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ANTIDIABETIC POTENTIAL OF ETHYL ACETATE EXTRACT OF *ADHATODA VASICA* NEES ON HIGH FAT DIET AND STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The present study is to investigate the antidiabetic effect of the ethyl acetate extract of *Adhatoda vasica* Nees on type 2 diabetes induced by high –fat diet and streptozotocin in diabetic male Wistar albino rats. Diabetes is a costly disease with high financial burden. This study is to treat the diabetes using natural resources. The hypoglycemic effect of ethyl acetate extract of *Adhatoda vasica* Nees was assessed by -using high fat diet (HFD)/ streptozotocin(STZ). The diabetic rats were randomly divided into four groups and treated orally by gavage with vehicle, ethyl acetate extract (200 mg/kg body weight and 400 mg/kg body weight), glibenclamide (10 mg/kg body weight) respectively, T2DM Induction through 3weeks of high fat diet (HFD) intervention was followed by single low dosage of STZ (30mg/kg dissolved in 0.1 mol/L citrate buffer at pH 4.5,i.p). Diabetic rat models showed a significant increase in blood glucose level as compared to the treated animal which was determined by OGTT.T2DM rats have decreased levels of liver glucose 6 phosphate dehydrogenase (G6PD), glutathione reduced (GSH), nitric oxide (No),and antioxidant enzymes. Furthermore, the present study has shown the hypoglycemic, hypolipidemic, and antioxidant the ethyl acetate extract of *Adhatoda vasica* Nees as confirmed by its ability for ameliorating most of the alteration caused in the studies parameters of diabetic rats.The ethyl acetate extract of *Adhatoda vasica* Nees may be useful as therapy against oxidative stress and liver damage in type2 diabetes mellitus and is therefore recommended for further studies.

KEYWORDS: T2DM, Ethyl acetate extract of *Adhatoda vasica* Nees, hyperglycemia, Lipid profile, Liver toxicity, Oxidative stress.

INTRODUCTION

The plants have been the basis for medical treatments through much of human history; traditional medicine is still widely used today. Modern medicine recognizes herbalism as a form of alternative medicine, as the practice of herbalism is not strictly based on evidence gathered using the scientific methods. Modern medicine, does, however, make use of many plant compounds are tested for pharmaceutical drugs. Scope of herbal medicine is sometimes extended to including fungal and bee products, as well as minerals, shells and certain animals. Among the 120 active compounds isolated from the higher plants widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived. Out of two thirds of the world 's plant species, at least 35,000 of which are estimated to have medicinal value that come from the developing countries. In modern pharmacopoeia in 7000 medical compounds are derived from plants. In many medicinal and aromatic plants (MAPs) significant variations of plants characteristics have been ascertained with varying soil traits, and the selective recovery and subsequent release in food of certain elements have been demonstrated. Plants had been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC. African and Native American cultures used herbs in their healing rituals, while others developed traditional medical systems such as Ayurvedic and Traditional Chinese Medicine in which herbal therapies were used.[1] Researchers found that people in different parts of the world tend to use the same or similar plants for the same purposes.

In the early 19th century, chemical analysis were for first time, became available, scientists began to extract and modify the active ingredients from plants. Later, chemists began making their own version of plant compounds and, over time, the use of herbal medicines had a decline in favour of chemical drugs. Almost one fourth of pharmaceutical drugs are derived from botanicals. In the recent past the World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care. In Germany, about 600 - 700 plant based medicines are available and are prescribed by some 70% of German physicians. In the past 20 years in the United States, public dissatisfaction

with the cost of prescription medications, combined with an interest in returning to natural or organic remedies, has led to an increase in herbal medicine use.

