primary metabolism [1]. Glucose is utilized by cells in body for the production of energy and it takes place in presence of hormones produced in pancreas called insulin, in the absence of which glucose gets accumulated in blood and later on excreted along with urine. Diabetes also gives rise to various secondary problems such as cataracts, micro vascular problems and neuropathy. These secondary problems commonly arise due to oxidative stress and DNA damage caused in cells by means of free radical generation [2]. It is also associated with high heart risks caused by means of improper cholesterol metabolism which in turn leads to hyperlipidemia [3] hence it is necessary to bring out an agent which is antidiabetic as well as the one which readily overcomes the oxidative stress and hyperlipidemic problems. This need prompted us the current investigation.

In the current scenario most of modern drugs have been isolated from natural sources such as medicinal plants containing a wide range of chemical compounds that serves as a leads for development of novel anti diabetic agents. The current study involves *Adhatoda zeylan ica*, Medic (Acanthaceae) which is a perennial shrub with dark green colour leaves and white flowers arranged in pedunculated spikes. The leaves were rich in quinozoline alkaloids namely vasicine, vasicinone, deoxyvasicine, vasicol, adhathodinine and vascinol. The plant has been choosen owing

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to its wide range of pharmacological activities which includes antiulcer [4], hepatoprotective [5], anti-inflammatory [6], antitussive [7], bronchodialator [8], antitubercular [9] and radioprotective [10] activities. The study not only involves the evaluation of antidiabetic activity of leaf extract but also antioxidant and antihyperlipidemic activities which may pave a way to overcome the secondary problems associated with diabetes.

MATERIALS AND METHODS

Plant Collection

The whole plant of *Adhatoda zeylan ica*, Medic leaves were collected from plant Anatomy Centre, Chennai, Tamil nadu, India and were authenticated by Dr. Jayaraman, Botanist, Plant anatomy research center, Tambaram, Chennai – 45.

Preparation of Extracts

The air dried and powdered leaves was mixed with hexane macerated and extracted to get the hexane extract(HE), the marc was then again extracted with chloroform and is concentrated by means of vacuum evaporation in order to get chloroform extract(CE). The remaining marc was then treated with methanol to get methanolic extract (ME). The extracts were then subjected to preliminary phytochemical evaluation.

Preliminary Phytochemical Analysis

All the prepared plant extracts were subjected to preliminary phytochemical screening for the presence of Alkaloids, Carbohydrates and Glycosides, Phytosterols, Flavanoids Fixed oils, Fats, Saponins, Tannins, Phenolic compounds, Proteins and free amino acids. The results were shown in table 1.

Table 1 Result of qualitative test

Sl. No.	Experiment	Hexane Extract	Chloroform Extract	Methanolic Extract
1.	Test for carbohydrates	-	-	-
2.	Test for gum and mucilage	-	-	-
3.	Test for Proteins	-	-	-
4.	Test for Alkaloids	-	+	+
5.	Test for Glycosides	-	-	-
6.	Test for Steroids	+	+	+
7.	Test for Tannins	-	-	-
8.	Test for Saponins	-	-	-
9.	Test for Flavanoids	-	+	+
10.	Test for Anthraquinones	-	-	-
11.	Test for Furanoids	-	-	-
12.	Test for Coumarin	-	-	-
13.	Test for Terpenoids	-	-	-

Table 2: Effect of oral administration of extracts in hyper glycemic rats.

Group	Treatment	Body Weight (gms)		Urine Sugar
		Initial	Final	
I	Normal	142.5± 7.73	145.6± 6.36	Nil
II	Alloxan Induced	145.2 ± 2.15	115.7± 5.2	+++
III	Diabetic+Glibenclamide	143.3± 7.74[b]	146.2± 6.62[a]	Nil
IV	Diabetic+HE (100mg/kg)	133.3± 3.1	130.2± 4.8	+++
V	Diabetic+ HE (200mg/kg)	141.4± 2.2	138.3±5.6	+++
VI	Diabetic+CE (100mg/kg)	138.3± 5.37[a]	142.6± 3.92[b]	++
VII	Diabetic+CE (200mg/kg)	145.8± 10.54[b]	160.2± 5.71[a]	+
VIII	Diabetic+ME (100mg/kg)	146.6± 9.29[b]	150.9± 5.01[a]	++
IX	Diabetic+ME (200mg/kg)	143.3± 4.04[b]	155.9± 4.9[b]	+

Values are given as ± S.E.M. for six rats in each group.
[a]P<0.001, [b]P<0.05 compared to diabetic control

Experimental animals

Albino rats (120 – 175gms) of either sex were maintained under laboratory conditions and were fed with standard laboratory diet and acclimatized for the period of 15days under standard environmental conditions of 55% humidity, temperature (22± 5°C) and 12hr dark/light cycle.