Medicinal herbs are significant source of synthetic and herbal drugs. In the commercial market, medicinal herbs are used as raw drugs, extracts or tinctures. Today there is growing interest in chemical composition of plant based medicine. Diabetes mellitus often simply referred to as diabetes, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). After you eating, various foods are broken down into sugars. The main sugar is called glucose which passes through your bloodstream. However, to remain healthy, your blood glucose level should not go too high or too low. When your blood glucose level begins to rise (after you eat), at the same time the level of a hormone called insulin also rise. Insulin works on the cells of your body and makes them take in glucose from the bloodstream for producing energy, and some is converted into stores of energy (glycogen or fat). When the blood glucose level begins to fall (between meals) at the same time the level of insulin also falls. Some glycogen or fat is then converted back into glucose which is released from the cells into the bloodstream. Insulin is a hormone that is secreted by beta cells of pancreas. These are part of little islands of cells (islets) within the pancreas. Hormones are chemicals that are released into the bloodstream and work on various parts of the body. Body's failure to produce insulin, and presently requires the person to inject insulin. Also called as insulin-dependent diabetes mellitus (IDDM). Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, also called insulin deficiency.[2-3] On the basis of ethno medical/tribal information *Adhatoda vasica Nees* has been used to treat and prevent diabetes.[4-6] *Adhatoda vasica Nees* possess a diverse number of pharmacological activities including antioxidant and free radical scavenging activity, anticholinesterase action[7-8] and anti-inflammatory property. However, the studies on anti-diabetic effects of *Adhatoda vasica Nees* extracts were not focused on the enzyme inhibitory activity thus, The present study is designed to evaluate the in-vivo anti-diabetic activity of ethyl acetate extract of *Adhatoda vasica Nees* and to understand how the extract acts against high –fat diet and streptozotocin.

MATERIALS AND METHODS

Preparation of extract

The whole plant of *Adhatoda vasica* Nees (Family: Acanthaceae) were collected from Doddabetta, Nilgiris, Tamilnadu and authenticated by Dr.S.Rajan, Ph.D. Field Botanist, Survey of Medicinal Plants & Collection Unit, Emerald, Nilgiris. A voucher specimen (JSSCPDP/2016-17/167) has been deposited at the Department of Phmarmacology, JSS College of Pharmacy, Udhagamandalam.

The plant material was air dried, coarsely powdered and extracted separately with ethyl acetate in a soxhlet extractor for 24 h. The extract was concentrated to dryness in a rotavapor (R-205, Buchi Laboratory equipment's, Flawil, Switzerland) under reduced pressure and controlled temperature (40-50^{o}C). The extract is stored in a refrigerator at 4^{o}C for further studies. Extract was concentrated at 40^{o}C, using a rotavapor and freeze-dried to yield about 10 g of ethyl acetate extract (EE).

Chemicals

All the chemicals used in the study are of analytical grade and are purchased from Sigma chemicals.

Animal

Healthy Male Wistar rats, weighing 180-220 g, were procured from the animal house, JSS College of Pharmacy, Ootacamund, India. The animal house was well ventilated and animals had 12±1 h day and night schedule. The animals were housed in large spacious hygienic cages during the course of the experimental period and room temperature was maintained at 25±1 °C. The animals were fed with standard rat feed and water *ad libitum*. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India (approval no. JSSCP/IAEC/PH./COLOGY-06/2016-17).

Induction of type 2 diabetes in rats

The rats were allocated into dietary regimens by feeding HFD (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) *ad libitum*, respectively, for the initial period of 2 weeks.[9] The composition and preparation of HFD were as described elsewhere. After the 4 weeks of dietary manipulation, the group of rats fed by HFD were injected intraperitoneally (i.p.) with low dose of STZ (30 mg/kg), while the respective control rats were given vehicle citrate buffer (pH 4.5) in a dose volume of 1mL/kg, i.p. The fasting blood

glucose was measured 72 houres after the STZ injection. The rats with the FBG of more than 300 mg/dL were considered diabetic and selected for further pharmacological studies. The rats were allowed to continue to feed on their respective diets until the end of the study.

Composition of high fat diet

S.No.	Ingredients	Diet g/kg
1	Powdered NPD	365
2	Lard	310
3	Casein	250
4	Cholesterol	10
5	DL-methionine	03
6	Yeast powder	01
7	Sodium chloride	01

Experimental design

In this experiment, a total of 30 rats (6 normal;24 STZ-induced diabetic rats) were used and were divided into five groups of 6 rats in each.Group I: normal control rats administered saline (NC); Group II: Diabetic control rats administered saline (DC); Group III: Diabetic + Glibenclamide (10mg/kg) (DG); Group IV: Diabetic + EEAV(200mg/kg)(DEEAR); Group V: Diabetic + EEAV(400mg/kg) (DEEAR); All the treatment groups were administered orally for 21 days. Body weight, fluid intake, food intake and glucose level were analyzed every week, Lipid and lipoprotein profile (TC, TG, HDL-c, LDL-c, VLDL-c) were analyzed in serum after 21 days.[10] The organs such as liver and kidney were isolated, weighed and stored. On the day of termination of the study, liver was used for the assay of hepatic glucose-6-phosphatase and glycogen content. Antioxidant parameters were carried in both liver and kidney.

OGTT in diabetic rats

Prior to an OGTT, diabetic rats were fasted for 16 h. Extracts and the reference drug, glibenclamide were orally administered to groups of 6 rats each. Thirty min later, glucose (3 g/kg) was orally administered to each rat with a feeding syringe. Blood samples were collected from the tail vein by tail milking at, 0 (just before the oral administration of glucose), 30, 60, 120 and 180 min after glucose load for the assay of glucose.[11]

Assay of glucose-6-phosphatase in liver

The assay of hepatic glucose-6-phosphatase activity was done by the method of Baginski *et al*.[12] Here, the glucose-6-phosphate in the liver extract is converted into glucose and

inorganic phosphate. The inorganic phosphate liberated is determined with ammonium molybdate; ascorbic acid is used as the reducing agent. Excess molybdate is removed by the arsenite citrate reagent, so that it can no longer react with other phosphate esters or with inorganic phosphate formed by acid hydrolysis of the substrate. Arsenite-citrate also stabilizes the system. The phosphate liberated per unit time as phosphomolybdous complex at 700 or 840 nm, is used to measure the activity of glucose-6-phosphatase. The glucose-6-phosphatase activity was expressed as mmol of phosphate released /min/ mg of protein.

Assay of liver glycogen content in liver

The liver glycogen content was determined by the enzymatic method.[13] Liver was homogenized with the Potter-Elvejhem homogenizer in ice-cold citrate buffer (0.1 mol/liter, pH 4.2). Immediately after homogenization, 10 ml of the mixture was used to determine free glucose in the tissue by the glucose oxidase method. The dry powder of Amyloglucosidase was mixed with the homogenate and allowed to stand for 2 hrs at room temperature. A 10 mL sample was used to determine total glucose. Initial free glucose was subtracted from the total glucose, and the resultant value was used to calculate the glycogen content. The glycogen content was expressed as mg/g wet tissue.

Estimation of antioxidant parameters

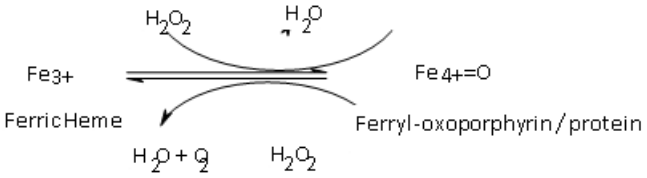
Preparation of tissue homogenate

Liver and kidney tissue parts were homogenized with 0.1 M tris-HCl buffer (pH 7.4) in Elvenjan homogenizer fitted with a teflon plunger at 600 rpm for 30 min. The homogenate was centrifuged at 2000 rpm for 10 min at 4 °C and the supernatants were used for *in vivo* antioxidant activity.

Estimation of CAT

The cells of plants, animals and aerobic bacteria contain Catalase enzyme. Catalase is located in a cell organelle called the peroxisome. In animals, catalase is present in all major body organs. The role of catalase is to scavenge hydrogen peroxide and prevent oxidative damage in the cell. Catalase is a heme containing protein that can convert hydrogen peroxide to water and oxygen in two-step reaction cycle. In the first step, one molecule of hydrogen peroxide is converted to water. The catalytic cycle begins with the oxidation of the ferric heme by two electrons by hydrogen peroxide to form the ferryl-oxo porphyrin/protein radical intermediate known as compound 1. The catalase cycle is completed by the reduction of compound 1 to

the ferric enzyme by hydrogen peroxide, resulting in production of molecular oxygen (Li and Goodwin, 2004).