Induction of Diabetes mellitus with Alloxan

The rats were injected with alloxan (100mg/kg) i.p. in 0.2 ml saline which resulted in decrease of endogenous insulin release and caused less utilization of glucose by tissues [11]. Diabetic rats with plasma glucose level above 150mg/dl were included in the study.

Experimental Design

The rats were divided into nine groups with six animals in each group. All the groups were induced with diabetes except the control group. The standard used was Glibenclamide and was given to one group. The remaining groups were treated with two different doses 100mg/kg and 200mg/kg body weight of hexane, chloroform and methanol extracts respectively.

- Group I Normal saline treated rats
- Group II Alloxan treated rats.
- Group III Diabetic rats given aqueous solution of Glibenclamide 10mg/kg per day
- Group IV Diabetic rats given suspension of HE (100mg/kg, per day p.o for 7 days)
- Group V Diabetic rats given suspension of HE (200mg/kg, per day p.o for 7 days)
- Group VI Diabetic rats given suspension of CE (100mg/kg, per day p.o for 7 days)

- Group VII Diabetic rats given suspension of CE (200mg/kg, per day p.o for 7 days)
- Group VIII Diabetic rats given suspension of ME (100mg/kg, per day p.o for 7 days)
- Group IX Diabetic rats given suspension of ME (200mg/kg, per day p.o for 7 days)

Experimental Procedure

After diabetic induction, the rats were administered orally with respective drugs according to experimental design for one week. On the 8th day blood samples were collected by retro orbital puncture and glucose levels were estimated by GOD/POD method.

Estimation of Glucose level by GOD/POD method

The GOD/POD methods [12] is simple and rapid method which utilizes two enzymes namely glucose oxidase (GOD) and Peroxidase (POD).The collected blood samples were mixed with chromogen 4-aminoantipyrine and phenol. They were incubated at 37° C for 10minutes and then the absorbance for standard and sample were measured against reagent blank within 60minutes on an autoanalyzer at 505nm.

Estimation of Total Cholesterol by modified Zak method

The method [13] involves the subsequent treatment of blood sample with ferric chloride, glacial acetic acid, sulphuric acid and measurement of absorbance at 540 nm. Cholesterol is used as a standard and

Table 3: Effect of ***Adhatoda zeylanica*** serum glucose and total cholesterol level in diabetic rats.

Group	Treatment	Glucose (mg/dl)	Cholesterol (mg/dl)
I	Normal	113.8± 6.37	85.9± 2.94
II	Alloxan Induced	312.6± 11.8	184.7± 4.79
III	Diabetic+Glibenclamide	156.5 ±15.47 [a] (78.45%)	98.3 ±3.03 [b] (91.48%)
IV	Diabetic+HE(100mg/kg)	272.3±9.02[b] (20.19%)	143.6±3.89[b] (41.65%)
V	Diabetic+HE(200mg/kg)	256.1±6.82[b] (28.20%)	138.4±4.23[b] (46.90%)
VI	Diabetic+CE(100mg/kg)	228.7 ±15.28 [b] (42.02%)	139.1 ±3.36 [a] (46.16%)
VII	Diabetic+CE200(mg/kg)	174.5± 8.8 [a] (69.37%)	120.8 ±4.12 [b] (64.66%)
VIII	Diabetic+ME(100mg/kg)	215.4±6.86 [a] (48.71%)	86.6± 4.19 [a] (99.3%)
VII	Diabetic+ME(200mg/kg)	180.3 ±9.6 [b] (66.41%)	79.8 ±2.04 [b] (106.2%)

Values are given as mean ± SEM for six rats in each group
[a]P<0.001, [b]P<0.05 compared to diabetic control

Table 4: The Anti oxidant activity of extracts of *Adhatoda zeylanica.*

Sl. No	Sample	% of inhibitory activity
1.	Hexane Extract	24.0%
2.	Chloroform Extract	36.2%
3.	Methanol Extract	67.8%
4.	Standard (α – tochopherol)	78.6%

Table 5: Anti- bacterial activity of ***Adhatoda zeylanica*** Extracts in three different concentrations.