Chemicals and reagents

Hydrogen peroxide (7.5 mM): 1.043 ml of 30% w/w H_2O_2 was made up to 100 mL with sodium chloride and EDTA solution (9 g of NaCl and 29.22 mg of EDTA dissolved in one litre distilled water). Potassium phosphate buffer (65 mM, pH 7.8): 2.2 g of potassium dihydrogen phosphate and 11.32 g of dipotassium hydrogen phosphate were dissolved in 250 ml and 1 L distilled water, respectively and mixed together. The pH was adjusted to 7.8 with KH_2PO_4. Sucrose solution: 10.95 g of sucrose was dissolved in 100 mL of distilled water.

Procedure

2.25 mL of potassium phosphate buffer (65 mM, pH 7.8) and 100 µL of the tissue homogenate/ sucrose (0.32 M) were incubated at 25^{o} C for 30 min. 0.65 mL of H_2O_2 (75 mM) was added to initiate the reaction. The change in absorption at 240 nm was measured for 2-3 min, and dy/dx for 1 min for each assay was calculated and the results are expressed as CAT units / mg of tissue.

$$\text{CAT (U) / 100 µlLof Sample} = \frac{[(dy/dx) \times 0.003]}{[38.3956 \times 10^{-6}]}$$

The dy/dx (change in absorbance/min) was calculated for each assay divided by 38.3956 x 10^{-6} (molar extinction coefficient of H_2O_2 at 240 nm) to obtain µM/l of H_2O_2 converted to H_2O per min, multiplied by 0.003 to obtain micromoles. H_2O_2 converted to H_2O per min in 3 ml by 0.1 ml sample.

SOD estimation

One of the most effective intracellular enzymatic antioxidants is superoxide dismutase (SOD). Superoxide dismutase is the antioxidant enzyme that catalyzes the dismutation of $O_2^{\bullet-}$ to O_2 and to the less reactive species H_2O_2. While this enzyme was isolated as early as 1939, it was only in 1969 that McCord and Fridovich proved the antioxidant activity of SOD.[14]

Superoxide dismutase exists in several isoforms, differing in the nature of active metal centre and amino acid constituency, as well as their number of subunits, cofactors and other features. In humans there are three forms of SOD: cytosolic Cu, Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD). SOD destroys $O_2^{\bullet-}$ with remarkably high reaction rates, by successive oxidation and reduction of the transition metal ion at the active site in a "Ping-Pong" type mechanism.[15]

Under physiological conditions, a balance exists between the level of reactive oxygen species (ROS) produced during normal cellular metabolism and the level of endogenous antioxidants, which serve to protect tissues from oxidative damage.

Disruption of this balance either through increased production of ROS or decreased levels of antioxidants produce a condition known as oxidative stress and leads to variety of pathological conditions. To protect against oxidative damage, organisms have developed a variety of antioxidant defenses that include metal sequestering proteins, use of compounds such as vitamin C, E and specialized antioxidant enzymes. One family of antioxidant enzymes, the superoxide dismutases (SOD) functions to remove damaging ROS from the cellular environment by catalyzing the dismutation of two superoxide radicals to hydrogen peroxide and oxygen.[16]

$$O_2^{-\cdot} + O_2^{-\cdot} \xrightarrow[2H^+]{SOD} O_2 + H_2O_2$$

SOD measurement was carried out on the ability of SOD to inhibit spontaneous oxidation of epinephrine to adrenochrome.

Chemicals and reagents

Sodium carbonate buffer (0.05 M, pH 10.2): 5.3 g of sodium carbonate and 1.2 g of sodium bicarbonate were dissolved separately in 1:1 of distilled water, which served as a stock solution. Buffer was prepared by mixing 64 mL of sodium carbonate and 70 mL of sodium bicarbonate solutions. The pH of the buffer was adjusted to 10.2 using the above stock solution accordingly.

Adrenaline (9 mM): 0.03 g of adrenaline was dissolved in distilled water and the final volume was made up to 10 mL with distilled water containing a drop of concentrated HCl (to bring pH down to 2). Adrenaline being sensitive, the vial was kept covered with aluminum foil at all times.

Sucrose (0.3199 M) solution: 10.96 g of sucrose was dissolved in distilled water and the volume was made up to 100 mL.

Procedure

2.8 mL of sodium carbonate buffer (0.05 mM) and 0.1 mL of tissue homogenate or sucrose (blank) was incubated at 30 ^{o}C for 45 min. The absorbance was then adjusted to zero to sample. Thereafter the reaction was initiated by adding 10 µL of adrenaline solution (9 mM). The change in absorbance was recorded at 480 nm for 8-12 min. The assay was maintained the temperature at 30 ºC. The SOD calibration curve was prepared the standard solution 10 unit/mL by taking.One unit of SOD produced approximately 50% inhibition of auto-oxidation of adrenaline. The results were expressed as unit (U) of SOD activity/mg of tissue.[17]

Thiobarbituric acid reactive substances (TBARS) estimation

Lipid peroxidation is commonly regarded as a deleterious process leading to structural modification of complex lipid protein assemblies, such as biomembranes and lipoproteins, and is usually associated with cellular malfunction. During lipid peroxidation, a polar oxygen moiety is introduced into the hydrophobic tails of unsaturated fatty acids. This process is of dual consequence: the presence of hydroperoxy group disturbs the hydrophobic lipid/lipid and lipid/protein interactions, which leads to structural alterations of biomembranes and lipoproteins; hydroperoxy lipids are sources for the formation of free radicals. When free radicals are generated, they can attack polyunsaturated fatty acids in cell membrane leading to a chain of chemical reactions called lipid peroxidation. As the fatty acid is broken down, the hydrocarbon gases and aldehyde are formed. The most common method used to assess malondialdehyde (MDA) is the thiobarbituric acid assay.[18]

Chemicals and reagents

Thiobarbituric acid solution: 0.8 g of thiobarbituric acid was dissolved in distilled water and volume was made up to 100 mL. The pH was adjusted to 7.4 with 1 N NaOH / 0.1 N HCl solution.

Acetic acid solution: 20 mL of acetic acid was dissolved in distilled water and the volume was made up to 100 ml with distilled water. The pH was adjusted to 3.5 with 1 N NaOH/0.1 N HCl solution.

Sodium lauryl sulfate solution: 8.1 g of sodium lauryl sulfate was dissolved in distilled water and the volume was made up to 100 mLwith distilled water.

Mixture of n-butanol and pyridine (15:1 v/v): 15 ml of n-butanol and 1 ml of pyridine were mixed together.

Reagents

Thiobarbituric acid solution: Thiobarbituric acid (0.8 g) was dissolved in distilled water and volume was made up to 100 ml with distilled water. The pH was adjusted to 7.4 with 1 N NaOH/0.1 N HCl solution.

Acetic acid solution: Glacial acetic acid, (20 ml) was dissolved in distilled water and the volume was made up to 100 ml with distilled water. The pH was adjusted to 3.5 with 1 N NaOH/0.1 N HCl solution.

Sodium lauryl sulfate solution: Sodium lauryl sulfate (8.1 g) was dissolved in distilled water and the volume was made up to 100 mL with distilled water.

Mixture of n-butanol and pyridine (15:1 v/v): n-butanol (15 mL) and 1 mL of pyridine were mixed together.

Procedure

To 1 mL of tissue homogenate, 0.2 ml of sodium lauryl sulfate solution (8.1%), 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of thiobarbituric acid solution (0.8% w/v, pH 7.4) were added. This incubation mixture was made up to 5.0 mL with double distilled water and then heated in boiling water bath for 30 min. After cooling, the red chromogen was extracted with 5 mL of the mixture of n-butanol and pyridine (15:1 v/v) and centrifuged at 4000 rpm for 10 min. The 532 nm was absorbance and measured by the organic layer.The results were expressed as nM of MDA/mL for serum and nM of MDA/mg for tissue using molar extension co-efficient of the chromophore ($1.56 \times 10^5\ M^{-1}\ cm^{-1}$).[19]

$$\text{nM of MDA/mg of tissue or ml of serum} = \frac{\text{OD} \times \text{volume of homogenate} \times 100 \times 10^3}{1.56 \times 10^5 \times \text{volume of extract taken}}$$