Extract	Zone of Inhibition (in mm)							
	Escherichia faecalis	*Staphylococcus epidermidis*	Actinomycin	*Vibrio para heamolysis*	*Serratries masciens*	*Staphylococcus heamophytenus*	*Bactere maserins*	*Vibrio cholorae*
HE 100µg/ml	13	15	16	12	14	15	12	13
HE 200µg/ml	15	18	18	14	14	17	13	16
HE 400µg/ml	18	19	19	17	17	27	14	17
CE 100µg/ml	14	14	15	15	17	13	12	15
CE 200µg/ml	16	14	15	15	19	15	14	16
CE 400µg/ml	18	15	17	16	20	17	16	19
ME 100µg/ml	14	15	14	12	12	14	13	12
ME 200µg/ml	15	16	16	14	14	16	15	14
ME 400µg/ml	16	18	17	17	16	18	16	15
Ampicillin	19	23	22	24	20	25	27	25

0.05%ferric chloride in concentrated sulphuric acid was used as a blank. The total cholesterol level was calculated.

Determination of antioxidant activity

The method [14] involves the use of α – tocopherol as standard. The blood sample and standard was treated with ferric chloride and 5mM ferrozine solution and absorbance was measured at 562 nm after 10 minutes. All the test and analysis was runned triplicate and averaged. The percentage of inhibition was calculated from the following formula,

Percentage inhibition = $[(A_0 – A_1/A_0] \times 100$

Where A_0 = Absorbance of the control

A1= Absorbance in presence of sample.

Determination of Antibacterial activity

The hexane, chloroform and methanolic extracts of *Adhatoda z eylanica* during the present work were studied for their antibacterial activity using different

clinically important strains at different concentration of 100, 200 and 400 µg/disc by agar diffusion method [15,16] against *Escherichia fa ecalis, S taphylococcus epidermidis, Acti nomycin, Vibrio paraheamolysis, Ser ratries masciens, Staphylo heamophytenus, Ba ctere ma serins* and *V ibrio cholorae.* The activity of different extracts was compared with the standard antibiotics, mentioned in table 5. The plates were incubated at 37° C for 48 hours. The zone of inhibition was calculated by measuring dimension of zone with number of microbial growth around the disc. For each value, the averages of three determinations were recorded in table 5.

Statistical Analysis

The experimental results were expressed as the mean ± SEM. The Dunnett's test was used to make a statistical comparison between groups. Results with $P< 0.05$ were considered statistically significant.

RESULTS

The presence of different phytoconstituents had been shown in table 1. The body weight and blood glucose levels of normal and experimental rats were explained in table 2. The effect of different extracts on serum glucose and cholesterol in diabetic rats had been shown in table 3. A highly significant reduction in blood glucose and cholesterol were observed in group V, VI, VII, VIII, IX. It was found that there was a significant reduction in body weight of diabetic control. The reductions in body weight of group VII animals were highly significant. The antioxidant activity of methanolic extract was found to be equal to standard hence the extract was found to posses the significant antioxidant activity. The antibacterial activity of hexane extract in the concentration of (400µg/ml) and chloroform extract (400µg/ml) has shown better activity than other extract.

DISCUSSION

In the present work, we have investigated the comparative hypoglycemic effects of the chloroform and methanolic extracts of *Adhatoda z eylanica* leaves in the alloxan diabetic model. Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas [17] *In vitro* studies have shown that alloxan is selectively toxic to pancreatic beta cells, causing cell necrosis [18] The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells [19] The results confirm the antioxidant activity of the extracts which would have also attributed to the antidiabetic activity by means of scavenging the oxygen free radicals. The preliminary phytochemical analysis shows the presence of many constituents which would have played a role in the pharmacological activities studied. The result of antibacterial studies confirms the antibacterial activity of extracts which helps in the treatment of many infections.

CONCLUSION

The study clearly shows that the extracts have either protected the cells from the toxic effect of alloxan or the cells recovered after the initial injury. The reported activities of the extracts make the leaves of plant more valuable in treating not only diabetes but also the associated secondary disorders. The plant can also be further explored for its activity against wide spectrum of microbes and can be developed into a powerful antibiotic.

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