RESULTS

Effect of extract on OGTT

The results of OGGT in diabetic rats are given in (Table 1). The data reveal that the extract cause a significant decrease in the blood glucose level at 90 min after oral administration to HFD and STZ-diabetic rats. The plant root ethyl acetate extract of the *Adhatoda vasica* Nees produce a significant attenuation ($p<0.001$) in the blood glucose level at 120 min to 180 min when compared with the diabetic control.

Effect of extracts on blood glucose level

The results of the blood glucose level are given in (Table 2). The data reveal that the extracts and fractions cause significant ($p<0.001$) time-dependent decrease in the blood glucose level after oral administration at 7, 14 and 21 days when compared to the diabetic control group. After 21 days of daily treatment with the plant root ethyl acetate extract of the *Adhatoda vasica* Nees (200 and 400 mg/kg) results show significant dose-dependent fall in blood glucose levels by 39, 53, 42 and 53%, respectively. Glibenclamide (10 mg/kg) treated rats also show significant decrease in the blood glucose level (51 %).

Effect of extract on body weight, fluid and food intake

The results of body weight, fluid and food intake are given in (Table 3). The data reveal that the normal control rats show an increase in body weight from 290.83±0.54 to 339±0.36 g. The diabetic control rats also show an increase in body weight from 284.33±0.61 to 316.50±0.43 g. The plant root ethyl acetate extract of the *Adhatoda vasica* Nees (200 and 400 mg/kg) treated diabetic rats show gradual increase in body weight from 286.50±0.50 to 324.50±0.62 g and 273.67±1.13 to 315.50±0.43 g, respectively.

In chronic treatment both the extract show a significant decrease in the fluid and food intake as compared to diabetic control ($p<0.001$).

Effect of extract on glucose-6-phosphatase and liver glycogen content

The results of glucose-6-phosphatase and liver glycogen content are given in (Table 4). The data reveal that the glucose-6-phosphatase activity in the liver show significant decrease ($p<0.001$, $p<0.01$) in the plant root ethyl acetate extract of the *Adhatoda vasica* Nees when compared to the diabetic control group. Similarly, liver glycogen content also show a significant increase ($p<0.001$, $p<0.01$) in the extract when compared to the diabetic control group.

Effect of extract on lipid and lipoproteins

The results of lipid and lipoproteins are given in (Table 5). The data reveal that the diabetic animals show a significant increase in the level of TC, TG, LDL and VLDL cholesterol and a decrease in the level of HDL cholesterol in serum, when compared to the normal animals ($p<0.001$). The levels of TG, TC, LDL and VLDL significantly decreases ($p<0.001$) whereas HDL significantly increases ($p<0.001$) in the plant root ethyl acetate extract of the *Adhatoda vasica* Nees will when compared to the diabetic control group.

Effect of extract on antioxidant parameters

The results of antioxidant parameters in liver and kidney are given in Table6-7.The data reveal that in diabetic animals, a significant decrease ($p<0.001$) in SOD and CAT enzyme levels are observed, whereas, the level of TBARS significantly increases ($p<0.001$) as compared to the normal control rats in liver and kidney. The plant root ethyl acetate extract of the *Adhatoda vasica* Nees will be treated animals, there is a significant ($p<0.001$) increase in SOD and CAT enzyme levels where as the level of TBARS decreases significantly ($p<0.001$) compared the diabetic control rats.

1. Effect of extract on the OGTT in HFD and STZ-diabetic rats

Values are expressed as mean±SEM (n = 6).

$^{###}p<0.001$ compared with normal control, ******* $p<0.001$ compared with diabetic control.

Two way ANOVA followed by Bonferroni's multiple comparison tests.

2. Effect of extract on the blood glucose level in HFD and STZ-diabetic rats

Groups	Treatment	Blood glucose level (mg/dL)			
		0 day	7th day	14th day	21th day
I	Normal control	89.50±0.84	90.77±0.67	90.93±0.87	90.73±1.05
II	Diabetic control	317.33±1.17	374.33±1.17$^{###}$	394.33±1.20$^{###}$	405.66±1.52$^{###}$
III	Glibenclamide (10mg/kg)	331.50±1.08	258.16±3.41***	134.16±1.51***	131.83±1.04***
IV	EEAV(200mg/kg)	335.33±0.91	275.67±0.99***	223.36±1.33***	162.16±1.04***
V	EEAV(400mg/kg)	320.16±1.15	264.66±1.08***	200.16±1.16***	150.50±1.31***

Values are expressed as mean±SEM (n = 6).

$^{###}p<0.001$ compared with normal control, ******* $p<0.001$ compared with diabetic control.

Two way ANOVA followed by Bonferroni's multiple comparison tests.

3. Effect of extract on body weight, fluid and food intake in in HFD and STZ-diabetic rats.

Groups	Treatment	Body weight (g)		Fluid (mL/rat/day)		Food (g/rat/day)	
		Before	After	Before	After	Before	After
I	Normal control	290.83±0.54	339±0.36	144.33±0.61	144.83±0.54	27.67±0.42	28±0.36
II	Diabetic control	294.33±0.61	326.50±0.43 ###	155.50±0.43	175.83±0.60 ###	37.67±0.49	49.16±0.47 ###
III	Glibenclamide (10mg/kg)	267±0.44	324.17±1.49 ***	142±0.57	148.16±0.54 ***	34.83±0.30	39.33±0.30 ***
IV	EEAV(200mg/kg)	296.50±0.50	354.50±0.62 ***	154.50±0.71	159.33±0.42 ***	38.50±0.43	48.66±0.33 ***
V	EEAV(400mg/kg)	283.67±1.13	335.50±0.43 ***	150.83±0.54	155.50±0.43 ***	36.33±0.55	45±0.57 ***

Values are expressed as mean±SEM (n = 6).

###p<0.001 compared with normal control, *** p<0.001 compared with diabetic control.

Two way ANOVA followed by Bonferroni's multiple comparison tests.

4. Effect of extracts on glucose-6-phosphatase and liver glycogen content in HFD and STZ-diabetic rats.

Groups	Treatment	Glucose -6-phosphatase (mmol/min/mg)	Liver glycogen content (mg/g wet tissue)
I	Normal control	0.28±0.04	23.50±0.76
II	Diabetic control	0.70±0.05###	26±0.25###
III	Glibenclamide (10mg/kg)	0.37±0.04***	21.50±0.34***
IV	EEAV(200mg/kg)	0.44±0.00***	28.87±0.22**
V	EEAV(400mg/kg)	0.49±0.04***	30.33±0.21***

Values are expressed as mean±SEM (n = 6).

###p<0.001 compared with normal control, *** p<0.001 compared with diabetic control.

One way ANOVA followed by Bonferroni's multiple comparison tests.

5. Effect of extract on serum lipid and lipoprotein profile in HFD and STZ-diabetic rats

Groups	Treatment	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
I	Normal control	98.17±0.65	89.83±1.04	61.50±1.15	28.50±1.23	25.97±0.20
II	Diabetic control	174.7±1.57###	187±1.31###	28.17±0.65###	46.17±0.75###	37.40±0.24###
III	Glibenclamide (10mg/kg)	106.3±1.20***	104.3±0.80***	40.83±0.74***	21±0.36***	22.97±0.56***
IV	EEAV(200mg/kg)	129±0.61***	135.3±1.23***	52.17±0.83***	35.50±0.95***	38.57±0.64***
V	EEAV(400mg/kg)	136.2±0.75***	140.3±0.95***	55±0.81***	39.50±0.80***	40.63±0.54***

Values are expressed as mean±SEM (n = 6).

###p<0.001 compared with normal control, *** p<0.001 compared with diabetic control.

One way ANOVA followed by Bonferroni's multiple comparison tests.

6. **Effect of extracts on antioxidant parameters in HFD and STZ-diabetic rats liver.**

Groups	Treatment	CAT (IU/min/mg of tissue)	SOD (IU/min/mg of tissue)	TBARS (nmole of MDA/mg of tissue)
I	Normal control	16.39±0.49	11.70±0.49	12.50±0.42
II	Diabetic control	7.33±0.33$^{###}$	5.67±0.33$^{###}$	34.20±0.60$^{###}$
III	Glibenclamide (10mg/kg)	11.30±0.21***	9.33±0.33***	15.20±0.30***
IV	EEAV (200mg/kg)	12.80±0.30***	10.20±0.26***	15.70±0.42***
V	EEAV (400mg/kg)	12.30±0.30***	11.08±0.81***	19.80±0.42***

Values are expressed as mean±SEM (n = 6).

$^{###}$p<0.001 compared with normal control, ******* p<0.001 compared with diabetic control.

One way ANOVA followed by Bonferroni's multiple comparison tests.

7. **Effect of extract on antioxidant parameters in HFD and STZ-diabetic rats kidney.**

Groups	Treatment	CAT (IU/min/mg of tissue)	SOD (IU/min/mg of tissue)	TBARS (nmole of MDA/mg of tissue)
I	Normal control	14±0.57	9.50±0.42	10.50±0.56
II	Diabetic control	5.83±0.30$^{###}$	4.33±0.21$^{###}$	22.50±0.42$^{###}$
III	Glibenclamide (10mg/kg)	11±0.21***	7.17±0.70***	12.50±0.30***
IV	EEAV (200mg/kg)	11.70±0.36***	8.43±0.28***	16.80±0.49***
V	EEAV (400mg/kg)	12.80±0.33***	9.10±0.38***	18.50±0.21***

Values are expressed as mean±SEM (n = 6).

$^{###}$p<0.001 compared with normal control, ******* p<0.001 compared with diabetic control.

One way ANOVA followed by Bonferroni's multiple comparison tests.

DISCUSSION

We have investigated that the *Adhatoda vasica* Nees which is used in traditional ayurvedic medicine for the treatment of several diseases, has been serving as anti-diabetic potential. Previously, this beneficial and priceless herb was not been investigated for its *in vivo* anti-diabetic activity. This herb has clearly recognized the potential of anti-diabetic activity.The treatment goal of diabetes patients is to maintain glycemic level in control, in both the fasting and post-prandial states. Many natural resources have been investigated for the suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine.[20-21] The inhibition of their activity, in the digestive tract of humans, is considered to be effective to control diabetes by diminishing the absorption of glucose decomposed from starch by these enzymes.[22-23] Therefore, effective have long been sought. In this study we have investigated the anti-diabetic potential of the *Adhatoda vasica* Nees, which is used in

traditional ayurvedic medicine for the treatment of several diseases.[24-25] This valuable herb was not previously investigated for its *in vivo* anti-diabetic activity. However, our study clearly established the anti-diabetic potential of *Adhatoda vasica* Nees, and revealed that the active principles responsible. Anti-oxidants, may prevent the progressive impairment of pancreatic beta-cell function due to oxidative stress and may thus reduce the occurrence of type 2 diabetes. Although, in the present study, the enzyme inhibitory activity of these extract were assayed *in-vivo*, the results from this work should be relevant to the human body. This supportive evidence further increases the medicinal importance of this *Adhatoda vasica* Nees,indicating that this herb is not only beneficial for diabetes but also may be useful to a number of other human health complications. To maintain glycemic level in control, in both the fasting and post-prandial states is the treatment goal of diabetes patients. The suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine have been investigated using natural resources.[26] The present study confirms the whole of plant ethyl acetate extract of the *Adhatoda vasica* Nees hypoglycemic effect and improved the lipid profile. The plant root extract of the *Adhatoda vasica* Nees efficiency in treating type2 diabetic mellitus was variable and depending on the estimated parameters.Furthermore, plant root extract of the *Adhatoda vasica* Nees may be useful as therapy against oxidative stress and liver damage in type 2 diabetic mellitus and is therefore recommended for further studies. These effects should be studies further in human volunteers and diabetic patients.

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